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Temperate oceanic treelines

Low temperature effects
on photosynthesis and growth

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A thesis submitted in partial fulfilment of the degree of

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Department of Biological and Biomedical Sciences

Durham University

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Abstract

Altitudinal treelines form where tree growth is limited by low growing season temperatures. However, exactly what aspects of temperature are critical remains unclear. Temperate New Zealand treelines are at a lower altitude than in comparable temperate regions elsewhere. Past studies have shown them to be warmer, and suggested that New Zealand montane trees are not capable of growing at cool temperatures.

A detailed study at six sites showed that New Zealand treelines are not anomalously warm, but instead are within the global range of growing season soil temperature at treeline. The thermal environment in summer did not differ between abrupt and gradual treelines, but winters were much colder at the former. The consistency of mean daily minimum air temperature during the growing season at 20 oceanic treelines across the New Zealand archipelago suggests that thermal thresholds to tree growth are better described by minimum temperature, rather than often-used mean temperature. Extreme freezing temperatures are unlikely to control treeline position through dieback of adult trees, as the frost tolerance of trees of all species studied was in excess of the extreme minimum temperatures experienced at the New Zealand treeline. Overall, the proposition that an absence of hardy taxa in New Zealand has resulted in low treelines appears incorrect.

In controlled environment experiments, the response of photosynthesis and growth to growing season temperatures differed between the six treeline species. Three species showed evidence of reduced growth activities at low temperature (carbon sink limitation), whereas one species showed reduced growth at low temperature because of limited carbon acquisition (carbon source limitation). Different measures of sink- and source activities provided support for both hypotheses for two other species. These results highlight species-specificity in the temperature response of photosynthesis and growth as well as the complexity in the interpretation of carbon sink and source limitation hypotheses.

A combined approach in the field will be required to untangle the processes and thresholds that interact to determine tree growth and treeline positions.

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Acknowledgements

It’s impossible to look into the future, but when I started, the PhD path ahead looked straight forward. The journey turned out a bit more rocky, and the tracks up to treeline definitely steeper, than anticipated, but it was an amazing experience nonetheless. Now, three years after I set off, I would like to take this opportunity to thank everybody who helped me along the way.

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…

doar steet ’n boom
doezend joar hef e doar ewes
stil en gewoon
veertig generasies hef edoar alleen estoan
hie hef in die tied heel wat an em vuurbie zeen goan
O, grote keal van holt
meer dan doezend joarn old
‘k wol daj proatn konn
dan zol ie oons kunn verteln
van de dingn die bunt ewes

…

Erik Dam
Oonder an ’n Fleringer Er – De Kroezenboom
(From: Taal tussen Oldemarkt en Oldenzaal)

Chapter 1 Introduction

1.1 Trees at their altitudinal limit

Trees at their distributional boundaries are limited by a combination of abiotic and/or biotic factors, including moisture, temperature, species-specific traits and competition with species better adapted to the local conditions (e.g., Woodward 1995, Loehle 1998). The altitudinal limit of trees is represented by the treeline, associated with an increasingly unfavourable climate at higher altitude.

Patterns in global treeline altitude, generally increasing with decreasing latitude and increasing continentality (Leuschner 1996, Körner 2003a, Caccianiga et al. 2008), have led to correlations of treeline altitude with climate, and in particular with temperature (see Tranquillini 1979). A comprehensive study by Körner and Paulsen (2004), described a remarkably consistent mean soil temperature during the growing season at altitudinal treelines across the globe. In contrast, other potential determinants (e.g., growing season length, thermal sums, thermal extremes, snow-pack duration and precipitation) varied considerably by site or region (Körner and Paulsen 2004). The latter study has provided support for the general consensus that some level of growing season warmth is required for tree growth, which is not attained at higher altitudes and that this low temperature, or heat deficiency, leads to the formation of treelines (Tranquillini 1979, Grace 1989, Wardle 1993, Körner 1998). However, it remains unclear exactly how temperature limits the growth of trees and affects treeline position.

1.2 Defining treeline

Over centuries of research in this distinct ecotone, ‘treelines’ have been variably referred to as tree-lines, timber-lines or forest-lines (Körner 1998). Many variations on the definition of treeline exist, but most include some threshold relating to tree height (see Holtmeier 2009). Setting an absolute minimum for tree height is arbitrary, as this may vary due to species-specific differences or variation in climatic conditions (e.g., within snow cover or not). However, in general, the climate decouples from the soil surface from around 2 m height, and most recent treeline definitions therefore have variable height thresholds of 2-3 m. A schematic representation of the conventions of

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Photo previous page: Overlooking the gradual treeline ecotone near Kelly Creek

Photo previous page: A cloudy day in the upper montane forest, 150 m below treeline at Camp Creek

terminology used in this study is presented in Figure 1.1. I will follow Körner and Paulsen (2004), and define ***treeline*** as the line between groups of (2 or more) trees that are at least 3 m tall (Figure 1.1). Isolated individuals of any stature may occur above that line, and where they cease is referred to as the ***tree species line***. For the upper limit of the closed canopy forest (where the > 3 m canopy covers more than 50% of the ground area), I will use the term ***upper forest limit*** rather than (upper) "timberline", to disassociate the terminology from that used by the commercial logging industry (Bakker et al. 2008). The ***treeline ecotone*** encompasses the zone from the upper forest limit to the tree species line. For the purpose of this study, a treeline ecotone will be considered an ***abrupt treeline ecotone*** if the distance from the closed canopy forest to a full cover of tussock grassland is < 10 m. Alternatively, the treeline ecotone may be diffuse, often extending over more than 100 altitudinal metres (and up to 500 m), in which case it will be referred to as a ***gradual treeline ecotone.***

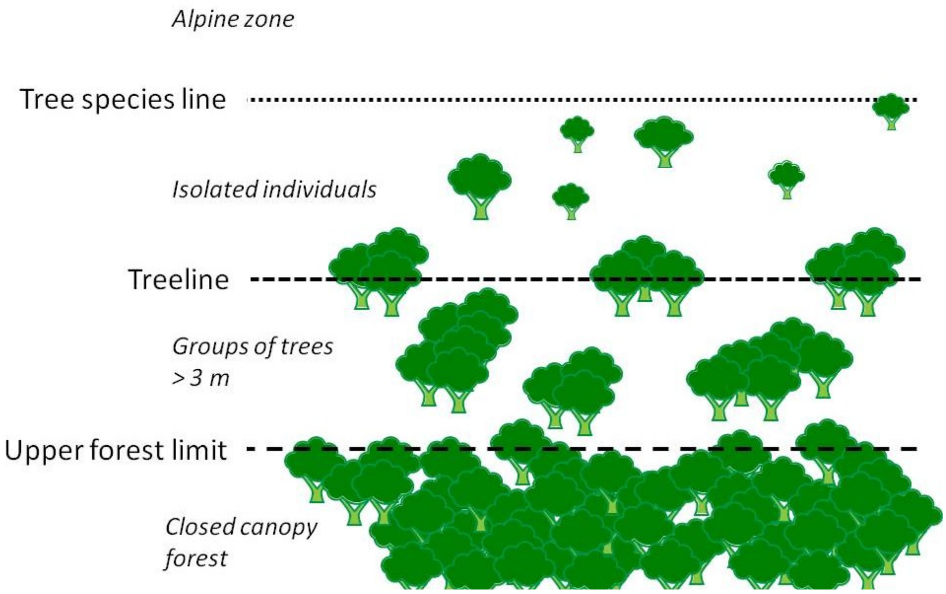

Figure 1.1. A schematic representation of treeline terminological conventions used in this study (after Körner and Paulsen 2004).

1.3 Treeline drivers

It is generally recognised that the treeline phenomenon is caused by low temperature (Wardle 1993, Körner 1998, 2007a, Holtmeier 2009, Richardson and Friedland 2009). However, it remains unknown through which exact mechanisms and processes low temperature limits tree growth, or which aspects of low temperature are most important (e.g., time of day or year) (Körner 1998). Interactions of abiotic and biotic factors result in complex differences in local microclimatic conditions; for example, wind, solar radiation, topography and surrounding vegetation can have strong modifying effects on the temperature experienced by trees (Grace et al. 1989, Smith et al. 2003, Körner and Paulsen 2004) (Figure 1.2).

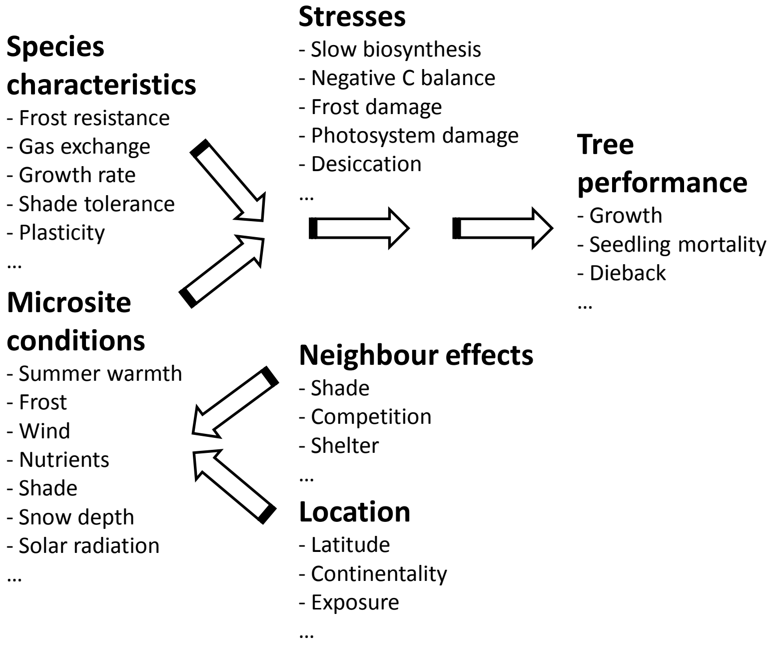

Figure 1.2. Multiple factors and processes interact to affect tree performance at treeline. Lists are not exhaustive (indicated by "..."). Modified from Harsch & Bader (2011).

Several, not necessarily mutually exclusive, theories of how low temperature affects treeline formation have been put forward, and they have been summarised in numerous studies (Tranquillini 1979, Stevens and Fox 1991, Wardle 1993, Körner 1998, Körner 2003a, Smith et al. 2003). Most recently, treeline studies have focussed on limitations to regeneration and tree establishment (Maher and Germino 2006, Ninot et al. 2008, Wesche et al. 2008, Batllori et al. 2009, Piotti et al. 2009), limitations caused by frost, or phototoxic effects after frost (Germino and Smith 1999, Piper et al. 2006, Molina-Montenegro et al. 2011), and on whether low temperature invokes a carbon-source or carbon-sink limitation. A carbon-source limitation involves restriction of photosynthate production, whereas a carbon-sink limitation restricts the ability of trees to grow by constraining incorporation of photosynthate into tissues (Körner 1998). Körner and Paulsen (2004) suggested these two carbon-balance related hypotheses could be tested in different ways. First, non-structural carbohydrates in tree tissues at treeline should increase with altitude if low temperatures restrict growth more than photosynthesis. Second, *in situ* enrichment with carbon-dioxide (CO_2) should increase the source but not the sink for photosynthate, and thus not increase growth at treeline.

Evidence is equivocal for both tests. Whilst some studies have found higher levels of non-structural carbohydrates at treeline than at lower altitudes, suggestive of a carbon-sink limitation (e.g., Hoch et al. 2002, Li et al. 2002, Hoch and Körner 2003, Piper et al. 2006, Hoch 2008, Shi et al. 2008, Fajardo et al. 2011), other studies have not (e.g., Li et al. 2008b, Bansal and Germino 2010a, Susiluoto et al. 2010, Sveinbjörnsson et al. 2010, Gruber et al. 2011). Likewise, a 9-year experimental enrichment of the ambient atmosphere with CO_2 at treeline in the Swiss Alps stimulated the growth of *Larix decidua* but not that of *Pinus mugo* (Dawes et al. 2011). In summary, whilst it has been demonstrated that many trees are not carbon-limited at treeline, some may be.

Although there has been an extensive search for one unifying explanatory factor of treeline (Körner 1998, Körner and Paulsen 2004), this approach may be overly simplistic (Smith et al. 2003) as factors controlling treeline position are strongly scale-dependent. Temperature may correlate with global treelines, but when looking at regional and local scales, other factors (e.g., precipitation or exposure) may become increasingly important (Holtmeier and Broll 2005, Kessler et al. 2007). Additionally, differing treeline forms (abrupt or gradual) suggest that different mechanisms are involved, which may result in diverse treeline dynamics, for example, in relation to global change (Harsch et al. 2009, Harsch and Bader 2011). The interplay of species characteristics with the conditions in which they grow is likely to be important, and there is a need to explore the importance of these regional and taxon-specific differences (Kessler et al. 2007).

1.4 New Zealand treeline

New Zealand treelines provide an interesting testing ground for the effects of temperature on trees in the ecotone, since they exhibit a number of anomalous features, two of which will be addressed in this study. Firstly, not only are Southern Hemisphere treelines in general lower in altitude than treelines at similar latitudes in the Northern Hemisphere (Körner 1998), but they have also been found to be anomalously warm compared to their northern counterparts (Körner and Paulsen 2004). An absence of taxa that can withstand the harsh conditions at potential treeline has been suggested to cause these low, and therefore warm, treelines (Körner and Paulsen 2004, Wardle

2008). This is supported by the observation that Northern hemisphere treeline conifers can grow and establish successfully above the native treeline (Wardle 1985a, 1998).

Secondly, unlike most natural treelines elsewhere, which are part of a gradual ecotone, New Zealand has treelines of contrasting form (Plate 1.1). Abrupt treelines are formed by species of *Nothofagus* (Southern beech), whereas in areas without *Nothofagus* (Figure 1.3) the mixed conifer-broadleaf forests form gradual treeline ecotones. This provides the opportunity to test whether species in these contrasting ecotones are affected by different limiting processes.

New Zealand, a small island archipelago in a large ocean, has a generally oceanic climate. The main mountain range is the Southern Alps, which extends most of the length of the South Island (Figure 1.3a). As a result of its orientation perpendicular to the prevailing westerly winds, the western side of the ranges experience high levels of cloud cover and precipitation. East of the Southern Alps, however, the winds bring much drier weather. The climate in this rain-shadow region is more continental, with dry, hot summers and cold winters (McCracken 1980, Salinger 1988). The altitude attained by native treelines in New Zealand varies from *ca.* 1500 m in the North Island to *ca.* 900 m in the south. At similar latitudes, abrupt treelines on inland mountains are about 200 m higher than gradual treelines on coastal mountains, reaching their highest altitude of *ca*. 1550 m at 42°S (Wardle 2008) (Figure 1.4). Overall, the oceanicity of the New Zealand archipelago results in highly variable climate all year round, distinct from the much more seasonal climate experienced in other temperate, more continental mountain regions (e.g., McCauley and Sturman 1999).

The New Zealand treeline has long been studied, most notably by the late Peter Wardle (e.g., Wardle 1965, 1971, 1973, 1985a, b, c, 2008). Most of the research has focussed on the abrupt *Nothofagus* type treelines, and mostly in one place: the Craigieburn Range in the South Island. The ecology of *Nothofagus* species at treeline here has received considerable attention, including studies of phenology, growth-climate relationships, disturbance effects and gas exchange (Benecke and Havranek 1980b, Benecke and Nordmeyer 1982, Norton 1984a, b, Norton and Schönenberger 1984, Wardle 1985a, b, c, Cullen et al. 2001a, 2001b). In contrast, there have not been such detailed and dedicated studies of the main tree species in the gradual ecotones, the processes limiting their growth, or the climate they experience. However, descriptions of the ecotone are available in altitudinal zonation or vegetation community studies (Stewart and Harrison 1987, Reif and Allen 1988, Druitt et al. 1990, Williams 1991, 1993), and some individual components have been described (Wardle 1960, 1963a, Haase 1986a, 1987, Payton 1989, Haase 1990b).

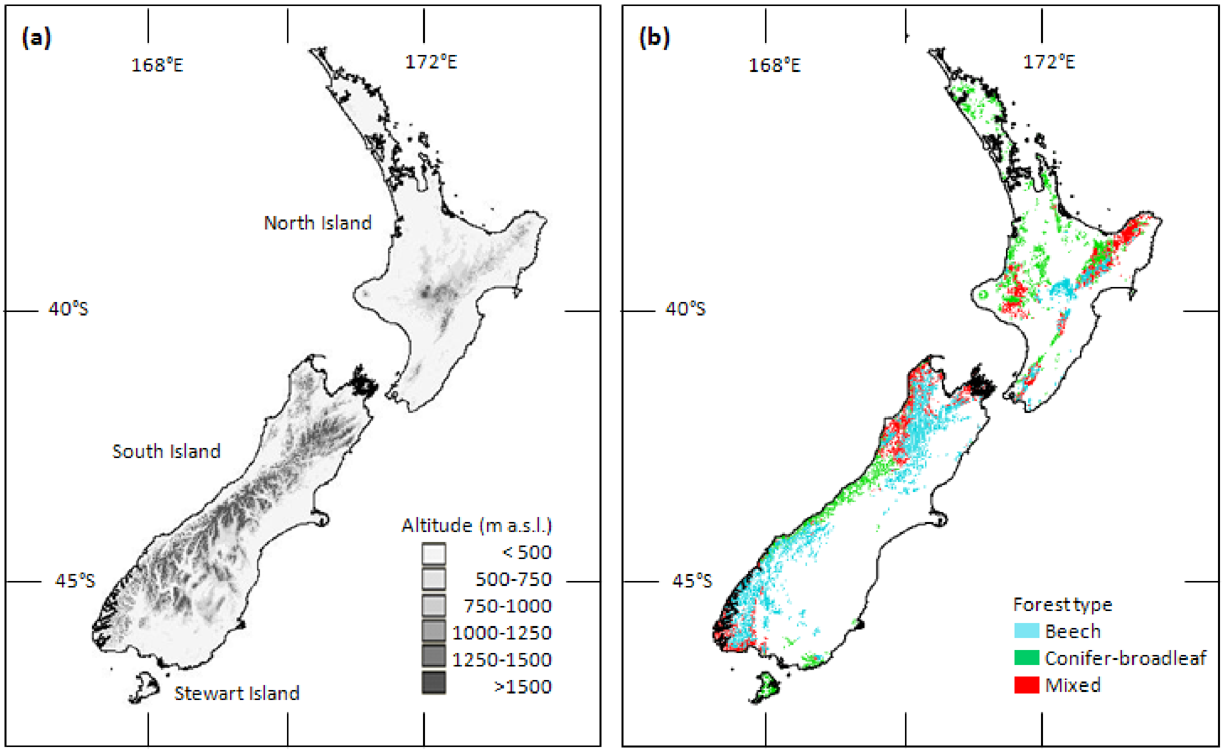

Figure 1.3. (a) Digital elevation model and (b) distribution of the main forest types in New Zealand (Newsome 1987).

1.4.1 *Nothofagus* treelines

Most New Zealand treeline forests are composed of one or other of two evergreen species of *Nothofagus*. *N. solandri* var. *cliffortioides* (Hook. f.) Poole (Mountain beech; further referred to by the species, not variety, name) dominates on the eastern sides of the North and South Island (Wardle 2008). It forms a clear abrupt treeline, where the closed canopy forest (of up to 12 m tall) is abruptly replaced by alpine tussock grassland (Plate 1.1). *N. menziesii* (Hook. f.) Oerst (Silver beech) forms an abrupt treeline on the western mountains of the North and South Island. In more humid, milder areas, these abrupt treelines may be replaced by broader shrubby treeline ecotones. For example, in the far north-west and far south-west corners of the South Island, where the forests consist of a combination of *Nothofagus,* conifers and broadleaved species (Figure 1.3b), a large altitudinal belt of shrubs extends beyond the *N. menziesii* treeline (Wardle 1963b, Williams 1991, Williams 1993). These more gradual *Nothofagus* treelines were not included in this study, due to their inaccessibility.

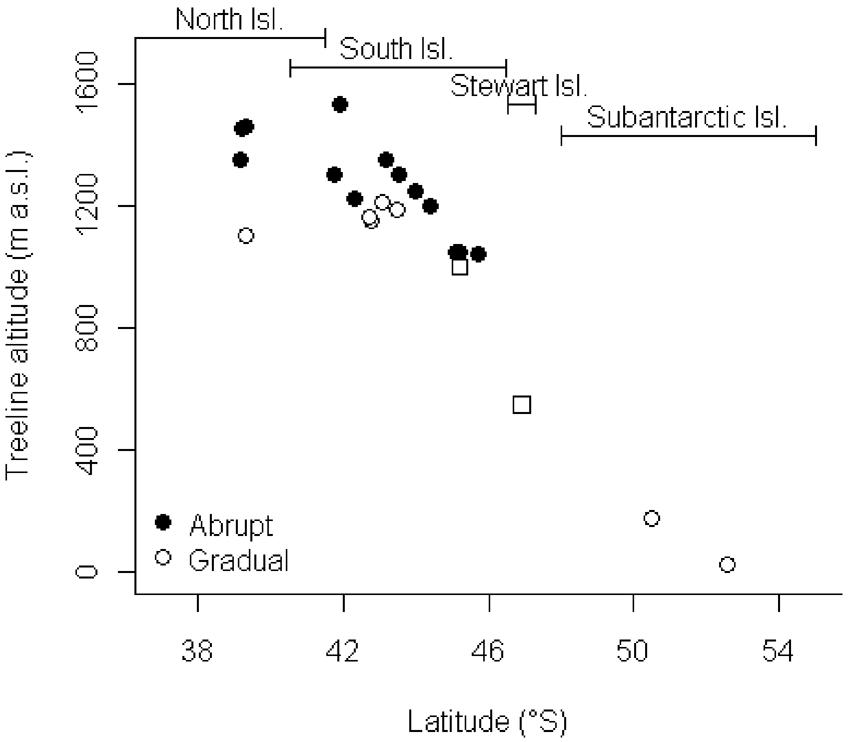

Figure 1.4. At the same latitude, gradual treelines (open symbols) are at lower altitudes than abrupt treelines (black symbols). Circles indicate treeline sites used in this study (Chapter 2 and 3); two additional gradual treeline sites (open squares) are included for illustration of the latitudinal trend.

On the East Coast of the South Island, the *Nothofagus* treeline may be depressed – especially on northern aspects – as a result of burning by Polynesian and European settlers (Wardle 1985c), for example, at Mt. Cockayne, near Craigieburn (Greer et al. 1989), and the Inland Kaikoura Range (Williams 1989). Relatively recent volcanic activity in the North Island may still be evident in a depressed treeline, and changed forest composition (McGlone et al. 1988). Abrupt treelines elsewhere around the world are often attributed to fire, herbivory, or other disturbances, as underlying environmental gradients fail to explain this pattern (Cairns and Moen 2004); however, the abruptness of almost all of the *Nothofagus* treelines in New Zealand appears to be natural.

1.4.2 Mixed conifer-broadleaved treelines

Mixed conifer-broadleaved hardwood forests dominate in central Westland between *ca*. 42°50'S and 43°40'S, where *Nothofagus* species are absent (Figure 1.3, Reif and Allen 1988, Haase 1990a) and these forests extend up to the treeline. Pockets of mixed conifer-broadleaved forests and treelines are also found on the North Island volcanoes Mt Taranaki and Mt Ruapehu. All are located on the western sides of New Zealand's main axial ranges and receive high annual rainfall (> 4 m). The treelines formed in these regions are of a gradual form: with increasing altitude, forest is replaced by lower-statured shrub vegetation, which in turn becomes shorter and more sparse and merges into grassland (Wardle 1973). This gradual ecotone supports a wide variety of tree and shrub species and can extend over several hundred altitudinal metres (Plate 1.1, Wardle 2008).

Just below treeline, even-aged forests formed by *Metrosideros umbellata* (Southern rata, Myrtaceae), *Weinmannia racemosa* (Kamahi, Cunoniaceae) and *Podocarpus hallii* (Hall's totara, Podocarpaceae) are typically replaced by shrub forest with emergent *Libocedrus bidwillii* (New Zealand cedar, Cupressaceae). Other common tree species near the treeline include *Halocarpus biformis* and *H. bidwillii* (Pink pine and Bog pine, respectively, Podocarpaceae) and *Phyllocladus alpinus* (Mountain toatoa, Podocarpaceae) (Reif and Allen 1988). Tree-statured (> 3 m tall) individuals of *Dracophyllum* species (including *D. longifolium, D. traversii,* Ericaceae) and *Olearia* species (*O. colensoi*, *O. ilicifolia, O. lacunosa,* Asteraceae) often form the actual treeline. All species in the ecotone are evergreen, with the exception of the deciduous *Hoheria* species (Malvaceae) which occur in gullies near treeline (Wardle 2008).

1.4.3 Exotic species treelines

The introduction of burning as common farming practice and over-stocking with domesticated herbivores, principally sheep, in the late 19th century led to the loss of topsoil in the high-country of New Zealand. In the 1950's, research was initiated to find tree species that could establish and grow on these eroded soils, assist in the restoration of soils and reforest depleted grasslands (Ledgard and Baker 1988). Many tree species, mainly exotic conifers, were planted in trials in the Craigieburn Range and elsewhere (Ledgard 1980). *Pinus* species proved especially successful. *Pinus mugo* was the hardiest conifer both climatically and edaphically, but it remained shrub-size. *Pinus contorta*, having the fastest growth rate and forming tall-statured trees, was the main species later planted on larger scales (Ledgard and Baker 1988). These plantings have since become the source of a wilding tree problem (Ledgard 2001). Tree-statured individuals of *P. contorta* are present up to 150 -200 m above the native *Nothofagus* treeline, whilst short-statured individuals may establish a further 150 m upwards (Plate 1.1, Wardle 1985a).

1.5 Research question and objectives

Although there is consensus that heat deficiency, or low temperature, is a major driver of treeline formation worldwide, it remains unclear exactly how low temperature limits tree growth at treeline. The main research question of this study is: *How does temperature affect the performance of trees at the New Zealand treeline?*

In this study, I will focus on two of the hypotheses that have been put forward regarding temperature limitations to tree growth: frost and carbon-balance. However, understanding potential temperature effects requires a sound knowledge of the year-round climatic conditions at the New Zealand treeline, and such data are lacking, most notably in the gradual ecotone.

Therefore, to answer the main research question, the following objectives were identified:

A. To characterise the thermal environment in the New Zealand treeline ecotone (Chapters 2 and 3);

B. To determine how frosts affect trees at treeline (Chapter 4); and

C. To determine whether low temperature limits the photosynthesis and/or growth of trees near the New Zealand treeline (carbon source- or sink-limitation) (Chapters 5 and 6).

1.6 Thesis outline

To address these objectives, the remainder of this thesis will cover the following topics:

In **Chapter 2**, I describe the results of a temperature measurement campaign at six treeline sites in New Zealand. I compare altitudinal trends in temperature with patterns in tree physiognomy and growth across abrupt and gradual treeline ecotones. These descriptions will provide the background to later address which aspects of temperature might influence the formation of treeline.

In **Chapter 3**, I use measured and estimated temperature at twenty treeline sites across the New Zealand archipelago to find the indicator that best describes the treeline thermal climate. A thermal descriptor which shows minimal variation across a wide range of natural treelines may provide valuable indications of the underlying physical processes that are limiting.

To assess what temperature-related processes are most limiting to the performance of treeline species, three studies are described. Firstly, in **Chapter 4**, I explore the effect of freezing temperature on the performance of trees across gradual and abrupt ecotones. Seasonal frost tolerance of treeline trees is compared to the temperature experienced at treeline.

Then in **Chapter 5**, I describe the effect of temperature on gas exchange of New Zealand treeline species, including a detailed study of the temperature response of limitations of internal conductances.

In **Chapter 6**, I discuss an experiment testing the carbon source- and sink-limitation hypotheses. This experiment addresses the effect of growing season temperature on gas exchange, growth and the carbon-balance of New Zealand treeline species.

Finally, in **Chapter 7**, I discuss the general conclusions from the previous chapters and suggest future research directions.

Plate 1.1. Three different types of treeline in New Zealand

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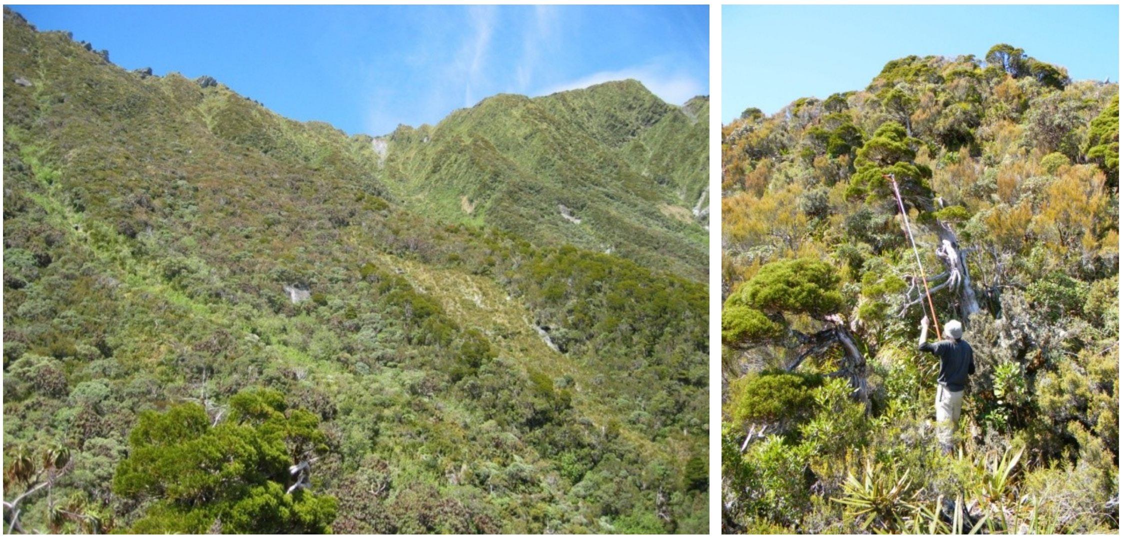
Abrupt *Nothofagus solandri* var. *cliffortioides* treeline at St Arnaud (left) and Craigieburn (right)



Gradual treeline ecotone at Camp Creek (left and right). Right: collecting foliage of *Libocedrus* near treeline

***Pinus contorta* established at and above the *Nothofagus* treeline at Craigieburn**

Chapter 2 Treeline thermal environment and tree performance

2.1 Introduction

Altitudinal treelines are relatively discrete boundaries in a landscape with a gradual gradient in the biophysical environment. Forest stature and density decrease before forest gives way with increasing altitude to isolated trees and stunted individuals and eventually to communities of shrubs or herbaceous species (Tranquillini 1979, Körner 2003a). It has been suggested that such different growth forms are an adaptation to increasing altitude and the accompanying environmental stresses, including low temperature and snow accumulation (Barrera et al. 2000, Körner 2003a). In New Zealand, the two main treeline types are of different forms: abrupt *Nothofagus* treelines form mostly in the eastern rain-shadow districts, whereas in western, more oceanic districts, gradual treelines of a diverse species composition are common. Such contrasting treeline forms suggest that treeline position might be driven by different processes and respond to climatic changes in a different way (Harsch et al. 2009, Harsch and Bader 2011).

Despite a long history of research at the New Zealand treeline (e.g., Zotov 1938), few measurements of the climate are available. A short-term intensive study examined air and soil temperature in different microclimates near a *Nothofagus* treeline (Wardle 1985b). More recently, a longer term (1-3 years) and larger scale picture is starting to develop, with temperature data available from four abrupt *Nothofagus* treelines around New Zealand (Mark et al. 2000, Körner and Paulsen 2004, Mark et al. 2008). In contrast to the relatively well-studied *Nothofagus* treelines, the available climatic data from mixed conifer-hardwood treelines consists of a single study of a broadleaf tree (*Metrosideros umbellata*) commonly found in the upper montane forest on the West Coast of New Zealand (Payton 1989). To estimate conditions at more treeline sites, data from low altitude climate stations and a generic lapse rate of 6°C km^{-1} have been used (Wardle 1998, 2008). However, these estimates may not reflect the temperature experienced at higher altitudes, because lapse rates change between regions, time of day (Grace et al. 1989) and year (e.g., Blandford et al. 2008). Hence there is a need for measurements of the thermal environment experienced at treeline sites in New Zealand.

Photo previous page: A cloudy day in the upper montane forest, 150 m below treeline at Camp Creek

In addition, vegetation may significantly alter the microclimate in which it grows, and the thermal regime experienced by trees at the treeline may differ substantially from the conditions in adjacent tussock grassland (Körner et al. 1986, Grace et al. 1989). The tall stature of trees at treeline means aboveground meristems are strongly coupled to the ambient air, and they therefore experience a colder climate than meristems of low-stature vegetation (shrubs or grasses). In addition, the tree canopy causes shading of the soil, and therefore keeps root temperatures cold (Körner and Paulsen 2004). Thus the architecture of trees cools the microclimate the trees grow in, and ultimately will limit the trees' growth. The trees will give way to shorter vegetation, which is more de-coupled from the ambient conditions, and can take advantage of the warmer conditions in the air near the ground surface, as well as increased soil heat flux through the sparser canopy. Documenting and understanding the effects of vegetation on the local microclimate is therefore an important step to understanding the key processes in the formation and position of treeline.

Different aspects of tree performance can be used to assess how tree growth changes towards the upper limit of trees. Often the most obvious change with altitude is stature (Körner 2003a), but trends in tree height may not be mirrored by radial growth (e.g., Paulsen et al. 2000). Trees may also become more multi-stemmed with altitude, as is the case for tropical and New Zealand forests (Reif and Allen 1988, Bellingham and Sparrow 2009). Decreases in specific leaf area (SLA) along an altitudinal gradient are common (Körner 2003a) and may reflect changes in relative growth rate and stress tolerance (Niinemets 2001, Wright and Westoby 2002). Additionally, the non-structural carbohydrate (NSC) content of plant organs along an altitudinal (and thus temperature) gradient can inform whether decreasing temperature limits photosynthesis or growth (source- , or sink-limitation, respectively) (Hoch et al. 2002, Li et al. 2002). Towards treeline, the NSC pool generally increases with altitude (Hoch et al. 2002, Hoch and Körner 2003, Körner 2003b, Piper et al. 2006, Shi et al. 2006, Shi et al. 2008), suggesting that trees at higher altitude are sink-limited.

In this chapter, I describe altitudinal trends in the thermal environment and tree performance across New Zealand treeline ecotones of different forms (abrupt and gradual). Together, such trends may provide indications of which environmental stressors are limiting tree growth at higher altitudes. These will be further examined in the remainder of this study.

2.2 Methods

2.2.1 Data collection

Site selection

To characterise the thermal environment of the New Zealand treeline ecotone, six field sites were selected, including different treeline types and spanning a large range of latitude. Additional selection criteria included accessibility and relative freedom from anthropogenic disturbance. Four gradual mixed conifer-hardwood treeline sites (Mt Fox, Mikonui, Camp Creek and Kelly Creek) were located west of the Southern Alps in Westland (referred to below as “West Alps” sites, Figure 2.1, Table 2.1). One abrupt *Nothofagus* site was located in each of the North and South Islands (Kaweka and Rainbow, respectively). An additional abrupt site (Craigieburn) was used for the collection of performance data along an altitudinal gradient, but data loggers were not deployed. At this site, the exotic conifer *Pinus contorta*, introduced to reduce soil erosion, has established and is spreading above the local *Nothofagus* treeline (Ledgard 2001). At all three beech sites, *Nothofagus solandri* var. *cliffortioides* forms an abrupt treeline, as it does elsewhere in the rain-shadow region. These sites are referred to below as “East Alps” sites. Due to limited accessibility, gradual *Nothofagus* treelines were not included.

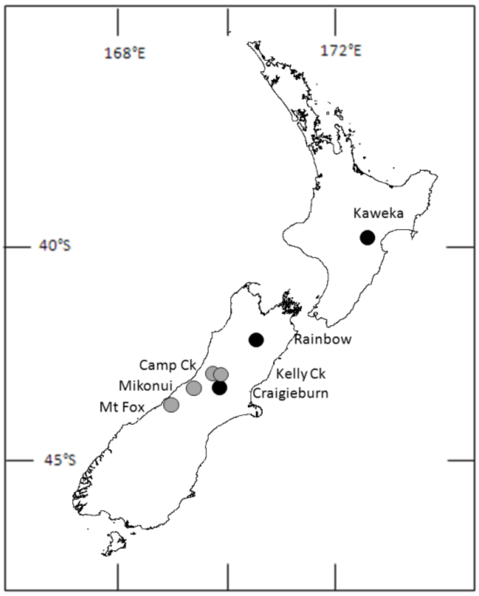


Figure 2.1. Location of field sites. Gray circles indicate gradual treelines (West Alps); black circles indicate abrupt treelines (East Alps). See Table 2.1 for site details.

Climatic data

Temperature measurements

To establish the thermal conditions across the ecotone at each of the six field sites, three altitudinal transects with temperature data loggers were set up. Transects were situated on topographically separate units (e.g., parallel ridges), about 100 m (horizontally) apart. Each transect consisted of four logger sites (Figure 2.2). First, the position of the local treeline was determined following the definition of Körner and Paulsen (2004): the line between groups of trees that are more than 3 m tall (see Section 1.2, Figure 1.1). Where the altitudinal transect crossed this virtual line, the treeline logger site was positioned. The lowest logger site was established in the upper forest 100 m (vertically) below this treeline site. At the gradual ecotone sites, logger sites were also established 100 m and 200 m above the treeline. At the abrupt beech treelines a pair of data loggers was established, with one set located within the treeline forest, and one in the associated tussock grassland (within 10 m of the treeline canopy); a logger site was also established 100 m above the treeline (Figure 2.2).

Tinytag Plus2 data loggers were used, which are precise to ± 0·2°C (Gemini Ltd, UK), and were programmed to record temperature hourly. The *soil* logger placement followed the protocol of Körner and Paulsen (2004) for montane forest and treeline sites. The loggers were buried in a location screened by the forest tree canopy throughout the day, with the sensor 10 cm below the soil surface. The litter layer was replaced after burial. At sites without a forest canopy cover (above treeline), loggers were placed at 10 cm depth in the soil on the south side of and beneath the canopy of a shrub or tussock to reduce the effect of direct radiation. The *ground* loggers were placed on the soil, with the location of the internal temperature sensor 1 cm above the surface. *Aerial* loggers were fixed to a metal pole at 1·3 m above the ground surface. They were placed outside the closed canopy (in the open canopy at gradual treelines; in the tussock grassland at abrupt treelines). The aerial and ground loggers were screened from direct sunlight using a white perforated plastic shield (see Appendix 1, Figure A1.1). Geographical positioning system (GPS) fixes of locations, combined with site markers, enabled relocation of the loggers, which were downloaded and restarted at least yearly.

Table 2.1. Details of the West Alps and East Alps treeline ecotone sites studied. Within region, sites are ordered by increasing latitude.

Treeline Site Name	Lon (°E)	Lat (°S)	Altitude (m a.s.l.)	Form	Main tree species in the ecotone[1]	Records Available (from d/m/y - to d/m/y) 1. Soil, 2. Ground, 3. Air
West Alps						
Camp Creek	171.57	42.71	1160	gradual	HAL, LIB, OLE, DRA	1. 31/01/2009-27/04/2011 2. 31/01/2009-27/04/2011 3. 31/01/2009-27/04/2011
Kelly Creek	171.58	42.78	1150	gradual	HAL, LIB, OLE, DRA	1. 13/05/2009-28/04/2011 2. 13/05/2009-28/04/2011 3. 13/05/2009-28/04/2011
Mikonui	170.87	43.06	1210	gradual	HAL, LIB, OLE, DRA	1. 17/01/2009-20/03/2011 2. 17/01/2009-20/03/2011 3. 17/01/2009-20/03/2011
Mt Fox	170.01	43.50	1185	gradual	HAL, LIB, OLE, DRA	1. 06/01/2010-22/03/2011 2. 06/01/2010-22/03/2011 3. 06/01/2010-22/03/2011
East Alps						
Kaweka	176.36	39.29	1460	abrupt	NOT	1. 26/11/2008-06/06/2011 2. 26/11/2009-06/06/2011 3. 26/11/2009-06/06/2011
Rainbow	172.86	41.89	1530	abrupt	NOT	1. 11/04/2009-09/04/2011 2. 11/04/2009-09/04/2011 3. 11/04/2009-09/04/2011
Craigieburn	171.71	43.15	1350	abrupt	NOT, (PHY), PIN	No temperature data recorded

[1] Treeline forming species: DRA = *Dracophyllum* spp. (Ericaceae); HAL = *Halocarpus biforme* (Podocarpaceae); LIB = *Libocedrus bidwillii* (Cupressaceae); NOT = *Nothofagus solandri* var. *cliffortioides*; OLE = *Olearia* spp. (Asteraceae); PHY = *Phyllocladus alpinus* (Podocarpaceae); PIN = *Pinus contorta* (Pinaceae). Tree species in brackets forms only shrub statured individuals (< 3 m tall) at that particular site.

Data verification

The data loggers were checked for stability and absolute accuracy in an ice-water bath and at several higher temperatures prior to and after deployment, and the recorded temperatures adjusted accordingly (deviation from zero was < 0·25°C for 95% of loggers; the highest anomaly was 0·6°C). Körner and Paulsen (2004) found that the 24-h amplitude of 10 cm soil temperature did not exceed 5·5°C at the treeline if the ground was permanently shaded. As *post-hoc* verification, the daily amplitudes of all treeline soil temperature data were checked and never exceeded this threshold, implying confidence in the placement of the loggers. Under open canopy or under grassland, the daily amplitude can be larger due to radiative heating of the soil. All measurements were obtained between November 2008 and May 2011, and for all sites at least 2 years of continuous data were available (Table 2.1). Overall, the data loggers proved very reliable, with only few malfunctions and short outages. Averaging across parallel transects enabled gap-filling of the datasets in the case of short outages, or when a site was not immediately fully established (e.g., at Kaweka, two of the three transects were established in the first year).

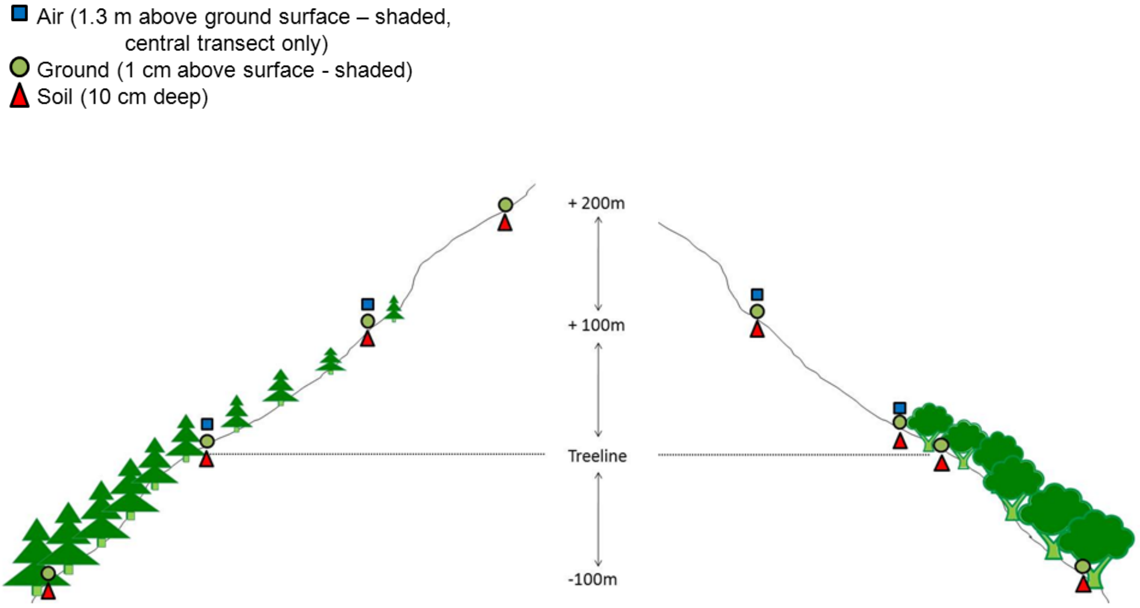


Figure 2.2. Schematic set-up of temperature data loggers along an altitudinal gradient for a gradual treeline (left) and an abrupt treeline (right). Three such identical transects were laid in parallel at *ca*. 100 m (horizontally) apart. Note the paired data loggers at the abrupt treeline (one set under the canopy, one set just above in the tussock grassland). At the same latitude, gradual treelines (on the western side of the Main Divide) are at lower altitudes than abrupt treelines (mostly on the eastern side of the Main Divide).

Snow

Although snow cover was not directly measured, its presence can be estimated from soil and ground temperatures. Because of its insulating effects, snow cover strongly dampens the daily amplitude of these temperatures; this measure, together with a ground and/or soil temperature around 0°C, can be used to estimate snow cover duration. This method was not fool-proof, with estimates from soil temperature often resulting in many more snow-covered days than when assessed from ground temperatures (data not shown). This may indicate that the snow cover was less than 10 cm, resulting in the ground logger screen exposed above the snow, but with less aeration due to snow cover blocking its air-vents, and potentially increasing temperatures within the screen. Due to this uncertainty, only soil temperature was used for the estimation of snow cover.

Additional climatic variables

In addition to temperature measurements, two climate stations (*DataHog2*, Skye, UK) were deployed at Kelly Creek (at the treeline and at the tree species limit); these stations recorded humidity, wind direction, wind speed and radiation at hourly intervals. The higher of the two stations malfunctioned after a couple of months and only data from the weather station situated at the treeline are shown here. Additionally, many of the maximum daily radiation values recorded were beyond physically possible values (higher than extra-terrestrial solar radiation input). The source of this error could not be identified, so the radiation data are not presented here.

Half-hourly data from a mid-elevation weather station on the east side of the Main Divide (Craigieburn, 914 m a.s.l.) were obtained from Tony McSeveny (Landcare Research). Another station directly uphill (1554 m a.s.l., Ski Basin) operated from 1967 to 1986 (data from National Institute of Water and Atmospheric Research, NIWA, *cliflo.niwa.co.nz*). Using data from the 20 years when both stations were operating, the conditions at the local *Nothofagus* treeline (at 1350 m, lying between the two stations on the same slope) were interpolated. Lapse rates were then calculated and applied to more recent data from the lower station to estimate treeline temperature. Monthly lapse rates were calculated for mean temperature and rainfall. Average monthly relative humidity differed little between these sites (< 5%), so the lower station data were used. Wind-direction and -speed data from the lower station were used, because they were not consistently reported at the higher altitude station. Mean daily maximum wind speed (km hr^{-1}) and wind run (a measurement of the 'amount' of wind passing the station during 24 hours, or 'kilometres of wind') were calculated on a monthly basis.

Tree performance

In this study, *Halocarpus biformis* (Hook.) Quinn (Podocarpaceae) and *Libocedrus bidwillii* Hook.f. (Cupressaceae) were measured and sampled in the gradual treeline ecotone at the West Alps sites; and *Nothofagus solandri* var. *cliffortioides* (Hook.f.) Poole (Nothofagaceae)*, Phyllocladus alpinus* Hook.f. (Podocarpaceae) and the exotic *Pinus contorta* Loudon (Pinaceae) were measured and sampled near abrupt treelines at the East Alps sites. These species will be referred to below by their generic names only. An altitudinal transect was established through the treeline ecotone (following the central transect of the data loggers, except at Craigieburn). Trees of the selected species within 10 m either side of this transect were measured at intervals of *ca.* 25 m (altitude) in the case of the stature measurements, and intervals of *ca.* 100 m for the collection of material for determination of the specific leaf area and non-structural carbohydrate content.

Stature

Tree height, diameter at breast height (DBH, 1·3 m) and the height of the first branch were recorded for trees across the treeline ecotone at selected sites. Tree height was estimated visually and estimates were regularly checked with a Vertex III Ultrasonic Hypsometer (Haglöf AB, Sweden).

Specific leaf area (SLA)

Foliage samples of adult trees (and in the case of *Libocedrus* and *Halocarpus* also juvenile foliage) were collected along the altitudinal gradient for assessment of SLA (leaf area per unit dry weight). Before being dried at 70°C, projected single-sided leaf area was determined by scanning the foliage on a flat-bed scanner and processing the images using WinFolia Pro 2004a (Regent Instruments Inc., Canada). For the flat leaves of *Nothofagus, Phyllocladus* and *Podocarpus,* this single-sided area equals the half-total leaf area. However, the other species have three dimensional foliage, where projected single-sided area does not approximate half-total leaf area (Cornelissen et al. 2003) (see Plate 2.1). Here, single-sided area (SSA) was converted to half-total area (HTA) using geometry (Equations 2.1 - 2.3). The measurements of dry weight and half-total leaf area were used to calculate SLA.

Halocarpus foliage was approximated by a cylinder (Equation 2.1). Each *Pinus* needle was approximated by half a cylinder (dissected through its long axis; each circular fascicle produces two needles that together form a cylinder, Plate 2.1) (Grace 1987) (Equation 2.2). *Libocedrus* foliage was assumed an elliptical cylinder (Equation 2.3); measurements of the foliage were made to establish a generic ratio of the two diameters of this cylinder. The mean first diameter and length of the foliage segments were calculated from the scanned images using WinRhizo Pro 2004a (Regent Instruments Inc., Canada), the second diameter was estimated using the above ratio.

Cylinder: $HTA = SSA * \pi / 2$ .... Equation 2.1

Half a cylinder: $HTA = SSA + (SSA * \pi / 2) / 2$ .... Equation 2.2

Elliptical cylinder: $HTA = ((\text{Diameter}_1 + \text{Diameter}_2) * \pi * \text{Length}) / 2$ ..... Equation 2.3

Radial growth

To assess the radial growth of trees across the ecotone, trees were cored along an altitudinal transect at three of the gradual treeline sites (Camp Creek, Mikonui and Mt Fox). Along each transect, sampling occurred within three altitudinal bands each of *ca*. 25-30 vertical metres. Within each band, 8-10 of the largest, and presumably oldest, trees were selected for increment core sampling. From each tree, one or two 5 mm diameter cores were extracted at *ca*. 0·8-1·2 m height. If possible, the cores were taken parallel to the slope contour, thus minimising the influence of compression wood. Cores were mounted, air dried, sanded and polished, and scanned using a flat-bed scanner at high (3600 DPI) resolution. Image analysis software CooRecorder 7·3 (Cybis Elektronik & Data AB, Sweden) was used to measure annual ring widths with a precision of 0·01 mm. Additional tree-ring data from Camp Creek were kindly provided by Pavla Fenwick (Fenwick 2003); all *Libocedrus* data and a subset of the *Halocarpus* data from this site were obtained from this source. Mean width of the 50 growth rings spanning the common period for all samples (1950-1999) was calculated for each tree (where data from multiple cores were available from the same tree, ring-widths were averaged for each year). Mean ring widths for the periods 1850-1899 and 1900-1949 were also calculated.

Tree age

To estimate the age of *Libocedrus* trees cored at Mt Fox as part of this study, the number of rings per tree was counted using cores that hit the tree centre. If cores narrowly missed the centre, the number of missing rings was estimated following Duncan (1989). Ages of *Libocedrus* and *Halocarpus* trees cored by Fenwick (2003) were taken as the total number of rings counted; this may be an underestimate of the true tree age, as the core may not have hit the tree centre, or the centre may have decomposed. The absolute age of the tree will always be older than the age of the pith of the tree at coring height, because of the years taken to reach the coring height. For instance, two 1.3 m tall saplings of *Libocedrus* 150 m below treeline at Mt Fox were cored at the base and their age was estimated at 45 years. Previous estimates of time to reach 50 cm tall were 40 and 25 years for *Libocedrus* and *Halocarpus,* respectively (Stewart and Rose 1988).

Non-structural carbohydrate content

To determine the NSC content across the ecotone, foliage was sampled along an altitudinal gradient at one East Alps and one West Alps site. Five tree species were sampled at 100 m vertical intervals across the ecotone, the exact altitudes depending upon each species' altitudinal distribution and the local treeline altitude (Table 2.2). If the tree-forming species were present above the treeline (< 3 m tall), these were also sampled. Fully expanded foliage was collected from fully sun-lit branches in the top half of the crown of five individuals of each species at each altitude. Samples were kept cool during transport and were heated in a microwave oven (in paper bags) at 1000 W for 60 seconds to denature enzymes, within 8 hours of collection. The material was dried to a constant weight at 60°C, ground to a fine powder, sealed and stored over silica gel at 4°C until extraction.

Table 2.2. Details of sites and species sampled for NSC content. Species abbreviations as in Table 2.1.

Site	Species	Altitudinal range sampled (m a.s.l.)	Altitude of highest occurring tree-statured (> 3 m) individuals (m a.s.l.)
East Alps: Craigieburn	NOT	1250 – 1450	1350[1]
	PHY	1250 – 1480	NA (no trees > 3 m present)
	PIN	1250 – 1550	1500[1]
West Alps: Camp Creek	HAL	850 – 1250	1150
	LIB	850 – 1120	1120

[1] In this study, "treeline at Craigieburn" will refer to the local native abrupt treeline at 1350 m a.s.l., even though trees of the exotic *Pinus contorta* occur at higher altitudes.

NSC content is defined here as the sum of free low-molecular-weight sugars (glucose, fructose and sucrose) plus starch (Chapin et al. 1990). NSCs were extracted following an established protocol of enzymatic extraction (Wong 1990, Hoch et al. 2002), with one alteration: the crude fungal amylase ("Clarase *g-plus*" from *Aspergillus oryzae*, Enzyme Solutions Pty Ltd., Australia) was purified using microfiltration (VivaSpin-20, Sartorius stedim, Germany) rather than dialysis (following Richter et al. 2009). Approximately 10 mg of fine ground plant material was extracted in distilled water above steam for 30 min. After cooling, invertase and isomerase were added to an aliquot of the extract to convert fructose and sucrose into glucose. Glucose was then enzymatically converted to gluconate-6-phosphate using gluco-hexokinase (Sigma Aldrich, Australia). Subsequent oxidisation of the gluconate-6-phosphate results in a reduction of NAD to NADH, increasing absorbance of the solution at wavelength λ = 340 nm in direct proportion to the free sugar concentration. Absorption was measured using a 96-well microplate spectrophotometer (FLUOstar Omega, BMG Labtech, Germany). The remainder of the original extracted plant material was incubated overnight at 40°C with the micro-filtered amylase to break down starch to glucose. An aliquot was extracted and centrifuged, isomerase added, and total NSC (TNC, free sugars plus starch) determined as describe above. Starch was then calculated as TNC minus free sugars.

Pure starch, fructose, glucose and sucrose were used as standards and standard plant powder (“*Orchard leaves*”, Leco, USA) was included to ensure the reproducibility of extractions. NSC concentrations were expressed both as a percentage of dry weight and relative to leaf area (g cm^{-2}). The ratio between free sugars and starch concentration was calculated for each sample to better distinguish between osmotic adjustments and growth-limitation (Fajardo et al. 2011). That is, one might expect an increasing sugar:starch ratio as a method of increasing frost tolerance (Öquist et al. 2001), whereas processes related with growth limitation would result in a decreasing sugar:starch ratio (Fajardo et al. 2011).

2.2.2 Data analyses

Climatic data

To condense the temperature data, for each field site, hourly readings from the three parallel transects were averaged, resulting in four datasets corresponding to the average hourly temperature at each altitudinal logger site for the recorded period. Daily T_{min}, T_{max} and the arithmetic T_{mean} were calculated. These data were then condensed to a 365-day dataset by averaging any data obtained for the same day in multiple years (Körner and Paulsen 2004).

The first year in this study (2009) was cold compared to the long-term mean, whereas 2010 was the 5th warmest year on record. Average anomalies from the long-term (1971-2000) national annual mean calculated from seven weather stations around the country were -0·22°C and +0·53°C for 2009 and 2010, respectively. The first few months of 2011 were near the long-term mean temperature for these months (source: NIWA). Temperature data were not adjusted for these anomalies. Since almost all records include data from the full length of 2009 and 2010, and these were averaged, data presented here should not be biased compared to where only one year’s record was available (with two notable exceptions: at Kelly Creek, the final loggers were established in May 2009, and the highest altitude loggers at Mt Fox recorded from January 2010).

To assess whether the seasonal course of temperature was significantly different at abrupt and gradual treelines, a stepwise procedure in linear mixed effects (LME) models for air and soil temperature was used. First, the basic model included the additive effects of treeline type (abrupt or gradual) and seasonality (as a sine/cosine function), with location as a random effect. This model was compared to the same model with serial correlation incorporated as an autoregressive moving average (ARMA) covariance structure with a lag of 1 or 2 months. AIC*c* (see below) was used to assess which model was most strongly supported by the data. The same procedure was undertaken for a model that included an interaction between seasonality and treeline type. The results section indicates how the final LME models for air and soil temperature were adjusted for seasonality and serial autocorrelation.

Tree performance

To analyse altitudinal trends in the various performance indicators, two simple models were fitted: a linear model (reflecting a linear response of the indicator with altitude, referred to as the "altitude model") and a null model with an intercept ("null model"). Only the response variable NSC 'sugar:starch ratio' required (log) transformation to fulfil normality assumptions. The two models in each candidate set were ranked using the Akaike's information criterion corrected for small sample sizes (AIC*c*) to determine the Kullback-Leibler (KL) best model (Burnham and Anderson 2002). The AIC*c* identifies the model(s) most strongly supported by the data based on the bias-corrected, maximized log-likelihood (*LogLik*) of the fitted-model with a penalty for the number of parameters used. The model with the smallest AIC*c* ($AICc_{min}$) is the most strongly supported, given the data. ΔAIC*c* is calculated for each model *i* as $\Delta_i = AICc_i - AICc_{min}$. As a rule of thumb, models with Δ_i of < 2 also receive substantial support, models with $2 < \Delta_i < 7$ receive considerably less support, whereas models with Δ_i > 8-14 receive essentially no support (Anderson 2008). A measure of the strength of evidence for either model (altitudinal trend or not) is described by the model probability (Akaike weights, w_i). This is the probability that model *i* is the KL best model, given the data and candidate set of models (Anderson 2008). The sum of w_i of the models in a candidate set equals 1. The evidence ratio (ratio of the model probabilities) then provides an assessment of the empirical support of the best KL model *vs*. another model.

All analyses in this study were performed in R v. 2.12.2 (R Core Development Team 2011), and included use of the packages *AICcmodavg* (Mazerolle 2011), *dplR* (Bunn 2010), and *nlme* (Pinheiro et al. 2011).

2.3 Results

2.3.1 Treeline climate

Temperature

Mean annual air temperature ranged from 4·7°C at 100 m above the treeline at Rainbow to 6·4°C at the treeline at Mt Fox. Mean annual ground temperature ranged from 4·1°C at 200 m above the Camp Creek treeline to 6·2°C at 100 m below treeline at the same site. Mean annual soil temperature ranged from 4·7°C at 200 m above the treeline at Camp Creek to 5·3°C at 100 m below the treeline at Rainbow. Monthly temperatures showed a distinct seasonal pattern, with January – March being the three warmest months, and June – August the three coldest (Figure 2.3). Altitudinal trends of mean air, ground surface and soil temperatures for the warmest three months are illustrated in Figure 2.4. Summarised temperature data for the altitudinal gradients at the six sites can be found in Appendix 1 (Tables A1.1, A1.2, and Figures A1.2, A1.3).

At the gradual treeline sites, soil, ground surface and air temperatures generally decreased with altitude (Figure 2.4a-d). Two obvious outliers are the extreme increase of soil and ground surface temperature 200 m above the treeline at the Mt Fox site (Figure 2.4d). These loggers were located on very steep slopes (> 60° inclination) with a high proportion of bare soil and rock. All other loggers were placed under more or less level surfaces. In contrast, at the abrupt treelines, the effect of logger location (under the forest canopy *vs*. under the tussock grassland) on ground and soil temperature was more pronounced than the effect of altitude alone (Figure 2.4e-f).

At the gradual treeline sites, the lapse rate of air temperature from treeline to 100 m above treeline was generally higher than those of ground and soil temperature for all sites (i.e., steeper slopes of the lines in Figure 2.4a-d), but at the abrupt Rainbow site, the three lapse rates were similar (Figure 2.4f).

The best LME models of mean monthly air and soil temperature at the treeline, given the data, included an interaction between time (as a sine/cosine function) and treeline type. There was substantial support for autocorrelation (with a lag of two months) in both models. Mean monthly air and soil temperatures were similar in the warm months at gradual and abrupt treelines. However, temperatures at the abrupt treelines dropped more rapidly at the end of summer than at the gradual treelines, and the colder months at the gradual treelines were much more mild (Figure 2.3).

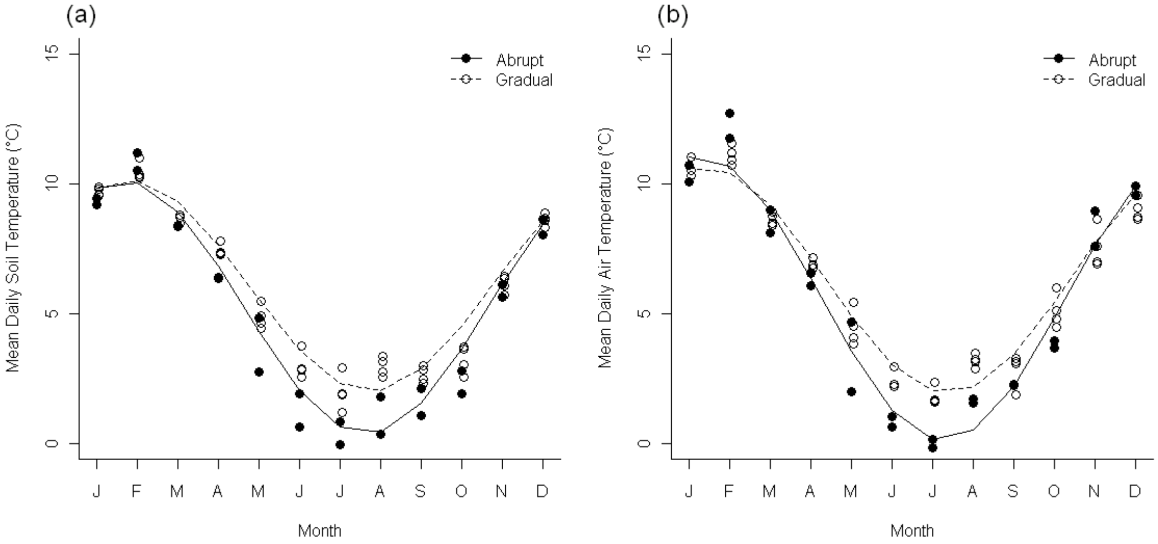


Figure 2.3. Mean monthly soil (a) and air (b) temperatures at the six treeline sites measured in this study. Soil and air temperatures differ between abrupt and gradual treelines in the colder months. Lines are predictions from LME models (see Section 2.2.2).

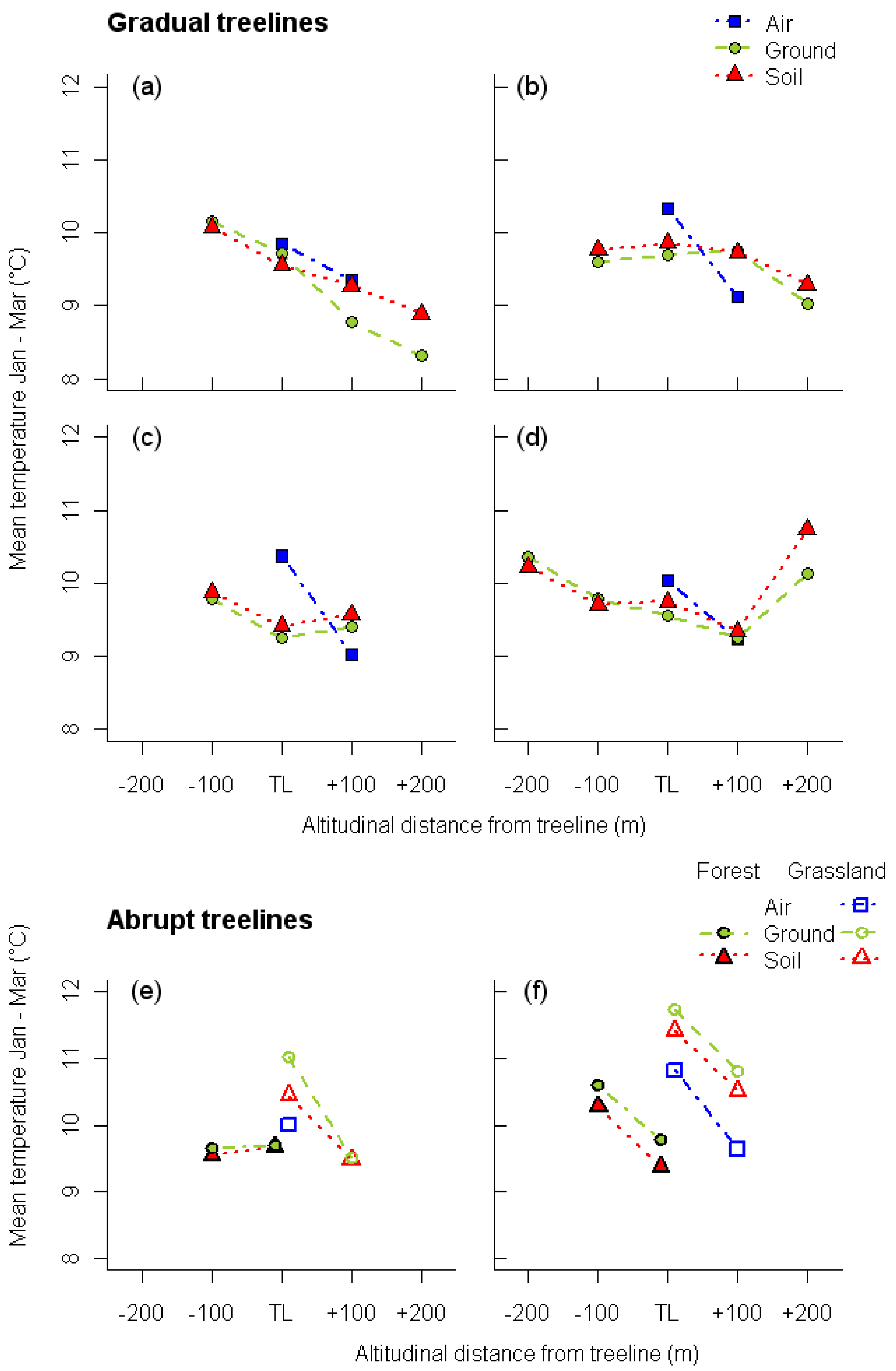


Figure 2.4. Mean temperature in January - March for the air, ground and soil loggers along an altitudinal gradient at the four gradual treeline sites (a) Camp Creek; (b) Kelly Creek; (c) Mikonui; (d) Mt Fox; and the abrupt treelines at (e) Kaweka; and (f) Rainbow. Note that at the abrupt treelines, a pair of logger sites was set up, one underneath the treeline forest canopy, and one in the alpine grassland within 10 m of the treeline.

Frost and snow

Absolute minimum air temperature at treeline was between -6·5°C and -6·0°C for all four gradual sites and the Kaweka site, whereas a much colder -9·0°C was recorded at the Rainbow treeline in 2009 (Figure 2.5). Extreme minima 100 m above the treeline were at least 0.9°C colder than at treeline, with -10·3°C the lowest recorded (100 m above the Rainbow treeline). Air frosts occurred on around 105 days per year at treeline (although up to 145 at Rainbow), and *ca*. 20 more days 100 m higher. Only the two warmest months were without any air frosts at the treeline of all sites, whereas frosts occurred during most nights in mid-winter. The months August to October shared a similar number of frosts for most sites (Figure 2.5).

With increasing altitude in the gradual ecotone, ground frosts were also more frequent (ranging from 40 to *ca*. 100 per year at Kelly Creek and Mt Fox, and up to 120 at Dickie Spur and Camp Creek). At the abrupt treelines, ground frosts were much more evenly spread along the altitudinal gradient (ranging from 78 to 100 days, and 100 to 150 days of the year at Kaweka and Rainbow, respectively) (Figure 2.5). In all cases ground extreme minima recorded were less severe than air temperature minima (see Section 2.4 Discussion).

Regardless of altitude, soils hardly ever froze at the gradual treeline sites. If they did freeze, it was only for up to a week, with an extreme low recorded of -0·2°C (across all gradients, sites and years) (Figure 2.5, Table A1.1, A1.2). At the abrupt treelines, freezing soil temperatures were recorded more commonly, with extreme minima of -2·5°C (in the tussock grassland directly above the Rainbow treeline). Soil above both abrupt treelines was frozen for *ca.* 20 days each year. Soil under the treeline canopy never froze at Kaweka, but froze an average 34 days every year at Rainbow (Figure 2.5f; these frosts were all associated with snow cover, and soil temperatures never fell below -0·5°C). Soil 100 m below the Kaweka treeline froze for one week, but not at Rainbow.

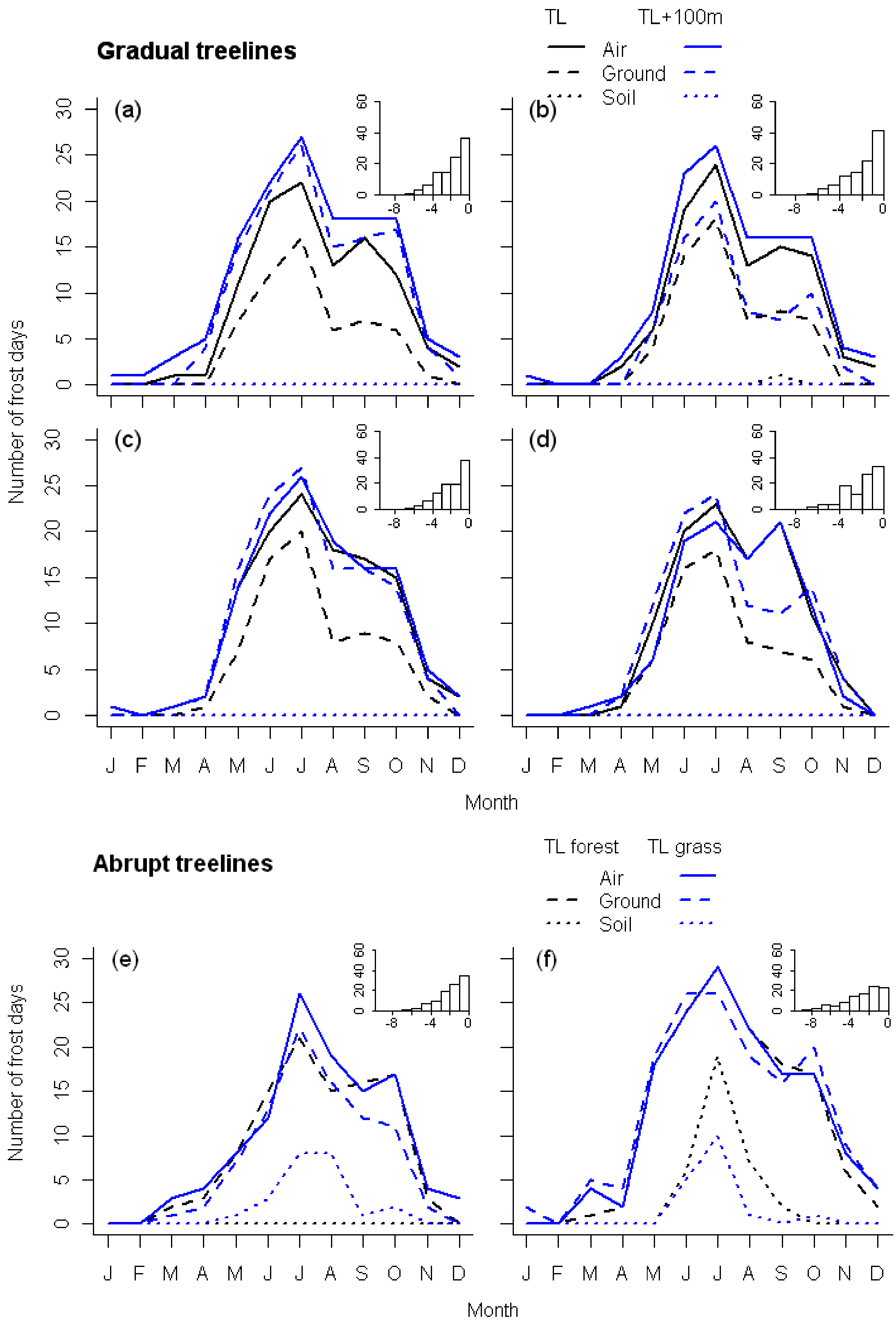


Figure 2.5. Average number of frost days per month at the four gradual treeline sites (comparing air, ground and soil frosts at treeline (TL) and at 100 m above treeline: (a) Camp Creek; (b) Kelly Creek; (c) Mikonui; (d) Mt Fox); and at the two abrupt treeline sites (comparing frosts under the treeline forest canopy *vs*. the adjacent treeline grassland: (e) Kaweka; (f) Rainbow). Insets show the relative distribution (%) of frosts (°C) at the treeline (air temperature only).

Snow cover duration, estimated from stable soil temperatures close to 0°C, was almost twice as long in the winter of 2009 compared to 2010, but similar patterns along the altitudinal gradient were visible in both years. In the gradual treeline ecotones, the average maximum duration of snow cover over these two measured winters was less than 14 days at all but the very highest altitude (either based on ground or soil temperatures). At this highest site (200 m above treeline, or *ca.* 1400 m a.s.l.), continuous snow cover lasted for about a month. In contrast, at the abrupt treelines snow cover duration was between 30 and 50 days across the ecotone.

Rainfall, humidity and wind

The weather station at the Kelly Creek treeline (West Alps) provided additional climate data, including humidity and wind speed and direction data, from May 2009 – April 2011. Simultaneous data from an established weather station at Craigieburn (East Alps, 914 m a.s.l., *ca.* 425 m below the local abrupt treeline) were obtained. Rain is evenly distributed throughout the year at both sites, but annual rainfall is approximately three times higher at West Alps compared to East Alps treelines (Figure 2.6a,b). Average relative humidity is steady at *ca.* 90% throughout the year in the west, whereas in the east it ranges between 70 and 80% with a distinct drop during the warmer months. In the west, winds most commonly come from a south- to westerly or a northerly direction. Mean wind run and maximum wind speed are higher in the warm months (220-350 km per day, with mean daily maxima of *ca.* 50 km h^{-1}), than in the winter months (wind run of < 100 km and mean daily maxima of 10 km h^{-1}) (Figure 2.6c). At the East Alps site, a north-east wind direction is most common. Mean maximum wind speed is evenly distributed through the year (*ca.* 20 km h^{-1}), but maximum wind runs of > 200 km occur in the spring months September, November and December (Figure 2.6d).

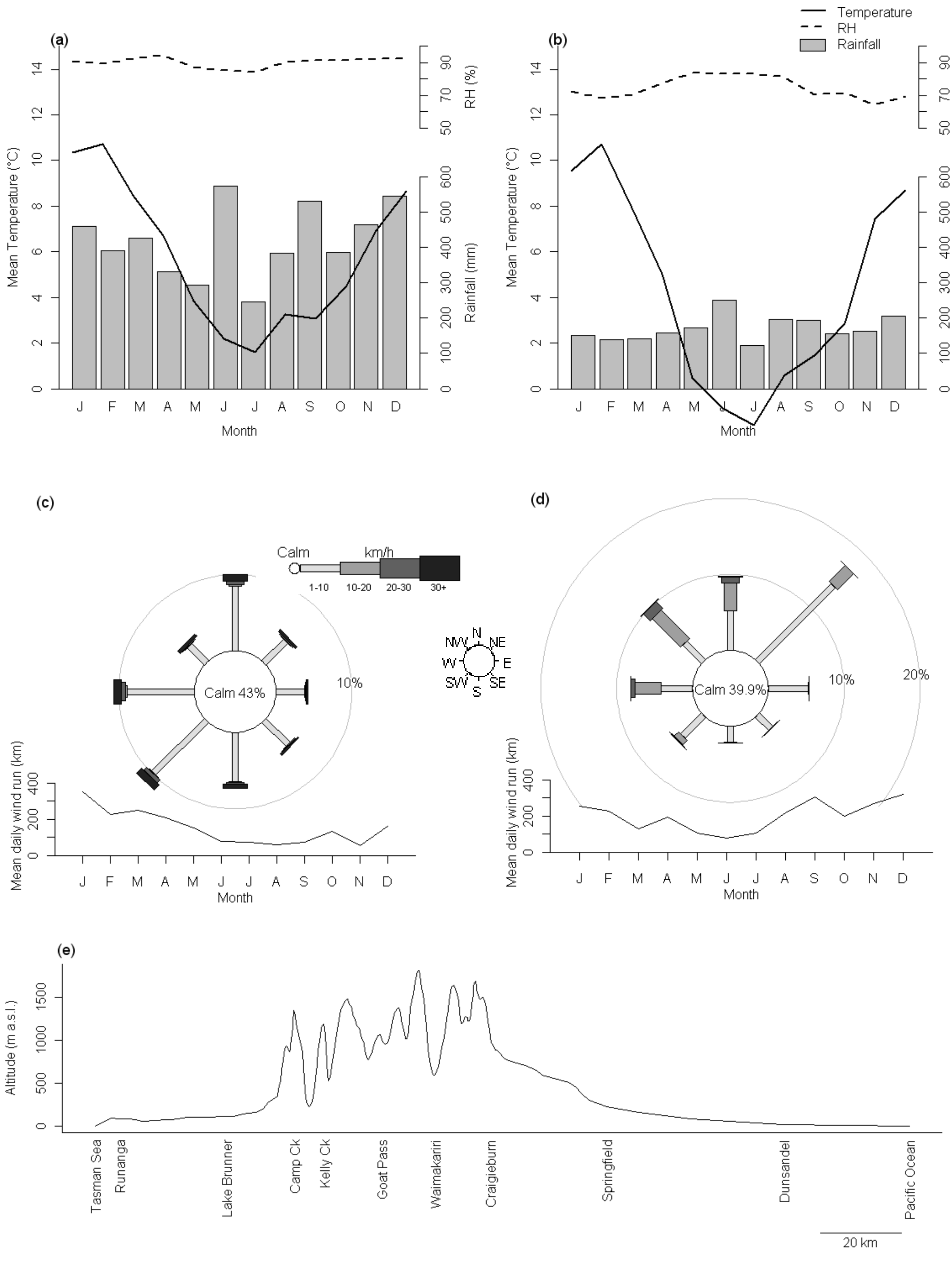


Figure 2.6. Climatic data from West Alps (left: a and c) and East Alps (right: b and d) treeline sites. (a, b) temperature, rainfall and relative humidity (RH); (c, d) wind rose and mean maximum daily wind speed; (e) altitude profile across the Main Divide from the West Coast (42.4°S) to the East Coast (43.6°S). For the West Alps, air temperature, relative humidity, wind direction and wind speed were collected at the Kelly Creek treeline, and the rainfall data were from Inchbonnie, located between Camp Creek and Lake Brunner (see e). All data for the East Alps were collected at Craigieburn (914 m a.s.l.); temperature and rainfall data were interpolated to treeline altitude (1350 m a.s.l., see Section 2.2.1 for more details).

2.3.2 Tree performance

All species of tree stature showed a clear decline of height and DBH with altitude (Figure 2.7, Figure 2.8); model probabilities w_i for the models including altitude (further referred to as “altitude models”) were at least 98%, compared to < 2% for the intercept only models (Table 2.3). Altitude models explained between 60-84% of the variation in tree height. The short-statured (< 2 m) *Phyllocladus* also showed an altitudinal decrease in height (altitude model $w_i = 90$, $R^2_{adj} = 0.08$), but not DBH (null model $w_i = 74$, Table 2.3). Decrease in height was equivalent to 1·9, 2·4, 3·4, 0·3 and 2·7 m per 100 m altitude, for *Halocarpus, Libocedrus, Nothofagus*, *Phyllocladus* and *Pinus* respectively. DBH decreased by 8·4, 6·6, 8·5 and 6·2 cm per 100 m altitude, for *Halocarpus, Libocedrus, Nothofagus* and *Pinus* respectively. Altitude accounted for between 11% (*Libocedrus*) and 60% (*Pinus*) of the variation in the DBH data (Table 2.3).

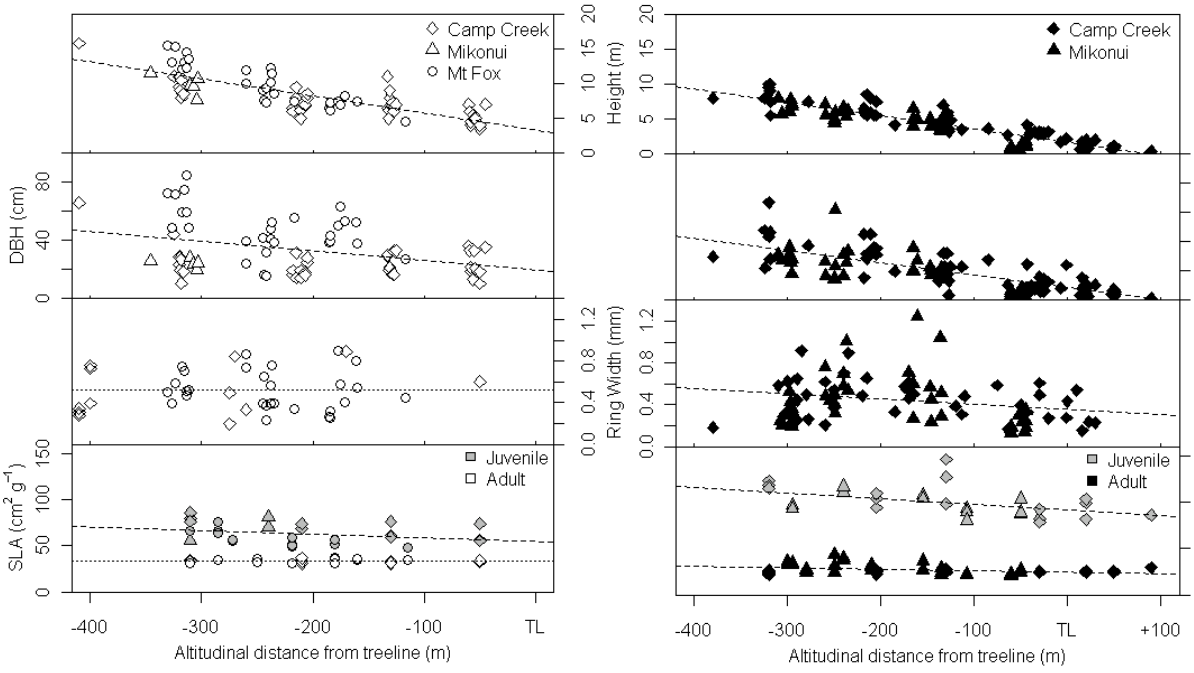


Figure 2.7. Altitudinal trends in *Libocedrus* (left) and *Halocarpus* (right) tree height, diameter breast height (DBH), mean tree ring width over the last 50 years, and specific leaf area (SLA) of adult and juvenile foliage. The highest individuals of *Libocedrus* are *ca*. 50 m below the treeline formed by *Halocarpus*. Lines present the best linear model given the data; a dashed line indicates an altitudinal is supported by the data, whereas a dotted line indicates no improvement over the null (intercept only) model by including altitude. Camp Creek tree ring data are partially sourced from Fenwick (2003) (see Section 2.2.1 for details).

The data for mean annual radial growth from 1950-1999 did not support a trend with altitude for *Libocedrus* (null model $w_i = 73\%$). However, there was substantial support for an altitudinal decrease in radial growth for *Halocarpus* (altitude model $w_i = 78\%$) (Figure 2.7, Table 2.3). Trends did not change when assessing earlier periods 1850-1899, or 1900-1949 (data not shown). The age of *Libocedrus* and *Halocarpus* trees decreased with altitude (altitude model $w_i > 78\%$).

SLA of juvenile foliage of *Halocarpus* and *Libocedrus* was twice as high as that of their mature counter parts. Both juvenile and mature *Halocarpus* foliage showed a negative trend in SLA with altitude, although the bulk of the variation (> 80%) remains unexplained. The SLA of mature foliage of *Libocedrus* did not change with altitude, and evidence for an altitudinal trend in juvenile foliage SLA was equivocal ($w_i = 51\%$ and 49%, respectively; Figure 2.7). SLA for *Pinus* and *Nothofagus* decreased with altitude ($w_i = 86$ and 99%, respectively), although there was substantial unexplained variation (R^2_{adj} of 0·24 and 0·51, respectively) (Table 2.3, Figure 2.8).

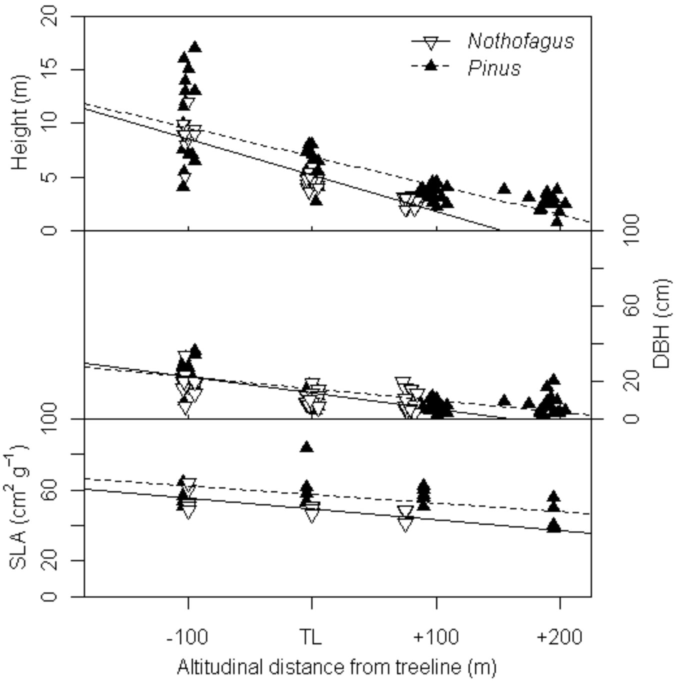


Figure 2.8. Altitudinal trends in *Pinus* and *Nothofagus* tree height, diameter breast height (DBH) and specific leaf area (SLA) at Craigieburn. The treeline (TL) is the native abrupt *Nothofagus* treeline, a lone island of trees of the same species exist *ca.* 80 m above this treeline, and the exotic *Pinus* was sampled up to 200 m above this treeline. Lines show the altitudinal trend in the indicator supported by the data (see Table 2.3).

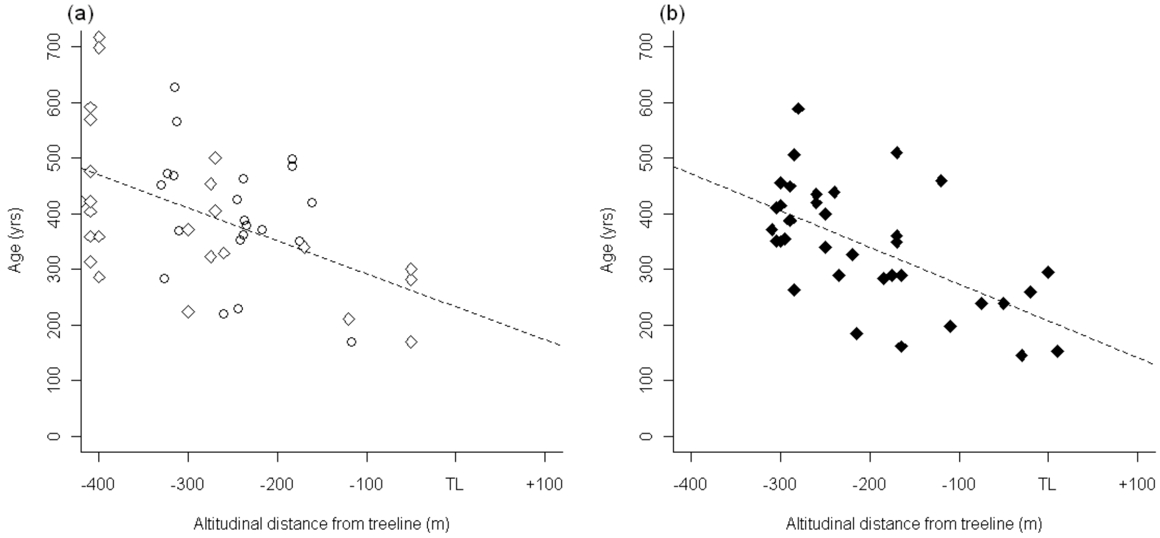


Figure 2.9. Altitudinal trend of age of *Libocedrus* (a) and *Halocarpus* (b) trees near the treeline (TL) at Mt Fox (circles) and Camp Creek (diamonds). Lines indicate the altitudinal trend supported by the data (see Table 2.3).

NSC concentration differed between the species (Figure 2.10, Table 2.4). The dry weight content of all NSC components measured in *Pinus* (*ca*. 10% free sugars and 20% starch) was twice as high as the concentrations found in the native species (*ca*. 2-4% free sugars, 6-10% starch). However, when expressed on a leaf area basis, the contents were comparable (*ca*. 2-4 mg cm^{-2},Figure 2.10). The data did not provide strong evidence of an altitudinal trend in the dry weight concentration of starch, free sugars or TNC (in all cases, altitude model $w_i < 0.55$, so the probability of the null model being the better model was at least 0.45, data not shown). However, when expressed on an area basis, the data supported altitudinal trends in NSC content for some species. The model probability for the altitude model for TNC of *Pinus* was $w_i = 0.90$ and the data also provided strong support for an altitudinal trend in the starch and sugar content, but not in the sugar:starch ratio (altitude model $w_i = 0.70$, 0.77 and 0.54, respectively). The starch content and sugar:starch ratio of *Nothofagus* each showed a slight trend with altitude (altitude model $w_i = 0.71$ and 0.67, respectively). The data did not support an altitudinal trend in NSC content for *Phyllocladus, Halocarpus* or *Libocedrus* foliage (expressed either on a dry weight or area basis) (Figure 2.10).

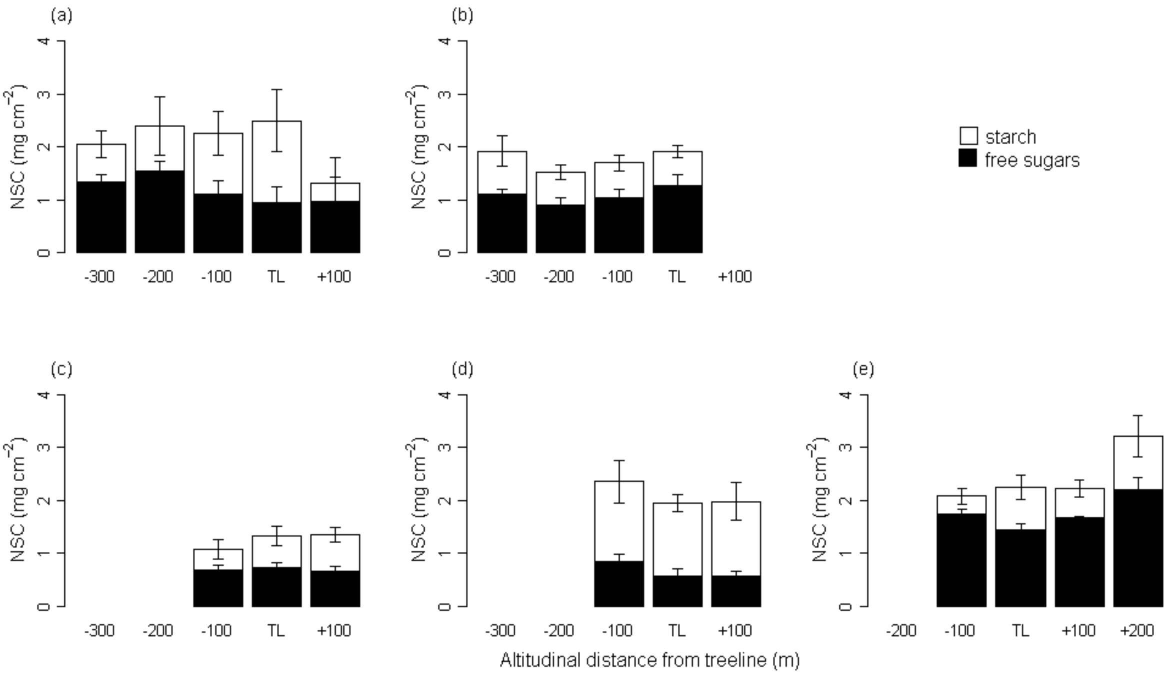


Figure 2.10. Non-structural carbohydrate (starch and free sugars) content (on a leaf area basis, means ± 1 SE) in foliage of treeline species at the West Alps site (a: *Halocarpus;* b: *Libocedrus*) and East Alps site (c: *Nothofagus,* d: *Phyllocladus*, e: *Pinus*) at the end of the 2010 growing season. *Nothofagus* (d) shows a weak trend of decreasing sugar:starch ratio; *Pinus* (e) shows a strong increase in NSC in stunted individuals 200 m above the native treeline (see Table 2.4).

Table 2.3. Linear model results for tree height, DBH, SLA, and mean tree ring width (1949-1999). Two models were tested for each indicator and species: a model with a linear altitudinal trend ("Altitude") and one including an intercept ("Null"), and ranked using *AICc*. Species abbreviations as in Table 2.1.

Indicator	Species	Model[1]	AICc[2]	Δ_i[3]	w_i[4]	LogLik[5]	R^2 [6]
Tree Height	HAL	Altitude	325.75	0	1.00	-159.76	0.84
		Null	522.87	197.12	0.00	-259.38	
	LIB	Altitude	289.86	0	1.00	-141.75	0.62
		Null	356.98	67.12	0.00	-176.4	
	NOT	Altitude	369.47	0	1.00	-181.59	0.77
		Null	500.36	130.9	0.00	-248.11	
	PHY	Altitude	112.44	0	0.90	-53.02	0.08
		Null	116.81	4.37	0.10	-56.31	
	PIN	Altitude	288.81	0	1.00	-141.2	0.60
		Null	344.29	55.48	0.00	-170.04	
DBH	HAL	Altitude	785.66	0	1.00	-389.72	0.60
		Null	885.48	99.81	0.00	-440.68	
	LIB	Altitude	609.43	0	0.98	-301.54	0.11
		Null	617.15	7.72	0.02	-306.49	
	NOT	Altitude	638.25	0	1.00	-315.99	0.52
		Null	703.01	64.76	0.00	-349.44	
	PHY	Null	241.38	0	0.74	-118.59	
		Altitude	243.49	2.11	0.26	-118.54	-0.01
	PIN	Altitude	403.01	0	1.00	-198.3	0.56
		Null	452.63	49.62	0.00	-224.21	
SLA	HAL adult	Altitude	256.98	0	0.92	-125.04	0.18
		Null	261.8	4.82	0.08	-128.68	
	HAL juv	Altitude	366.96	0	0.98	-180.27	0.13
		Null	374.75	7.78	0.02	-185.27	
	LIB adult	Null	132.1	0	0.77	-63.84	
		Altitude	134.52	2.42	0.23	-63.83	-0.03
	LIB juv	Altitude	216.71	0	0.51	-104.86	0.05
		Null	216.79	0.08	0.49	-106.16	
	NOT	Altitude	92.23	0	0.99	-42.02	0.51
		Null	100.91	8.68	0.01	-47.96	
	PHY	Null	75.31	0	0.73	-35.15	
		Altitude	77.25	1.95	0.27	-34.53	0.08
	PIN	Altitude	150.71	0	0.86	-71.6	0.24
		Null	154.38	3.67	0.14	-74.83	
Tree Ring Width 1950-1999	HAL	Altitude	-12.4	0	0.78	9.36	0.04
		Null	-9.91	2.49	0.22	7.03	
	LIB	Null	-11.04	0	0.73	7.68	
		Altitude	-9.07	1.98	0.27	7.87	-0.02
Tree age	HAL	Altitude	427.47	0	1.00	-210.36	0.35
		Null	441.74	14.21	0.00	-218.69	
	LIB	Altitude	556.84	0	0.99	-275.13	0.22
		Null	566.95	10.1	0.01	-281.33	

[1] Number of estimated parameters (K) is 2 and 3 for the Null and Altitude models, respectively;

[2] AIC*c*: Aikaike Information Criterion corrected for small sample size;

[3] Δ_i: Difference in AICc between *i*th model and the top-ranked model;

[4] w_i: Model probability given the data and candidate set;

[5] LogLik: Maximised log likelihood;

[6] R^2: adjusted coefficient of determination.

Table 2.4. Linear model results of foliar starch, sugar, and total non-structural carbohydrates (TNC = sum of starch and free sugars), on a leaf area basis (mg cm^{-2}), and the sugar:starch ratio for each species. Species abbreviations as in Table 2.1. Explanation of table contents, see Table 2.3 footnotes.

Species	Component	Model	AICc	Δ_i	w_i	LogLik	R^2
HAL	TNC	Altitude	70.66	0	0.60	-31.79	0.09
		Null	71.46	0.79	0.40	-33.47	
	Starch	Null	67.44	0	0.60	-31.46	
		Altitude	68.26	0.82	0.40	-30.58	0.03
	Sugar	Null	37.04	0	0.66	-16.26	
		Altitude	38.35	1.32	0.34	-15.63	0.00
	Sugar:Starch	Null	42.01	0	0.82	-18.55	
		Altitude	45.09	3.08	0.18	-18.55	-0.07
LIB	TNC	Null	24.30	0	0.74	-9.80	
		Altitude	26.36	2.06	0.26	-9.43	-0.02
	Starch	Null	26.93	0	0.80	-11.11	
		Altitude	29.67	2.74	0.20	-11.09	-0.05
	Sugar	Null	17.81	0	0.76	-6.55	
		Altitude	20.08	2.27	0.24	-6.29	-0.03
	Sugar:Starch	Null	44.38	0	0.81	-19.79	
		Altitude	47.30	2.91	0.19	-19.79	-0.06
NOT	TNC	Null	18.54	0	0.55	-6.77	
		Altitude	18.98	0.44	0.45	-5.40	0.10
	Starch	Altitude	15.11	0	0.71	-3.46	0.23
		Null	16.87	1.76	0.29	-5.93	
	Sugar	Null	-3.84	0	0.79	4.42	
		Altitude	-1.23	2.61	0.21	4.71	-0.04
	Sugar:Starch	Altitude	27.74	0	0.67	-9.67	0.23
		Null	29.18	1.44	0.33	-12.04	
PHY	TNC	Null	45.48	0	0.80	-20.24	
		Altitude	48.27	2.79	0.20	-20.04	-0.05
	Starch	Null	41.62	0	0.83	-18.31	
		Altitude	44.77	3.15	0.17	-18.29	-0.07
	Sugar	Null	9.28	0	0.58	-2.14	
		Altitude	9.90	0.62	0.42	-0.86	0.09
	Sugar:Starch	Null	33.43	0	0.78	-14.17	
		Altitude	35.98	2.54	0.22	-13.79	-0.03
PIN	TNC	Altitude	44.90	0	0.90	-18.70	0.26
		Null	49.31	4.41	0.10	-22.30	
	Starch	Altitude	27.08	0	0.70	-9.79	0.16
		Null	28.80	1.72	0.30	-12.05	
	Sugar	Altitude	25.55	0	0.77	-9.02	0.19
		Null	27.97	2.42	0.23	-11.63	
	Sugar:Starch	Altitude	24.68	0	0.56	-8.49	0.12
		Null	25.12	0.44	0.44	-10.16	

2.4 Discussion

2.4.1 Treeline climate

Annual precipitation and relative humidity was much higher at western, gradual, treelines than at the abrupt, eastern treelines, consistent with the arrangement of the main axial ranges and predominant west to southwest airflow patterns in New Zealand and associated rain-shadow effects (Salinger 1988). A scarcity of measured temperature records at higher altitudes have led to engrained assumptions of similarly striking differences between temperature regimes between these regions. For example, summers in subalpine forests in the high rainfall regions on the West Coast have long been considered shorter and cooler (e.g., Wardle 1973, Veblen and Stewart 1982) than summers in similar forests in the eastern rain-shadow region. Temperature measurements in the current study show that the assumption of difference in summer warmth is overstated. Mean air temperature during the three warmest months was 9·9-10·5˚C at the four gradual treeline sites in this study, only slightly cooler than the 10·6-11·0˚C of the abrupt *Nothofagus* treelines in the rain-shadow region.

New Zealand treelines have previously been argued to equate with a warmest month mean air temperature of *ca*. 10°C (Zotov 1938, Wardle 1973, 2008), largely based on extrapolated weather station data. Overall, the sites in this study had a higher average warmest month mean of 11·5°C (Figure 2.3). The only other published measured data from the gradual Camp Creek ecotone (Payton 1989) and from two abrupt *N. menziesii* treelines in the wetter western regions of South Westland (Mark et al. 2000) and Fiordland (Mark et al. 2008) provided slightly lower means of between 9·9 and 10·6˚C.

Warm months may not differ much in temperature between treeline types, but differences become clear when comparing the climate in the colder months of the year. Measured mean coldest-month air temperature at the two abrupt treelines were -0·1˚C and +0·2˚C. Previously, Wardle (2008) found a correlation of New Zealand *Nothofagus* treelines with a coldest-month mean temperature of around 0˚C, using extrapolation of low altitude meteorological data. These values are in accordance with the limits of broadleaved evergreen trees globally at 0˚C (Ohsawa 1990). In contrast, mean coldest-month air temperature in this study was warmer at the mixed conifer-broadleaf gradual treelines at between 1·6˚C and 1·9˚C, comparable to the 1·5˚C recorded at Camp Creek in 1979-1983 (Payton, unpublished). Absolute air temperature minima were also lower at the abrupt treeline (-9˚C at Rainbow) than the gradual treelines (-6˚C).

Soil frosts were very rare in the gradual treeline ecotone (maximum of 3 days per year 200 m above Camp Creek treeline), whereas at the abrupt *N. solandri* var. *cliffortioides* treelines, they occurred around 20 days per year. At an abrupt *N. menziesii* treeline ecotone in south Westland, however, soil only froze near the upper limit to tussock (at 1550 m, approximately 500 m above the local treeline) (Mark et al. 2008). There was no clear difference in the number of air frosts at the different treeline types: air frosts occurred around 105 days per year at treeline (although abrupt Rainbow, at 145, had many more frosts), and *ca.* 20 more days 100 m above treeline. In this study, ground extreme minima were always less severe than air temperature minima, contrary to common belief. The ground loggers were placed in screens under small shrubs or tussock grasses. The warmer temperatures suggest that the vegetation cover or logger screens caused some heat retention or decreased radiative cooling, or that the screens were not sufficiently aerated to allow for advective cooling to occur. Extreme maxima recorded at the ground surface were higher than the air temperature.

The presence of snow cover estimated from a stable soil temperature around 0˚C indicates a continuous snow duration of only about two weeks at and around gradual treeline, and of up to one month 200 m above these treelines, as had been reported for Camp Creek by Payton (1989). At the two abrupt treelines studied, continuous snow cover lasted between one and two months. Strong winds may cause considerable snow redistribution (McCracken et al. 1985), and more than 30 additional days with snow cover (up to 124 days, not necessarily continuous) were recorded under the treeline canopy compared to the adjacent tussock grassland. It has been suggested that in places where the snow accumulates in the treeline margin, krummholz *Nothofagus* trees prevail (Norton and Schönenberger 1984, Wardle 1985b); however, these are not found at the *Nothofagus* sites in this study.

The mean soil temperature during the growing season has been used to describe the treeline thermal environment globally (Körner and Paulsen 2004) and in New Zealand (Mark et al. 2008). In their survey of soil (10 cm) temperature at 46 treeline sites worldwide, Körner and Paulsen (2004) found a remarkably consistent growing season temperature of 6·7˚C ± 0·8 S.D. However, they found that two abrupt *Nothofagus* treelines in New Zealand (and several other highly oceanic sites, including sites in the Andes) were anomalously warm – with temperatures of 8-10˚C. At another abrupt

Nothofagus treeline in the southern region of New Zealand, temperature data gathered using the same protocol also suggest a warm treeline at 8·7˚C (Mark et al. 2008). In Chapter 3, the growing season thermal environment at the New Zealand treeline will be discussed in detail and put in an international context. Here, it suffices to say that soil temperature during the growing season from the six New Zealand treeline sites in the current study fall within the range published by Körner and Paulsen (2004) and provide support for the notion that the New Zealand treeline is not warm compared to global patterns (see Chapter 3).

Altitudinal temperature trends

The altitudinal temperature trends found in this study reflect the interaction of temperature with vegetation stature and cover (Körner 2003a): across the gradual treeline ecotone, the gradual decrease in vegetation stature with altitude (see below) was matched by a general decrease in temperature. In contrast, this simple altitudinal pattern was not visible across the abrupt treeline ecotone: abrupt changes in vegetation stature are accompanied by abrupt changes in the temperature regime (Figure 2.4).

Across the gradual treeline ecotone, soil and ground temperatures gradually decreased with altitude. At the treeline, air temperature during the three warmest months was warmer than the ground and soil temperature, but at 100 m above treeline, the air was equally warm or cooler than the ground and soil (Figure 2.4a-d). The highest altitude site at Mt Fox (Figure 2.4d) was much warmer than lower sites, presumably due to the effect of the very steep (> 60˚ inclination) slope at this site and lack of vegetation, providing an example of the effect of land surface characteristics and vegetation stature on temperature (Körner 2007b). However, this may have been augmented by the lack of recording at this site during the cool year of 2009 (see Section 2.2.2, p. 26), biasing the data to the warmer 2010 and 2011. I have not found a satisfactory reason why the ground surface temperature at 100 and 200 m above the Camp Creek treeline (Figure 2.4a) are so low compared to soil temperature, as both were placed under the same vegetation, and were recording during the years 2009-2011.

Grace and others (1989) showed that apical meristem temperature in tall forest was similar to the ambient air temperature, whereas there was considerable difference between these temperatures for krummholz and dwarf shrubs. Although short vegetation is warmer than tall vegetation on sunny days with little wind (especially under a continental climate regime), in areas of high wind, high humidity and/or where cloud cover reduces insolation with altitude (e.g., oceanic climate), short vegetation has less of a thermal advantage. However, even at the highly oceanic West Alps sites decoupling of atmospheric temperature within lower stature vegetation was evident: lapse rates of ground and soil temperatures were lower than air temperature lapse rates during the three warmest months (slope of the trends in Figure 2.4). Similarly, air temperature at the grassland adjacent to and above the abrupt Rainbow treeline was much cooler than the ground and soil temperatures at the same sites, suggesting an effective buffering of the ambient climate closer to the ground surface.

At the two abrupt treeline sites, soil supporting *Nothofagus* forest was cooler than soil of nearby sites where trees were absent, as has been found by others (Wardle 1985b, Körner et al. 1986, Mark et al. 2008). Mean soil and ground temperature within 10 m either side of the treeline (i.e., in the forest margin and in the alpine grassland) during the warmest months differed by 1-2·5°C (Figure 2.4e-f). The forest canopy shades the soil upon which it grows, and the small soil heat flux and radiative warming keeps the root zone temperature cold (Körner 2003a). These results emphasise the role that the architecture of trees can play in creating a cool microclimate and hence in limiting the growth of trees themselves.

2.4.2 Tree performance

Growth

All tree species showed a clear decline in tree height and DBH across the treeline ecotone (Figure 2.7, Figure 2.8), as is commonly found along such altitudinal gradients (Paulsen et al. 2000, Massaccesi et al. 2008). New Zealand treelines are at low altitudes compared with continental sites at the same latitudes (*ca*. 1800–3300 m; Körner & Paulsen 2004; Wardle 2008), and forest stature therefore declines over a narrower altitudinal range (Bellingham and Sparrow 2009). However, the average decrease of tree height of 2·6 m per 100 m altitude towards the New Zealand treeline is much lower than that of *Pinus* and *Picea* in the European Alps (*ca*. 7 and 5 m, respectively) (Paulsen et al. 2000). Presumably, this reflects the overall smaller stature of trees in New Zealand compared to species in other temperate regions (McGlone et al. 2010a). Indeed, when expressed as a proportion of tree height in the montane forest (e.g., 300 m below treeline), the decrease in stature per 100 m altitude is similar at the European and New Zealand treeline (26% and 18-25%, respectively). The height of the first branch also decreased with altitude for all species (data not shown), supporting findings of more frequent multi-stemmed trees at higher altitude in New Zealand *Nothofagus* forests (Bellingham and Sparrow 2009) and mixed conifer-broadleaf forests in New Zealand (Reif and Allen 1988).

At the abrupt treeline, *Nothofagus* trees *ca*. 5 m tall gave way to tussock grassland. In areas near their introduced range, scattered tree-statured individuals of exotic *Pinus* were found up to 150 m higher than the native *Nothofagus* treeline, with short-statured individuals occurring even higher. In effect, the scattered *P. contorta* individuals form a higher altitude gradual treeline (Figure 2.8). Interestingly, a similar phenomenon was found at the gradual West Alps treeline. Here, trees (> 3 m tall) of *Halocarpus*, *Olearia* and/or *Dracophyllum* species form the treeline, but individuals of *Halocarpus* of decreasing stature extend to more than 200 m higher (Figure 2.7). Emergent *Libocedrus* trees of 3-8 m tall are part of the mixed-conifer forest up to the species' altitudinal limit close to the local treeline (Veblen and Stewart 1982, Haase 1986b). At none of the sites studied, or visited, were any *Libocedrus* individuals smaller than tree stature (< 3 m) present at higher altitudes, and thus it reaches an abrupt limit as a tree 50 m below the local treeline. This species' strong monopodial growth form, may not support development of a shrub growth form, perhaps because of the lacking ability to produce epicormic shoots after injury (Haase 1990b) or phylogenetic constraints.

The decrease in tree stature and DBH with altitude of *Libocedrus* was not matched by a decrease in radial growth rate (Figure 2.9). Similarly, Paulsen et al. (2000) found that *Pinus* and *Picea* trees decreased in stature with altitude in the European Alps, but that recent tree-ring width was similar across altitudes. This discrepancy could not be explained in terms of age (trees higher up were not simply younger), rather, they found that a decreasing trend in tree-ring width had been evident in previous times (pre-1950). Since that time, tree ring width has been similar across the 250 m treeline ecotone. The timing of these changes does not favour explanations of increases in ambient CO_2 concentrations and temperature over the recent decades. Instead, they suggest the results are consistent with an elevational response of tree-rings that includes a threshold component, probably related to a minimal seasonal temperature (Paulsen et al. 2000). In the current study however, trees were younger at higher altitudes, which could explain the reduced stature with altitude. In addition, mean tree-ring width over consecutive 50 year periods (1850 onwards) showed the same patterns. *Halocarpus* showed reduced height, DBH as well as radial growth with altitude (Figure 2.7). In addition, as for *Libocedrus*, the largest individuals of this species are younger (*ca*. 220

years old) near treeline than at lower altitude (*ca*. 400 years at 300 m below treeline) (Figure 2.9).

These tree ages are consistent with, but do not prove, an upward extension of the treeline starting in the late eighteenth century, coinciding with the retreat of New Zealand's glacier after the "Little Ice Age" from *ca.* 1740 AD (Winkler 2004). The timing of the upward extension of gradual treelines in New Zealand warrants more study due to the uncertainties in the crude age estimates: for the majority of the samples, age was taken as the total count of tree-rings, even though it is not certain that the cores hit the centre of the tree. Additionally, almost all mature individuals of *Libocedrus* suffer from heart-rot (Dunwiddie 1979, Norton 1983), resulting in uncountable older rings. Therefore, at best, the age data presented here refer to the tree's minimum age; and, at worst, if heart-rot varies with altitude, should be considered invalid. As *Halocarpus* hardly suffers from heart-rot (Dunwiddie 1979), age estimates should be more reliable than for *Libocedrus*. In any case, the age estimates from cores about 1 m above the ground will underestimate the actual time from germination by several decades.

Annual radial growth of *Nothofagus* and *Pinus* were not assessed in this study, as they have been already documented. Wardle (1984) showed a strong decline of stem diameter increment in *Nothofagus* with altitude, but a clear *in*crease in radial growth of trees close to the treeline forest margin (*ca*. 8 mm/annum), presumably due to reduced competition. Norton (1985) found *Nothofagus* trees towards treeline were more responsive to climatic variations than trees at lower altitude. In contrast, in a study of co-generic *N. menziesii* near treeline, Cullen *et al.* (2001a) found no changes in sensitivity towards treeline. At the same altitude, radial and height growth of *Pinus contorta* is approximately twice as fast as that of *Nothofagus* (Scott 1972, Benecke and Nordmeyer 1982). Nordmeyer (1980a) found that stand mean annual increment (m^3 ha^{-1}) of *Pinus* was almost four times that of *Nothofagus* at 1200 m a.s.l.. Most of the above studies did not present absolute values of tree-ring width for direct comparison with the current study. Nonetheless, both these species have much faster growth rates than *Libocedrus* and *Halocarpus* (average 0·5 mm $annum^{-1}$).

Specific leaf area (SLA) is an indicator of several ecophysiological characteristics, including relative growth rate, stress tolerance and leaf longevity (Wright and Westoby 2002). It is closely related to leaf thickness, leaf nitrogen, light-saturated leaf photosynthetic rate per unit mass and leaf lifespan. These co-variations can be explained by the hypothesis that plants maximise their canopy carbon export over the lifespan of leaves (McMurtrie and Dewar 2011). The presence of old leaves requires robustness which only occurs in long-lived species with slow growth rates and low carbon gain requirements from individual leaves (Richardson et al. 2010). The low SLA of the West Alps species in this study concurs with their low growth rate and long leaf life span. Most scale-like leaves of *Halocarpus* live around ten years, although some may live > 50 years; *Libocedrus* foliage has a life span of six to eight years (Wardle 1963a). This contrasts with the higher SLA and growth rates and lower leaf life span of *Nothofagus* and *Pinus*: *Nothofagus* leaves are shed in the season after they are produced, although slower growing shoots may retain their leaves for two years (Wardle 1963a), whereas most needles of *Pinus* at Craigieburn are retained for around three years, although some may live up to seven years (Benecke and Nordmeyer 1982). To my knowledge, no studies have been published detailing changes in leaf life span with altitude for these species.

Generally, SLA decreases with altitude as environmental stress increases and leaves become more robust, i.e., thicker and denser (Körner et al. 1986, Cordell et al. 1999). In this study, all species showed a decrease in SLA with altitude (Figure 2.7, Figure 2.8); similar trends elsewhere have been attributed to a different radiation (Poorter et al. 2009) or thermal (Körner 2003a) environment. The limited data available for New Zealand higher altitude sites suggest that radiation changes with altitude are small (at Craigieburn, incoming solar radiation changes of around 1% per 100 m, McCracken 1980), so any changes in SLA with altitude are more likely to be a response to temperature (Körner 2003a). For *Libocedrus,* only juvenile foliage SLA decreased with altitude, whilst that of adult foliage remained constant. *Halocarpus* adult foliage showed a weak but significant negative trend in SLA with altitude, whereas the trend for juvenile foliage was much more pronounced. At all altitudes, SLA of juvenile foliage for both species was higher than the adult foliage, reflecting their different shapes, as well as the light environment they experience (adult foliage was gathered specifically from the sun-lit canopy, whereas juvenile trees and/or foliage were only found in the shade). These results suggest that for these two species, juvenile foliage is more plastic and perhaps more sensitive to changes along the altitudinal gradient than the more robust adult foliage.

Carbon balance

Observations show that low temperatures exert a stronger dampening effect on growth and developmental processes than on photosynthesis (Grace et al. 2002, Körner 2003a). Such low-temperature induced imbalance between growth and photosynthesis is expected to cause an accumulation of non-structural carbohydrates (NSC) in plant tissues (Susiluoto et al. 2007). Assessing the NSC pool size along an altitudinal (and temperature) gradient can thus inform the debate surrounding carbon sink and source limitation (Hoch et al. 2002, Li et al. 2002). So far, most of the published studies of altitudinal gradients of NSC concentration at the end of the growing season have shown an increase in NSC towards treeline, which has been interpreted as evidence for the carbon sink limitation (Hoch et al. 2002, Hoch and Körner 2003, Körner 2003b, Shi et al. 2006, Shi et al. 2008). In this study, the exotic *Pinus contorta* showed a significant increase of NSC and its components sugar and starch with altitude, driven by an increase of *ca*. 25% of sugar and almost doubling of starch concentration at the highest site. This suggests that this species, at high altitude sites, is sink-limited, as has been found for con-generic species (Hoch and Körner 2003); i.e., photosynthetic assimilates accumulate because cold temperatures limit the activity of growth processes, resulting in individuals no longer reaching tree height (3 m).

Nothofagus showed an increasing altitudinal trend in starch content, and a decreasing sugar:starch ratio with altitude, which may be interpreted as evidence of a carbon-sink limitation (Fajardo et al. 2011). In contrast, none of the native conifer species in this study showed an altitudinal trend in total NSC or its components.

2.5 Preliminary conclusions and research questions

This chapter described the results of an intensive temperature measurement campaign across the ecotones of abrupt and gradual treelines, providing information about the climate experienced at New Zealand treeline. Western New Zealand, gradual, treelines experience higher precipitation and relative humidity than the abrupt, eastern, rain-shadow treelines. The different treeline forms have a similar temperature regime in the warmer months of the year, but they differ markedly in their winter season: the abrupt treeline ecotone experiences colder temperatures, more frosts and a longer lasting snowpack than the gradual treeline ecotone.

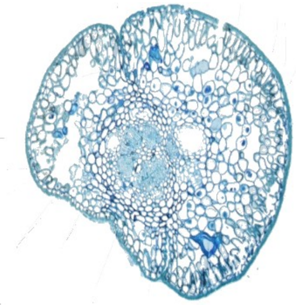
Altitudinal gradients can be used as natural experiments of the responses of plants to environmental stresses. Across the New Zealand treeline ecotone, tree stature decreased with altitude for all species studied, presumably as an adaptation to the higher altitude and accompanying environmental stresses. At the abrupt *Nothofagus* treeline, trees of *ca.* 5 m tall give way to tussock grassland. This treeline form could result from positive feedback processes mediated by the differences in the microclimate between forest and adjacent grassland. The gradual decrease in stature and DBH with altitude at the West Alps sites can at least partially be explained by tree age, with younger trees near the treeline apparently having established after the maximum of the "Little Ice Age". Specific leaf area reflected the leaf longevity and growth rates of the species and generally decreased with altitude. Carbon storage in foliage of native conifer species near treeline did not provide clear evidence as to whether cold temperatures may be limiting photosynthesis or growth process (source- or sink-limitation, respectively), whereas the native *Nothofagus* and exotic *Pinus contorta* showed evidence of the sink-limitation hypothesis towards their upper limit.

Trends in climate and tree performance across the ecotone may provide indications of which environmental stresses or processes are limiting tree growth at higher altitudes. Linking to the patterns highlighted in this chapter, the remainder of this study will assess how temperature may limit the growth of tree species at the New Zealand treeline: Chapter 3 will explore the warmth of the growing season at treeline; Chapter 4 will assess whether freezing air temperatures at any time of the year are likely to affect the performance of treeline trees; Chapter 5 and Chapter 6 will look at the effects of temperature on photosynthesis and growth assessed in controlled environment experiments; finally in Chapter 7, all findings will be drawn upon to assess which mechanisms are important in limiting the growth of different tree species near treeline in New Zealand.

Plate 2.1. Examples of trees (left), foliage (middle) and foliar cross sections (right) of the main species in this study.

Halocarpus biformis

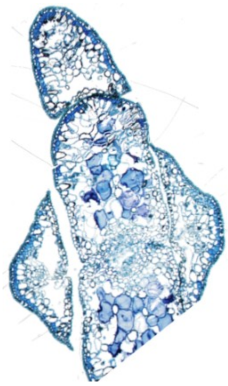
Shrub-statured individual near Camp Creek treeline



Libocedrus bidwillii

Emergent individual in the upper montane forest at Camp Creek

Photo: John Hunt



Nothofagus solandri

Trees at the abrupt treeline at Rainbow

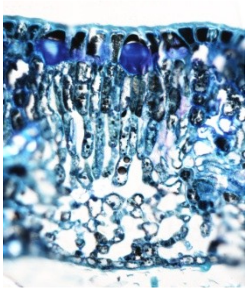
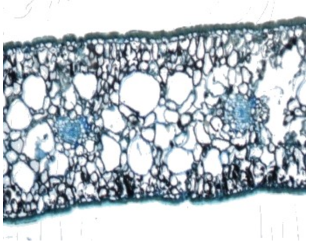


Plate 2.1 continued.

Phyllocladus alpinus

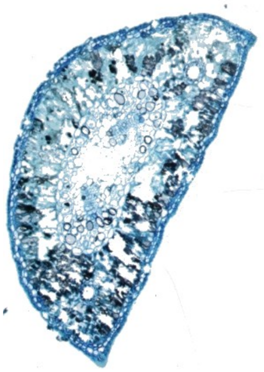

© O2landscapes.com

Shrub-statured individual in Hooker Valley



Pinus contorta

Individual *ca.* 50 m above the native treeline at Craigieburn



Podocarpus hallii


© M Cosgrove

Tall-statured individual on Mt Taranaki

Chapter 3 Re-evaluating growing season warmth at oceanic treelines

3.1 Introduction

Despite a long research history, the exact physical controls of the upper altitudinal limit of trees remain unclear (see e.g., Holtmeier and Broll 2005). The general consensus is that growing season warmth provides the best explanation of the formation of treelines, convincingly demonstrated in an extensive global study of treeline temperatures (Körner and Paulsen 2004). Studies at several treelines suggest that the ability to invest in growth, rather than acquisition of photosynthates, is limiting tree growth at higher altitudes (Hoch et al. 2002, Piper et al. 2006, Shi et al. 2006, Fajardo et al. 2011). However, a mechanistic explanation is lacking and exactly what aspect of the growing season temperature regime is most important to alpine tree growth remains uncertain. Given the central importance of growing season warmth, it is puzzling that standard measures such as growing degree days (GDD) and growing season length, which have long been used as accurate predictors of primary productivity in lowland agricultural systems (Goudriaan and Van Laar 1994, Bonhomme 2000), have no explanatory power with regard to treeline position worldwide (Körner and Paulsen 2004).

In the global assessment of treeline soil temperatures, some locations stood out as anomalous. In particular, Mediterranean and south temperate zone treelines (including two in New Zealand) were much warmer than the global mean, and this was attributed to ‘genus-specific boundaries’ (Körner and Paulsen 2004). This implies that certain tree lineages are held either unable of evolving species capable of growing at treeline, or have had insufficient time to do so (Wardle 2008). These explanations seem improbable. To take the New Zealand example, there has been at least 2.6 million years during which alpine treelines have been present, and at least 13 tree species (in nine genera) are present in the treeline ecotone (Wardle 2008). That all these species failed to evolve as great a tolerance of cool growing season conditions as trees elsewhere is, of course, possible. What is less explicable is that all 13 reach a common altitude at any given mountain site where they are present, forming a natural-appearing treeline. This requires the assumption that they have encountered some fundamental limitation that comes into play at growing season temperatures warmer than the global treeline norm. This may be so, but since most tree lineages at the New Zealand treeline have close

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Photo previous page: View down the Mikonui gradual treeline ecotone

relatives that form high, cold treelines in the tropics (Wardle 2008), this explanation is improbable.

Instead of perpetuating these suppositions, the possibility that the climate data have not been adequately considered in the light of a highly variable oceanic climate should be considered. For instance, oceanic treelines, such as those of the New Zealand archipelago, are characterised by long, highly variable growing seasons making it difficult to apply growing season concepts developed primarily for use in more continental, north temperate and boreal regions. More fundamentally, we could be mistaken in our assumption that soil temperature is, at each and every treeline, a reliable surrogate for air temperature. Until comprehensive temperature data have been analysed for a range of treelines, we will not be able to narrow down the fundamental relationship between temperature and treeline. This is a vital first step before accurate prediction of the response of treeline to climatic change can take place (Holtmeier and Broll 2007).

In this study, I used detailed soil and air temperature at six alpine treeline sites (described in Chapter 2) and supplemented this dataset with previously collected air temperature data from different sources for a further 14 sites near treeline. These twenty sites include abrupt and gradual treeline ecotones and range from the most southern treeline in the south-western Pacific Ocean (sub-antarctic Campbell Island at 52·6°S) to the central North Island at 39·2°S, and from sea level to 1530 m. This study has three interlinked aims:

1. To establish if New Zealand treelines, as an example of southern temperate treelines, are significantly warmer than the global average;
2. To find a metric that most consistently describes the thermal environment at treelines in New Zealand, and that will permit comparisons between oceanic and continental treelines;
3. To assess which component of the growing season thermal environment is most likely to control growth of alpine trees.

3.2 Methods

Temperature data

Air and soil temperature were measured for *ca*. 2 years at six treeline locations in New Zealand (Table 3.1). The placement of the data loggers is described in Chapter 2. Here, I used air and soil temperature data from the data loggers located exactly at the treeline only. The daily amplitudes of all soil temperature data did not exceed 5·5°C, which would indicate direct sunlight on the soil surface above the logger (Körner and Paulsen 2004).

Table 3.1. Details of 10 New Zealand treeline sites with soil (10 cm depth) temperature data. Measurements were taken underneath the canopy at the treeline, except at Campbell Island, where they were taken approximately 10 m below the treeline in the open. Sites are ordered by decreasing latitude. Site numbers are consistent in the following tables.

Site No.	Treeline Site Name	Altitude (m a.s.l.)	Longitude (°E)	Latitude (°S)	Form	Data Source	Records Used (from d/m/y - to d/m/y)
1	Campbell Island	25	169.15	52.55	gradual	NIWA	01/01/1985-31/12/1994
2	Mt Burns	1040	167.37	45.73	abrupt	Mark et al. 2008	01/01/2003-14/12/2005
3	Mt Fox	1185	170.01	43.50	gradual	This study	06/01/2010-22/03/2011
4	Mikonui	1210	170.87	43.06	gradual	This study	17/01/2009-20/03/2011
5	Kelly Creek	1150	171.58	42.78	gradual	This study	13/05/2009-28/04/2011
6	Camp Creek	1160	171.57	42.71	gradual	This study	31/01/2009-27/04/2011
7	Mt Haast	1220	172.08	42.33	abrupt	Körner & Paulsen 2004	01/12/1998-15/04/2000
8	Rainbow	1530	172.86	41.89	abrupt	This study	11/04/2009-09/04/2011
9	Kaweka	1460	176.36	39.29	abrupt	This study	26/11/2009-06/06/2011
10	Kaimanawa	1350	175.85	39.17	abrupt	Körner & Paulsen 2004	23/12/1998-12/03/2000

Soil temperature data acquired in this study were augmented with data from Campbell Island (obtained from the New Zealand National Institute of Water and Atmosphere website, NIWA, cliflo.niwa.co.nz), and from three New Zealand treeline sites previously published (Körner and Paulsen 2004, Mark et al. 2008); these data were kindly provided by the authors (Table 3.1). All soil temperature data presented here are based on arithmetic means of hourly values, except the soil data from the sub-antarctic Campbell Island. Soil temperatures there were from a standard meteorological station within the treeline ecotone, but not under a canopy. They are based on daily 9 am readings of soil (10 cm) temperature. The island is cloudy and direct radiation received is very low (*ca*. 650 hours annually, with less than one hour of sunshine on 59% of days of the year).

Average annual rainfall is 1450 mm, falling over 325 days a year. Low solar radiation combined with a permanently saturated soil results in a strongly dampened temperature comparable to that experienced underneath the canopy.

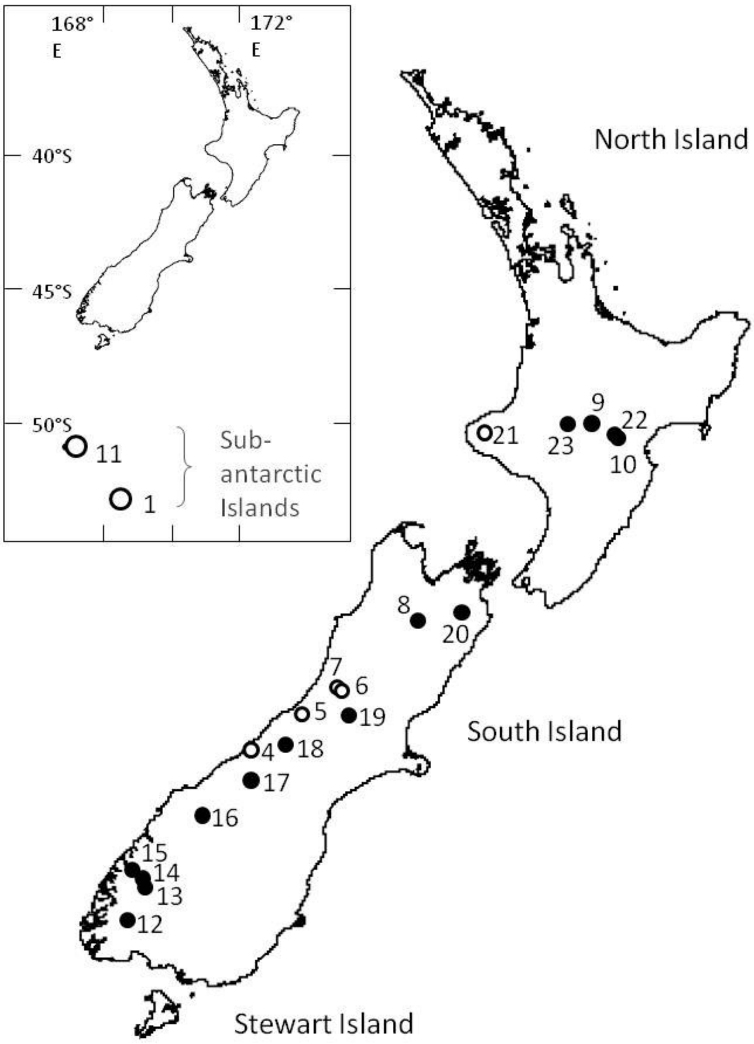

Figure 3.1. Location of all treeline sites with soil and/or air temperature data included in this study. Black circles are abrupt treelines, white circles are gradual treelines. Numbers relate to the site numbers in Tables 3.1-3.3.

To assess the thermal environment at a wider range of treelines, a dataset of air temperature from 14 additional sites near treelines was collated from multiple sources (Figure 3.1, Table 3.2). The air temperature data consisted of daily minimum (T_{min}) and daily maximum (T_{max}) temperature. If possible, records spanning the 2001-2010 period were selected (Table 3.2). The altitude of treeline closest to each weather station was determined from the literature, topographic maps or Google Earth. Only data from weather stations less than 500 m vertically from treelines were selected to reduce errors associated with lapse rate calculation and extrapolation. For similar reasons, weather stations located on valley floors prone to cold air drainage were excluded. Since lapse rates vary substantially throughout the year (e.g., Blandford et al. 2008), monthly temperature lapse rates for T_{min} and T_{max} were separately calculated from additional weather stations on the same mountain range. Daily mean temperatures were then calculated using the meteorological convention: $(T_{min} + T_{max}) / 2$. For each measured and estimated site, mean data for a 365-day year were obtained by averaging data collected for the same day in different calendar years. Together the 20 sites spanned the length of the New Zealand archipelago, and an altitudinal range of 1520 m (Figure 3.1).

New Zealand treeline soil temperature in a global context

To compare the six measured treeline sites in this study with the global study of soil temperature at treeline (Körner and Paulsen 2004), the average temperature of the growing season was calculated following the conventions in that study: the growing season starts on the date when the soil temperature at 10 cm depth exceeds 3·2°C in spring, and ends when this falls below 3·2°C for the first time in autumn (further referred to as MEANSOIL3·2 growing season). In continental regions, snowpack accumulation or melt often results in abrupt transitions between seasons. However in more oceanic settings, a more gradual transition is common and the 3·2°C daily threshold may be crossed multiple times. To reduce variability, the threshold of 3·2°C was applied to the 7-day mean of the soil temperature and the growing season was not allowed to begin in the three mid-winter months (June – August) nor end before the 1st of May. In their study, Körner and Paulsen (2004) applied similar logic to prevent biologically unrealistic outcomes (Jens Paulsen, pers. comm.).

Table 3.2. Details of the 20 treeline sites with screened air temperature data used in the analyses. Sites are ordered by decreasing latitude. Site numbers in Tables 3.1 and 3.2 are consistent for sites with measurements of both soil and air temperature (Sites 2, 5, 9 in Table 3.1 did not have air temperature measurements, so are not included here).

Site No.	Treeline Site Name	Altitude (m a.s.l.)	Altitude difference TL – WS1 (m)	Lon (°E)	Lat (°S)	TL Form	Data Source	Records Used (from d/m/y - to d/m/y)
1	Campbell Island	25	+10	169.15	52.55	gradual	NIWA	01/01/2001-30/12/2010
11	Auckland Islands	180	+140	166.30	50.48	gradual	NIWA	01/01/2001-31/12/2010
12	Borland Saddle	1040	+49	167.37	45.73	abrupt	NIWA	01/01/1972-30/09/1976
13	Takahe Valley	895	0	167.68	45.30	abrupt inverted	NIWA	01/06/2009-31/12/2010
14	Murchison Mountains	1050	-90	167.63	45.22	abrupt	NIWA	01/07/2008-31/12/2010
15	Doon	1050	-161	167.48	45.09	abrupt	Meridian	01/01/2001-31/12/2010
16	Albert Burn	1200	-80	168.93	44.38	abrupt	NIWA	01/02/2008-31/12/2010
17	Hopkins	1250	-152 & +449[2]	169.79	43.99	abrupt	Meridian	01/01/2001-31/12/2010
18	Godley	1300	-209 & +425[2]	170.48	43.52	abrupt	Meridian	01/01/2001-31/12/2010
*3	Mt Fox	1185	0	170.01	43.50	gradual	This study	06/01/2010-22/03/2011
19	Craigieburn	1350	+436	171.71	43.15	abrupt	NIWA	01/01/2001-31/12/2010
*4	Mikonui	1210	0	170.87	43.06	gradual	This study	17/01/2009-20/03/2011
*5	Kelly Creek	1150	0	171.58	42.78	gradual	This study	13/05/2009-28/04/2011
*6	Camp Creek	1160	0	171.57	42.71	gradual	This study	31/01/2009-27/04/2011
*8	Rainbow	1530	0	172.86	41.89	abrupt	This study	11/04/2009-09/04/2011
20	Black Birch Range	1300	-96	173.80	41.75	abrupt	NIWA	01/01/1972-01/10/1976
21	Mt Taranaki	1100	+254	174.12	39.31	gradual	NIWA	01/01/1976-31/12/1985
*9	Kaweka	1460	0	176.36	39.29	abrupt	This study	26/11/2009-06/06/2011
22	Kaweka Makahu Spur	1460	-18	176.39	39.28	abrupt	NIWA	01/01/1972-01/09/1973
23	Mt Ruapehu	1450	+353	175.54	39.20	abrupt	NIWA	01/01/2001-31/12/2010

1 Difference in altitude between TL (Treeline) and WS (weather station) sites; where '+' indicates TL is above the WS, '-' TL is lower than the WS.

2 Temperature data for the treeline were interpolated from two weather stations were availabe, one above and one below treeline.

* Sites measured as part of this study, exactly at treeline.

Finding a consistent thermal descriptor of treeline

To find a metric that most consistently describes the thermal environment of New Zealand treeline, a series of thermal descriptors were calculated. First, some metrics that previously have been associated with treeline altitude were calculated: mean air temperature of the warmest and coldest month, and growing degree days (sums of the daily mean air temperature above bases of 0°C and 5°C; GDD0 and GDD5, respectively). Secondly, a series of alternative growing seasons were defined using different thresholds of daily mean and daily minimum air temperature. The same mean temperature may reflect different combinations of minimum and maximum temperature, and the biological relevance of mean temperature remains poorly understood (Körner 2003a, Hoch and Körner 2009). The use of thresholds based on minimum temperatures reflects the observations that carbon accumulation and growth processes may be restricted by overnight chilling temperatures (Johnson et al. 2004, Rossi et al. 2008).

Eleven potential growing seasons were calculated using a threshold on the mean 7-day daily air mean temperature (T_{mean} thresholds were set at intervals of 1°C within the range 0°C to 10°C). Similarly, eleven potential growing seasons were calculated using thresholds based on the mean 7-day daily minimum air temperature (T_{min}, ranging from -2°C to 8°C). The overall warmth of these different growing seasons was calculated as the average T_{mean}, T_{min} or T_{max} during each growing season.

The highly variable climate in oceanic regions is likely to give rise to a non-continuous growing season, in that cold periods could result in a ‘stop-start’ growing season. Therefore, these cold periods (where the 7-day temperature fell below the threshold) were excluded from the growing season metric. In a more continental setting with consistently warm temperatures in the warmer months, applying this same definition would give rise to a continuous growing season.

A strong test of whether or not a given temperature metric is likely to have a direct relationship with biological processes at treeline is that it shows minimal variation with latitude, altitude or species composition of natural treelines across a wide range of sites. To assess the degree of convergence across sites, we used the standard deviation (S.D.) and the coefficient of variation (C.V.). The C.V. is the ratio of the standard deviation to the mean (expressed as a percentage), and hence is independent of the size of the mean. As C.V. can only be usefully calculated for values on a ratio scale with a nonarbitrary, absolute zero, temperature descriptors (°C) were expressed in degrees Kelvin, prior to calculating C.V.

In the search for the component of the growing season thermal environment which is most likely to control growth of trees at treeline, it is important that the results of any selection process as described above should not contradict data from field observations. For example, any defined growing season should at least encompass the growing season as identified by phenological studies at treeline. Convergence of the different descriptors was assessed across the six treeline sites that received simultaneous temperature measurements as part of this study. Concentrating on these measurements gathered exactly at the treeline reduced potential errors introduced through the estimation of treeline temperature by use of lapse rates. Linear models were used to assess the relationship of the different thermal descriptors with latitude and altitude.

Applying this thermal descriptor to a wider range of treelines

After identifying the best descriptor of the thermal environment across the six measured sites, I then calculated the same metric for the additional 14 treeline sites across the New Zealand archipelago, to determine whether this descriptor is also relevant and consistent across sites with a wider altitudinal, latitudinal and compositional range.

Lastly, I assessed whether this metric also consistently described treeline temperature around the world. To this end, daily T_{min} and T_{max} air temperature data from treeline sites in the major biomes were kindly provided by Bob Baxter and the ABACUS project (Abisko – boreal), Gerhard Wieser (Austria – northern temperate), Guenter Hoch (Bolivia – tropics), Nick Pepin (Kilimanjaro – tropics), Lohengrin Cavieres (Chile – southern temperate/mediterranean), and the Australian Meteorological Bureau (Australia – southern temperate/mediterranean). Thermal descriptors were calculated as described previously.

3.3 Results

Treeline soil temperature

Growing season soil temperatures (MEANSOIL3.2) at the New Zealand treeline sites in this study ranged from 6·6 to 7·6°C (Table 3.3). MEANSOIL3.2 growing season length was shorter and mean soil temperature was higher at abrupt (221 days, 7·4°C) than gradual (278 days, 6·8°C) treelines. Mean soil temperatures were similar between the two treeline forms if equal time intervals were compared (Appendix 2, Figure A2.1).

The temperature data from the two New Zealand *Nothofagus* sites as presented by Körner and Paulsen (2004) are not consistent with the other New Zealand sites. On inspection of their raw data, it became clear that only one year of data was included although several more months were available (see their Table 1). In addition, for Mt Haast, the seasonal threshold determining the onset of the growing season did not follow protocol; despite two weeks of daily $T_{mean} > 5°C$ from the end of September, the onset of the growing season was placed in mid-October (see their Figure 5). The recalculation from their raw hourly data, taking these two factors into account, yields a growing season soil temperature for Mt Haast of 6·9°C (cf. 8.7°C in Körner and Paulsen 2004), a result close to the global mean and the *Nothofagus* sites in the current study. Despite these corrections, the Kaimanawa site (Urchin Track) is still markedly warmer at 10·2°C (cf. 10·7°C in Körner and Paulsen 2004). The measurement period (December 1998 – March 2000) at these sites was anomalously warm (nationally, 1998 and 1999 were the warmest and second warmest years on record since 1910; in central North Island 1998, 1999 and 2000 were 0·81, 0·72 and 0·30°C warmer, respectively, than the 1961-1990 mean). Whilst this may account for part of the discrepancy, the corrected Mt Haast site is not warmer than the other *Nothofagus* sites, which suggests other factors are involved at the Kaimanawa site.

As there is no reason to suspect the Kaimanawa treeline site has been lowered by disturbance, the most likely explanation is that the logger placement did not follow protocol. The low daily temperature ranges at this site suggests the logger was at the correct depth, but the high maximum temperature recorded on several occasions (16°C) exceeds Körner and Paulsen's (2004) 15°C maximum for soil temperature under a forest canopy at treeline. Perhaps the canopy altered after logger placement skewing the results to a warmer mean.

Table 3.3. Means, extremes and sums of growing-season of 10 cm soil temperatures from 10 treeline sites (for site details, see Table 3.1). Units are °C unless indicated.

		Annual						MEANSOIL3.2 Growing season	
Site No.	**Treeline Site Name**	**T_{mean} (T_{median})**	**Extr. T_{min}**	**Extr. T_{max}**	**Warmest Month T_{mean}**	**GDD0 (sum °C > 0)**	**GDD5 (sum °C > 5)**	**T_{mean} (T_{median})**	**Length (d)**
1	Campbell Island	6.7 (6.6)	-0.8	14.8	10.4	2456	777	6.7 (6.6)	365
2	Mt Burns[1]	6.4 (6.5)	2.0	16.9	10.0	2307	721	7.1 (7.2)	298
*3	Mt Fox	6.1 (5.9)	0.2	15.1	10.4	2235	692	6.9 (7.4)	292
*4	Mikonui	5.5 (5.1)	-0.2	14.0	10.2	2002	610	6.6 (7.3)	275
*5	Kelly Creek	5.7 (5.4)	0.0	15.3	11.0	2090	695	7.0 (7.6)	265
*6	Camp Creek	5.8 (5.3)	0.1	14.8	10.3	2106	655	6.8 (7.4)	279
7	Mt Haast[2]	4.2 (3.8)	0.7	12.8	9.6	2058	567	6.9 (6.5)	278
*8	Rainbow	4.6 (4.4)	-0.9	13.9	10.5	1637	557	7.6 (7.9)	197
*9	Kaweka	5.8 (5.3)	0.3	15.2	11.2	1929	656	7.1 (7.4)	245
10	Kaimanawa[2]	8.1 (8.3)	1.2	16.0	12.7	2969	1216	8.6 (8.8)	336
Mean[3]		5.5 (5.2)	-0.1	14.5	10.4	2064	651	7.0 (7.3)	275
S.D.		0.8 (0.9)	0.5	0.9	0.5	236	73	0.3 (0.5)	47
C.V. (%)[4]		0.3 (0.3)	0.2	0.3	0.17	11	11	0.11 (0.2)	17
Range		2.5 (2.8)	1.6	2.5	1.6	819	220	1.0 (1.4)	168

[1] Annual values for site 2 are recalculated from Mark et al. (2008) raw data, see text.

[2] Values for sites 7 and 10 are recalculated from Körner & Paulsen (2004) raw data.

[3] Data for site 2 and 10 are excluded from summary statistics at bottom of the table, due to probable calibration and placement errors, respectively, see text.

[4] Coefficient of variation for temperature measurements is based on degrees Kelvin.

*Sites measured as part of this study, exactly at treeline.

Recalculation of the raw data from the Mt Burns treeline (Mark et al. 2008) resulted in a different mean soil temperature during the growing season than the published value (7·1 vs. 8·6°C, respectively), whilst annual and warmest mean temperatures were identical to the published values. The reported length of the growing season ("c. 180 days") could not be reconciled with the raw data (> 226 days, mean 298). Moreover, the extreme minimum soil temperature was 2·0°C. This is warm compared to all other sites, and is unlikely, especially since temperatures fell down to this value repeatedly over four winters; such trends are generally seen around 0°C. It appears likely that the uncalibrated temperature data from this study do not represent the actual soil temperature.

Given these uncertainties, I exclude the Kaimanawa and Mt Burns sites from the compilation of treeline temperatures, but include the re-calculated values from the Mt Haast site.

Table 3.4. Summary of air temperature data from the six sites exactly measured at treeline, all 20 sites, and subsets of the abrupt and gradual treelines (for site details, see Table 3.2; for results per site, see Appendix Table A2.1). Units are °C, unless specified.

	Annual	Warm-est Month	Three Warmest Months	Coldest Month Mean		Frost Days	Growing Degree Days		Potential Growing Season MINAIR2 T_{min} threshold = 2°C				Days with T_{min} ≥ 5°C
	Daily T_{mean}	Daily T_{mean}	Daily T_{mean}	Daily T_{mean}	Extr. T_{min}.	Sum (d)	GDD0 (sum °C > 0)	GDD5 (sum °C > 5)	Length (d)	Daily T_{mean}	Daily T_{min}.	Daily T_{max}.	Sum (d)
Treeline sites measured in this study (n = 6)													
Mean	5.9	11.5	10.4	1.1	-6.7	116	2200	853	188	8.9	4.9	14.4	98
S.D.	0.3	0.7	0.4	0.9	1.2	18	95	53	14	0.5	0.1	1.3	13
C.V.%	0.12	0.25	0.13	0.32	0.43	15	4	6	8	0.18	0.04	0.44	14
All sites (n = 20)													
Mean	5.9	11.0	10.2	1.3	-8.1	110	2181	832	198	8.8	4.9	13.1	107
S.D.	0.6	1.0	0.7	1.6	2.2	38	238	123	61	0.8	0.4	1.8	34
C.V.%	0.23	0.34	0.26	0.57	0.84	35	11	15	31	0.27	0.13	0.62	32
Min.	4.7	9.0	8.5	-1	-12.2	9	1747	536	129	7.0	4.2	9.2	66
Max.	7.3	12.7	11.4	5.3	-4.5	169	2644	1038	365	9.7	5.5	15.9	204
Range	2.6	3.7	2.9	6.3	7.7	160	897	502	236	2.7	1.3	6.70	138
Abrupt treelines (n = 13)													
Mean	5.6	11.1	10.3	0.4	-9.3	128	2073	810	174	9	4.8	13.4	96
S.D.	0.5	1.0	0.7	0.7	1.7	21	189	141	24	0.6	0.4	1.3	16
C.V.%	0.17	0.35	0.26	0.24	0.63	16	9	17	13	0.2	0.16	0.44	17
Gradual treelines (n = 7)													
Mean	6.5	10.7	10.1	2.8	-5.8	77	2383	871	244	8.4	5.0	12.8	126
S.D.	0.5	0.9	0.8	1.6	0.7	43	187	74	84	0.9	0.2	2.5	49
C.V.%	0.20	0.32	0.27	0.58	0.28	56	8	8	34	0.33	0.07	0.89	40

Comparing the effectiveness of thermal descriptors of treeline

At the six simultaneously measured treeline sites in this study (indicated with * in Table 3.2 and Table 3.4), the mean daily air temperature for the warmest month and warmest three months were 11·5°C and 10·4°C, respectively. The mean daily temperature of the coldest month was 1·7°C and 0°C, for gradual and abrupt treelines respectively, with absolute minima for both between -6°C and -9°C (Table 3.4, or see Appendix Table A2.1 for results per site). MEANSOIL3.2 growing season temperature, annual mean air temperature and mean air temperature of the three warmest months were more consistent across sites than the other metrics, as indicated by the coefficient of variation (C.V. of 0·1% compared to ≥ 0·3%). Annual thermal sums (growing degree days) were highly variable between sites, with a variation of almost 20% around the mean.

When assessing the convergence of air temperature across the six sites for growing seasons defined by different threshold temperatures, mean daily minimum temperature of seasons based on T_{min} thresholds had the lowest C.V. (Figure 3.2). When the growing season was defined by a 7-day T_{min} of ≥ 2°C (criterion MINAIR2), variation across sites was smallest (C.V. = 0·04%). Mean T_{min} for the thus defined growing season was 4·9°C (± 0·1 S.D.). The mean length of this season was 188 days. For comparison, a mean air temperature threshold of 0°C (for which the 3·2°C mean soil temperature in Körner and Paulsen (2004) was a proxy) had twice the variation between sites, and resulted in a near year-round growing season.

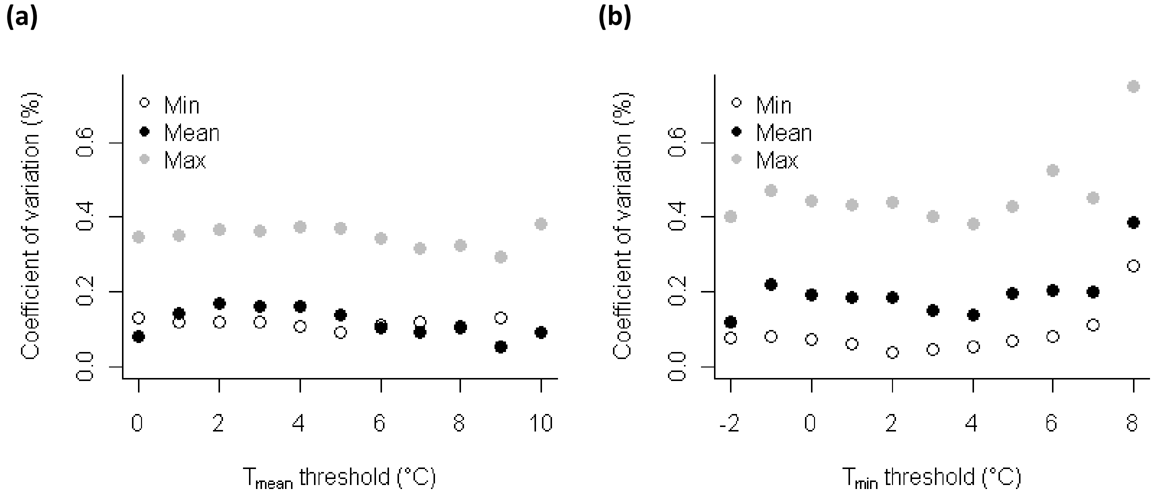

Figure 3.2. The convergence of daily T_{min}, T_{mean} and T_{max} across six treeline sites when multiple growing seasons are calculated on the basis of a range of T_{mean} (a) or T_{min} (b) thresholds. A potential growing season calculated using a T_{min} ≥ 2°C threshold resulted in the lowest coefficient of variation, and thus the most consistent values between sites.

From the different soil and air temperature metrics assessed, the mean daily minimum air temperature in the MINAIR2 growing season showed the highest convergence across the six measured treeline sites. Mean daily minimum temperature during this growing season did not show a significant relationship ($p > 0·7$) between treeline altitude or latitude, whereas daily mean and maximum temperatures were significantly correlated with both altitude and latitude ($p < 0·01$, see Figure 3.3). This growing season will further be referred to as the MINAIR2 season or “potential growing season” (see Section 3.4 Discussion).

Applying thermal descriptors to a wider range of treelines

Assessing all 20 sites (consisting of the six measured treeline sites and the 14 sites with estimated treeline temperatures) resulted in similar values for all metrics (Table 3.4). Again, the mean daily minimum in the MINAIR2 season was most consistent across sites 4·9°C (± 0·4 S.D.), whereas growing degree days showed the most variation.

Air temperature from a number of treelines around the world showed mean daily minima for the MINAIR2 season consistent with the New Zealand data (Table 3.5). Mean T_{min} in the growing season was 5·3°C at an Austrian treeline, and 5·4°C at both a sub-arctic treeline in Sweden, and a south-temperate treeline in Australia. The Chilean treeline was warmer at 6·1°C, however, this was calculated from temperature data of less than six months. At the two tropical treelines (Bolivia and Tanzania), overnight air temperatures were often much lower than the 2°C threshold. In Bolivia, during a year of measurements in 2008, there was only one day with a $T_{min} \geq 2$°C. This makes it impossible to apply the growing season threshold. At the Kilimanjaro treeline, $T_{min} \geq 2$°C were recorded on 274 days, but only four days with $T_{min} \geq 5$°C, resulting in an overall mean minimum temperature in the MINAIR2 growing season of a low 3·1°C.

3.4 Discussion

New Zealand treeline (soil) temperature in a global context

Soil temperatures at nine New Zealand tree lines (four *Nothofagus*, four conifer-broadleaf, and one *Dracophyllum*) provide international comparability with the Körner and Paulsen (2004) data set (Table 3.3). Using their definition of the growing season (MEANSOIL3.2), the four gradual conifer-broadleaf and the sub-antarctic *Dracophyllum* treelines have mean growing season root zone temperature of 6·8°C (± 0·2 S.D.), close to the global isotherm of 6·7°C (± 0·8 S.D.) and somewhat cooler than northern temperate treelines (range 7 - 8°C). The growing season at the abrupt treelines was shorter and warmer than at gradual treelines (mean 7·4°C over 222 days; mean 6·8°C over 278 days, respectively).

These results suggest that New Zealand treelines are not anomalously warm by the global soil temperature criteria (cf. Körner and Paulsen 2004). Similarly, although two Chilean *Nothofagus* treelines were also previously classed as warm (Körner and Paulsen 2004), recent data suggest that such a treeline in Patagonia also has growing season root zone temperature close to the global norm had a mean of 6·9°C, also very close to the global norm (6.9°C; Hoch unpublished, in Fajardo et al. 2011).

Troublesome application of growing season definitions in oceanic regions

Care needs to be taken when applying growing season definitions and interpreting the resulting means (Gehrig-Fasel et al. 2008). As long ‘growing seasons’ necessarily extend into cooler months and, as little growth is actually possible in these shoulder months, the ‘growing season’ means may be misleadingly cool. The longer ‘growing season’ at the New Zealand gradual treeline (278 days, compared to 222 days at abrupt treelines) is the only reason for their lower mean temperature. When successive periods of equal duration are compared, the abrupt and gradual treelines experience a similar mean climate, except over the winter months (Appendix Figure A2.1). It is probable that this phenomenon is partly responsible for the spread of MEANSOIL3.2 temperatures at the global scale. If the warmer sites (> 1 S.D. warmer than the mean) are excluded from the Körner and Paulsen (2004) data set (most of these have site-specific explanations for their deviation from the mean), there is a significant negative correlation between season length and temperature (MEANSOIL3.2 Temp = 7·36 - 0·0052 * Season length; $R^2 = 0.24$, $p = 0.01$).

Following Körner and Paulsen (2004), a mean soil temperature above 3·2°C is taken as a signal of the beginning of the (MEANSOIL3.2) growing season, whilst a mean soil temperature 3·2°C or below indicates its end. However, an isolated mean soil temperature above 3·2°C during winter is unlikely to result in a premature start to the growing season, nor is a similar cold period in summer indicative of the season’s end. In order to create a workable definition for oceanic sites that was consistent with continental situations, the criterion was expanded to a 7-day mean above or below the 3·2°C threshold. Even so, in these oceanic environments, mid-winter 7-day means of over 3·2°C were sometimes reached, and means below this threshold were seen in early autumn, although warmer periods of substantial length occurred later. Therefore, in practice, some investigator interpretative liberty is needed to apply the thresholds. Körner and Paulsen (2004) refer to a ‘first time in spring’ and ‘first time in autumn’ criterion for the 3·2°C to be activated and this was subjectively determined from the graphed temperature trends (Jens Paulsen, pers. comm.). Instead, I consistently applied a time restriction: the growing season could not begin in the three mid-winter months (June – August), or end before the 1st of May.

These definition modifications would not lead to different results at continental treelines because of the generally more abrupt transitions, but permitted reasonably comparable estimates to be made for the New Zealand oceanic settings. Although the modified soil temperature thresholds permitted calculation of an objective growing season, the resulting growing seasons still seemed too long to be realistic. For instance, the gradual treelines averaged 278 days, or approximately 9 months (see below).

Comparing the effectiveness of thermal descriptors of treeline

In order to find the air temperature factor with the best match to treeline position, a number of thermal summaries derived from treeline air temperature data were explored. These included warmest and coldest month mean temperature, growing degree days (GDD0, GDD5), and warmth during the growing season. Different growing seasons were defined based on a range of air temperature thresholds. The metrics were assessed across treeline sites of a range of latitude, altitude and species composition, and the metric with the least variation around the mean was selected. The notion is that a metric that most consistently describes the thermal conditions across such a wide range of treeline environments is most likely to have a direct relationship with the biological processes that limit growth at treeline, an assumption also implicit in Körner and Paulsen's study (2004).

New Zealand treelines have previously been argued to equate with a warmest month mean air temperature of *ca*. 10°C (Zotov 1938, Wardle 1973, 2008), largely on the basis of extrapolated weather station data. Our six measured treeline sites give an average warmest month mean of 11·5°C (noted with an asterisk * in Table 3.4), and the full data set of 20 sites is slightly cooler at 11·0°C. These values are comparable with other temperate treelines, but not with Arctic or tropical treelines (see review in Körner 2003a). Therefore, the biological significance of this indicator as relating to global treeline formation is unconvincing, and it probably owes what validity it does have, to its relationship with the overall warmth of the growing season. Annual thermal sums (GDD0, GDD5) varied by a factor of two (C.V. of 10-15%) between the sites and are therefore poor predictors of treeline position, as noted by Körner and Paulsen (2004).

The most consistent thermal descriptor of the treeline sites was based on a growing season defined by a 7-day mean air T_{min} of ≥ 2°C. For the six sites with temperature measurements exactly at treeline, the mean T_{min} during this potential growing season was 4·9°C (± 0·1 S.D., or C.V. = 0.04%). For all twenty sites in this study, the mean T_{min} during this potential growing season was also 4·9°C (± 0·4 S.D.), and did not show a relationship with treeline altitude or latitude (Figure 3.3). This alternative growing season threshold provides biologically realistic results for the New Zealand mainland sites, with growing season lengths of approximately 6 months, which is supported by phenological studies, indicating growing seasons of *ca*. 6-7 months for various species present at treeline (Wardle 1970, Benecke and Havranek 1980b, Payton 1989). In comparison, applying a threshold of a mean air temperature of 0°C (for which the 3·2°C soil threshold chosen by Körner and Paulsen (2004) was a proxy) resulted in long growing seasons (means of 310 days and 365 days for abrupt and gradual treelines, data not shown).

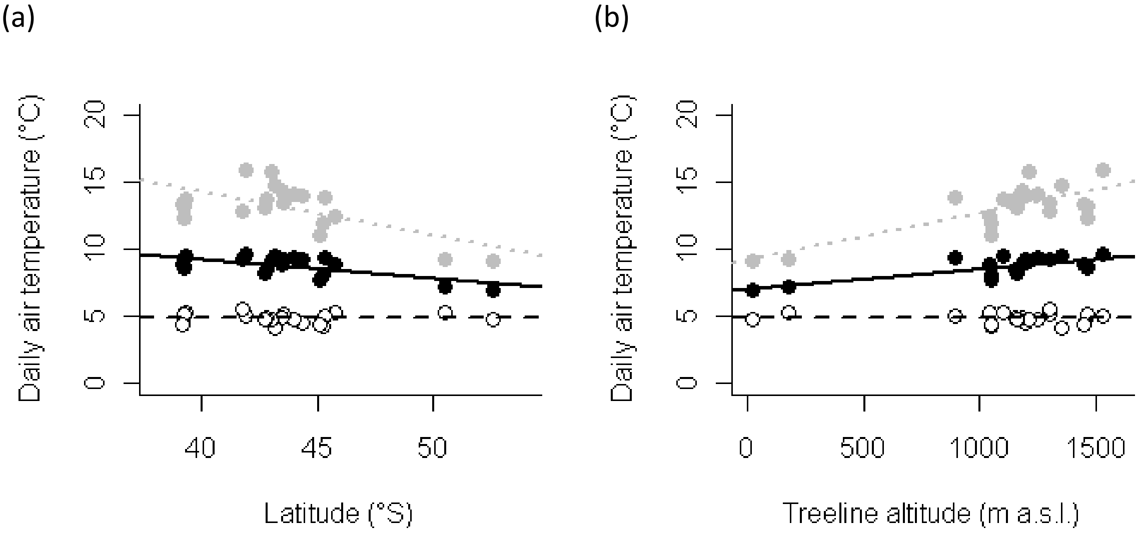

Figure 3.3. Relationship between mean daily air temperatures during the MINAIR2 potential growing season and latitude (a) and altitude (b) of the treeline sites in this study. Open circles indicate mean daily minimum (T_{min}) temperature, black circles indicate mean daily temperature (T_{mean}) and grey circles indicate mean maximum air temperature (T_{max}). There is no significant trend with latitude or altitude for daily minimum temperature ($p > 0{\cdot}7$, $p > 0{\cdot}8$, respectively). The decrease in daily mean and maximum temperature with latitude is significant (T_{mean}: $p = 0{\cdot}002$, $R^2_{adj} = 0{\cdot}39$, T_{max} : $p = 0{\cdot}003$, $R^2_{adj} = 0{\cdot}37$), and the increase in daily mean and maximum temperature and treeline altitude is significant (T_{mean}: $p < 0{\cdot}001$, $R^2_{adj} = 0{\cdot}54$; T_{max} : $p < 0{\cdot}001$, $R^2_{adj} = 0{\cdot}57$).

Mean minimum temperature across a growing season with $T_{min} \geq 2$°C was the most consistent descriptor across treeline sites in this study. This is supported by the notion that overnight chilling is sufficient to disrupt normal plant processes and therefore may provide a biologically defensible basis for estimating growing season warmth and length. Low (but not freezing) overnight temperatures in summer have been shown to decrease photosynthesis of some high-elevation species (Germino and Smith 1999, Cavieres et al. 2000, Öquist et al. 2001, Johnson et al. 2004). A recent study of conifers near treeline in the Rocky Mountains, USA, found a reduction in photosynthesis to *ca*. 30% of normal after an overnight temperature below 2°C (Johnson et al. 2004), consistent with the T_{min} threshold found in the current study.

The resulting growing season then, may be called the “potential growing season”, as it encompasses the times when minimum air temperature allows for photosynthetic rates and growth processes to occur. The “realised growing season” is likely to be shorter, since growth (e.g., wood formation) is often reduced at warmer temperatures than photosynthesis. In a study of conifers from a wide geographic range in Europe and Canada, Rossi (2007, 2008) found that the onset and ending of xylogenesis converged to average daily minimum, mean, and maximum temperature of 4-5, 8-9 and 13-14°C, respectively (see also Swidrak et al. 2011).

Indeed, mean T_{min} of the potential growing season roughly coincides with the T_{min} that initiates xylogenesis in temperate regions. If only days of $T_{min} \geq 5°C$ allowed wood formation to occur, this would result in 98 true growing days (± 13 S.D.) for the six sites directly measured, and 107 days (± 20 S.D.) for all 20 sites (Table 3.4). Interestingly, this is very close to the generally accepted minimum number of days needed to attain tree stature, of *ca*. 90 days (Körner 2003a, Körner 2007a, Rossi et al. 2008).

International comparisons

To assess whether the best thermal indicator of New Zealand treeline position, had validity elsewhere, I collated air temperature data from six treeline sites across both hemispheres (Table 3.5). Mean T_{min} of the potential growing season (MINAIR2) at the north-temperate, south-temperate (Australian) and sub-antarctic treelines were highly consistent (5·3°C, 5·4°C, and 5·4°C, respectively), and slightly higher than the mean value of 4·9°C for the New Zealand treelines. The Chilean treeline was warmer at 6·1°C, however, this was calculated from an incomplete dataset, potentially not covering the full growing season (November 2004 – March 2005). The temperate treeline regions also showed comparable number of days with $T_{min} \geq 5°C$ (average 96 days ± 4 S.D.). The sub-arctic site (Sweden) had substantially fewer, whereas treelines on the sub-antarctic islands of New Zealand had substantially more days with $T_{min} \geq 5°C$.

The tropical treelines in Bolivia and on Mt. Kilimanjaro had a very different thermal environment than all other investigated treeline sites. At the Bolivian *Polylepis* treeline, in 2008 there was only one day with a $T_{min} \geq 2°C$ (and only 63 days when frosts did not occur, see Hoch and Körner 2005), and thus the MINAIR2 growing season could not be applied (Table 3.5). This highlights the complexity of growth processes at treeline: they are unlikely to respond to one simple temperature threshold, which has been the implicit assumption in the search for a single predictor of global treeline position.

Table 3.5. Comparisons of calculated “potential growing season” (MINAIR2) at treelines worldwide

					MINAIR2 growing season		
Region	**Treeline**	**Latitude**	**Longitude**	**Treeline altitude (m a.s.l.)**	**Mean T_{min} (°C)**	**Length (days)**	**No. days $T_{min} \geq 5$°C**
Sub-arctic	Abisko, Sweden	68.38°N	19.28°W	700	5.4	126	66
North-temperate	Patscherkofel, Austria	47.22°N	11.40°E	1950	5.3	151	92
Tropical	Kilimanjaro, Tanzania	3.07°S	37.35°E	3650	3.1	274	4
Tropical	Nevado Sajama, Bolivia	18.12°S	68.95°W	4810	NA	0	0
South-temperate/ Mediterranean	Farellones, Chile	33.32°S	70.25°W	2200	6.1*	141*	100*
South-temperate/ Mediterranean	Thredbo, Australia	36.49°S	148.29°E	2000	5.4	146	92
South-temperate	New Zealand (18 sites)	39.20-45.73°S	167.37-176.39°E	1040-1530	5.0	192	98
Sub-antarctic	New Zealand (2 sites)	50.48-52.55°S	166.30-169.15°E	25-180	4.9	365	199

* The data from Chile are incomplete, see text.

Critical thermal components controlling growth of treeline trees

Even though phenological measurements would permit an uncontested estimate of the start and end of a given growing season, they are difficult to obtain and therefore generally lacking. Moreover, they inevitably are species-specific and thus violate the condition for a universal treeline metric. Körner and Paulsen (2004) and others have used mean soil temperature of the growing season as a practical proxy for the more complex temperature environment to which alpine trees are subjected.

Although soil temperature has been shown to be correlated to tree growth at treeline (Kirdyanov et al. 2003, Körner and Hoch 2006, Alvarez-Uria and Körner 2007, Gruber et al. 2009), other studies have suggested that soil temperature is less critical than air temperature with respect to tree-building processes such as tracheid production and xylogenesis (Rossi et al. 2007, Rossi et al. 2008, Gruber et al. 2009, Swidrak et al. 2011). The above-ground meristems have been called the tree’s “thermal sensors” for height growth (Petit et al. 2011). Instead perhaps, there are multiple temperature thresholds that all need to be satisfied before growth can occur, and once one of them is overcome, the others become limiting. That is, growth cannot occur if the soil is too cold (e.g., Körner and Hoch 2006), or if the above-ground meristems are too cold; and neither one can be traded off against the other.

In any system, woody plant growth is dependent upon three fundamental physiological processes: carbon acquisition, transport of solutes and xylogenesis. If light, nutrients and water are not limiting, temperature is the main variable affecting these processes (Figure 3.4). Carbon acquisition through photosynthesis is least affected by low temperature, and it can proceed at low rates at temperatures close to zero (Körner 2006), provided there is sufficient light. Cell production and xylogenesis are more sensitive to low temperature. Little growth is possible when mean temperatures fall below 5-7°C (e.g., Vapaavuori et al. 1992, James et al. 1994, Körner 2006), although some of the most cold-adapted species (e.g., winter cereals) still show some growth at 2-3°C (see Körner 2006). Similarly, water and solute movement across the soil-water interface into plant roots is constrained at mean soil temperatures lower than 8°C (Mellander et al. 2004).

Previously experienced minimum temperatures may negate the effects of ambient temperature, through what I will call “chilling legacy effects”. For example, low (but non-freezing) overnight temperatures in summer have been shown to decrease photosynthesis of some high-elevation species (Germino and Smith 1999, Cavieres et al. 2000, Öquist et al. 2001, Johnson et al. 2004). Exposure to one frosty night can cause damage to photosystems, and may lower photosynthetic rates for several days (Öquist and Huner 1991). Similarly, overnight chilling of the soil or meristems may reduce solute movement and growth (Domisch et al. 2002). Low soil temperature (< 8°C) can restrict water and nutrient uptake and sap flow, especially if such cold soils persist long into the growing season (Mellander et al. 2004). In an experiment at treeline in Sweden, an increase in soil temperature affected growth of the seedlings in a similar way to an increase in nutrient supply, because of increased nitrogen uptake under warmer conditions (Weih and Karlsson 2001). Although nitrogen is not generally considered limiting at treeline sites, uptake of other minerals crucial for wood formation will be constrained if water movement is limited. For example, calcium uptake by roots occurs through passive mass flow driven by transpiration of the leaves (McLaughlin and Wimmer 1999, Gilliham et al. 2011).

As growing degree days and growing season length have proved useful in predicting growth in lowland ecosystems (Goudriaan and Van Laar 1994), the lack of correlation with treeline position, both globally and in New Zealand, initially seemed puzzling.

However, the direct and “chilling legacy effects” of temperature together may explain why lowland tree growth can be adequately predicted by GDD, whereas high altitude growth cannot. During the growing season in lowland systems, soils and foliage are generally well above the minimum temperature to ensure solute movement and growth. Daily warmth and number of days in the growing season will translate more or less directly into more growth. In contrast, at the alpine treeline, trees are close to the soil and/or air temperature thresholds for solute movement and growth. The thresholds are often crossed by chilling, which will result in little subsequent growth, even during the growing season on days that are otherwise suitable for growth, because of the chilling legacy effect.

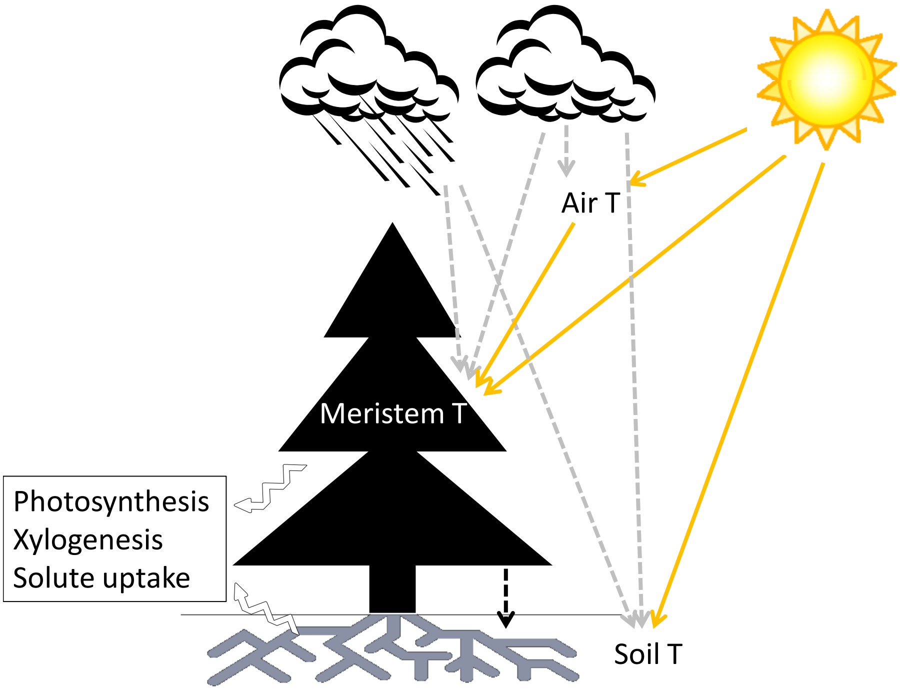

Figure 3.4. Schematic effects of direct radiation, cloud cover, wind and humid conditions on air, soil (root-zone) and above-ground meristem temperatures. Self-shading by the tree canopy will decrease soil temperature. Positive effects are indicated with solid lines, negative effects with dashed lines. Zig-zag arrows indicate temperature thresholds.

Shorter growing seasons at tree line must be more suitable for growth processes (through warmer temperature and/or less legacy effects) than that of long growing seasons, otherwise trees would establish at higher altitudes. Tropic treelines (with few days of $T_{min} \geq 2°C$) have intense, usually daily, legacy effects. However, these high altitude treelines are often also the driest, and sun-exposed rocky, bare soils, low cloud cover (Hoch and Körner 2005, Duane et al. 2008) and high radiation (Bader et al. 2007), result in strong diurnal heating of canopies and soil (Figure 3.4), which may reduce the strength or duration of legacy effects. The growing season at these sites is long, and the cool mean soil temperatures (Körner and Paulsen 2004) and overnight low temperatures support slow, continuous radial growth and monthly leaf production (Velez et al. 1998, Biondi and Hartsough 2010), rather than the relatively rapid but short duration of leaf flush found at temperate treelines.

Exactly the opposite may be happening in the sub-antarctic, where the growing season is long, and there are few days with low overnight temperature. The extremely windy, cloudy, low light, high humidity environment results in much lower leaf and meristem temperatures (Meurk 1984, Grace et al. 1989, Grace 2006). Although the two sub-antarctic island treelines in this study had many more days with overnight lows ≥ 5°C (average 195 days) than all other sites, they shared near identical MINAIR2 T_{min} (5·0°C cf. 4·9°C). This suggests that environmental factors may mitigate or exaggerate the strength or importance of the temperature thresholds and legacy effects.

3.5 Conclusions

The first aim of this study was to assess whether New Zealand treelines are significantly warmer than the global average, as has been suggested. Using detailed soil temperature data from seven treeline sites, it is shown that these sites fall within the international norm of global growing season root-zone temperatures. However, mean growing season temperatures thus calculated are sensitive to how abruptly the growing season starts and finishes, and are thus difficult to apply in more oceanic regions, like New Zealand.

Warmth of the growing season has long been understood to be critical to treeline position. However, exactly which temperature metric best conforms to the biology underlying treeline has long been obscure. The Körner and Paulsen (2004) global soil temperature survey obtained an impressively consistent result across a great variety of treelines and thus must, in some fashion, relate closely to the underlying fundamental drivers. The answer to our second aim of this study, the quest for the most consistent descriptor of the treeline thermal environment over a wide range of oceanic sites, proved to be a growing season based on daily minimum temperatures.

Air temperature thresholds for photosynthetic and growth rates have been described, however, these are often based on mean temperatures. Instead, this study suggests that overnight low temperatures are likely to play a disproportionate role due to the chilling legacy effect on these rates. Daily minimum air temperature proved highly consistent during the growing season across southern and northern hemisphere temperate treeline sites, suggesting similar thresholds and legacy effects are operating in concert, and are likely to be involved in the control of alpine tree growth. However, legacy effects may be overcome more quickly when other conditions are favourable. This is exemplified by the different results when applying threshold temperatures to tropical and sub-antarctic treeline sites. It appears that, whilst air temperature crucially affects meristem temperature, and thus photosynthesis and growth, low soil temperatures may constrain growth by inhibiting the uptake of essential minerals or root-meristems. For growth to occur, both thresholds need to be met.

Chapter 4 Seasonal frost tolerance

4.1 Introduction

Most species forming northern hemisphere treelines are extremely frost tolerant, whereas their equivalents in the southern hemisphere are not (Bannister and Neuner 2001). Temperate treeline environments are cold only in winter, and trees acclimate to these conditions by becoming dormant. As local species are accustomed to annually recurring mid-winter frosts, temperate treelines only rarely are determined by annual minimum temperatures (Körner 2003a). Nevertheless, it is also recognised that frosts occurring in the shoulder seasons, rather than mid-winter, are likely to be more damaging, because foliage is already de-hardened or not yet fully hardened (Taschler and Neuner 2004). Additionally, some limitation of tree growth with increasing altitude may occur due to the inability of trees to complete summer growth, with subsequent death caused by winter desiccation and frost damage of immature growth (Tranquillini 1979). The lack of frost tolerance of native tree species has been suggested to be the main reason for the low altitude of New Zealand treelines (Wardle 1985a, 2008)

Many of the trees that grow in New Zealand’s subalpine zone have greater affinities with montane tropical and subtropical vegetation than with other temperate regions (Mark et al. 2000, Wardle 2008). The mountain climate in New Zealand also shares similarities with that of the high altitude tropics, with many frosty nights even in the growing season (especially on the eastern side of the Main Divide). In addition, the maritime climate results in small seasonal temperature amplitudes compared to similar latitudes in the northern hemisphere. Introductions and experimental plantings at and above the native New Zealand treeline have shown that several exotic treeline species that are generally considered to have great tolerance to low temperatures, including *Picea engelmannii* and *Larix decidua*, were highly susceptible to summer frosts (Benecke et al. 1981, Wardle 1985a). *Pinus contorta*, a native of north-western North America, has proved the most vigorous invader among exotic high altitude species (Ledgard 2001), with trees occurring up to 200 m above the native treeline. This species too, however, has been

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Photo previous page: Damaged foliage *Nothofagus solandri* var. *cliffortioides* at the Craigieburn treeline in spring 2010.

observed with signs of frost damage (Wardle 1985a). Foliar damage of the native *Nothofagus solandri* var. *cliffortioides* has been attributed to frost exposure of immature growth (e.g., Wardle 1985a, 1998, 2008).

It appears, therefore, that New Zealand's treeline species may be more likely to be exposed to frosts all year round than are trees at temperate treeline elsewhere. However, although frost tolerance has been measured for most tree and shrub species that form New Zealand treeline (Wardle 1991), the material tested is often from lower altitudes and seasonal frost tolerance remains poorly studied (Bannister 2007). Additionally, comparisons between previous studies are difficult because different techniques were used to assess frost damage and/or material was collected from different locations and/or in different seasons.

This study investigates whether frosts may significantly affect the performance of trees near treeline in New Zealand. It addresses two questions with regard to four native and one exotic treeline species:

1. Does frost tolerance of foliage increase, and photosystem efficiency decrease, along an altitudinal gradient towards treeline?
2. Do seasonal temperature minima at treeline exceed the frost tolerance for that season?

4.2 Methods

Plant material

Foliage of five tree species was sampled at 100 m vertical intervals along the treeline ecotone at two sites in New Zealand, as described for the non-structural carbohydrate sampling in Chapter 2 (see Section 2.3.2 *Tree performance,* Table 2.2). *Halocarpus biformis* (Hook.) Quinn (Podocarpaceae) and *Libocedrus bidwillii* Hook.f. (Cupressaceae) were collected at Camp Creek on the west side of the Southern Alps, and *Nothofagus solandri* var. *cliffortioides* (Hook.f.) Poole (Nothofagaceae)*, Phyllocladus alpinus* Hook.f. (Podocarpaceae) and the exotic *Pinus contorta* Loudon (Pinaceae) were collected at Craigieburn on the east side of the Southern Alps (Figure 2.1). The species will be referred to by their generic name only, and the sites as West Alps and East Alps, respectively. Where the species were present above the treeline (< 3 m tall), samples were also taken. In the case of *Phyllocladus*, no trees > 3 m were present, so shrubs were sampled along the whole gradient.

Material was collected from the sites three times during the year: at the end of the growing season when hardening had started (autumn – 4 and 11 April 2010 for West Alps and East Alps respectively); in spring, before leaves had flushed (30 and 31 October 2010); and in early summer (9 and 15 January 2011). A mid-winter collection at the sites was not feasible because difficulties with access. On a north-west facing slope of *ca.* 30° inclination, at each altitude, five mature individuals were selected (different trees each season). From each individual, a sample was taken consisting of eight short shoots with the most recent, fully-expanded foliage. Foliage was consistently collected from fully sun-lit branches in the top half of the crown (shotgun sampling where necessary), as frost tolerance and stress levels may differ between sun and shade leaves (e.g., Stecher et al. 1999). Samples were kept in polythene bags in an insulated container during transport to the laboratory, where they were held in a refrigerator at *ca.* 4°C overnight. Shoots were then allocated randomly to eight frost treatments (see below).

Freezing treatments

Freezing treatments followed Bannister et al. (2005). Small shoots (*ca.* 5 cm) with foliage were placed on damp paper towel inside polythene bags and cooled to seven treatment temperatures (0, -3, -6, -9, -12, -15 and -18°C) at a rate of 5°C h^{-1}. Control samples were held in a refrigerator at +4°C. The range of target temperatures was selected so that the least severe treatment was likely to inflict no damage, whereas the most severe treatment would be at a temperature lower than those experienced in the field. The target temperature was held for four hours (Taschler et al. 2004); the combination of this length of time and the presence of damp towel with the sample prevented super-cooling (Bannister and Lord 2006). The material was then thawed to +4°C at a rate of *ca.* 5°C h^{-1}, before being placed at room temperature (*ca.* 20°C) in the dark. Freezer air temperatures were measured using a calibrated thermocouple (type T) and logged at one-second intervals using a data logger (CR10X, Campbell Scientific Inc., Logan, UT, USA). An on/off control relay, activated by the data logger, was used to keep two freezers within 0·3°C of their respective target temperatures. Cold air stratification inside the freezers was eliminated by the use of a small fan. The seven frost treatments were allocated randomly to the two freezers, and were conducted over two days.

Damage assessment

Foliage damage was assessed by chlorophyll-*a* fluorescence measurements three days after frost treatments. The ratio of variable to maximum fluorescence of the sample (F_v/F_m) of dark-adapted photosynthetic systems (> 30 min) (Maxwell and Johnson 2000) was determined using a portable infra-red gas analyser with chlorophyll fluorescence attachment (Li6400 and Li6400-40 LCF, Lincoln, Nebraska, USA). As dead material effectively has an F_v/F_m of zero, the degree of damage was calculated as the photoinactivation ratio (*PhI*) as described by Larcher (2003, Equation 4.1):

$PhI = (1 - F_{fT}/F_{max})$ .... Equation 4.1

where:
F_{fT} is the F_v/F_m of the sample exposed to a freezing temperature T; and
F_{max} is the maximum value of F_v/F_m for all samples of each tested species.

The temperature producing 50% damage (LT_{50}) was determined by linear interpolation using the temperature causing the highest *PhI* of < 50% and the temperature causing the lowest *PhI* of > 50% (Bannister et al. 1995, Bannister et al. 2005, Sierra-Almeida et al. 2009). Extrapolation was used only if the F_v/F_m from the coldest treatment approached 50% of F_{max}. If the lowest temperature treatment (-18°C) caused less than 50% damage to a sample, that temperature was taken as the best estimate of freezing tolerance. The F_v/F_m of control (unfrozen) dark-adapted samples measured the day after collection provided a measure of the efficiency of photosystem II. F_v/F_m values for healthy plants are typically around 0·83 (Bjorkman and Demmig 1987) and lower values indicate photo-inhibition.

Alternative methods of frost damage assessments include electrolyte leakage and visual estimates. Electrolyte methods have been tried on New Zealand sub-alpine plant species, but with limited success, as a number of the species tested (including *Phyllocladus alpinus*, also in this study) showed no perceptible electrolyte release, probably due to their thick cuticle (Reitsma 1994). Others have shown that although chlorophyll fluorescence measurements may overestimate the degree of frost resistance, particularly if readings of F_v/F_m are taken soon after thawing, they are similar to the visual and electrolyte leakage methods when leaves are allowed to develop damage over several days after thawing (see review in Bannister 2007).

Climatic data

Frost tolerance (LT_{10} and LT_{50}) data were compared with temperatures experienced at treeline to assess if (and if so, how frequently) these temperatures exceed the frost tolerance and can thus result in lethal foliar damage. As no long-term climatic data are available from New Zealand treeline sites, existing data from nearby weather stations (downloaded from the National Institute of Water and Atmosphere website, *cliflo.niwa.co.nz*) were used to construct models predicting the temperature at higher altitudes. Data from lower altitude stations and a generic lapse rate of 6°C km^{-1} have been used in the past to estimate temperatures experienced at treeline (e.g., Wardle 2008). However, treeline temperature estimates so calculated can deviate markedly from actual treeline temperatures. Reasons for this include the positioning of stations in inland basins with more extreme conditions (due to cold air drainage and pooling), as well as lapse rates that differ dramatically throughout the year (e.g., Blandford et al. 2008). Therefore, instead of using a generic lapse rate, models of daily minimum temperature (T_{min}) at treeline were constructed using long-term, low altitude climate data and overlapping shorter-term treeline data, and including factors such as temperature, rainfall, atmospheric pressure, solar radiation, wind-speed and wind-direction. These models were then used to predict treeline temperature for longer time series for which measured treeline data were not available.

For the East Alps site, frost tolerance data were compared with temperature data from a weather station at Craigieburn (less than 1 km from the collection site, at 914 m a.s.l.). From 1967 to 1986 there was another station directly uphill (at Ski Basin, 1554 m a.s.l.). T_{min} at treeline (at 1350 m, lying between the two stations on the same slope) was interpolated from the 20 years of data when these stations were both operating. Interpolated treeline T_{min} were then used to construct a model predicting treeline T_{min} from data at the lower (Craigieburn) station during this time, using a linear model approach. A model consisting only of Craigieburn T_{min} explained 90% of the variation in interpolated treeline T_{min}. This was improved (as indicated by Akaike's Information Criterion, AIC, see Burnham and Anderson 2002) by adding the following parameters: insolation, mean daily wind speed and month (as a factor). This best model explained 91% of the variation, and was used to estimate treeline T_{min} from the 914 m weather station data from the last 40 years (1971 – 2010). The extreme T_{min} for each month was summarised for three periods (2001 – 2010, 1991 – 2010 and 1971 – 2010).

For the West Alps site, a similar approach was adopted: temperature data were collected at a weather station at the Camp Creek treeline (*ca*. 100 m from the treeline collection site in this study) between March 1978 and April 1984 (kindly provided by Ian Payton, see Payton 1989). These data were modelled using data recorded by the Hokitika weather station (*ca.* 40 km to the west, 39 m a.s.l.). A model containing Hokitika T_{min} explained 54% of the variation in treeline T_{min}, whereas a more comprehensive model (selected using AIC) explained 71% of the variation. The latter model included the following significant parameters: Hokitika T_{min}, wind direction (included as a combination of cosine and sine to account for the circularity of this variable), wind speed, atmospheric pressure and month (as a factor). This model was used to predict T_{min} at the Camp Creek treeline from the Hokitika data for 1971 – 2010, and the results again summarised by month for the previously mentioned periods.

Statistical analyses

The effects of altitude and time of sampling (season) on frost tolerance and photosystem efficiency were assessed using linear models (using the function *lm()* in R). For each species and each response (frost tolerance and photosystem efficiency), a full model was constructed including the variables altitude and season (as a categorical variable) and their interaction. Following Crawley (2002), each full model was then simplified by progressively removing non-significant terms and selecting the minimum adequate model. At each step of this backward selection procedure, the non-significant term with the smallest associated effect size was eliminated. Main effects were removed only after relevant interactions had been eliminated. Differences in photosystem efficiency and frost tolerance between treeline individuals and short-statured individuals occurring above the treeline were assessed using *t* tests.

4.3 Results

Frost tolerance

At the East Alps site, all species were more tolerant of frosts in autumn than in spring or summer (Figure 4.1, Table 4.1). Altitude did not affect the frost tolerance of *Nothofagus* or *Phyllocladus*, although both species showed significant seasonal variation in frost tolerance (autumn LT_{50} -11·2°C and -15·6°C for *Nothofagus* and *Phyllocladus*, respectively, compared to -10.8°C and -12·9°C in spring, and -5·5°C and -5·7°C in summer) (Figure 4.1). *Pinus* had a frost tolerance of at least -18°C for most sites in autumn (i.e., no sign of damage at the coldest temperature measured), although decreased tolerance at the highest altitude site resulted in a slight but significant positive altitudinal trend. In summer, altitude did not affect frost tolerance of *Pinus* (-6·8°C), whereas in spring tolerance increased significantly with altitude, from -10·5°C to -16·5°C (Figure 4.1, Table 4.1). For all species and sites, the temperature at which foliage damage was initiated (10% damage, LT_{10}) followed the same trends with altitude and season as described for LT_{50} (data shown only for treeline altitude in Figure 4.2). Examples of foliar damage at the different frost treatment temperatures are presented in Plate 4.1.

At the West Alps site, altitude did not affect LT_{50} of either conifer species (Table 4.1). However, there was a strong seasonal effect: species were most tolerant to frosts in spring and least in summer. *Halocarpus* could withstand frosts down to -8·7°C and -9·0 in autumn and spring, respectively, but only -6·6°C in summer. *Libocedrus* was least tolerant to frosts in summer (-5·7°C), whilst it could withstand frosts of -7·3 and -8·8°C, in autumn and spring, respectively (Table 4.1, Figure 4.1).

The LT_{50} of all species was substantially below the extreme minimum monthly air temperatures experienced at the treeline in all seasons in the last 20 years (Figure 4.2). However, at the East Alps site, a rare (1 in 40 year) extreme frost event in January (-4·8°C) was close to temperatures causing 50% foliar damage. At the same site, regular frost events in spring, and perhaps also summer, approach the LT_{10} for all species. At the West Alps site, temperatures causing 10% damage were encountered in the last 20 years only for *Libocedrus* in spring (Figure 4.2).

Table 4.1. Linear model results for frost tolerance (LT_{50}) for each species.

	df	Sum of Squares	Mean Squares	F	p
West Alps					
Halocarpus					
Season	2	93.7	46.8	12.6	<0.001
Residuals	70	260.2	3.9		
Libocedrus					
Season	2	90.3	45.2	10.0	<0.001
Residuals	55	248.1	4.5		
East Alps					
Nothofagus					
Season	2	310.3	155.1	96.2	<0.001
Residuals	42	67.7	1.6		
Phyllocladus					
Season	2	785.5	392.7	251.2	<0.001
Residuals	43	67.22	1.6		
Pinus					
Altitude	1	43.8	43.8	31.1	<0.001
Season	2	1229.6	614.8	436.8	<0.001
Altitude:Season	2	80.1	40.1	28.5	<0.001
Residuals	55	77.4	1.4		

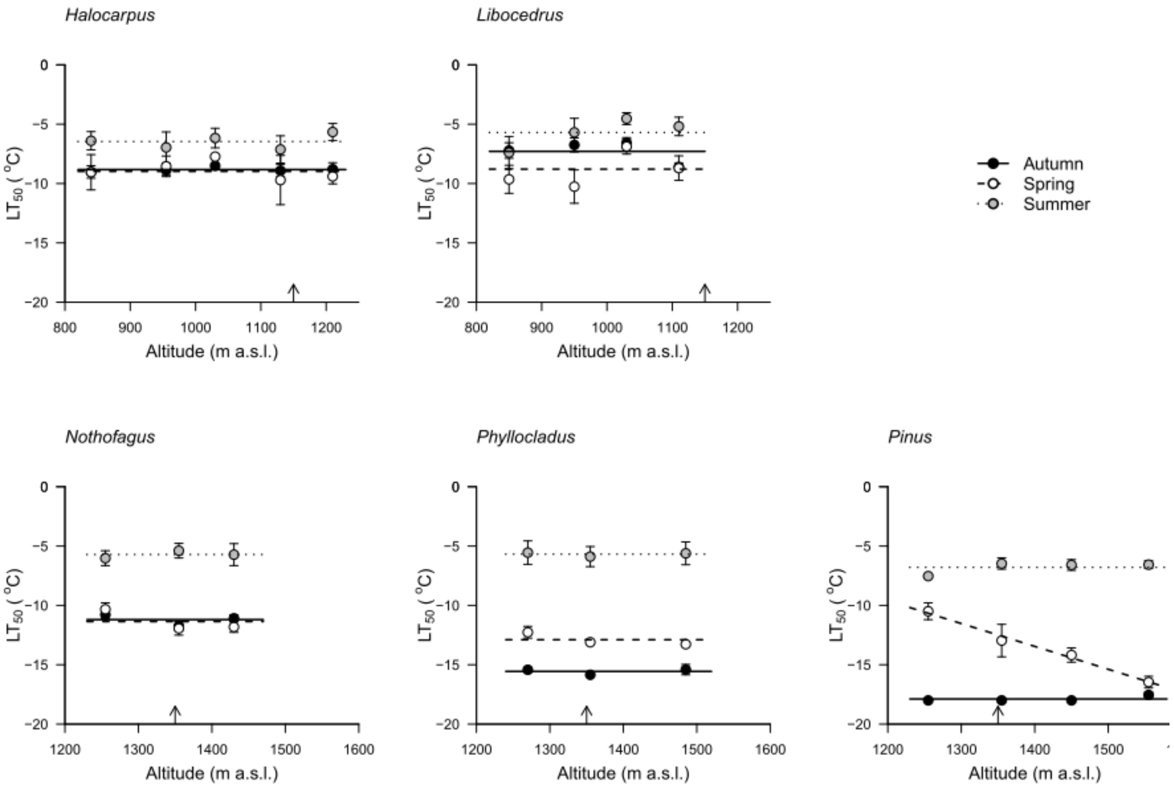


Figure 4.1. Effect of altitude and season on the frost tolerance (mean LT_{50}, temperature that causes 50% lethal damage to the photosystem, ±1 standard error) of treeline species at the West Alps (top row) and East Alps (bottom row) sites. Arrows indicate the altitude of the local (native) treeline.

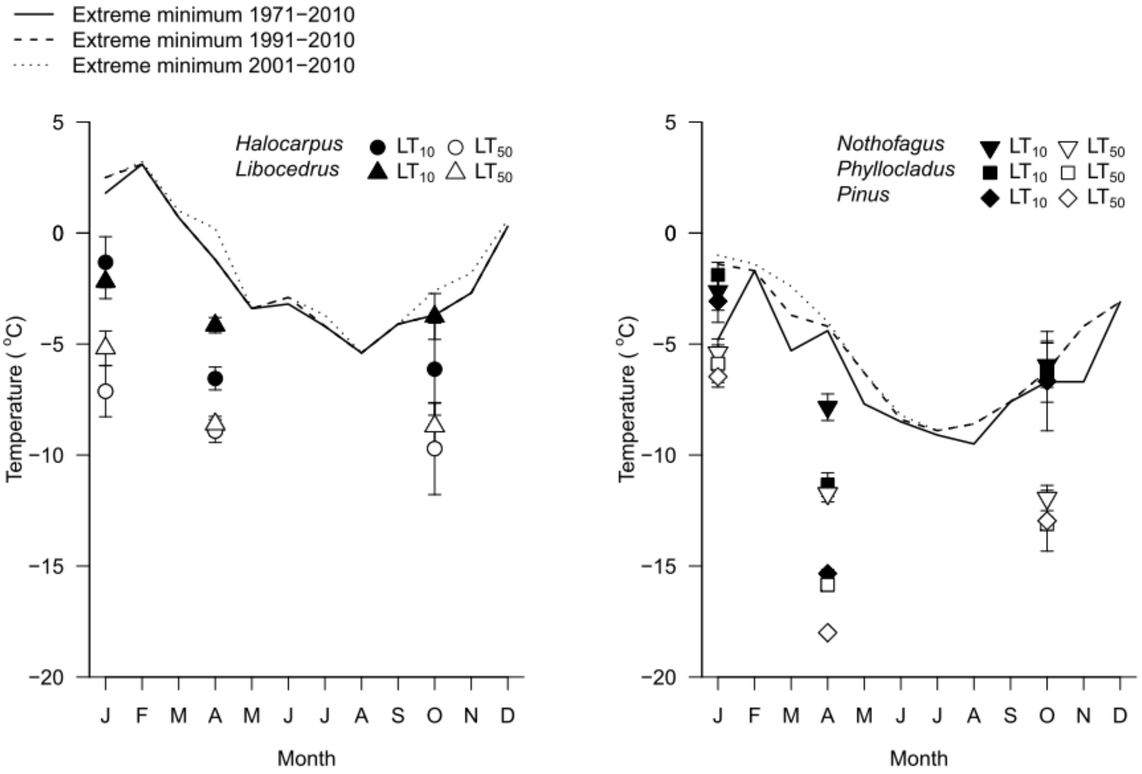


Figure 4.2. Frost tolerance temperature (mean temperature at which 10% and 50% of the foliage is damaged, LT_{10} and LT_{50} respectively, ± 1 standard error) of trees of five species at treeline in relation to estimated monthly extreme minimum air temperatures at the West Alps (left) and East Alps (right) sites for the last 10, 20 and 40 years.

Photosystem efficiency

All species showed decreasing photosystem efficiency (F_v/F_m) in spring compared to autumn, and an intermediate level in summer (Figure 4.3, Table 4.2). For two species (*Halocarpus* at West Alps and *Nothofagus* at East Alps), there was a significant negative trend of F_v/F_m with altitude. For *Halocarpus,* the time of sampling affected this trend, with a smaller difference between altitudes in autumn than in other seasons. For *Pinus,* F_v/F_m did not differ significantly over the entire altitudinal range. However, if the highest altitude site (where individuals were < 3 m tall) was excluded, F_v/F_m decreased significantly with altitude in all seasons (data not shown). Only in spring was F_v/F_m of low-statured *Pinus* at the highest altitude site significantly different from F_v/F_m at the treeline site (*t* test: mean F_v/F_m at treeline = 0·586, mean 100 m above treeline 0·670; *t* = -3·70, df = 5·62, *p* = 0·01). Photosystem efficiency did not differ between treeline individuals and low-statured *Halocarpus* 100 m above treeline in any season.

Table 4.2. Linear model results for photosynthetic efficiency (F_v/F_m) for each species.

	df	Sum of Squares	Mean Squares	F	p
West Alps					
Halocarpus					
Altitude	1	0.0290	0.0290	47.58	<0.0001
Season	2	0.0634	0.0317	51.98	<0.0001
Altitude*Season	2	0.0047	0.0024	3.86	0.026
Residuals	66	0.0403	0.0006		
Libocedrus					
Season	2	0.0365	0.0183	10.93	0.0001
Residuals	54	0.0902	0.0017		
East Alps					
Nothofagus					
Altitude	1	0.0094	0.0094	5.47	0.024
Season	2	0.0812	0.0406	23.66	<0.0001
Residuals	41	0.0704	0.0017		
Phyllocladus					
Season	2	0.1155	0.0578	16.042	<0.0001
Residuals	42	0.1512	0.0036		
Pinus					
Season	2	0.3166	0.1583	38.767	<0.0001
Residuals	58	0.2369	0.0040		

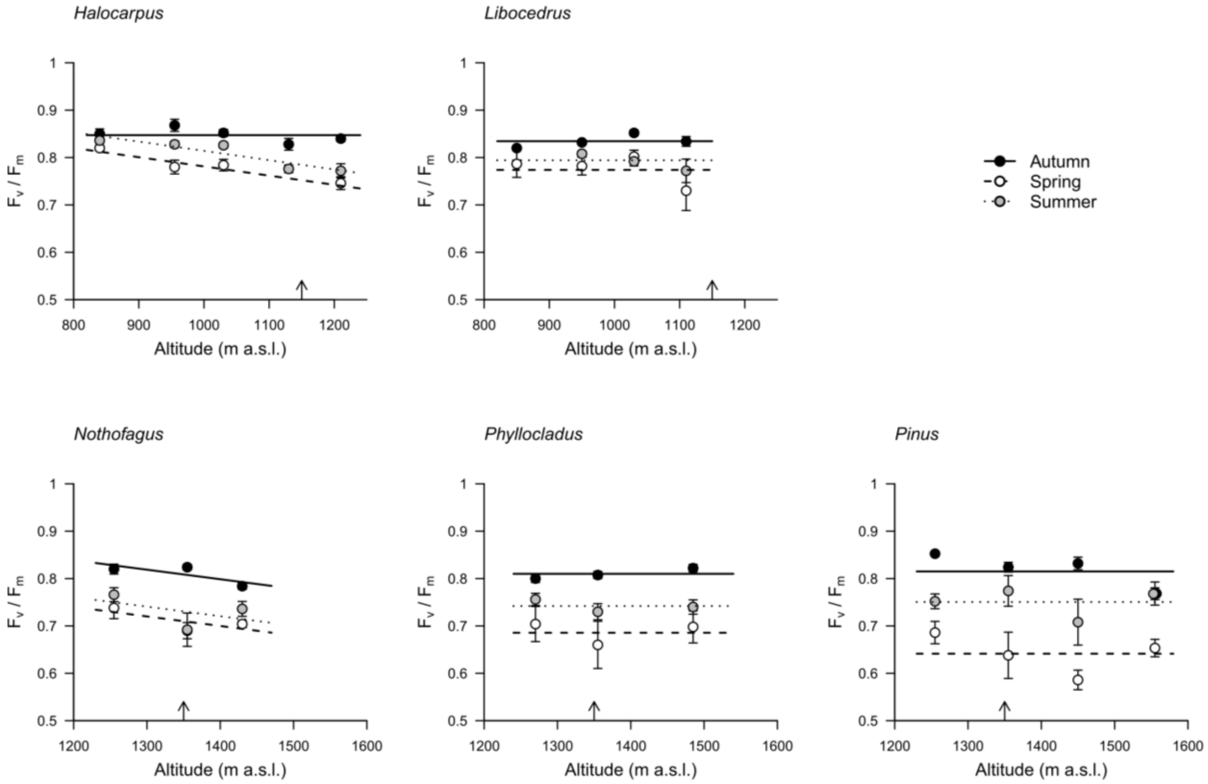


Figure 4.3. Effect of season and altitude on photosystem efficiency (F_v/F_m) of treeline species at the West Alps (top) and East Alps (bottom) sites. Efficiency is highest for all species in autumn and lowest in spring. *Nothofagus* and *Halocarpus* show a significant decline in efficiency with altitude. Arrows indicate the altitude of the local (native) treeline.

4.4 Discussion

All five New Zealand treeline species in this study were least frost tolerant in early summer, during the most intensive growth period (Benecke and Havranek 1980b), as has been described by others (Tranquillini 1979, Bannister and Neuner 2001, Larcher 2005). Although exact comparisons with previous frost tolerance studies are difficult because of different damage assessment techniques, collection location and timing, LT_{50} values generally concurred with those from previous studies. The maximum frost tolerance for *Nothofagus solandri* var. *cliffortioides* in this study (-11·2°C) fell within the range of -10 to -13°C reported by previous studies (Wardle and Campbell 1976, Sakai and Wardle 1978, Sakai et al. 1981, Greer et al. 1989). The summer tolerance of *ca.* -5·5°C fell between -3·5 and -7°C, previously reported for seedlings (Greer et al. 1989) and adults (Wardle and Campbell 1976), respectively. *Phyllocladus alpinus* tolerated temperatures down to -15·6°C in autumn, close to -16·3°C reported by Reitsma (1994) but not as extreme as the -18 to -20°C recorded by Sakai and Wardle (1978). In its native range, *Pinus contorta* can withstand temperatures down to -35°C in winter and -9°C in summer (Bigras et al. 2001); in this study, the species showed no damage in autumn at the lowest temperature measured (-18°C), but LT_{50} increased up to *ca*. -7°C in summer.

The maximum frost tolerance values in this study for the West Alps conifers, *Halocarpus biformis* and *Libocedrus bidwillii,* were *ca*. 4°C warmer (-9·0°C and -8·8°C, respectively) than the -13°C recorded for both species by Sakai and Wardle (1978). Collection locations are likely to explain this difference, as their material came from east of the main divide, and thus was exposed to more extreme winter conditions, likely resulting in increased hardening. Additionally, the maximum frost tolerance in this current study was determined in autumn, whereas the species may continue to harden further into the winter (Sakai and Wardle's samples were collected mid-winter).

The frost tolerance of native species in this current study did not vary with altitude. In contrast, the exotic *Pinus contorta* showed a significant altitudinal trend in frost tolerance in this current study, but only in spring. At this time of sampling, the de-hardening process had begun at the lower altitudes, with frost tolerance values approaching summer values. In contrast, at higher altitudes, lower temperature postponed de-hardening, resulting in similar spring and autumn values of *ca*. -16°C for the shrub-statured individuals 200 m above the native treeline.

At all times, the average LT_{50} of all species far exceeded (by at least 4°C) minimum air temperatures that trees would have experienced at the respective treeline locations over the past 20 years (Figure 4.2). Of course, onset of damage (indicated by LT_{10}) occurs at warmer temperatures, closer to the air temperatures experienced at treeline. Such minor frost-damage may be expected regularly for all species at the East Alps site in spring (when LT_{10} are within 0·5°C of T_{min}) and less regularly in summer (when LT_{10} is between 0·6°C and 1·8°C colder than T_{min} for these species). This concurs with personal field observations and damage descriptions (e.g., Wardle and Campbell 1976, Wardle 1985a). Additionally, a longer time series of 40 years at East Alps showed that minima of -5°C do occur in the summer months; these would result in foliar damage approaching 50% for all species at this site (Figure 4.2). The canopy, however, has a buffering effect and only the small proportion of foliage in the outer canopy thus will be exposed to these temperatures (McGlone et al. 2004). Hence, the risk of a severely damaging frost during summer is low, and even extreme episodic frosts are unlikely to kill adult trees of the five species at either location. Lack of frost tolerance in adult trees has been implicated as a driver of treeline altitudes in tropical regions (Cordell et al. 2000, Rada et al. 2001). In contrast, this study suggests that, although some damage occurs, it is unlikely that such occasional frosts control the temperate New Zealand treeline position through dieback of adult trees.

Elsewhere, frosts at treeline have been found to result in damage through *winter desiccation*, or *frost drought*, occurring when water uptake to feed evaporative demand is blocked because of frozen soil and stems (Tranquillini 1980, Mayr et al. 2006, Kullman 2007). The resulting formation of embolisms may affect the tree's water transport, and result in irreversible damage (Mayr et al. 2006). Since soils at gradual treelines in New Zealand do not freeze (see Section 2.3.1), effects of winter desiccation are unlikely to be significant at such sites. Even at abrupt treelines, where soils may freeze for a few days at a time (see Section 2.3.1), studies of water relations of *Nothofagus* have shown that throughout the winter, even without snow protection, foliage develops water potentials no lower than experienced during normal diurnal fluctuation in summer (McCracken et al. 1985). This suggests that winter desiccation is not an important limiting factor at either type of New Zealand treeline.

Overall, it appears that the New Zealand treeline species, at least in winter, have excess frost tolerance relative to the risk of damaging frosts at treeline. In winter, the differences in LT_{50} between the five species are largest (-18 to -8·6°C). In summer, despite the very different leaf and tree structures, biogeographic and ecological distributions of the species, they show a much narrower range in LT_{50} (-7·1 to -5·2°C). This suggests that a minimal level of tissue adaptation secures protection down to -5 to -7°C, which apparently is relatively easily achieved by most trees capable of growing in the alpine zone. Almost 80% of the 58 New Zealand trees assessed for winter freezing resistance of their leaves can tolerate -5°C or colder (Wardle 1991). *Phyllocladus alpinus* is the only native species in this study whose range extends out to the drier, eastern basins where extreme low winter temperatures are experienced (annual minimum < -10°C). The ability to achieve such levels of winter tolerance, as in *Phyllocladus alpinus* and *Pinus contorta*, may be linked to the permanent structural protection provided by the robust anatomical features of these conifers, as has been suggested for other conifers and winter cereals (Savitch et al. 2002, Öquist and Huner 2003).

However, frost resistance of mature trees, as measured in this study, may be less important than the effects of temperature on the survival and growth of seedlings (Bannister and Neuner 2001). Indeed, Wardle (1973, 1985c) suggested that limiting factors for treeline are probably met in the seedling stage, and frost damage of seedlings has been shown to affect treeline in Chile (Piper et al. 2006). Seedlings are generally less frost tolerant than adults (Sakai & Larcher 1987), although seedlings of some Southern Hemisphere species have been shown to be more tolerant than their mature counterparts (see Bannister et al. 1995). Seedlings occupy space closer to the ground surface, resulting in foliar temperatures much more extreme than the air (1·5 m) temperatures measured here or foliar temperatures in the canopy, especially during calm clear weather (Wilson et al. 1987). This is especially the case for seedlings established in the open beyond the canopy. On the other hand, seedlings that establish beneath the canopy may be more susceptible to frosts when they do occur because of their general lack of exposure to cold temperatures.

No direct comparisons of frost tolerance of seedling and mature foliage exist for New Zealand treeline species. A study of the seasonal frost tolerance of *Nothofagus* seedlings near treeline (Greer et al. 1989) showed only a *ca.* 2°C higher frost tolerance (LT_{10} and LT_{50}) compared to that of the adult foliage used in the current study. In summer, seedling LT_{50} was *ca*. -3·5°C, suggesting that in many years they would experience some damage, this value being close to minimum summer temperatures experienced near treeline (Greer et al. 1989).

The small number of seedlings of the slow-growing conifer species at the West Alps site made destructive sampling at multiple intervals inadvisable. Nevertheless, the > 4°C window between adult foliage LT_{50} and extreme minimum temperatures at treeline (Figure 4.2) indicates that seedlings would have to be very much more sensitive to frost for this factor to affect their survival. However, seedlings do not have the advantage of a buffering canopy that adult trees have, and with much of their foliage exposed to the extreme cold, the onset of frost damage, LT_{10}, may be a more applicable indicator for seedling damage. This would suggest that seedlings of all treeline species, except perhaps *Halocarpus*, are likely to suffer slight damage by frosts in spring and/or autumn (Figure 4.2).

This study does not take into account exacerbating factors, for example frost desiccation or cold-induced photo-inhibition. In other locations, these processes affect trees near treeline (e.g., Hadley and Smith 1986, Bader et al. 2007). If combinations of frost and other environmental factors, such as high radiation or strong winds, are damaging processes to tree foliage, and the severity of such combinations increases with altitude, one would expect an altitudinal trend of decreasing performance. This can be measured by fluorescence measurements that indicate changes in photosystem efficiency, F_v/F_m, with altitude (Richardson et al. 2001). In this study, photosystem efficiency decreased towards treeline for some species: *Nothofagus* in all seasons, and *Halocarpus* in autumn and spring (Figure 4.3). In contrast, *Phyllocladus* and *Libocedrus* showed no change in F_v/F_m with altitude. In the Himalayas, De Lillis et al. (2004) also found that fluorescence measurements of trees in the treeline ecotone were more strongly related to species than to altitude. In the current study, exotic *Pinus contorta* showed a pattern of decreasing photosystem efficiency towards treeline in spring (or slower recovery after winter), but individuals at the highest site had higher photosystem efficiency than at the treeline site (overall resulting in a non-significant linear altitude effect). A similar pattern has been reported in the USA, where *Picea* and *Abies* showed decreasing photosystem efficiency with altitude towards treeline, although at the highest altitude site efficiency was increased, probably related to the prostrate plant architecture and/or stress-tolerant physiology at this site (Richardson et al. 2001). As the trees at the highest site in this study were not more sheltered, nor prostrate, it seems likely that a more stress-tolerant physiology of *Pinus contorta* may explain this decrease in F_v/F_m measurements at the highest site.

F_v/F_m measurements were lower in spring and summer than in autumn for all species. Similarly, in a study of photosynthetic capacity of five conifers species near treeline in the Rocky Mountains, Koh et al. (2009) found that lower temperatures in autumn were initially correlated with increases in maximal photosynthetic capacity, “as long as the declining temperatures remained in a range that should likely permit further net carbon gain" (p. 320). However, when winter encroached and freezing events became common, strong photosynthetic down-regulation followed (Koh et al. 2009). Stecher et al. (1999) also found an extreme reduction in fluorescence in *Picea abies* and *Pinus cembra* near treeline in winter (down to 10%), and a slow recovery towards summer.

4.5 Conclusions

The results of this study suggest that frost is not a major factor limiting the performance of adult trees near treeline in New Zealand. Frost tolerance did not show a trend with altitude along the gradient studied here for four native species, and an altitudinal decrease of photosystem efficiency was found only for some species. There was a significant trend in frost tolerance of the exotic *Pinus* with altitude, but only in spring. Although occasional frosts, especially in spring, may damage some of the foliage, this would affect only a small portion of the tree's foliage; such frosts are unlikely to control treeline position through dieback of adult trees. The window of at least 4°C between extreme minimum temperatures in the last 20 years and the LT_{50} of mature foliage is likely to allow at least a moderately reduced seedling frost tolerance without seriously damaging effects.

However, combinations of frost and other environmental factors, such as high light or strong winds, may still damage foliage and affect mature tree and seedling performance at high altitudes. This may explain the decreased photosystem efficiency found along the altitudinal gradient for trees of *Nothofagus* in all seasons, and for *Pinus contorta* and *Halocarpus* in summer and/or spring.

Plate 4.1. Examples of frost damage at the different treatment temperatures for all species, in autumn, early April 2010. Panel (f) shows the layout of the samples.

(a) *Halocarpus*

(b) *Libocedrus*

(c) *Nothofagus* (back of leaf shows damage clearer)

(d) *Phyllocladus*

(e) *Pinus*

(f) Sample lay out

0°C	-3°C	-6°C	-9°C
-12°C	-15°C	-18°C	Control +4°C

Chapter 5 Temperature response of photosynthesis

5.1 Introduction

Photosynthesis requires CO_2 to diffuse from the atmosphere to the sites of carboxylation. Along this path, there are a series of resistances that result in a decrease in the CO_2 concentration in the atmosphere (C_a) to the carboxylation site (C_c). First, CO_2 must diffuse from the atmosphere across the boundary layer in the air above the foliage surface (C_s), then through the stomatal opening into the sub-stomatal cavity (C_i); CO_2 then dissolves into the cell wall water of the mesophyll cells and diffuses across the plasma membrane, cytosol and finally across the stroma to the sites of carboxylation (see Evans et al. 2009, Terashima et al. 2011). Significant resistances to CO_2 diffusion occur through the stomata and through the mesophyll, and result in drops in concentration from C_s to C_i and C_i to C_c. Resistances are more commonly referred to as the inverse; boundary layer, stomatal and mesophyll conductances (g_b, g_s and g_m, respectively).

The mesophyll (or internal) conductance, g_m, is affected by leaf morphology and anatomy, as well as by external factors including temperature and water availability (Niinemets et al. 2009a, Niinemets et al. 2009b). The diffusion pathway through intercellular airspaces can be affected by leaf thickness, cell shape, and packing relative to the position of stomata (Evans et al. 2009), and is therefore species-specific. Low g_m may significantly limit photosynthesis to a degree equal to or larger than g_s. The extent of these limitations has been measured in an increasing number of species, but very few conifers (six conifer species in a review of 81 species across 60 studies; Niinemets et al. 2009b). No studies of the temperature response of g_m for conifers have yet been published. For the angiosperm *Eucalyptus regnans*, the relative limitations of both stomata and mesophyll appear to increase with temperature, but this increase did not happen until at higher temperatures (Warren 2008a).

It has been suggested that plants growing in harsher environments are more strongly controlled by limited conductances, especially mesophyll conductance, than species in more favourable conditions (Flexas et al. 2008, Warren 2008b, Niinemets et al. 2009b).

Photo previous page: Measuring gas exchange in a controlled environment chamber

Species occurring at treeline may be of special interest given their strong limitation by temperature (Körner 2003a).

Here, I assess the temperature response of photosynthetic capacity (V_{cmax}, J_{max}) and stomatal and mesophyll conductances of native tree species that occur near the New Zealand treeline and compare with an exotic conifer *Pinus contorta* found above the native treeline. I hypothesised that the native treeline species have reduced photosynthetic capacity compared to the exotic. I investigated whether any differences may be explained by the responses of stomatal and mesophyll conductances to temperature, using a combination of stable isotope techniques and conventional gas exchange measurements in a short-term controlled environment pot study.

5.2 Methods

Plants and controlled environment conditions

The species tested include *Libocedrus bidwillii* Hook. f. (Cupressaceae)*, Halocarpus bidwillii* Kirk (Quinn)*, Nothofagus solandri* var. *cliffortioides* (Hook.f.) Poole (Nothofagaceae), *Phyllocladus alpinus* Hook. f. (Podocarpaceae), *Podocarpus hallii* Kirk (Podocarpaceae)*,* and *Pinus contorta* Loudon (Pinaceae). Below these species will be referred to by their generic name only.

All plants were sourced from nurseries, except the *Pinus*. This exotic pine is classified as a ‘noxious weed’ in New Zealand and seedlings were taken from invasions near Craigieburn. All plants were potted into > 2.5 litre pots with standard potting mix at least three months before the first measurements were taken. Seedling heights ranged from 15-90 cm, but were more consistent within species. Plants were (short-term) acclimatised to treatment conditions in the controlled environment chambers for at least seven days before measurements (following Atkin et al. 2000, Ow et al. 2008). Treatments were temperatures of 25/15, 20/15, 15/15, 10/10 and 5/5°C (day/night, day length = 14 hrs). Relative humidity (RH) was maintained at 70-80% for all treatments. However, the same RH at a different temperature will result in a different vapour pressure deficit (VPD), and since stomatal conductance is reduced at higher VPD, this may affect photosynthetic rates measured. However, the relatively low VPD for all treatments (70% RH would result in a VPD between 0·26 and 0·95 kPa for temperatures between 5 and 25°C, respectively) is unlikely to affect photosynthetic rates of temperate trees (Cunningham 2005). Photosynthetically active radiation (PAR) at the plant level was *ca*. 700 µmol $m^{-2}s^{-1}$, above or near saturation values (see Appendix 3, Figure A3.1). Plants were watered every second day and nutrients were added monthly.

Gas exchange measurements

Photosynthetic CO_2 response curves (A-C_i) were determined by measuring the response of net photosynthesis (A) to varying intercellular CO_2 partial pressure (C_i) on fully expanded foliage of six plants for each species, using a Li-COR 6400 portable gas exchange system (Lincoln, NE, USA). CO_2 partial pressure (C_a) of the air stream entering the chamber was supplied in 16 steps, decreasing the partial pressure from 370, to 350, 300, 250, 200, 200, 125, 75, 50, 20, and 0 µmol CO_2 mol $^{-1}$, then increasing from 370 to 450, 600, 800, 1000, and 1500 µmol CO_2 mol $^{-1}$. Increasing the number of steps at lower C_a decreases the error in V_{cmax} and J_{max} estimates (Miao et al. 2009). PAR was maintained at a saturating value of 1350 µmol $m^{-2}s^{-1}$ (red-blue), provided by a Li-COR 6400-18 RGB light source (Lincoln, NE, USA). All measurements were made in Lincoln, New Zealand, at 30 m above sea level, and atmospheric pressure varied between 101 and 104 kPa.

Measurements and calculations, including net photosynthesis rate, C_i and leaf temperature were recorded automatically at each C_a set point when photosynthesis had stabilised, which was typically less than 3 min. A custom-built chamber was used, consisting of milled aluminium, nicklised then teflon-sprayed, with a clear top to allow enclosure of up to 38 cm^2 leaf area (Barbour et al. 2010). The large leaf area of the chamber maximises the difference in concentration and isotopic composition between incoming and outgoing chamber air, which reduces errors in calculated $\delta^{13}C$ and photosynthetic discrimination (see below). Measurements were made with the leaf fan set to fast, the flow rate of air through the leaf chamber at 300 µmol s^{-1} and the chamber humidity maintained close to ambient (80 ± 10%). If necessary, the flow was increased to 600 µmol s^{-1} to maintain the humidity. The block (chamber) temperature was maintained at the constant treatment temperature. Due to the complex foliage structure, the leaf thermocouple could not always touch the foliage. Instead, the thermocouple was recessed slightly and measured the air temperature in the leaf chamber. I assumed that the combination of high flow rate and small and/or scale-like foliage resulted in a low boundary layer resistance and used this measured air temperature as an approximation of leaf temperature.

Estimates of V_{cmax} and J_{max} from A-C_i curves are affected by CO_2 diffusion into and out of the leaf chamber which is proportional to the difference in [CO_2] between the air inside and outside of the chamber and the air flow rate through the system (Flexas et al. 2007). These diffusion leaks were incorporated by measuring A-C_i curves for an artificial leaf in the custom-built chamber at the different flow rates, and adjusting the LiCOR data as outlined in Flexas et al. (2007).

Net photosynthetic rates were recalculated based on half-total leaf area. For all species, the foliage samples were harvested after the gas-exchange measurements and single-sided leaf areas were determined by scanning the foliage on a flatbed scanner and processing using WinFolia Pro 2004a (Regent Instruments Inc., Canada). For *Nothofagus, Phyllocladus* and *Podocarpus,* single sided area approximates the half-total leaf area. For the other species with more three dimensional foliage, half-total leaf area was estimated as described in Section 2.2.1. The foliage samples were dried at 70°C and measurements of dry weight and half-total leaf area were used to calculate the leaf mass per area (LMA). For each plant, the foliage samples of the 25°C measurement were analysed for nitrogen and carbon content and $\delta^{13}C$ on a dry weight basis using stable isotope mass spectrometry performed by the Waikato University Stable Isotope Unit (Hamilton, New Zealand).

Estimation of V_{cmax} and J_{max} from the CO_2 response of photosynthesis

First, A-C_i curves were recalculated to A-C_c curves using the measured mesophyll conductance, g_m, (see below), using:

$$C_c = C_i - \frac{A}{g_m} \quad \text{……………………………………………………………} \quad \text{Equation 5.1}$$

where:
C_c and C_i are the concentrations of CO_2 in the chloroplast and in the intercellular spaces, respectively;
A is the net photosynthesis; and
g_m is the mesophyll conductance.

The mesophyll conductance was measured at C_a = 450 on the same leaf as the corresponding A-C_i curve. If g_m was not available for this leaf, the average value for the species (at the treatment temperature) was used. Although there have been suggestions that g_m change with CO_2 concentration (see Flexas et al. 2008), more recent evidence suggests that this may not be the case (e.g., Tazoe et al. 2009, Tazoe et al. 2011). In many studies of gas-exchange, g_m measurements are not available and are instead modelled, with current fitting procedures fitting one modelled g_m value and excluding the potential effect of differing [CO$_2$] during the A-C_i curve. In this study, the g_m value measured at C_a = 450 was used in the fitting procedure outline below.

According to the photosynthesis model of Farquhar et al. (1980), net leaf photosynthesis (A) can be expressed as the minimum of two limiting rates: A_c, the rate of photosynthesis when Rubisco activity is limiting, and A_j, the rate when ribulose-1,5-*bi*phosphate (RuBP)-regeneration is limiting, minus R_d which is the rate of mitochondrial respiration:

$$A = min(A_c, A_j) - R_d \quad \text{.........} \quad \text{Equation 5.2}$$

When photosynthesis is Rubisco-limited (A_c), this can be described by:

$$A_c = V_{cmax}\left[\frac{C_c - \Gamma^*}{C_c + K_c(1 + \frac{O}{K_o})}\right] - R_d \quad \text{.........} \quad \text{Equation 5.3}$$

where:
V_{cmax} is the maximum rate of Rubisco activity;
C_c and O are the concentrations of CO$_2$ and O$_2$ in the chloroplast (at the Rubisco site), respectively;
K_c and K_o are the Michaelis-Menten coefficients of Rubisco activity for CO$_2$ and O$_2$, respectively;
Γ^* is the CO$_2$ compensation point in the absence of mitochondrial respiration; and
R_d is the rate of mitochondrial respiration

When photosynthesis is RuBP-regeneration limited (A_j), this can be described by:

$$A_j = J_{max}\left[\frac{C_c - \Gamma^*}{4C_c + 8\Gamma^*}\right] - R_d \quad \text{.........} \quad \text{Equation 5.4}$$

where:
J_{max} is the maximum rate of electron transport, assuming four electrons per carboxylation and oxygenation.

The derivations of the equations have been presented on numerous occasions, see for instance (Long and Bernacchi 2003).

For each A-C_c curve, the above equations were fitted using non-linear least square regression in the *nls2* package in R (Grothendieck 2010). Fitting parameters (including K_c and K_o, scaling constants, enthalpies of activation and deactivation) were taken from Sharkey et al (2007). The *nls2()* function allows a coarse grid-search based on all plausible combinations of V_{cmax}, J_{max} and R_d within specified ranges ($0 < V_{cmax} < 80$ µmol m^{-2} s^{-1}; $0 < J_{max} < 160$ µmol m^{-2} s^{-1}; $0 < R_d < 4$ µmol m^{-2} s^{-1}). The combination with the lowest error term is identified, after which increasingly fine grids can be searched around the obtained output (Ritz and Streibig 2008). Rather than *a priori* identifying regions of Rubisco and RuBP limitations, all A-C_c values were fitted simultaneously, which is appropriate for non-stressed plants (Niinemets et al. 2009a).

To assess the temperature response of V_{cmax} and J_{max}, the Arrhenius function was fitted to these values at different temperatures for each plant:

$$f(T_k) = k_{25} exp\left[\frac{H_a(T_k - 298)}{(298\, R\, T_k)}\right] \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad \text{Equation 5.5}$$

where:
T_k is the leaf temperature in degrees Kelvin;
k_{25} is the value at 25°C;
H_a is the activation energy or exponential rate of rise of the function; and
R is the universal gas constant (8.314 J mol^{-1} K^{-1}).

In addition, a peaked function (modified Arrhenius curve) was fitted (following Medlyn et al. 2002):

$$f(T_k) = k_{opt} \frac{H_d exp\left[\frac{H_a(T_k - T_{opt})}{(T_k\, R\, T_{opt})}\right]}{H_d - H_a\left(1 - exp\left[\frac{H_d(T_k - T_{opt})}{(T_k\, R\, T_{opt})}\right]\right)} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad \text{Equation 5.6}$$

where:
T_k is the leaf temperature in degrees Kelvin;
H_a is the rate of exponential increase of the function below the optimum;
H_d is the rate of decrease above the optimum; and
T_{opt} is the optimum temperature.

Stable isotope method to estimating mesophyll conductance

On-line photosynthetic ^{13}C discrimination was measured by coupling the open leaf gas exchange system described above to a tunable diode laser (TDL) spectroscopy system (TGA100A; Campbell Scientific, Inc.) (Barbour et al. 2007, Barbour et al. 2010). The TDL measures concentrations of $^{13}CO_2$ and $^{12}CO_2$ of the air going into and coming out of the leaf chamber. Each three minute TDL measurement cycle consisted of two calibration tanks and the reference and sample gas streams for two LiCOR 6400 instruments, each measured for 30 s, from which average isotopologue concentrations over the last 15 s of each inlet cycle were calculated. Measurement conditions were as described above, except that [CO_2] was controlled within the leaf chamber at 450 µmol mol^{-1}. Each leaf was allowed to adjust to the chamber conditions for at least 10 minutes before gas exchange and online discrimination measurements were made.

The TDL measured concentrations of $^{13}CO_2$ and $^{12}CO_2$ values are converted to the carbon isotope composition $\delta^{13}C$ notation (in ‰) using:

$$\delta^{13}C = \frac{R_s}{R_{VPDB}} - 1 \quad \dots\dots\dots\dots\dots\dots \quad \text{Equation 5.7}$$

where:
R_s is the $^{13}C/^{12}C$ ratios of the sample; and
R_{VPDB} is the $^{13}C/^{12}C$ ratios of the VPDB (Vienna Pee Dee belemnite) standard.

The photosynthetic carbon isotope discrimination (Δ_{obs}) was then calculated following (Evans et al. 1986):

$$\Delta_{obs} = \frac{\xi(\delta_0 - \delta_e)}{1 + \delta_0 - \xi(\delta_0 - \delta_e)} \quad \dots\dots\dots\dots\dots\dots \quad \text{Equation 5.8}$$

where:

$$\xi = \frac{c_0}{c_e - c_o}; \quad \dots\dots\dots\dots\dots\dots \quad \text{Equation 5.9}$$

c_e and δ_e are concentrations and isotope compositions of CO_2 entering the leaf chamber; and
c_o and δ_o are concentrations and isotope compositions of CO_2 leaving the leaf chamber.

All air streams were passed through a Nafion drying tube prior to entering the TDL, so values presented are at zero water vapour concentration. g_m was estimated from the difference between calculated carbon isotope discrimination assuming infinite g_m (Δ_i) and that measured by the coupled system (Barbour et al. 2010):

$$g_m = \frac{\left(b - a_l - b_s - \frac{e' R_d}{A + R_d}\right) A}{C_a\,(\Delta_\mathrm{i} - \Delta_\mathrm{obs})} \quad \dots\dots\dots\dots\dots\dots \quad \text{Equation 5.10}$$

$$\Delta_i = a_b \, \frac{C_a - C_s}{C_a} + a \, \frac{C_s - C_i}{C_a} + b \, \frac{C_i}{C_a} - f \, \frac{\Gamma^*}{C_a} - e' \, \frac{R_d}{A + R_d} \, \frac{C_i - \Gamma^*}{C_a} \quad \dots\dots \quad \text{Equation 5.11}$$

where:
a (4.4‰), a_b (2.8‰) and a_l (0.7‰) are fractionations associated with diffusion through the stomata, leaf boundary layer and leaf water, respectively;
b (28‰) and b_s (1.1‰ at 25°C) is the net fractionation of CO_2 during carboxylation and as CO_2 moves into solution, respectively;

e' (-0.5‰) is the fractionation associated with mitochondrial respiration in the light [corrected for source $^{13}CO_2$, taking into account both isotope effects of -3‰ (Bickford et al. 2009) and the ^{13}C disequilibrium of +2.5‰ between growth and measurement CO_2 (Wingate et al. 2007, Tazoe et al. 2009)];

f (11.6‰) is the fractionation associated with photorespiration (Lanigan et al. 2008);

C_a, C_s, C_i and C_c are the concentrations of CO_2 in the ambient air, at the leaf surface, leaf intercellular spaces and at the sites of carboxylation, respectively; and

Γ^* is the CO_2 compensation point in the absence of mitochondrial respiration (R_d).

g_m was calculated using Equations 5.10 and 5.11 for each coupled measurement of gas exchange and TDL, and three sequential measurements were used to calculate mean and standard error of relevant parameters for each leaf. Errors around the above fractionations (a, a_b, a_i, b, b_s, e', f) and due to instrument precision of CO_2 concentration and isotopic composition (e.g., Bickford et al. 2009, Barbour et al. 2010) will increase the uncertainty of the calculated g_m; however in the following analyses, such propagation of errors was not taken into account. The presented g_m values therefore only represent an approximation of the mesophyll conductance. Again, the half-total leaf area of the foliage sample was determined and used to recalculate the concomitant gas exchange variables (including A and g_s) accordingly.

Analysis of limiting conductances

The limitation of photosynthesis imposed by finite g_m and g_s were estimated following Warren (2008a). Rates of net photosynthesis were estimated assuming g_m and g_s were as measured (A, the net light-saturated photosynthetic rate at $C_a = 450$), assuming g_m was infinite and g_s as measured (A_{ml}: the light-saturated photosynthetic rate at $C_c = C_i$), or assuming g_m as measured and g_s was infinite (A_{sl}: the light-saturated photosynthetic rate at $C_i = 450$). The relative limitations due to mesophyll (L_m) and stomatal (L_s) resistances were estimated as:

$$L_m = \frac{A_{ml} - A}{A_{ml}} \quad \text{.......} \quad \text{Equation 5.12}$$

$$L_s = \frac{A_{sl} - A}{A_{sl}} \quad \text{.......} \quad \text{Equation 5.13}$$

Statistical analyses

Differences in leaf-level characteristics (including leaf mass per area and nitrogen content, as well as A_{max}, conductances and photosynthetic nitrogen use efficiency measured at 15°C) between species were tested using ANOVA and *a posteriori* Tukey's Honest Significant Difference ($p < 0.05$). Differences between the native conifers (*Halocarpus, Libocedrus, Phyllocladus* and *Podocarpus*) and exotic conifer (*Pinus*), and between the native conifers and the native broadleaf tree (*Nothofagus*) were compared using *a priori* contrasts ($p < 0.05$).

As discussed above, the temperature response of gas exchange parameters A_{max}, V_{cmax} and J_{max} may be described by a simple Arrhenius function (increasing with temperature) or a peaked function (with an optimum temperature). To determine which better described the data, *F* tests were performed (Medlyn et al. 2002). In contrast, since it is not known what the shape of the temperature response curve of the stomatal and mesophyll limitations may be, polynomial contrasts in ANOVA were used to test for a linear or quadratic trend across the temperature range. This procedure assessed a quadratic trend by contrasting the low and high temperatures with the middle two temperatures.

5.3 Results

Leaf-level characteristics

Photosynthesis on an area basis (A_{max}.area), measured at 15°C, saturating light and an ambient CO_2 concentration of 450 µmol mol^{-1} was marginally higher ($p = 0.06$) for *Pinus* than the native conifer species (Table 5.1). Native broadleaf *Nothofagus* had significantly higher photosynthetic rates than the native conifers. When expressed on a mass basis (A_{max}.mass), photosynthetic rates of *Nothofagus* far exceeded all other species, due to its very low LMA. LMA of *Pinus* was lower than LMA of the native conifers. Stomatal and mesophyll conductances under these circumstances were highest in *Nothofagus* and *Pinus.* Due to the variability in foliar nitrogen content of some species (most notably *Podocarpus*), there were no significant differences between the species. Photosynthetic nitrogen use efficiency (PNUE) was highest for *Nothofagus*, but differences between other species were not significant (Table 5.1).

Table 5.1. Leaf-level characteristics of the six species. Values are means of 4-6 replicates (± 1 SE in parentheses). Values within a row followed by the same superscript are not significantly different between the species.

Characteristic (units)	***Halo carpus***	***Libo cedrus***	***Notho fagus***	***Phyllo cladus***	***Pinus***	***Podo carpus***	**Con1‡**	**Con2‡**
A_{max}.area† (µmol CO_2 $m^{-2}s^{-1}$)	5.07^{ab} (0.36)	4.57^{ab} (0.34)	8.64^{ab} (1.87)	3.81^{a} (0.99)	7.19^{ab} (1.54)	4.95^{ab} (0.61)	.	**
LMA (g m^{-2})	305.18^{c} (5.13)	262.94^{c} (5.86)	108.69^{a} (15.53)	138.24^{a} (11.36)	198.38^{b} (10.99)	286.11^{c} (18.52)	***	*
A_{max}.mass† (nmol CO_2 $g^{-1}s^{-1}$)	16.65^{a} (1.27)	17.54^{a} (1.64)	81.09^{b} (14.92)	27.38^{a} (6.72)	36.77^{a} (8.1)	17.64^{a} (2.89)	NS	***
$g_m^{\dagger}$ (mmol m^{-2} s^{-1})	46.06^{a} (4.53)	26.85^{a} (1.79)	*NA*	24.41^{a} (4.36)	67.16^{b} (14.81)	*NA*	**	*NA*
$g_s^{\dagger}$ (mmol m^{-2} s^{-1})	38.1^{a} (3.95)	42.16^{a} (5.24)	119.83^{b} (31.65)	32.35^{a} (11.15)	72.28^{ab} (26.11)	42.63^{ab} (4.32)	NS	**
Nitrogen (% dry wt)	1.07^{a} (0.02)	2.36^{a} (0.06)	1.60^{a} (0.07)	1.20^{a} (0.08)	1.53^{a} (0.09)	2.07^{a} (0.30)	NS	NS
PNUE† (µmol CO_2 mol^{-1} N s^{-1})	21.88^{a} (1.78)	10.44^{a} (1.02)	68.61^{b} (13.74)	33.30^{a} (8.69)	34.60^{a} (8.61)	13.23^{a} (2.55)	NS	**

† Steady-state measurements of A_{max} (expressed on an area or mass basis as indicated), g_m, g_{sc} and photosynthetic nitrogen use efficiency (PNUE, photosynthesis per mol nitrogen) were measured at 15°C, saturating photon flux density (1500 µmol m^{-2} s^{-1}) and ambient CO_2 concentration of 450 µmol mol^{-1}.

‡ Significance level of *a priori* contrasts between

Con1: native conifers (*Halocarpus, Libocedrus, Phyllocladus* and *Podocarpus*) *vs*. exotic conifer *Pinus*

Con2: native conifers (*Halocarpus, Libocedrus, Phyllocladus* and *Podocarpus*) *vs*. native broadleaf *Nothofagus* (. $p < 0.10$, * $p < 0.05$, ** $p < 0.01$, NS not significant, and NA not available).

Temperature response of A_{max}, V_{cmax} and J_{max}

The peaked function best described the temperature response of A_{max} for the native conifers *Libocedrus* and *Halocarpus* (optimum temperature was *ca.* 20°C; Figure 5.1a). The exotic conifer *Pinus contorta* showed increasing maximum photosynthesis with temperature, as described by the Arrhenius function. In contrast, there was very little change across the measured range (5-25°C) in the A_{max} of *Phyllocladus.* Measurements of A_{max} of *Nothofagus* and *Podocarpus* were variable and the temperature response was best described by the Arrhenius function for both species, although there may be a hint of an optimum temperature around 20°C for *Podocarpus* (Figure 5.1b).

Due to very low fluxes, measurements of g_m at 5°C were not successful. Additionally, even at higher temperatures, two species, *Nothofagus* and *Podocarpus* did not provide interpretable measurements of g_m. Since these values are required for the calculation of V_{cmax} and J_{max} using the A-C_c method, further results and discussion exclude this lowest temperature and these two species (other than A_{max}, Figure 5.1b). For all other species, V_{cmax} showed an increase with temperature, albeit a small trend for the native species compared to *Pinus*. The temperature response of V_{cmax} was best described with the Arrhenius function for all species, except a peaked function for *Halocarpus* (Figure 5.1c). J_{max} intially also increased with temperature, with the native species showing an optimum within the measured range, all around 20°C. In contrast, *Pinus'* J_{max} optimum again fell outside the measured range, and was best described by the Arrhenius function (Figure 5.1d). The J_{max}:V_{cmax} ratio decreased with temperature, with values ranging from 3·8 to 1·2.

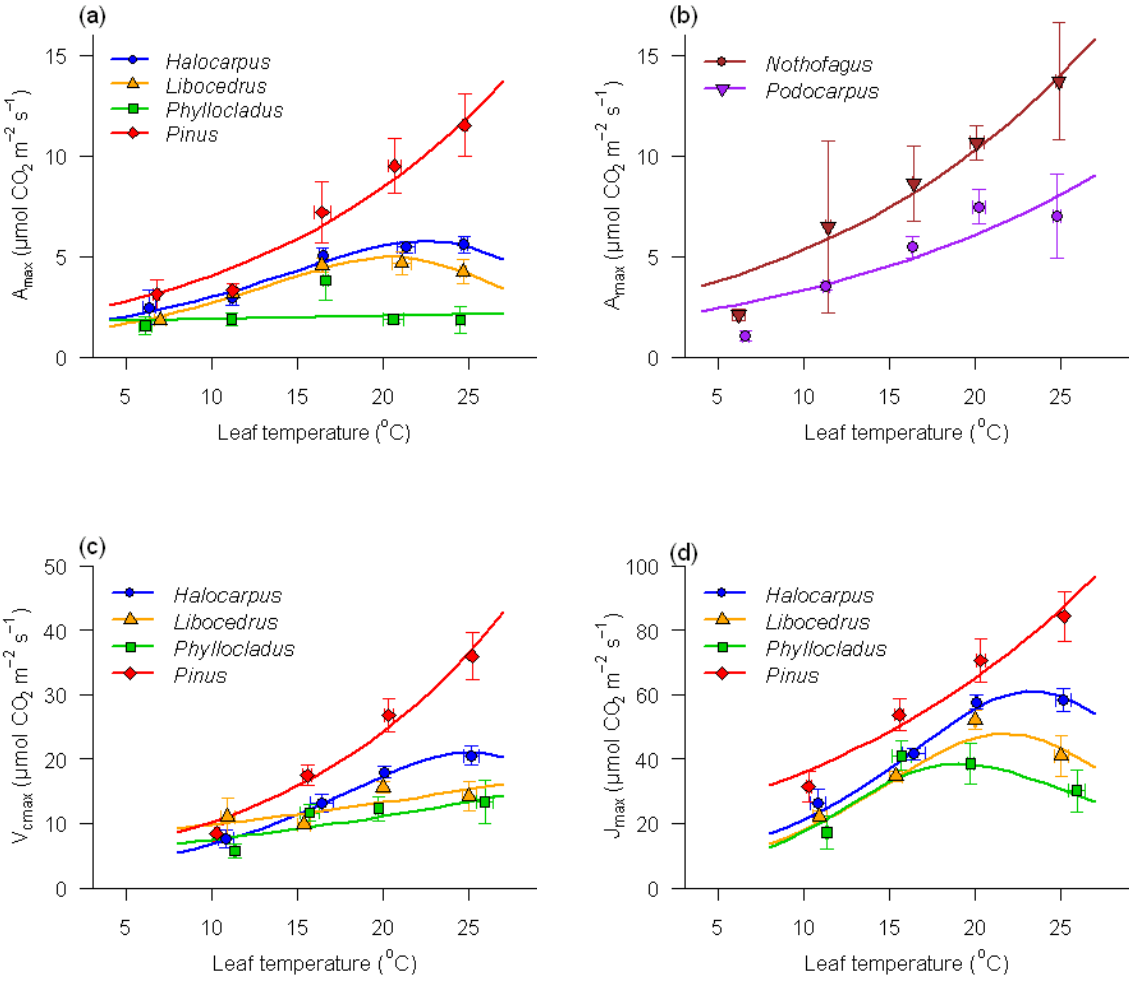


Figure 5.1. Temperature responses of gas exchange parameters: (a,b) maximum rate of net photosynthesis (A_{max}); (c) maximum rate of carboxylation (V_{cmax}) ; and (d) maximum rate of RuBP limited electron transport (J_{max}) for four species with successful measurements. (b) shows temperature response of A_{max} for the two species for which isotope method of internal conductance was not successful and for which V_{cmax} and J_{max} could not be established using the *A*-C_c method. A_{max} was measured at saturating irradiance and CO_2 = 450 ppm as described in Methods and Table 5.1; V_{cmax} and J_{max} were determined from *A*-C_c curves. Data are means of 4 – 6 replicates (± 1 SE). Peaked and Arrhenius functions were fitted through all replicates per species and parameter, and the lines represent the best-fitting function as indicated by an *F* test.

Temperature response of stomatal and mesophyll conductance

Stomatal and mesophyll conductances responses to temperature (Figure 5.2) were different between the native and exotic conifers. The native species only showed a clear positive response of stomatal conductance to temperature at the highest temperature measured (25°C), whereas mesophyll conductance increased from 10-15°C, but remained steady at higher temperature. The exotic *Pinus* showed higher and more temperature-responsive conductances (Table 5.1, Figure 5.2). Across species, g_s and g_m were highly correlated with A ($R^2 = 0.95$ and 0.94, respectively, $p < 0.0001$, Figure 5.3a, b)

The relative limitation of internal conductance (L_m) was higher than that of stomatal conductance (L_s) for all species at all temperatures (Figure 5.4). Limitation values ranged from L_s of around 10% for all species at 10°C to an L_m of almost 60% for *Phyllocladus* at 20°C. A quadratic contrast (showing an optimum at mid-temperatures) was significant for L_s of *Halocarpus*, *Phyllocladus* and *Pinus.* L_s of *Libocedrus* also showed an increase at 20°C, but with a lower value at 15°C, this resulted in a non-significant quadratic trend. There was a significant linear trend of L_m with temperature for *Halocarpus*, *Libocedrus* and *Phyllocladus*. In contrast, L_m of *Pinus* showed no significant trend with temperature (Figure 5.4).

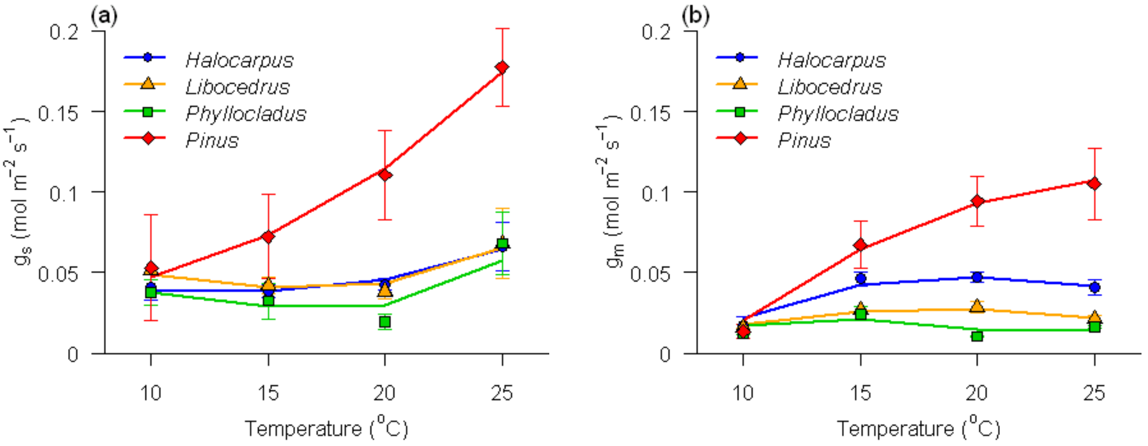


Figure 5.2. Temperature response of stomatal conductance (a) and mesophyll conductance (b) to CO_2. Data are means of 4-6 replicates (± 1 SE); lines are smooth splines.

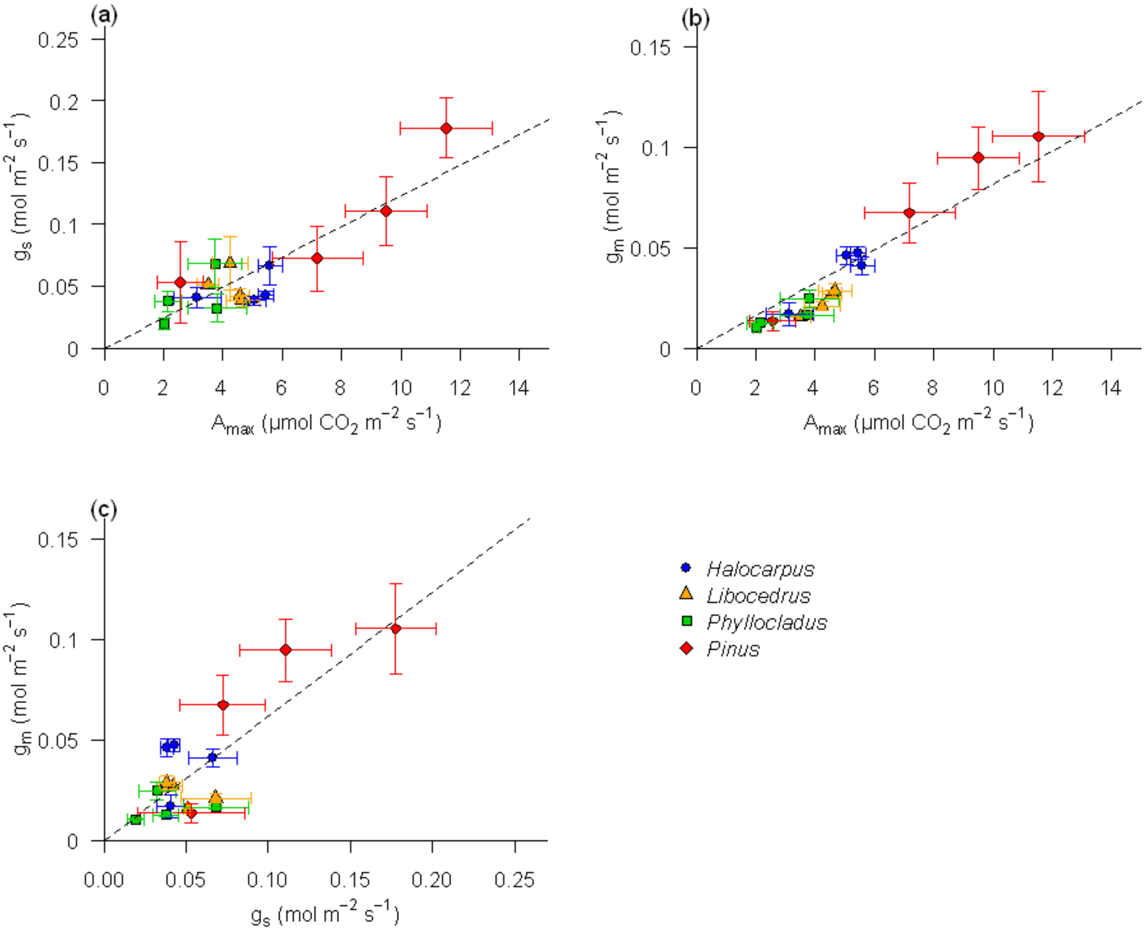
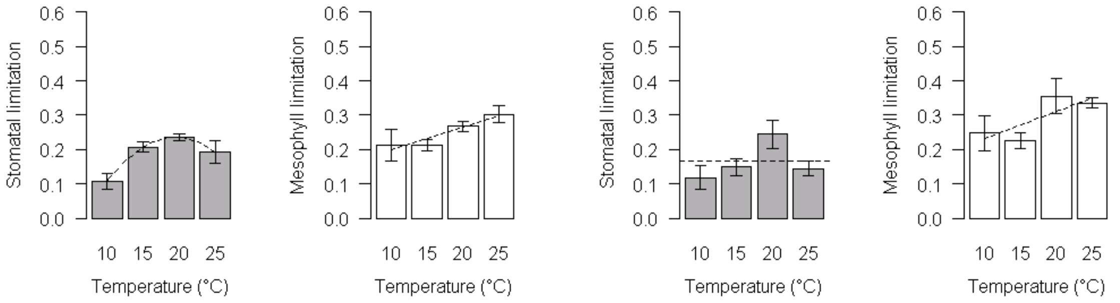


Figure 5.3. Relationship between photosynthetic rate and stomatal conductance (a); photosynthetic rate and mesophyll conductance (b); and (c) mesophyll and stomatal conductances. Data are mean values (± 1 SE) of 4-6 plants per species per temperature (10, 15, 20, 25°C) under steady state conditions of saturated irradiance and C_a of 450 µmol mol^{-1}. Lines are linear regressions through the origin of the means of all species and temperatures: (a) $g_s = 0{\cdot}0123 * A$, $R^2 = 0{\cdot}94$, $p < 0{\cdot}0001$; (b) $g_m = 0{\cdot}00819 * A$, $R^2 = 0{\cdot}95$, $p < 0{\cdot}0001$; (c) $g_m = 0{\cdot}617 * g_s$, $R^2 = 0{\cdot}88$, $p < 0{\cdot}0001$.

(a) *Halocarpus*

(b) *Libocedrus*



(c) *Phyllocladus*

(d) *Pinus*

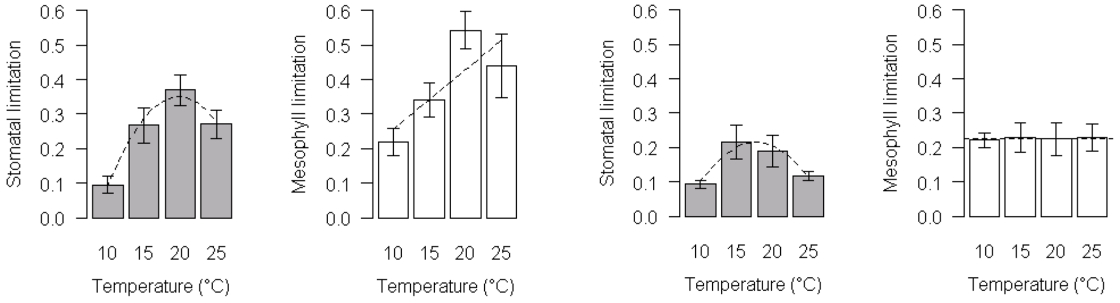


Figure 5.4. Temperature response of the relative limitation by stomata (L_S, grey bars) and mesophyll (L_M, white bars) for the four species: *Halocarpus* (a)*;* *Libocedrus* (b); *Phyllocladus* (c); and *Pinus* (d). Limitations are calculated at each temperature for individual plants from A-C_c curves and isotope measurements. If only one of these types of data was available for a plant at a given temperature, then the species' average at the respective temperature was used in the calculation. Since it is not known what temperature response is expected, simple bar graphs (means ± 1 SE) are plotted here. Lines are significant linear or quadratic trends with temperature, or if both insignificant, the mean (horizontal line) is plotted.

5.4 Discussion

Temperature response of A_{max}, V_{cmax} and J_{max}

The native conifers *Halocarpus, Libocedrus* and *Phyllocladus* showed a remarkably small temperature response of photosynthesis (A_{max}), and an overall low photosynthetic rate (Figure 5.1a). The optimum photosynthetic temperature of the first two species was *ca.* 20°C, similar to conifer species occurring near treeline in Tasmania (Read and Busby 1990), but lower than has been recorded for lowland conifer species in New Zealand (Hawkins and Sweet 1989). In contrast, the exotic conifer *Pinus contorta* showed highly temperature responsive photosynthetic rates, with an optimum above the measured highest temperature of 25°C. The measured optimum for *P. contorta* in its native range is 13-25°C (Smith and Carter 1988), and 17°C and 11°C for plants growing in New Zealand at altitudes between 890 to 1130 m a.s.l., respectively (Benecke and Havranek 1980a). Photosynthetic rates for *Nothofagus* and *Podocarpus* were variable, but higher than the other native species, and continued to increase with temperature across the measured range. There was some evidence of an optimum temperature for *Podocarpus* around 20°C, higher than recorded for a con-specific species near tropical treelines in Venezuela (Cavieres et al. 2000). Optimum photosynthetic temperature of *Nothofagus* below treeline in New Zealand has previously been reported around 20°C (Benecke and Havranek 1980a, Hawkins and Sweet 1989), but the results from this controlled environment study suggest a higher (> 25°C) optimum.

Maximum carboxylation efficiency (V_{cmax}) and maximum electron transport rate (J_{max}) showed an increase with temperature for all species towards an optimum between 20-25°C for the native species compared to an optimum of > 25°C for *Pinus contorta* (Figure 5.1b). Published optima of J_{max} for five other conifers, including four other pine species, have ranged between 28 and 38·5°C (Medlyn et al. 2002, Kattge and Knorr 2007). However, *Pinus sylvestris* from cold conditions in Finland had a much lower optimal J_{max} temperature of 19·9°C (Medlyn et al. 2002), more comparable to the values found in this study for the New Zealand native conifers.

Temperature response of stomatal and mesophyll conductance

Stomatal conductance was greatest at the highest temperature (25°C) for all species, whereas mesophyll conductance showed a clear temperature response only for *Pinus* (Figure 5.2). Across temperature treatments and species, g_s and g_m were positively correlated with each other and with photosynthesis (Figure 5.3), as has been found in many, but not all, cases (Evans and Von Caemmerer 1996, cf. Warren and Adams 2006).

The relative limitation of mesophyll (L_m) was larger than that of stomata (L_s) for all species at all temperatures in this study (Figure 5.4). This supports the findings of several studies that photosynthesis in robust leaves, such as evergreen sclerophylls and conifers, is less limited by stomatal resistance and more so by mesophyll resistance (e.g., see reviews by Flexas et al. 2008, Niinemets et al. 2009b, Niinemets et al. 2011). Lower L_s than L_m was recently also found for the evergreen broadleaf *Nothofagus* growing near the treeline in New Zealand (Whitehead et al. 2011).

The findings of this study suggest that the temperature response of conductance limitations to photosynthesis, especially of L_m, is species-specific (Figure 5.4). Mesophyll limitation increased with temperature for the native conifers, whereas L_m of *Pinus* showed no temperature trend. Niinemets (2009b) suggested mesophyll limitation is increased at lower temperature, which may be related to changes in leaf structure with cooler temperatures (e.g., less leaf expansion, greater leaf thickness). However, in this current study, stronger mesophyll limitation was found at *higher* temperatures for all native species (but not exotic *Pinus*). One obvious difference is that the plants were not grown under different temperature regimes. Instead, fully expanded mature foliage of saplings grown at *ca*. 15°C (average summer temperature at Landcare Research nursery, Lincoln, New Zealand) was measured consecutively at five treatment temperatures, acclimated to each temperature for at least 7 days. Therefore there were no changes in leaf structure (e.g., leaf expansion, leaf thickness) between the treatments. Although additional changes in leaf structure with temperature will be important (e.g., with respect to long-term climate warming), these results relate to the potential for these species to cope with mid-term (days – weeks – months) temperature variations once foliage is formed.

Although intra-species variation was large, there were significant differences in $\mathrm{g_m}$ between species which may be explained by variation in leaf anatomy, for example, the size of air spaces in the mesophyll, which increases the area for CO_2 dissolution (Terashima et al. 2011). A comparison of g_m for nine conifer and ten broadleaved tree species found that the conductance was approximately 1·9 times lower for the conifer species (Manter and Kerrigan 2004). Warren (2008b) suggested that thicker cell walls may be required for long-lived stress tolerant leaves. Low g_{m} values in evergreen tree leaves and alpine plants have been attributed to thick mesophyll cell walls (see Terashima et al. 2005).

The short-term temperature response of conductances provides information about important components of the conductance. For example, g_m and g_s of *Pinus contorta* show a strong temperature response (Figure 5.2), but their relative limitation remains low (Figure 5.4). This suggests that the strongest limitation to photosynthesis is a biochemical limitation, which is highly temperature responsive, as supported by the strong temperature response of V_{cmax} for this species (Figure 5.1c). In contrast, the conductances of the native conifers have a markedly smaller response to temperature (Figure 5.2), which suggests that leaf anatomy, or some other process independent of temperature, plays a larger role in limiting photosynthetic rates. At higher temperature, the relative size of such structural limitations increases, because enzymatic activity increases with temperature and the biochemical limitation is reduced (Figure 5.4). Although favourable conditions may allow relatively high stomatal conductance, the leaves of native conifers have inherently low g_m and consequently a large CO_2 drawdown, which would result in depressed photosynthesis.

The combination of the low photosynthesic rates in New Zealand evergreen conifers and their relatively high foliar levels of nitrogen (and presumably rubisco) results in a low photosynthetic nitrogen use efficiency (PNUE). It has been suggested that this low PNUE may be caused by low g_m (De Lucia et al. 2003). In this study however, there was no difference between the native and exotic conifers in PNUE; suggesting that low PNUE may be a feature more of evergreen species and conifers in general, rather than a feature of the New Zealand conifer flora. In contrast to PNUE, g_m values were significantly higher for the exotic conifer compared to the native species (Table 5.1). However, the nitrogen contents in this fertilised pot experiment may have been higher than found in the field. For example, the consistently high nitrogen concentration found in *Libocedrus* foliage (2·36 % of dry weight) was around twice the levels found in foliage of naturally occurring trees (*ca.* 1-1·2%; Sarah Richardson, unpublished).

There are few studies with data on the relative limitations of g_m and g_s, and to my knowledge, this is the first study including conifers. Some studies report g_{m} and g_{s}, however, additional values required to calculate their relative limitations in a comparable fashion to the current study, including V_{cmax} and R_d, are often not available. Instead, the ratio g_m:g_s has been used to provide a surrogate measure of their relative importance. In this study, all species showed the highest ratio at intermediate temperature (between 0·7-1·3 at 15°C and 20°C). These ratios are consistent with previously published values. For example, in a survey of 50 species (Warren 2008b) found g_m:g_s to be 1·3 in conifers and ranging from 0·52 to 1·5 in angiosperms. De Lucia *et al.* (2003) found the ratio to range between 0·6 and 1·1 in conifers and between 1·0 and 4·2 in angiosperms. Importantly, in the current experiment the ratio at high (25°C) temperature, the ratio was lower (0·4-0·8; 25°C), whereas at low temperature (10°C) the ratio ranged from 0·25-0·4, much lower than the previously published range. It is important to note that the contribution of any biochemical limitations cannot be assessed when using this ratio, and that there is a strong temperature dependence of the ratio, as found in this current study. This finding suggests that any studies reporting g_m:g_s ratios should clarify at what temperature the measurements were made, and any meta-analyses should incorporate the temperature response of the ratio.

5.5 Conclusions

This study of the temperature response of relative limitations of stomatal and mesophyll conductances in New Zealand treeline species suggests the photosynthesis of saplings of three New Zealand conifer species was strongly limited by g_m at higher temperatures in the short-term, whereas an exotic conifer showed no increase in mesophyll limitation. Overall, the native conifers showed reduced photosynthetic potential (V_{cmax} and J_{max}) and therefore growth potential, suggesting that they cannot take advantage of temporarily higher temperatures as much as the exotic *Pinus contorta* can. This lack of responsiveness of the native conifers to higher temperatures (at least partially due to high mesophyll limitation), in combination with lower photosynthetic potential could contribute to their limiting performance at the seasonal climate at higher altitudes near treeline. Similar photosynthetic rates and temperature responses of *Pinus contorta* and *Nothofagus solandri* var. *cliffortioides* suggest that their differential performance near treeline is not caused by an inherent lack of photosynthetic capacity.

Chapter 6 Long-term temperature effects on photosynthesis and growth

6.1 Introduction

Towards the climatic treeline, environmental conditions become harsher, and tree growth declines over a short altitudinal distance. A long-running debate has seen two contrasting carbon-related hypotheses put forward explaining this decline in growth with altitude/temperature (Körner 2003a): the *carbon source limitation* hypothesis suggests that net carbon uptake is insufficient to support maintenance and minimum growth of trees. The *carbon sink limitation* hypothesis proposes that growth processes (cell division and expansion) are limited by low temperatures, even though sufficient photosynthates have been acquired.

Photosynthesis is not as strongly dependent upon temperature as is growth. Other factors such as sunshine hours and sums of solar radiation appear to be more influential to photosynthesis than temperature itself (Tranquillini 1979). Growth and developmental processes, on the other hand, depend strongly on temperature (Grace et al. 2002, Körner 2003a). The imbalance between growth and photosynthesis that may be inflicted by low temperature can cause an accumulation of photosynthates (non-structural carbohydrates, NSC) in plant tissues (Susiluoto et al. 2007). An increasing trend in NSC with altitude has been found across many treeline ecotones (Hoch et al. 2002, Hoch and Körner 2003, Körner 2003b, Piper et al. 2006, Shi et al. 2006, Shi et al. 2008), which has been interpreted as evidence that low temperature induces a carbon sink-limitation in trees. However, other studies have not found trends in NSC with altitude (Li et al. 2008a, Li et al. 2008b, Sveinbjörnsson et al. 2010). Complications in interpreting altitudinal gradients in NSC arise when carbohydrate contents change along the gradient for reasons other than a sink limitation. For example, phenological changes occurring differentially along an altitudinal gradient have been implicated in the lack of NSC trend when assessed at any time other than at the end of the growing season (Hoch and Körner 2009). Carbohydrate concentrations are also known to change in concert with changes in frost tolerance (Öquist et al. 2001), or it could reflect the adaptive importance of maintaining stored carbon reserves in response to extreme and dynamic environments (Ogren et al. 1997, Sveinbjörnsson 2000), where reserves for respiratory-driven processes ensure survival in particularly harsh years (Chapin et al. 1990).

Photo previous page: *Dracophyllum longifolium* near the gradual treeline at Dickie Spur Hut (Mikonui Valley).

Additionally, trends in carbohydrate contents may be obscured if internal feedback processes responding to accumulating carbohydrates cause down-regulation of photosynthesis (Ainsworth and Bush 2011) and a decrease in the temperature sensitivity of photosynthesis (Susiluoto et al. 2007), both potentially obscuring an NSC trend.

Gradual treelines have been suggested to be more limited by growth than abrupt treelines, which respond to additional constraints (Harsch et al. 2009, Harsch and Bader 2011), but this has not yet been tested. Here, I assess the sink (growth) and source (net carbon exchange) activities of saplings when exposed to experimental conditions of different growing season temperatures, using six tree species occurring in abrupt and gradual treeline ecotones in New Zealand. When considering evidence for the sink- and source limitation hypotheses for these species in this controlled environment experiment, I assume that:

A. Evidence for the carbon sink limitation hypothesis includes:

   1. photosynthetic rates are similar across the temperature gradient, yet growth is reduced at low temperature;
   2. down-regulation of photosynthesis occurs at low temperature;
   3. temperature sensitivity of photosynthesis is decreased at low temperature;
   4. NSC increases with decreasing temperature.

B. Evidence for the carbon source limitation hypothesis includes:

   1. temperature trend of growth is positively correlated with photosynthesis;
   2. NSC is stable or decreases with decreasing temperature.

6.2 Methods

Plant material

The same six species tested in Chapter 5 were included in this study: four New Zealand native conifer species (*Halocarpus bidwillii, Libocedrus bidwillii*, *Phyllocladus alpinus* and *Podocarpus hallii*), one native broadleaf species (*Nothofagus solandri* var. *cliffortioides*) and one exotic conifer (*Pinus contorta*). The species will subsequently be referred to by their generic name only. Nursery-grown saplings of the New Zealand native species were imported into the United Kingdom from New Zealand in early May 2009. Plants were bare-rooted (for phytosanitary import requirements) and total transit time was four days. The saplings of the six species ranged in height from 40 to 90 cm tall (with high within-species consistency). Upon arrival in Durham, UK, the saplings were potted into 2 litre containers of a mixture of two parts organic bark-based compost, one part sand and one part grit. *Pinus contorta* was sourced from a nursery in the UK, potted up in the same soil. All plants recuperated in controlled environment chamber under a mild climate (daily mean temperatures of 15°C) for a period of 5 months. They were then subjected to mild winter conditions (daily mean temperatures of 3°C, for *ca*. 5 months). Subsequently, the temperature was gradually ramped up to the respective treatment temperatures (including a diurnal component, see below) over a period of four weeks. The treatments commenced in early April 2010, *ca*. 11 months after importation.

Growing conditions and experimental design

All plants were watered so as to keep the soil near field capacity at all times. To minimise any possible effect of different vapour pressure deficits (VPD) among the temperature treatments, a high air humidity was maintained (at 80% relative humidity) in all treatments throughout the experiment. Light (photosynthetic photon flux density, PPFD, 400-700 nm) was maintained constant across temperature treatments (700 µmol photons m^{-2} s^{-1} at the plant level for 12 hours each day). At 20°C, these light levels are above saturation for plants of the native species, and close to saturation level for *Pinus* (Figure A3.1 in Appendix 3). . Since photosynthesis is light-saturated at lower levels when plants are grown at lower temperature (Benecke and Nordmeyer 1982), I assume that all plants are not limited by the day-time light-levels in any of the treatments (all < 20°C, see below). Dawn and dusk were simulated by applying a lower irradiance (300 µmol photons m^{-2} s^{-1}) during one hour at the start and end of each day. The light received by the plants reduced with distance from the lamps, so small plants were placed on pedestals so that the light received by plants of different heights was comparable. Nutrients (NPK, 6:5:5, plus trace elements; one-half strength proprietary liquid fertilizer, Miracle Grow; Scotts Company, Godalming, Surrey, UK) were added monthly.

The experiment had a factorial design with three temperature treatments, plants of six species and 5-8 replicates at the experimental unit level (plant). The exact number of replicates varied between species, due to differential mortality before the experimental set-up (seven plants, 4.7%) and a subsequent malfunction of the growth chambers (23%). Some plants, especially *Nothofagus* saplings, continued to show signs of stress more than a year after the importation, including significant amounts of foliage senescence.

The temperature treatments for the experiment were selected using temperature data from New Zealand treeline sites collected in 2009 (see Chapter 2). The mid-temperature treatment, with a daily mean of 10°C, represents the approximate temperature during the warmest three months of the growing season at the New Zealand treeline. A colder and a warmer treatment were added, with daily mean temperatures of 5°C and 15°C, respectively. For all three temperature treatments, a 5°C diurnal variation was applied with temperature rises/drops occurring over a couple of hours around dawn/dusk. This resulted in a day-time temperature 2·5°C higher and a night time temperature 2·5°C lower than the mean daily treatment temperature (Figure 6.1). For simplicity, the treatments will further be referred to as 5, 10 and 15°C, respectively.

The controlled environment chambers were fairly successful in reaching their target conditions, especially for the mid and warm treatments (Figure 6.1). The mean daily temperature achieved for the 5°C treatment was somewhat higher than the target, at 5·9°C, and overcompensation at the start of a night cycle often resulted in small spikes in the air temperature (Figure 6.1).

Pseudo-replication is inevitably associated with controlled environment experiments. To account for possible chamber effects, treatments were rotated between chambers such that each of the three chambers was used once for each temperature treatment during the length of the experiment. Additionally, the location of plants within the chambers was re-randomised every few weeks to reduce potential effects of any variation in climatic conditions within a chamber.

The main experiment lasted six months (April to September 2010), the approximate length of the growing season at New Zealand treeline (see Chapter 2 and Chapter 3). However, the plants remained at the respective treatment temperatures for another 11 months (August 2011), before a final biomass harvest was completed.

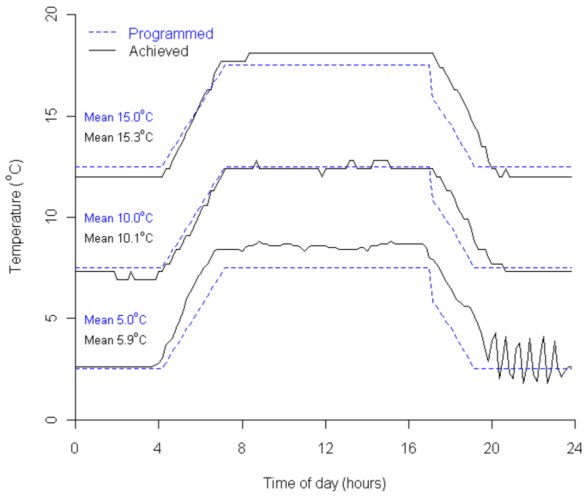


Figure 6.1. Programmed (blue, dotted lines) and actual, achieved (black, solid lines) controlled environment temperatures for the three temperature treatments on a typical day in summer.

Measurements

Carbon uptake

Five to six months into the experiment, measurements of instantaneous light-saturated net photosynthesis (*A*) were taken using a portable gas exchange system (LiCOR 6400, LiCOR, Lincoln, Nebraska, USA) and the standard 30 x 20 mm chamber. Photosynthetically active radiation (PAR) was maintained at a saturating value of 1350 µmol $m^{-2}s^{-1}$ using a LED light source (red-blue; 6400-02B, LiCOR, Lincoln, Nebraska, USA). The chamber humidity was maintained close to ambient (80 ± 10%), incoming CO_2 concentration was maintained at 390 ppm and the flow was maintained at 100 µmol s^{-1}. All measurements were made in Durham, United Kingdom, at 100 m above sea level, and atmospheric pressure during measurements varied between 99.7 and 101.4 kPa.

Net photosynthesis of newly-formed, fully expanded foliage of at least three plants for each species and temperature treatment, was measured at the respective growing season temperature (GrowT). Foliage was acclimated in the LiCOR chamber for 10 minutes prior to taking the measurements. Additionally, to assess potential feedback processes of sink activity on net carbon uptake (down-regulation), instantaneous (measured over a few hours) net photosynthesis of the same plants was measured at the two other treatment temperatures. The Li-COR chamber temperature was set to the respective day-time temperature. Due to the complex foliage structure, the leaf thermocouple could not always touch the foliage. Instead, the thermocouple was recessed slightly and thus measured the air temperature in the leaf chamber, which was then used to calculate the leaf temperature using the manufacturer's energy-balance equations.

Net photosynthetic rates were recalculated based on half-total leaf area to allow fair comparison between species with different leaf morphologies. Chapter 2 described the methodology for converting projected single-sided area of non-flat leaves to half-total area (Section 2.2.1, including Equations 2.1 – 2.3).

Growth and biomass allocation

Sapling height and root collar diameter (further referred to as 'diameter') were measured at the beginning and end of the main experiment. Growth was calculated as the difference in height and diameter over these six months. After 17 months under the same treatment conditions, the plants were cut at the root collar and dried at 70°C for at least five days. The above-ground components of main stem, branches and foliage were separated and their biomass (dry weight) determined. The proportion of above-ground biomass allocated to foliage was calculated. The specific leaf area (SLA) of foliage was determined (as dry weight per unit half-total leaf area).

NSC content

At the end of the main experiment, current-year foliage for five plants per temperature treatment and species was collected at noon, and immediately dropped in liquid nitrogen, dried at 70°C, and ground to a fine powder. NSC extractions of current-year foliage were carried out following the protocol outlined in Section 2.2.1.

Carbon isotope discrimination

Foliage samples of three randomly selected *Libocedrus* and *Pinus* growing at 5°C and 15°C were collected, dropped immediately into liquid nitrogen, dried at 70°C, and ground to a fine powder. Analysis of carbon isotope discrimination was carried out using stable isotope mass spectrometry at Cambridge University (UK). The $\delta^{13}C$ discrimination values of the plant material ($\delta^{13}C_p$) were converted to integrated plant carbon discrimination (Δ_p) values using the Farquhar et al. (1989) equation:

$$\Delta^{13}C = \frac{\delta^{13}C_{atm} - \delta^{13}C_p}{1+\frac{\delta^{13}C_p}{10^3}} \quad \text{Equation 6.1}$$

where: $\delta^{13}C_{atm}$ is the free atmospheric deviation, -8 ‰.

Statistical analyses

The effect of growing temperature (*GrowT*) on net photosynthesis and plant growth (height and diameter) and above-ground biomass was tested by fitting three models to the data. The models included a linear temperature response, a quadratic response (reflecting an optimum growth temperature at mid-temperature) and a model with only an intercept (null model, to compare as a measure of lack of fit).

To assess the effect of long-term acclimation and potential down-regulation of photosynthesis at lower temperatures, five linear mixed effect models were fitted reflecting plausible hypotheses about the relationships between photosynthesis and the acclimated and instantaneous measurement temperatures. Photosynthesis may be influenced by growing temperature (*GrowT,* reflecting long-term acclimation), by measurement temperature (*MeasT,* reflecting an instantaneous effect of temperature), or an additive (*GrowT + MeasT*) or interactive effect of the long- and short-term temperature responses (*GrowT * MeasT*). An intercept-only (null) model was again included for comparison. Repeated measurements were accounted for by including plant ID as a random effect in all models.

Plant-level relationships between net-photosynthesis at GrowT and different measures of plant growth were again assessed by fitting a linear model and a null model with only an intercept to data from plants for which both measurements were available.

All models in each candidate set were fitted and then ranked using Akaike's information criterion corrected for small sample sizes (*AICc*) to determine the Kullback-Leibler (KL) most parsimonious model (Burnham and Anderson 2002), as described in Chapter 2 (Section 2.2.2). The evidence ratio discussed in the text provides a measure of the strength of the evidence for two competing models. It is calculated as the ratio of w_i (model probability) of the best model to the w_i of another model. If the evidence ratio of the best vs. the second-best model was ≤ 2, the second model was also indicated in brackets. Predictions of the KL best model are presented in the figures to show the overall trend in the response variables with changing temperature. Full model tables (including *K, ΔAICc, LogLik*, and w_i etc) are placed in Appendix 3, and will be referred to as Table A3.1 etc. in the text.

Differences in isotopic discrimination between the cool and warm temperature treatments were assessed using a *t* test ($p < 0{\cdot}05$).

6.3 Results

Gas exchange

For three of the four native conifers, net photosynthesis increased linearly with GrowT across the measured range (model probability $w_i > 0{\cdot}72$, Figure 6.2 and Table A3.1). For *Podocarpus* and *Pinus*, the quadratic model far out-performed the other models ($w_i > 0{\cdot}97$, Table A3.1), resulting in an optimum temperature for *Podocarpus* of around 10°C; with a temperature between 5-10°C representing a minimum for *Pinus* (Figure 6.2e, f). The most parsimonious KL model for photosynthesis in the native broadleaf *Nothofagus*, included no effect of GrowT. However, a linear effect of GrowT on photosynthesis also received strong support ($w_i = 0{\cdot}48$ and 0·34, respectively, Table A3.1)

The presence and extent of long-term temperature acclimation and photosynthetic down-regulation differed between species (Figure 6.3 and Table A3.2). Overall, models including GrowT and/or MeasT explained the majority of the variation in photosynthesis ($R^2 > 0{\cdot}79$ for all species). Photosynthesis of *Halocarpus* was best described by GrowT + MeasT, although the GrowT-only model also received support ($w_i = 0{\cdot}53$ vs. 0·22). For *Libocedrus,* the MeasT model outperformed the other models ($w_i = 0{\cdot}93$). For *Nothofagus* and *Podocarpus* a simple model, containing MeasT or GrowT only, respectively, performed best; although the interaction model also received considerable support (*Nothofagus* $w_i = 0{\cdot}54$ vs. 0·31; *Podocarpus* $w_i = 0{\cdot}62$ vs. 0·23). For *Pinus* and *Phyllocladus,* the top-ranked KL model included the interaction of GrowT * MeasT ($w_i = 1{\cdot}00$): the slope of the effect of measurement temperature increased with increasing growth temperature (Figure 6.3 and Table A3.2).

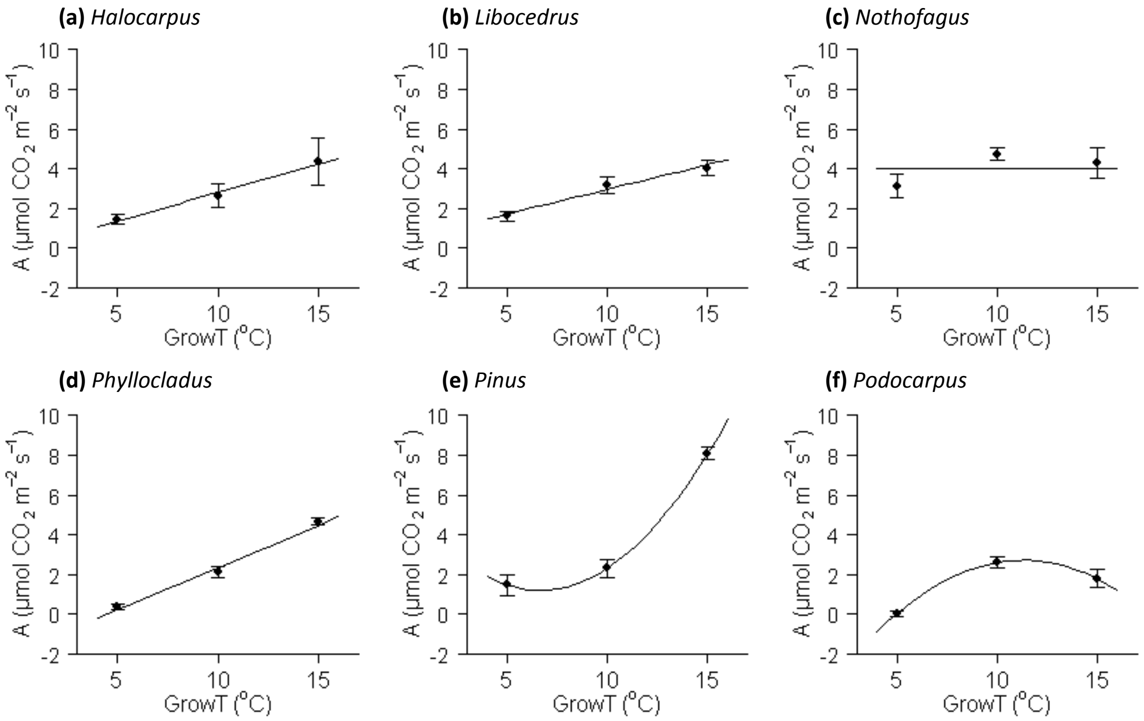


Figure 6.2. Effect of mean growing season temperature (GrowT) on net photosynthesis (*A*). *A* was measured at the respective growing temperature (points are means of 5 plants ± 1 SE). Lines represent the most parsimonious KL model (Table A3.1).

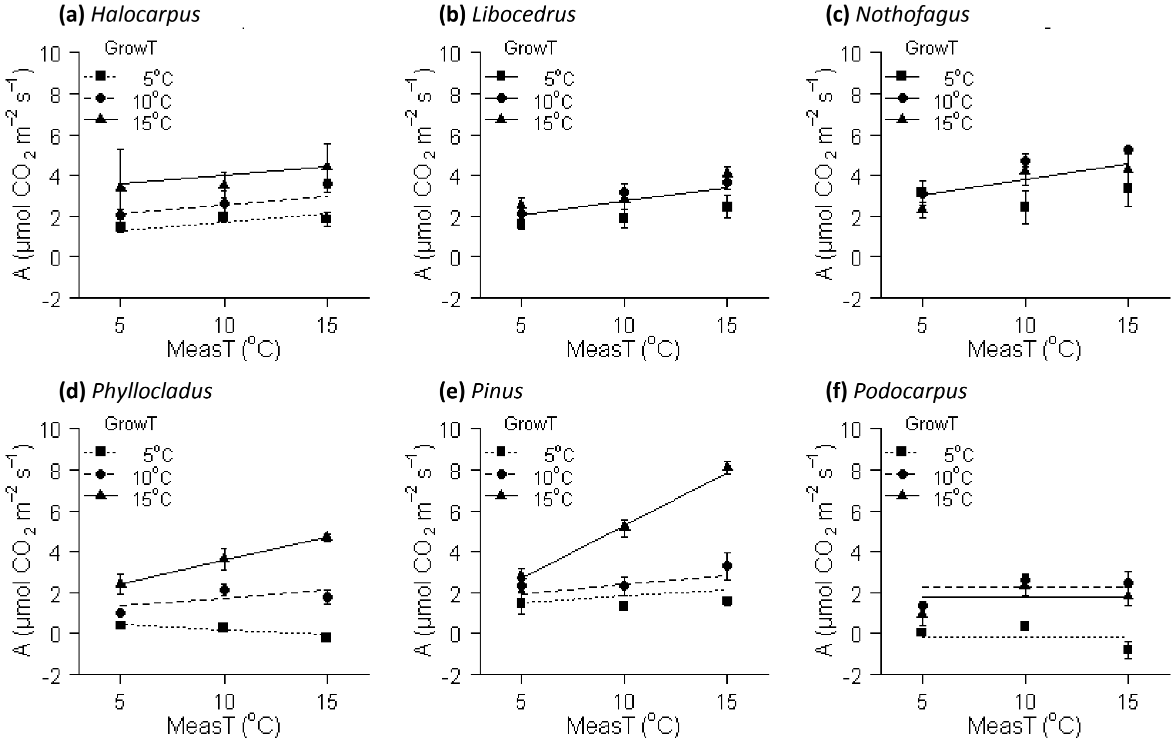
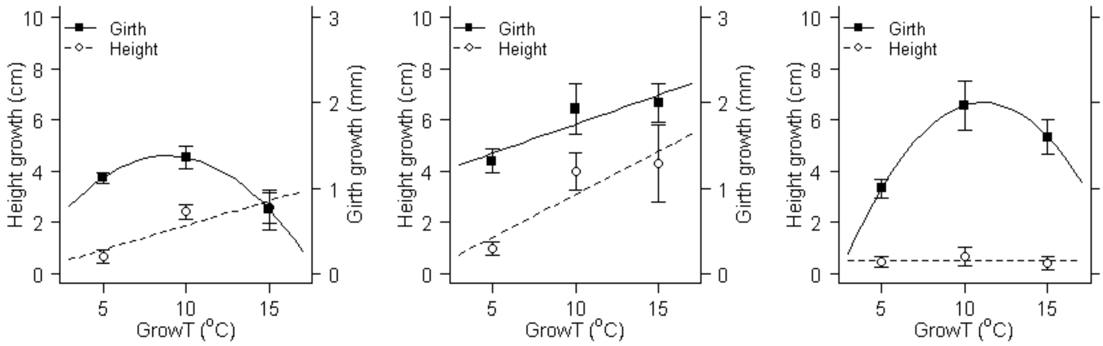


Figure 6.3. Effect of mean growing season temperature (GrowT) and measurement temperature (MeasT) on net photosynthesis (A). Plotted are means (± 1 SE) of 3-5 replicates and lines represent the KL best model (Table A3.2). A GrowT effect results in three lines, corresponding to the response of A to MeasT at each GrowT. One line indicates an effect of MeasT only.


(a) *Halocarpus* **(b)** *Libocedrus* **(c)** *Nothofagus*

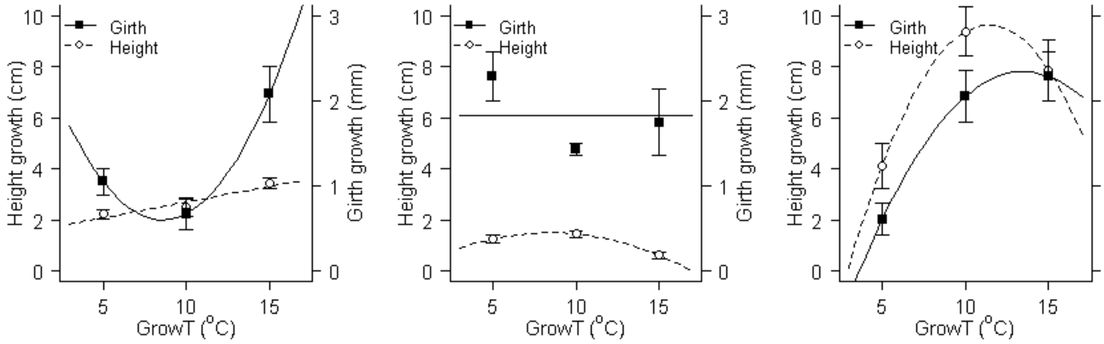
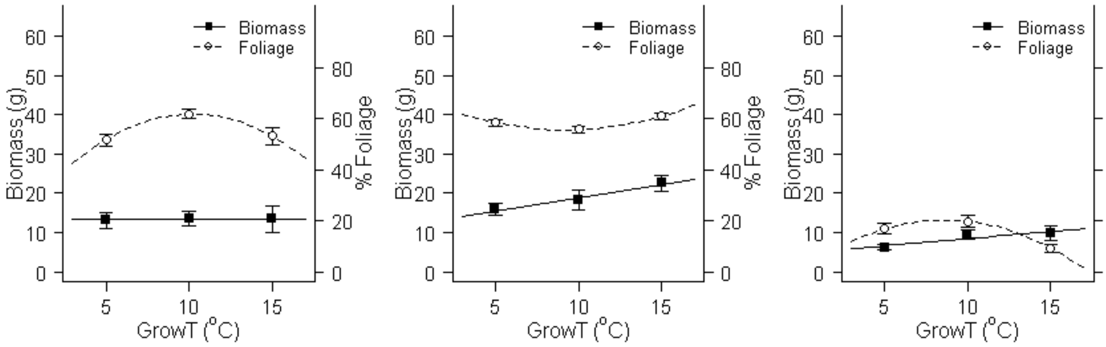

(d) *Phyllocladus* **(e)** *Pinus* **(f)** *Podocarpus*

Figure 6.4. Effect of mean growing season temperature (GrowT) on diameter growth (solid squares and lines) and height growth (open circles and dashed lines) for six species over six months (points are means of 5-8 plants ± 1 SE). Lines represent the most parsimonious KL model (Table A3.3).


(a) *Halocarpus* **(b)** *Libocedrus* **(c)** *Nothofagus*

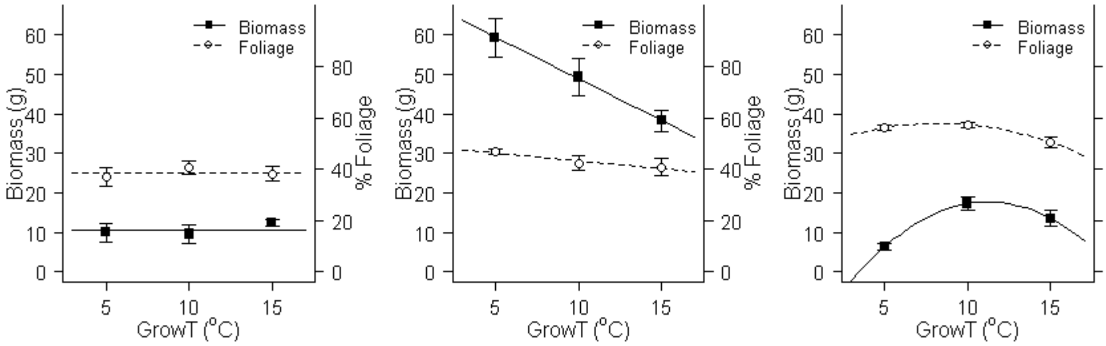

(d) *Phyllocladus* **(e)** *Pinus* **(f)** *Podocarpus*

Figure 6.5. Effect of mean growing season temperature (GrowT) on above-ground biomass (solid squares and lines) and proportion of above-ground biomass allocated to foliage (open circles and for six species over six months (points are means of 5-8 plants ± 1 SE). Lines represent the most parsimonious KL model (linear, quadratic or intercept only, see Table A3.5).

Growth and allocation

Overall, there was considerable variation in height and diameter growth within treatments, and the variation explained by all models (R^2) was < 60% (Table A3.3). The KL most parsimonious models of height growth for *Halocarpus, Libocedrus* and *Phyllocladus* included a linear response to growing temperature (Figure 6.4). The response of both *Pinus* and *Podocarpus* was best described by a quadratic trend, as they were tallest at mid-temperature (10°C). Many *Nothofagus* saplings suffered dieback of their apical shoots, resulting in apparent 'negative growth'. There was no treatment effect associated with this dieback (data not shown). Plants with dieback were further analysed as having zero height growth; this resulted in very low growth rates (Figure 6.4) and no trend in height growth with temperature. For all species, the top-ranked model of height growth well outperformed the other models ($w_i \approx 0{\cdot}8$, Table A3.3).

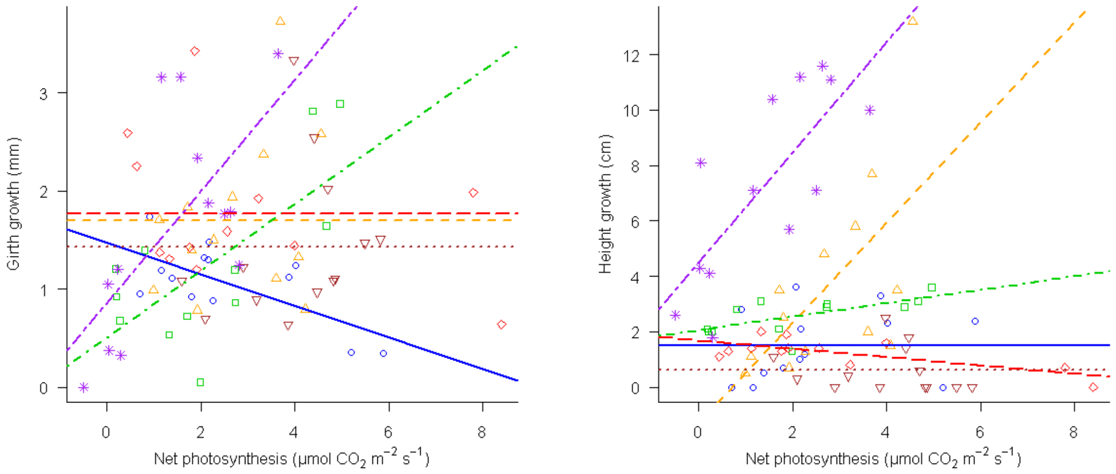
In contrast, for four of the species, trends in diameter growth were inconclusive, with multiple models receiving support (w_i of top model = 0·4-0·5). Only for *Nothofagus* and *Phyllocladus* was there strong support for a quadratic trend (Table A3.3). However, the temperature trends were opposite: the mid-temperature treatment (10°C) resulted in the highest diameter growth for *Nothofagus*, but the smallest diameter growth for *Phyllocladus* (Figure 6.4). Diameter growth of *Pinus* showed no clear trend with temperature, whereas for *Halocarpus* and *Podocarpus* evidence supported an optimum temperature, and *Libocedrus* showed a linear response (Figure 6.4, Table A3.3). Initial (pre-treatment) height and basal diameter did not differ significantly between treatments for any of the species, and the trends described above did not change when height and diameter growth were expressed as relative to the initial height and basal diameter of the plant (data not shown).

The effect of growing temperature on specific leaf area (SLA) was best described by a linear trend for *Libocedrus, Phyllocladus* and *Podocarpus* (w_i = 0·74, 0·54 and 0·62, respectively, Table A3.4; *Podocarpus* SLA decreased with temperature, whereas the other species increased). *Pinus* showed a quadratic trend, with the lowest SLA at mid temperatures (w_i = 0·99). There was no trend in SLA with temperature for *Nothofagus* (w_i = 0·65), and support for the null model and a linear increase for *Halocarpus* was similar (w_i =0·44 vs. 0·40). These SLA trends correspond to changes in new foliage formed under the experimental growing conditions (GrowT) for all species except for *Halocarpus* and *Libocedrus.* For these two species, the combination of slow growth rates and growth occurring both at the apex and through elongation (Plate 6.1), only allowed for measuring old and new foliage simultaneously. Even after more than five months, new foliage of *Podocarpus* at the lowest treatment temperature was thin, almost translucent and bruised easily (Plate 6.1). At the coldest temperature, foliage failed to mature, which resulted in much higher SLA compared to foliage from warmer growing season temperatures.

Above-ground biomass was highest for *Pinus* saplings, but the top-ranked model supported a linear *de*crease with increasing GrowT (w_i = 0·83, Figure 6.5, Table A3.5). In contrast, there was an increase in biomass for *Libocedrus* and *Nothofagus* (w_i = 0·67 and 0·55). There was strong evidence for an optimum temperature for above-ground biomass at 10°C for *Podocarpus* (w_i = 0·98), whereas *Halocarpus* and *Phyllocladus* showed no trend in biomass with GrowT (w_i > 0·70, Figure 6.5, Table A3.5).

The proportion of above-ground biomass allocated to foliage was between 40-60% for all the conifer species, but only accounted for 20% for the broadleaf *Nothofagus* (Figure 6.5). There was no change in this allocation for *Phyllocladus* with temperature (w_i = 0·80), and an indication of a decreasing allocation for *Pinus* (the linear and null models received similar support, w_i = 0·46 and 0·44, respectively, Table A3.5). The data supported a quadratic trend with temperature for *Halocarpus, Nothofagus* and *Podocarpus* (highest allocation to foliage at mid-temperature, w_i = 0·80, 0·86 and 0·56, respectively) and *Libocedrus* (lowest allocation to foliage at mid-temperature, w_i = 0·67).

Relationships between net photosynthesis and various measures of growth also differed between species (Figure 6.6, Table A3.6). *Podocarpus* was the only species with a positive correlation between photosynthetic rates and all three measures of growth (diameter, height and above-ground biomass). *Phyllocladus* showed no relationship between photosynthesis and above-ground biomass, but diameter and height growth increased when photosynthesis increased. *Libocedrus* showed a positive relationship between photosynthesis and height growth only. Photosynthesis and the various growth measures showed no trend for *Nothofagus*; these relationships were also absent, or negative, for *Halocarpus* and *Pinus.*



(a) Diameter growth

(b) Height growth

(c) Biomass

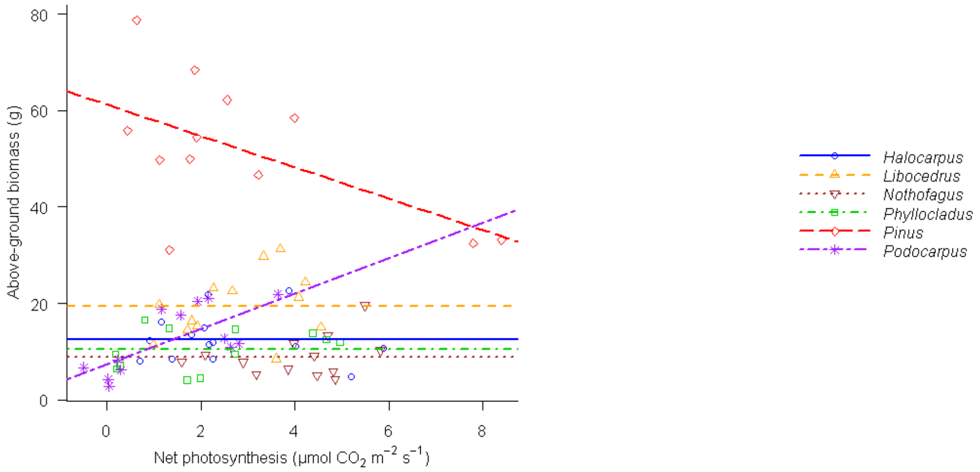


Figure 6.6. Relationships of net photosynthesis with diameter growth (a), height growth (b) and biomass (c) for each species. Lines represent the best KL model for each species (Table A3.6).

NSC content

NSC extractions were not successfully completed, because of unforeseen circumstances related to the series of earthquakes that hit Christchurch in 2010 - 2011. The preliminary data of the free sugar content are presented in Figure 6.7, but starch data are unavailable. These tentative results suggest a decreasing trend in free sugars with temperature for *Nothofagus* and *Pinus*, but not for the other species. However, since both the starch and sugar components of NSC are important in determining the carbohydrate reserves and carbon balance in response to temperature, these data are tentative and will not be discussed in detail.

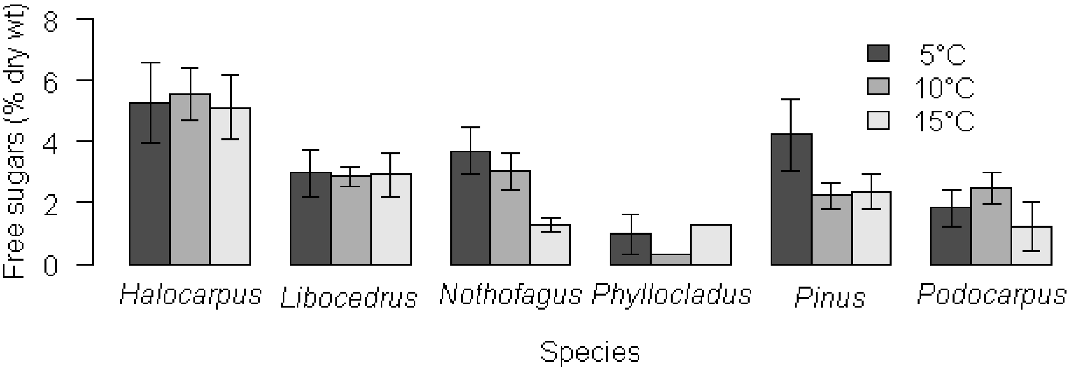


Figure 6.7. Foliar free sugar content (glucose, fructose and sucrose) for the six species grown at the three temperature treatments. Bars are means of 4-6 plants (except for *Phyllocladus*, only one successful extraction at 10 and 15°C), error bars are 1 S.E.

Carbon isotope discrimination $\Delta^{13}C$

Foliage of a subset of two species and the cool and warm treatments were sampled for carbon isotope discrimination. *Libocedrus* showed no difference in whole plant integrated $\Delta^{13}C$ values between the treatments. In contrast, *Pinus* had a significantly lower $\Delta^{13}C$ at warm temperatures (Figure 6.8).

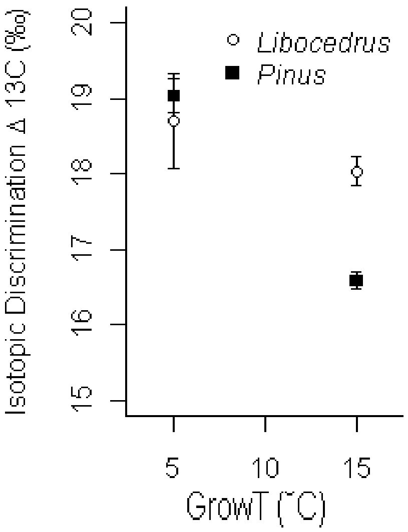


Figure 6.8. Foliage integrated carbon isotope discrimination ($\Delta^{13}C$) of *Libocedrus* (white circles) and *Pinus* (black squares) grown at 5°C and 15°C. Points are means (± 1 SE) of three samples. Discrimination was significantly different between temperature treatments for *Pinus* only.

Table 6.1. Summary of growth and performance responses to temperature for the six species. Values indicate the trend with increasing growing temperature for each of the different response variables best supported by the data. '*+*' indicates an increasing trend, '*-*' a decreasing trend, '*o*' an optimum at mid-temperature, '*m*' a minimum at mid-temperature, and '*x*' no clear evidence for any trend with temperature. Values in parantheses indicate trends that were also strongly supported by the data (evidence ratio of the top and second-best KL model ≤ 2).

		Species					
		Halo carpus	*Libo cedrus*	*Notho fagus*	*Phyllo cladus*	*Pinus*	*Podo carpus*
Photosynthesis	GrowT[1]	+	+	x (+)	+	m	o
	MeasT[2]	+	+	+	+	+	o
	Down-regulation[3]	Y	N	N (Y)	Y	Y	Y
	T sensitivity[4]	N	N	N (Y)	Y	Y	N
Growth	Diameter[5]	o (x)	+	o	m	x (-)	o (+)
	Height[5]	+	+	x	+	o	o
	Biomass[6]	x	+	+	x	-	o
Photosynthesis vs Growth[7]	Diameter	N	N	N	Y	N	Y
	Height	N	Y	N	Y	N	Y
	Biomass	N	N	N	N	N	Y
Sink / Source evidence	Sink[8]	A2	x	A1	A2,3	A2,3	A2
	Source[8]	x	B1	x	B1	x	B1
	Overall[9]	Sink	Source	Sink	?	Sink	?

[1] Trend respective to growing season temperature

[2] Trend respective to measurement temperature (overall trend in MeasT, Table A3.2)

[3] Down-regulation of photosynthesis occurs at lower growing temperatures (*Y*es or *N*o)

[4] Instantaneous temperature sensitivity increases with growing season temperature (interaction between GrowT and MeasT; *Y*es or *N*o)

[5] Height and diameter growth over 6 months growing season

[6] Above-ground biomass at harvest (18 months)

[7] Is there a *positive* relationship between photosynthesis and diameter growth, height growth and biomass (*Y*es or *N*o)?

[8] Do the above trends represent evidence for the sink- or source limitation hypotheses? Codes refer to the type of evidence presented in Section 6.1 and they are repeated below. 'x' indicates no evidence found.

A: sink-limitation hypothesis:
A1: photosynthesis similar across temperature gradient, yet growth reduced at low temperature
A2: down-regulation of photosynthesis at low temperature
A3: temperature sensitivity of photosynthesis is decreased at low temperature
A4: NSC increases with decreasing temperature

B: source-limitation hypothesis:
B1: growth is positively correlated with photosynthesis
B2: NSC is stable or decreases with decreasing temperature

[9] Taken together, do the data support the carbon sink- or source limitation hypothesis; a "?" indicates equivocal evidence.

6.4 Discussion

Growth at low temperature

Exposure to a mean temperature of 5·9°C (Figure 6.1) during a six month growing season reduced growth for most species, although there were large differences between the species and responses of various growth measurements (e.g., height and diameter growth and final above-ground biomass). For all species, growth was still measurable at this lowest mean temperature, thus complete cessation of growth must occur at an even lower temperature. Hoch and Körner (2009) also found that growth of two European treeline species was still measurable at a growing season mean temperature of 6°C.

Gas exchange and growth – Source and sink

There were species-specific temperature responses of growth and photosynthesis, and these provided sometimes contradictory evidence for the carbon source- and sink limitation hypotheses (a summary of the various relationships and trends is provided in Table 6.1).

Net photosynthesis did not differ between growing temperatures for *Nothofagus* (Figure 6.2c), yet it responded to instantaneous temperature increases (Figure 6.3c). This suggests acclimation of photosynthesis to growing season temperature in the long-term. There was no agreement in the temperature response of the different measurements of growth for this species. Many saplings of this species suffered damage to the tops of shoots, resulting in a lack of height growth at all temperatures, whereas diameter growth showed optimum growth at mid-temperature, and biomass an overall increase (Figure 6.4c). In this controlled environment experiment, photosynthetic rates were similar across the temperature gradient, yet growth was reduced at low temperature. In the field, there was an increasing starch content and decreasing sugar:starch ratio in *Nothofagus* foliage with altitude (Figure 2.10, Section 2.4.2), and an indication of decreasing sugar content with temperature under experimental conditions (Figure 6.7). All these lines of evidence suggest that *Nothofagus* is sink-limited at lower temperature, as has been proposed for con-generic *N. pumilio* in Chile (Fajardo et al. 2011, Molina-Montenegro et al. 2011).

In contrast, *Libocedrus* showed evidence consistent with the carbon source limitation hypothesis. There was a small positive response of net-photosynthesis with instantaneous and long-term temperature, but no signs of down-regulation or acclimation. At the treatment level (mean of 3-5 plants), the increasing trend in photosynthesis with temperature was mirrored by consistent increases in height, diameter and biomass. When directly relating photosynthesis with the growth measurements for each plant, variation was higher, although there was still a significant correlation between height growth and photosynthesis ($R^2 = 0{\cdot}33$). In the field, this species showed no trend in radial growth and NSC along the altitudinal gradient (Figure 2.7, Figure 2.10, Section 2.4.2), which is inconsistent with the sink limitation hypothesis, and may be interpreted as evidence for the source limitation hypothesis.

Podocarpus showed the highest values for each of the growth and photosynthesis measurements at mid-temperature, suggesting the optimum growth and photosynthesis temperatures are around 10°C. There was a clear relationship between photosynthesis and all measures of growth ($R^2 = 0{\cdot}40 – 0{\cdot}55$), supportive of the carbon source limitation hypothesis. However, at low temperature, photosynthesis was down-regulated (consistent with the sink limitation hypothesis), which resulted in negligible photosynthetic rates, yet saplings grew both in height and diameter (Figure 6.2, Figure 6.4). This suggests there were alternative (stored) sources of carbon to draw from. NSC data could help interpret these results. However the tentative carbohydrate data also showed the highest free sugar content at mid-temperature, and hence also do not provide clear evidence for either hypothesis. Although NSC in foliage is only considered in this study (Section 2.3.2 and the current Chapter), these are unlikely to give an accurate representation of whole-tree carbohydrate reserves, as starch storage in woody tissue is also substantial (Hoch et al. 2002). Therefore, for a comprehensive study of such whole-tree reserves inclusion of both foliage and woody tissues in NSC assessments is suggested.

All other species showed evidence of a carbon sink-limitation at lower temperatures, including down-regulation of photosynthesis. This down-regulation was not always accompanied by a decrease in temperature sensitivity (Figure 6.3), which was expected (Susiluoto et al. 2007). *Pinus* showed both down-regulation and decreased temperature sensitivity of photosynthesis at lower temperature; both are evidence that growth, rather than photosynthesis, is limited by low temperature. Short-statured individuals of the exotic *Pinus contorta* above the native treeline showed increased foliar NSC compared to individuals at lower altitude (Figure 2.10), and tentative results show similar trends for free sugars under experimental conditions. Other *Pinus* species at treeline in their native range in Mexico, Sweden and Switzerland have also been found with increasing NSC with altitude, supporting the sink-limitation hypothesis (Hoch et al. 2002, Hoch and Körner 2003).

Under experimental conditions, *Halocarpus* showed down-regulation of photosynthesis at low temperature (consistent with the sink limitation hypothesis); however, there was no increase in NSC content with altitude (at lower temperature) in the field (Figure 2.10). In the controlled environment, *Phyllocladus* and *Podocarpus* showed significant correlations of photosynthesis with growth (R^2 = 0·36 - 0·55), consistent with the source limitation hypothesis, but also down-regulation of photosynthesis with decreasing temperature, consistent with a sink limitation (Table 6.1).

Phyllocladus saplings showed increased height and diameter growth with increasing temperature, but this was not mirrored by a trend in final biomass. This counter-intuitive result could be caused by new tissues formed at higher temperature being less dense than at lower temperature. However, since there was no trend in new foliage SLA with temperature (data not shown), this seems unlikely. Instead, variation in the initial biomass of the saplings may have masked any temperature effects, especially if this differed between treatments. Since the initial biomass of the saplings (or average per treatment) is unknown, this cannot be ruled out definitively, although initial height and diameter did not differ significantly between treatments for any of the species (data not shown).

Pinus showed higher growth and biomass at *low* temperature, although photosynthetic rates continued to increase with temperature. This suggests a low optimum growth temperature of ≤ 5°C contrasted with a photosynthetic optimum ≥ 15°C (negative relationship between growth and photosynthesis, R^2 = 0·48). Near the New Zealand treeline (890-1330 m a.s.l.), this species had a late-summer photosynthetic optimum of 13-17°C (Benecke and Havranek 1980a), and tree growth declined with altitude (Benecke and Havranek 1980b). Given both its native and introduced ranges, this species is obviously capable of growing at low temperature (Thompson et al. 1999, Chuine et al. 2001, Chuine et al. 2006); however, it is likely that the extremely low growth optimum found here is an artefact of the experiment. Drought stress at higher temperature seems unlikely, given the watering-regime, the strong positive effect of growing temperature on photosynthesis, and the carbon isotope data (see below). The effect of pot-bound roots at higher temperature can be ruled out since this would have resulted in decreasing sink activity and subsequent down-regulation of photosynthesis at high temperature, which was not found.

Alternatively, high overnight temperatures (12·5°C in the warm temperature treatment) may have resulted in high respiratory carbon loss. *Pinus* has the highest potential growth-rate and photosynthetic rates of the species studied; both processes scale positively with dark-respiration (*Rd*) (Reich et al. 1998), and may therefore more negatively affect *Pinus* than the native species. This would suggest that respiration of this species does not fully acclimate to temperature. In contrast, full acclimation of respiration has been found for con-generic species, often resulting in a shift of the balance of photosynthesis and respiration in favour of carbon uptake (Teskey and Will 1999, Ow et al. 2008, 2010). The ratio of net photosynthesis to dark respiration (*A*:*Rd*) of *P. radiata* is *ca*. 30 (Ow et al. 2008), compared to *ca.* 7 at 15°C for *P. contorta* in this study. These preliminary measurements of *Rd* were taken within 10 minutes of placing foliage in the dark, and may be higher than respiration of fully dark-acclimated leaves (effect of light-enhanced dark respiration, Atkin et al. 1998). Although *A*:*Rd* did not change with temperature for *P. radiata* (Ow et al. 2008), stand-level *A*:*Rd* of *P. contorta* was higher at a treeline site than at a lower altitude site in New Zealand (Benecke and Nordmeyer 1982, Ryan et al. 1994), indicating that perhaps acclimation is not complete for this species. In the current study, the native conifers and native broadleaf *Nothofagus* had *A*:*Rd* values of 3.8-5.6 and 10, respectively. These are comparable to the results of Turnbull et al. (2005), who found that the *A*:*Rd* ratio of other native New Zealand species ranged between 15-18 for early successional broadleaf species and was around 6 for a late successional broadleaf (*Metrosideros umbellata*, which also occurs in the treeline ecotone) and a native conifer *Dacrydium cupressinum* (Turnbull et al. 2005, Whitehead et al. 2005).

Carbon isotope discrimination

Discrimination against $^{13}CO_2$ ($\Delta^{13}C$) in C_3 plants is positively related to the ratio of intercellular to ambient CO_2 concentration (C_i/C_a) and thus relates to the balance between supply and demand for CO_2. $\Delta^{13}C$ varies with stomatal conductance, g_s, and photosynthetic rate, *A* (Brugnoli and Farquhar 2000); an increase in g_s will increase $\Delta^{13}C$ (if *A* is constant), whilst an increase in *A* will decrease $\Delta^{13}C$ (if g_s is constant). Since temperature affects both g_s and *A*, measurements of $\Delta^{13}C$ may help determine the balance between these two responses.

The preliminary $\Delta^{13}C$ data presented in this study suggest that C_i/C_a is lower for *Pinus* grown at 15°C compared to 5°C. This could be due to increased *A* or decreased g_s or both; g_s actually increased with temperature (data not shown, but see also Chapter 5), but apparently this was compensated for by a stronger temperature response of *A* (Figure 6.2f). The reduced $\Delta^{13}C$ at higher temperature suggests an increase in the foliage-intrinsic water-use efficiency. However, this did not lead to faster growth rates.

Libocedrus showed no significant change in $\Delta^{13}C$ and thus C_i/C_a between growing temperatures, which could mean that *A* and g_s did not change with temperature, or that they did so proportionally. Measurements of *A* (increases with temperature, Figure 6.2b) and g_s (decreases, data not shown) suggest that these two properties change proportionally with temperature.

Contrasting evidence for two hypotheses

In the current study, temperature trends in growth and photosynthesis did not always provide unequivocal evidence for source- or sink limitation at low temperatures. Although the majority of the literature has been polarised over the sink- and source hypotheses, more recently it has been suggested that rather than being driven by *either* sink- or source limitation, source-sink relations of trees at high altitudes vary along a continuum in space, and probably time (Bansal and Germino 2008, Bansal and Germino 2009, Sveinbjörnsson et al. 2010). This seems a reasonable proposition, since source- and sink activity are closely coordinated (see Ainsworth and Bush 2011 for a review; note there is some evidence for decoupling at the alpine treeline, at least for some seedlings, Bansal and Germino 2010).

Previous studies and the current experiment show that separating the different components of sink and source may be important in solving this puzzle of treeline formation hypotheses. Focussing on one aspect, for example, direct (e.g., photosynthesis) or indirect (e.g., NSC) does not suffice, because various inter-linked processes may obscure expected trends. For example, drought effects obscured trends in NSC at a Chilean treeline (Fajardo et al. 2011), and both nutrient limitation and cold temperature led to increased NSC in saplings near treeline in Japan (Kabeya 2010).

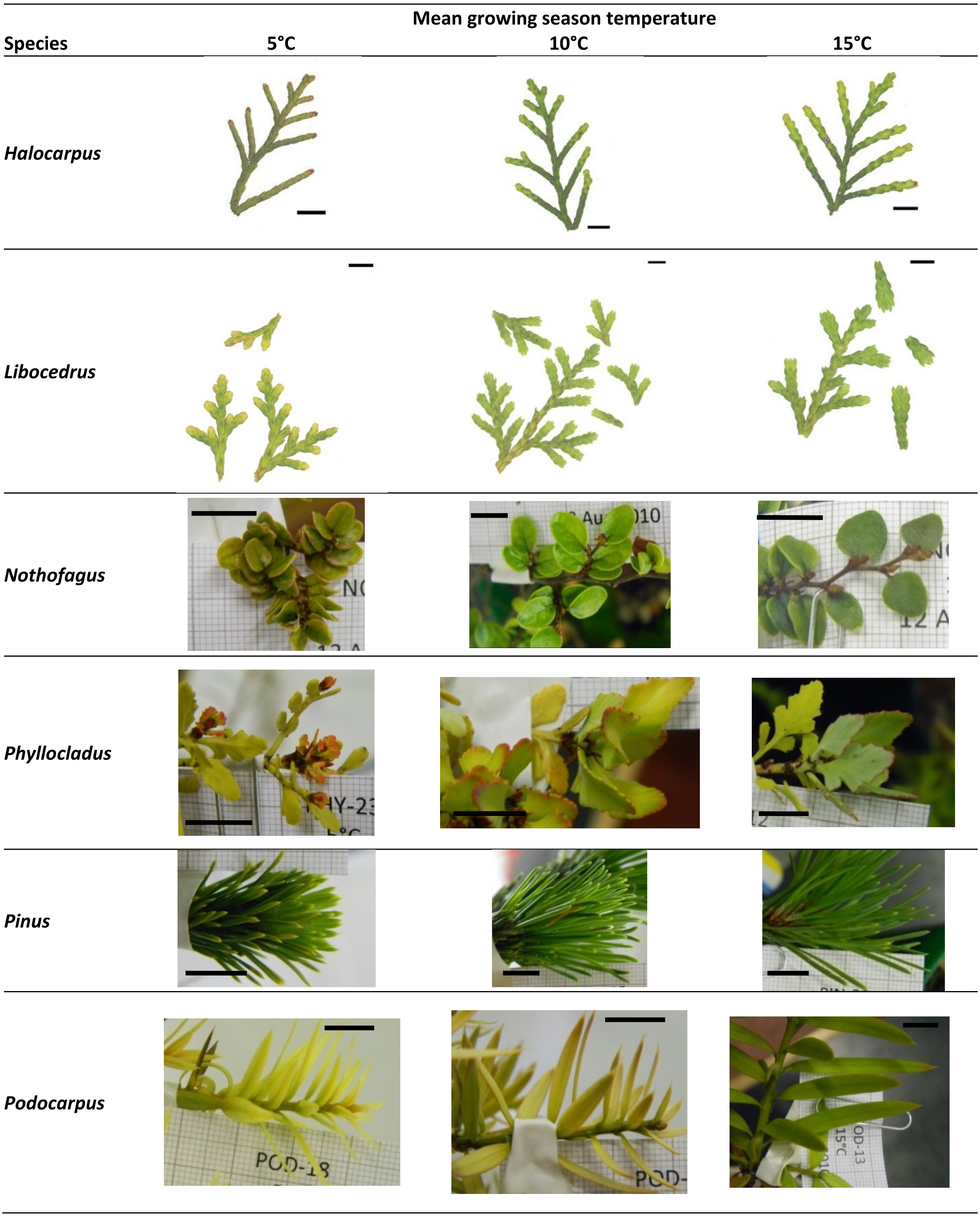
The current study showed that various measures of sink activity (e.g., diameter or height growth) may show different trends. Such observations are critical, as it is important to consider that increased growth may not be the best proxy of fitness or persistence of trees at treeline (Susiluoto et al. 2010). In addition, trees may adjust their biomass allocation patterns to maintain their carbohydrate reserves at fairly constant levels, even when under stress (Susiluoto et al. 2010). Similarly, measures of dark-respiration may also be important (Bansal and Germino 2008), as may be the case in explaining the trends found for *Pinus* in this study.

Carbon source limitation can be imposed by drought stress, impoverished soil nutrient status (Chapin et al. 1990, Millard et al. 2007), or a direct effect of low temperature on gas exchange (Sveinbjörnsson et al. 2010). To acknowledge these other indirect effects, this hypothesis has also been called the *re*source limitation hypothesis. In this study, the plants were well-watered and regularly provided with nutrients, so the observed changes in photosynthetic rates are likely to relate to temperature directly. However, growth may still be limited at lower temperature due to nutrient limitation, if nutrient uptake is impaired by lower temperature. An investigation of the nutrient status of saplings under such experimental conditions would allow the separation of the source- and *re*source limitation hypotheses.

6.5 Conclusions

Overall the results of this controlled environment study suggest the response of photosynthesis and growth to growing season temperature is species-specific. One of the native treeline conifers (*Libocedrus*) showed evidence consistent with carbon source limitation at lower temperatures. *Halocarpus, Nothofagus* and the exotic *Pinus* showed evidence consistent with sink-limited growth. However, contradictory evidence for two other native conifer species did not allow for a determination of the most likely limiting process. This and other studies show that multiple measures of carbon balance, growth and photosynthesis may not necessarily agree, and that it is important to gather evidence from several processes related to the limitations, rather than drawing conclusions from one set of data. In addition, rather than being driven by either source or sink limiting processes, trees at growing at higher altitude and/or lower temperatures may be operating in a more ‘plastic’ fashion, along a continuum between these hypotheses previously regarded as being clearly distinct.

Plate 6.1. Images of foliage with new growth of the six species after five months at three different growing temperatures. Colour and morphology of foliage of *Halocarpus* and *Libocedrus* can help discern new and old growth. New growth of *Halocarpus* occurs at the tip as well as through stem elongation (extending space between scales). New growth of *Nothofagus* and *Phyllocladus* show fluorescing at low temperature. Foliage of *Podocarpus* failed to mature at the coldest temperature (new growth was very thin and yellow, almost see-through, and very easily bruised). Approximate size of scale bar for *Halocarpus* and *Libocedrus* is 0.5 cm; and for *Nothofagus, Phyllocladus,Pinus* and *Podocarpus* 1 cm.



Chapter 7 General discussion

The aim of this research was to investigate the effects of low temperature on tree performance near the New Zealand treeline, in an effort to improve the understanding of the drivers of treeline position in New Zealand and elsewhere. The objectives were (1) to determine the temperature environment at the New Zealand treeline; and (2) to evaluate how low temperature affects the performance of trees across the treeline ecotone, focussing on (a) the effects of frost and (b) the effect of low temperature on source- and sink-activities. This study combined a variety of techniques and approaches to fulfil these objectives, ranging from deploying temperature data loggers in the field and measuring leaf-level gas exchange in controlled environment experiments, to assessing the growth and stature of trees across the treeline ecotone.

Although limitations of this study have been noted throughout the relevant chapters, I will repeat three important points here: firstly, this study presents the most comprehensive dataset to-date of air, ground and soil temperatures from treeline ecotones New Zealand (Chapter 2 and Chapter 3). However, such temperature data collected over 2 to 3 years inevitably do not constitute a full representation of the conditions encountered over longer periods in a temperate oceanic region. In addition, key plant metabolic processes respond to meristem temperatures rather than air or ground temperature (see Section 7.2.3, below).

Secondly, this study assessed the effects of low temperature on the performance of mature individuals (Chapter 4) and saplings (Chapter 5 and Chapter 6) of treeline species. However, key limiting factors for treeline may act differentially upon different life-stages. Additionally, many other factors may also be important in determining tree performance and treeline position, including climatic, edaphic and orographic factors, nutrients, and biotic interactions (see Figure 1.2).

Lastly, one underlying assumption of this study is that growth is a good indicator of the fitness or persistence of trees near treeline. However, as Susiluoto et al. (2010) point out, this may not be the case at the transition of the tree life-form near its distributional limits. Although a suitable alternative has not yet been proposed in the literature, regeneration, assessed relative to the lifespan of the trees present, will be a likely candidate.

In the remainder of this final chapter, I discuss some of the key findings of this study and suggest avenues for future research.

7.1 New Zealand treeline climate

Körner and Paulsen (2004), in their study of global treeline temperatures, included four Southern Hemisphere *Nothofagus* sites (two in New Zealand and two in Chile). All four treelines were anomalously warm (mean 9·2°C) compared to the global mean (6·7°C ± 0·8 S.D.), and it was suggested that they represented a genus-specific boundary. Körner and Paulsen (2004) recognised that data from additional locations were needed to verify this finding. In this study (Chapter 3), I used detailed temperature data collected at six treeline sites for two years or more to show that New Zealand treelines, as an example of southern temperate treelines, are *not* anomalously warm by the global soil temperature criterion (cf. Körner and Paulsen 2004). This conclusion of 'normal' temperatures at Southern Hemisphere treelines is supported by recent data from a *Nothofagus* treeline in Patagonia (Chile), which has a mean growing season soil temperature of 6·9°C, also very close to the global mean (Hoch unpublished, in Fajardo et al. 2011).

Continued data collection across multiple sites will increase the understanding of inter-annual and inter-site variation; these factors are likely to explain the discrepancy between temperature data presented for the Southern Hemisphere treelines by Körner and Paulsen (2004) and this current and other recent studies. Nonetheless, it is clear that exotic species grow and establish above the *Nothofagus* treeline in both New Zealand and Chile (Wardle 1998), and thus that these treelines do not represent the ultimate limit to tree growth, and hence represent a taxon-limitation. However, the present study suggests that the Southern treelines are not as climatically depressed as previously thought (Wardle 1998, Körner and Paulsen 2004).

This is the first study to assess the thermal regime experienced at New Zealand treelines of a different form than the relatively well-studied abrupt treelines; i.e., the gradual ecotone formed by the diverse species of mixed conifer-broadleaved forests. The gradual nature of these ecotones and their normal treeline warmth suggest that these treelines represent the ultimate limit to tree growth. Overall, the proposition that taxon-limitations cause a low treeline altitude in New Zealand (Körner and Paulsen 2004) appears incorrect; whilst, to a limited degree, it may apply to the *Nothofagus* treeline, it is not true for all treelines in New Zealand. However, instead of a taxon-limitation preventing treelines from forming at growing temperature close to the global norm in any region, it may be true that some trees - and very few - have capabilities of exceeding the typical treeline altitude, but not in all places. Although it has yet to be tested, *Pinus contorta* probably would not be able to exceed the current gradual mixed treelines on the New Zealand west coast mountains, because the combination of open ground, high light intensity, dry and severe frost environments in which it is competitive, is lacking.

The present study highlighted that a careful analysis of the effect of the growing season definition at a global scale is required, particularly regarding the effect of differences in abruptness of season transitions, as found when comparing continental with oceanic environments (Grace 1997, Crawford 2000). Gehrig-Fasel et al. (2008) suggested that comparing growing seasons of different lengths may create artificially different mean temperatures. Indeed, I found that seasons of differing lengths between abrupt and more oceanic gradual treelines resulted in different mean growing season air and soil temperatures. This was an artificial difference, caused by applying a threshold definition of growing season in a variable oceanic climate, because assessing periods of similar lengths of time did not result in differences in mean temperatures between treelines of abrupt and gradual forms.

7.2 New Zealand treeline drivers

7.2.1 Frost damage

New Zealand treeline species have low frost tolerance compared to their Northern Hemisphere counterparts (Bannister 2007), and Wardle (1985c) suggested this is an important driver of the treeline altitude. Chapter 4 presented a study assessing the seasonal frost tolerance of high-altitude foliage and the extreme minimum temperatures experienced at the treeline. Whilst frosts were much less severe than at European treelines (Sturman and Wanner 2001), trees of all treeline species studied (including the exotic *Pinus*) were susceptible to minor damage caused by frosts outside the winter season, especially in spring. Nonetheless, frost tolerance was generally well in excess of the minimum temperatures experienced at treeline, suggesting that frosts alone are unlikely to cause sufficient damage to control treeline position through dieback of adult trees.

However, if seedlings are more sensitive to frost, this mechanism could clearly be important for treeline formation and position. The window of at least 4°C between extreme minimum temperatures in the last 20 years and the temperature that causes 50% mortality of mature foliage, however, suggests that, even if seedlings were significantly more frost-sensitive, they would suffer few seriously damaging effects. This window exceeds the previously published difference between the frost tolerance of mature trees and seedlings of *Nothofagus* species of less than 2°C (Greer et al. 1989, Neuner et al. 1997).

Although frost alone is unlikely to kill seedlings or trees at treeline, several factors may exacerbate the effects of frost. Foliage may be damaged by wind-blasting, especially if augmented by ice (such as hail in wind storms or re-distributed rime ice, Grace 1997). Elsewhere, seedlings at or above treeline are vulnerable to cold-induced photo-inhibition caused by low temperature in combination with high solar radiation (Ball et al. 1991, Germino and Smith 1999, Piper et al. 2006, Bader et al. 2007, Danby and Hik 2007, Bansal and Germino 2010b).

Temperature measurements in this study (Chapter 2) reiterate previous observations of a strong increase in summer temperature in grasslands just above abrupt treelines, compared to under the *Nothofagus* canopy only a few metres away (Wardle 1985b, Körner et al. 1986, Mark et al. 2008). The data highlight how stature and shade of a forest canopy can create its own microclimate, potentially affecting the trees' own performance (Körner 2003a). Although the summer temperatures are much warmer only just beyond the shade of the canopy, few *Nothofagus* seedlings are found there. Experimental plantings suggest that seedlings are unable to exploit this summer warmth, except if shelter is provided (Wardle 1985a, Harsch 2010). It has been suggested that shelter (whether artificial or by neighbouring vegetation) positively affects *Nothofagus* seedling establishment by reducing damage from cold-induced photo-inhibition and/or frosts (Ball 1994, Wardle 2008). Whilst there is considerable support for this notion, such effects have not been quantified and it remains to be determined which of these two processes is most important in limiting the survival and establishment of seedlings beyond the *Nothofagus* canopy.

The lack of seedlings near the gradual treeline suggests that limitation at the recruitment stage may be important here too. In lowland and montane forests, the regeneration of many native conifer species is disturbance-limited (Wardle 1978, Veblen and Stewart 1982, Stewart and Rose 1988), but studies have not specifically assessed regeneration at their altitudinal distribution limit.

Specific leaf area (SLA) of seedlings of *Libocedrus* and *Halocarpus* decreased with altitude, whereas that of mature foliage did not show a strong trend with altitude (Chapter 2). Such changes in SLA with altitude may enhance the seedlings' tolerance to light and freezing stress (Bansal and Germino 2010a), and suggest that protection may be more important at higher altitude. Fewer frosts, and warmer nights with increased cloud cover at the gradual treelines compared to abrupt treelines would suggest decreased effects of frosts and photo-inhibition. Research focussing on such potential factors limiting seedling survival and establishment across the gradual treeline ecotone will be important in gaining a full understanding of the drivers of this treeline.

7.2.2 Carbon source *vs*. sink limitation

The native treeline conifer species showed reduced photosynthetic capacity, and therefore growth potential when compared to the native broadleaf *Nothofagus* and exotic *Pinus contorta* (Chapter 5). Although dominant in the gradual ecotone, these native conifer species can also be present in the abrupt treeline ecotone, but they are uncommon and fail to reach tree height. The combination of reduced photosynthetic and growth potential and a lack of responsiveness to higher temperatures (at least partially due to high mesophyll limitation, Chapter 5), could contribute to their limiting performance in such a seasonal climate. Additionally, xylem constraints may limit their distribution because of the vulnerability of Podocarpaceae to cavitation under the much drier conditions in the rain-shadow regions compared to wetter western regions (Brodribb and Hill 1998, 1999) of New Zealand.

Studies at several alpine treelines suggest that low temperatures decrease the ability of trees to invest in growth (carbon sink limitation hypothesis), more so than the ability to acquire photosynthates (carbon source limitation hypothesis) (Hoch et al. 2002, Piper et al. 2006, Shi et al. 2006, Fajardo et al. 2011). This carbon balance debate has mostly been assessed on the basis of non-structural carbohydrate (NSC) contents. However, this approach is neither direct evidence for either hypothesis, nor is it without complications (e.g., Smith et al. 2003, Johnson et al. 2004). For example, altitudinal differences in phenology may cause NSC trends unrelated to either hypothesis (Hoch and Körner

2009), increased frost tolerance may result in higher NSC (Öquist et al. 2001), and feedback mechanisms between source and sink activities may obscure trends in NSC (Ainsworth and Bush 2011). Growth and carbon acquisition processes are generally considered to be tightly coupled (Ainsworth and Bush 2011), but some independence of growth, respiration and NSC content of root and stem tissue has been found in treeline seedlings (Bansal and Germino 2010a).

The present study included the first assessment of the carbon sink- and source-limitation hypotheses for New Zealand treeline species. Rather than relying solely on NSC measurements to assess these hypotheses, I also used direct measures of growth and photosynthetic activity, combining evidence from the field (Chapter 2) and controlled environment experiments (Chapter 5, Chapter 6). Evidence from both field and controlled environment measurements suggest that *Nothofagus cliffortioides* var. *solandri* and *Pinus contorta* are sink-limited when grown at low temperatures, as has been found for other closely related species (e.g., Hoch and Körner 2003, Fajardo et al. 2011, Molina-Montenegro et al. 2011). In contrast, the lack of a trend in radial growth and NSC of *Libocedrus* across the treeline ecotone did not support the sink limitation hypothesis. Moreover, in the controlled environment experiment, the positive correlation of growth and photosynthesis with increasing temperature is consistent with source-limited growth. For the other native conifers (*Phyllocladus* and *Podocarpus*), multiple measures of sink- and source activities resulted in support for both hypothesis.

These results suggest that, although low temperature limits growth, rather than carbon-acquisition, of some (perhaps most) species, this is not the case for all (see also Bansal and Germino 2010a, Dawes et al. 2011). Further research is needed to confirm whether the performance of the native conifer species may be limited by source- or sink-activity. Firstly, assessing NSC of different plant organs (roots, branch- and stem-wood) may prove valuable, since these may be more indicative of whole-plant carbohydrate reserves than foliar NSC content (Hoch et al. 2002). Secondly, the complexity of source- and sink-activities should be acknowledged and deserves attention in a multi-faceted approach in the field. In recent examples of such an approach at Chilean *Nothofagus* treelines, it was shown that increased frost tolerance results in reduced height growth (Molina-Montenegro et al. 2011), and that local environmental conditions, such as drought, may mask trends associated with sink-limited growth (Fajardo et al. 2011).

7.2.3 Which aspects of temperature matter to treeline trees?

Woody plant growth involves three fundamental physiological processes: transport of solutes, carbon acquisition (photosynthesis) and xylogenesis (wood production, including cell division and differentiation). Growth and photosynthetic processes are both limited by temperature, and, rather than a strictly linear relationship, threshold temperatures are involved (Paulsen et al. 2000, Körner and Paulsen 2004, Körner 2006). However, trees are unlikely to respond to one simple temperature threshold, which has been the implicit assumption in the search for a single predictor of global treeline position. Either soil or air temperatures can provide fundamental thresholds, affecting below- or above-ground meristems and nutrient and mineral uptake. Ultimately, all thresholds have to be crossed for growth to occur. This was clearly demonstrated in a study of trees growing in and near permafrost soils; although all trees were subjected to the same air temperature, growth of trees in the permafrost was strongly limited because their roots experienced low temperatures (Körner and Hoch 2006, Hoch 2008).

The standard metric for growing season has been mean temperature, although it is not well understood how mean temperature can affect underlying mechanistic processes (Hoch and Körner 2009). Key photosynthetic and growth thresholds occur at low and not high temperatures. The failure of thermal sums (GDD) to correlate with treeline position suggests that additional heat above a given temperature threshold (e.g., 0°C or 5°C) has little influence on a season's growth. For these reasons, I explored the possibility that minimum temperatures would relate to treeline position better than mean temperatures (Chapter 3). Indeed, daily minimum temperature values were more consistent across multiple treeline sites than daily mean or maximum values. Overnight low temperatures (T_{min}) are well-established negatively to affect photosynthesis and growth for periods longer than these temperatures are themselves experienced, through what I have called *chilling legacy effects* (Chapter 3). Given the greater prevalence of low temperatures at higher altitude, such effects are more likely to be more critical for trees growing at their upper distributional limit than for lower altitude trees. However, the extent and duration of such legacy effects requires study. In addition, there may be a trade-off between overnight minimum and daily maximum temperatures; higher temperatures during the day could possibly offset the negative effects of a low overnight temperature.

A limitation in many studies involving temperature measurements, including this one, is that air and soil (10 cm) temperatures do not represent the full range of temperatures experienced by plant tissues. To provide comparable measurements, temperature loggers are generally sheltered from direct radiation or buried at a standard depth in the soil, but the plant meristems, where growth activity occurs, experience a different thermal environment. Local weather conditions (wind, cloud), as well as the degree to which topography and the nature of the soil surface (e.g., rocks versus vegetation) modify these conditions, can significantly affect meristem temperatures (Wilson et al. 1987, Grace 2006).

Diurnal nuances (for example, short periods of high temperature or brief pre-dawn frosts) may strongly affect meristem temperatures and plant growth. Strong buffering effects of the soils prevent such nuances from being captured by, or estimated from, soil temperature measurements. Therefore, I suggest that air, or better still, meristem temperatures, will play a key part in understanding treeline temperature thresholds.

In this thesis, I have focussed on temperature as the principle variable affecting growth and carbon acquisition processes, which assumes that other main variables including light, water and nutrients are non-limiting. Saturated light-levels for photosynthesis are often reached at treeline, at least at the abrupt treeline (Benecke and Havranek 1980a). Given the lower light-saturation of the native conifer species, the light environment at western gradual treeline is unlikely limiting on sunny days, however, the high cloudiness may result in light not reaching saturation point for photosynthesis on many days. In contrast to many treelines globally, trees at the oceanic New Zealand treeline are not water-limited (Wardle 2008).

Soil nutrient status is unlikely to be a major limitation to growth, at least at *Nothofagus* treelines (Nordmeyer 1980b, Clinton et al. 2002, Davis et al. 2004). However, soil temperature may crucially affect the uptake by the roots of minerals essential for growth. For example, calcium, a mineral important for many metabolic processes and crucial for wood-formation (McLaughlin and Wimmer 1999, Gilliham et al. 2011), has been implicated as important in the productivity of montane *Nothofagus* forests (Clinton et al. 2002). Calcium uptake from the soil is dependent on the passive mass flow of water within the tree, which is driven by transpiration (McLaughlin and Wimmer 1999, Gilliham et al. 2011). Such processes could provide a rate-limited (rather than temperature limited) step, which could go some way to explaining the lack of importance of measures of daily warmth (such as GDD).

Up-scaling the leaf-level measurements of photosynthetic capacity and carbon uptake to stand-level would be a useful next step, as ultimately, whole-tree and stand-level processes will affect the position of treeline. This up-scaling can be achieved with an integrated process-based model of carbon uptake and allocation (e.g., Landsberg and Sands 2011), using data collected in this study. Such an approach can help identify and quantify the limiting factors to growth (e.g., Whitehead et al. 2002) at treeline in New Zealand.

7.3 Treelines and climatic change

The well-established relationship between treeline position and temperature has resulted in the expectation of an upward shift in response to climatic warming (Tranquillini 1979, Körner 1998, Grace et al. 2002, Holtmeier and Broll 2005, Smith et al. 2009). However, in a meta-analysis of treeline advance over recent decades, Harsch et al. (2009) found that not all treelines have responded to increases in mean temperature over this time. If instead minimum temperature plays a key role in limiting tree performance near treeline (Chapter 3), this is likely to have different global change implications (e.g., with minimum temperatures generally warming faster than mean temperatures) – and these effects would need to be assessed.

In addition, Harsch et al. (2009) found that gradual treelines were more likely to advance in response to warming than abrupt treelines. This was suggested to be caused by different underlying mechanisms that determine the position of these treelines; temperature may strongly limit growth at gradual treelines and hence they respond more to warming, whereas abrupt treelines are subjected to additional constraints that may not have been alleviated by warming (Harsch et al. 2009, Harsch and Bader 2011). In the current study, I did not find stronger evidence of growth limitation at gradual treelines than at abrupt treelines; however, it should be noted that such comparisons are problematical since different species form the different types of treelines. Evidence for low temperature-limited growth was found for *Pinus* forming an exotic gradual treeline above the native abrupt treeline, for *Halocarpus* forming a gradual treeline in the western regions, and for *Nothofagus solandri* which forms an abrupt treeline. *Nothofagus menziesii* (not included in this study) forms both abrupt and gradual treelines, and would therefore be a suitable model-species to test this hypothesis.

Studying the underlying processes and conditions that lead to both forms of treelines would increase the understanding of the mechanisms that lead to these differing treeline types, without confounding species-specific responses. In turn, this would provide indications of the potential response of both treeline types to climatic warming.

Abrupt *Nothofagus* treelines have advanced approximately 1.6 m over recent decades (Wardle and Coleman 1992, Wardle et al. 2005, Harsch 2010); to date, there has not yet been a similar examination of gradual treelines in New Zealand. Any advances in treeline in New Zealand may be slower than elsewhere because of the limited climatic warming that has occurred here (McGlone et al. 2010b), compared to more continental settings. Additionally, slow growth rates and/or regeneration limitations, especially of the native conifers, may result in vegetation responses that are slower and smaller than the magnitude of the climatic changes.

7.4 Final words

Many studies have assessed aspects of treelines around the world, with the most notable and invaluable insights gained into drivers of global and New Zealand treelines by Christian Körner and the late Peter Wardle, respectively. However, a full understanding of many of the underlying processes is still lacking. It is becoming increasingly clear that there is no simple explanation for the formation of treeline, and that it likely involves multiple thresholds and processes, in addition to species-specific limitations. The effects of minimum temperatures (chilling and freezing of soil, air and meristems) and solar radiation on carbon acquisition and growth, and how these factors may be mediated by neighbouring vegetation require further detailed study. Rather than assessing potential driving factors singly, the interaction of such factors needs to be considered to untangle the complexity of factors that determine treeline positions, at local and global scales, and to understand the potential response of treelines to climatic change,

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Appendix 1. Temperature data

(Supplement to Chapter 2)

Temperature data loggers and radiation shields

The *Tinytag Plus2* temperature data loggers (Gemini Ltd, UK) used for air and ground surface temperature measurements were screened from direct radiation using perforated white plastic shields (Figure A1.1). The shields were constructed from white PVC downpipe (8 cm diameter, 8 cm high), which was perforated with dozens of 0.5 cm diameter holes. A 2 mm thick gloss white PVC sheet was glued to the top of the cylinder. The data logger was suspended inside the shield, so that the logger's temperature sensor (internally mounted behind the green dot visible in the picture of the data logger below) was just within the shield (1 cm higher than the bottom rim). For the ground temperature measurements, the shield was placed on the ground surface (so the sensor was 1 cm above the surface); for air temperature measurements, the shield was mounted on a post such that the temperature sensor was at 1.3 m above the ground surface. Data was downloaded from each logger using a laptop at least yearly (the screw cap shown in the picture below protects the download connection).

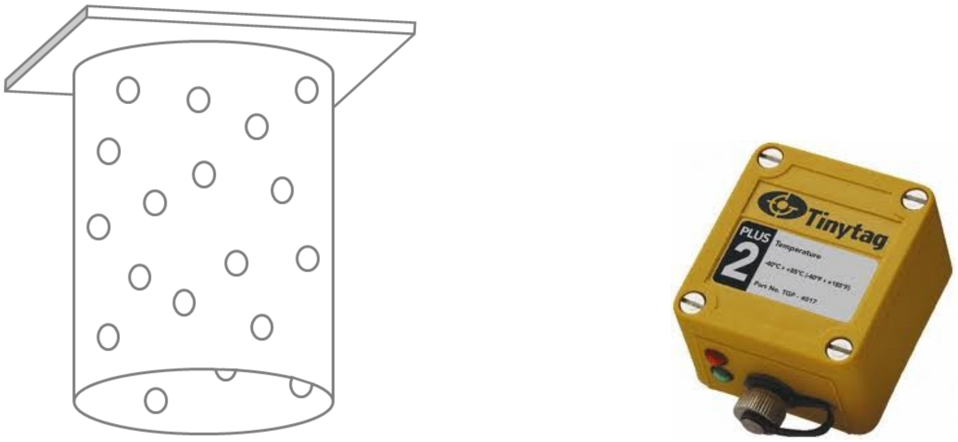
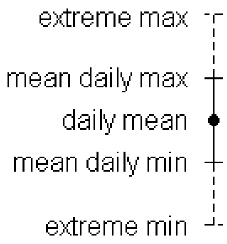


Figure A1.1. Sketch of the radiation shield (left) used to shade the temperature data loggers (right).

Temperature data summaries

The following figures (Figures A1.2 and A1.3) represent summarised temperature data. Figures A1.2a-f shows monthly soil, ground surface and air temperatures recorded across the ecotone at the six treeline sites during 2009-2011. Monthly mean daily and minima, maxima and extremes are indicated, for sites across the ecotone by a bar like this:



For each month, (up to) four bars are presented, indicating measurement sites across the ecotone by increasing altitude. Please note that the location of the sites above the treeline varies between the gradual and abrupt treeline sites, as indicated by the legends and described in Chapter 2 (see Figure 2.2).
Figure A1.3a-f are graphs of daily mean soil and air temperatures from treeline for the whole logging period.

Additional summary data, including annual and extreme temperatures for all sites, are presented in Tables A1.1 and A1.2 (p.182-183).

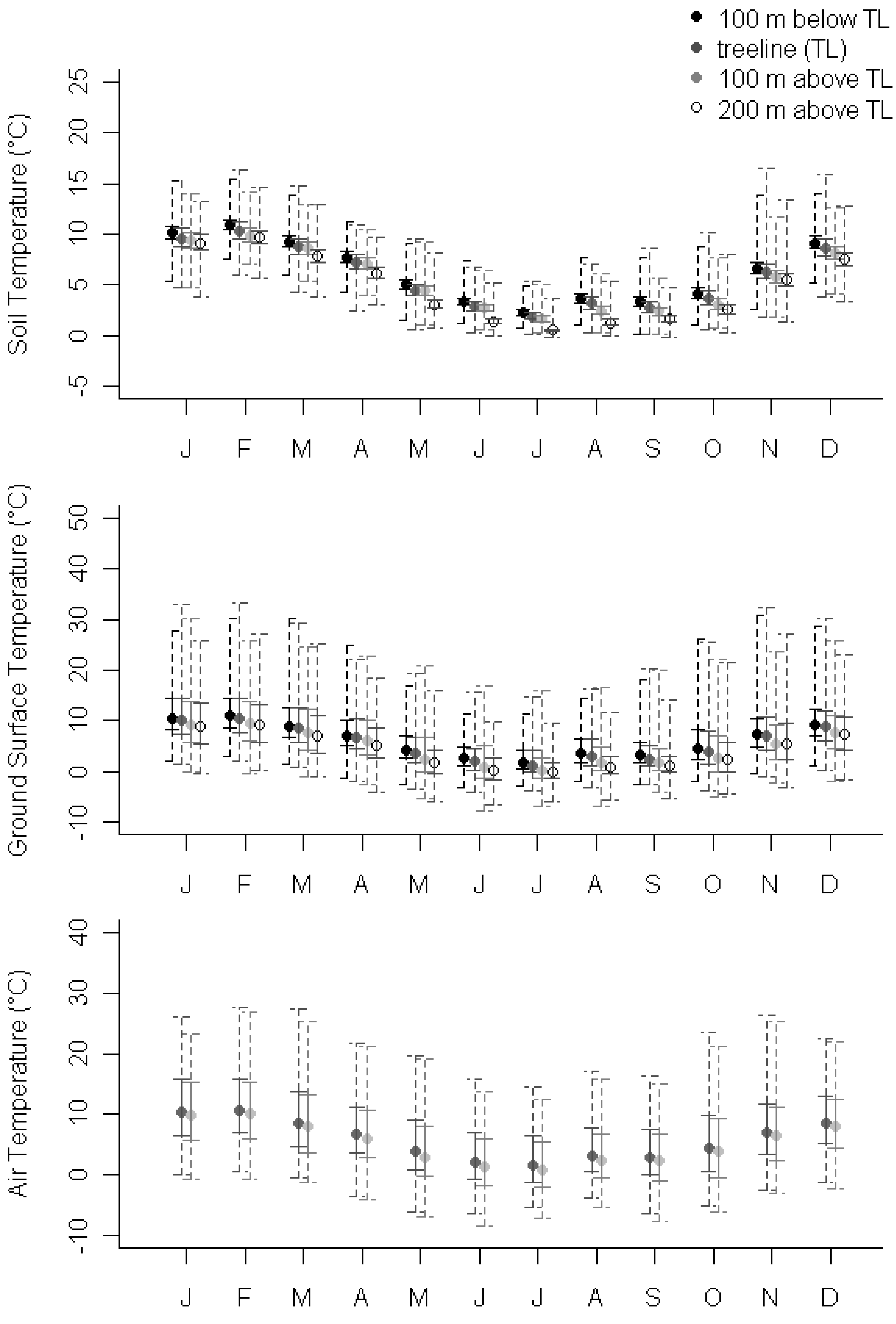


Figure A1.2 Monthly soil, ground and air temperature across the treeline ecotone: (a) Camp Creek. The bars indicate monthly mean daily means, minima, maxima and extremes for each logger site across the treeline ecotone (see previous page).

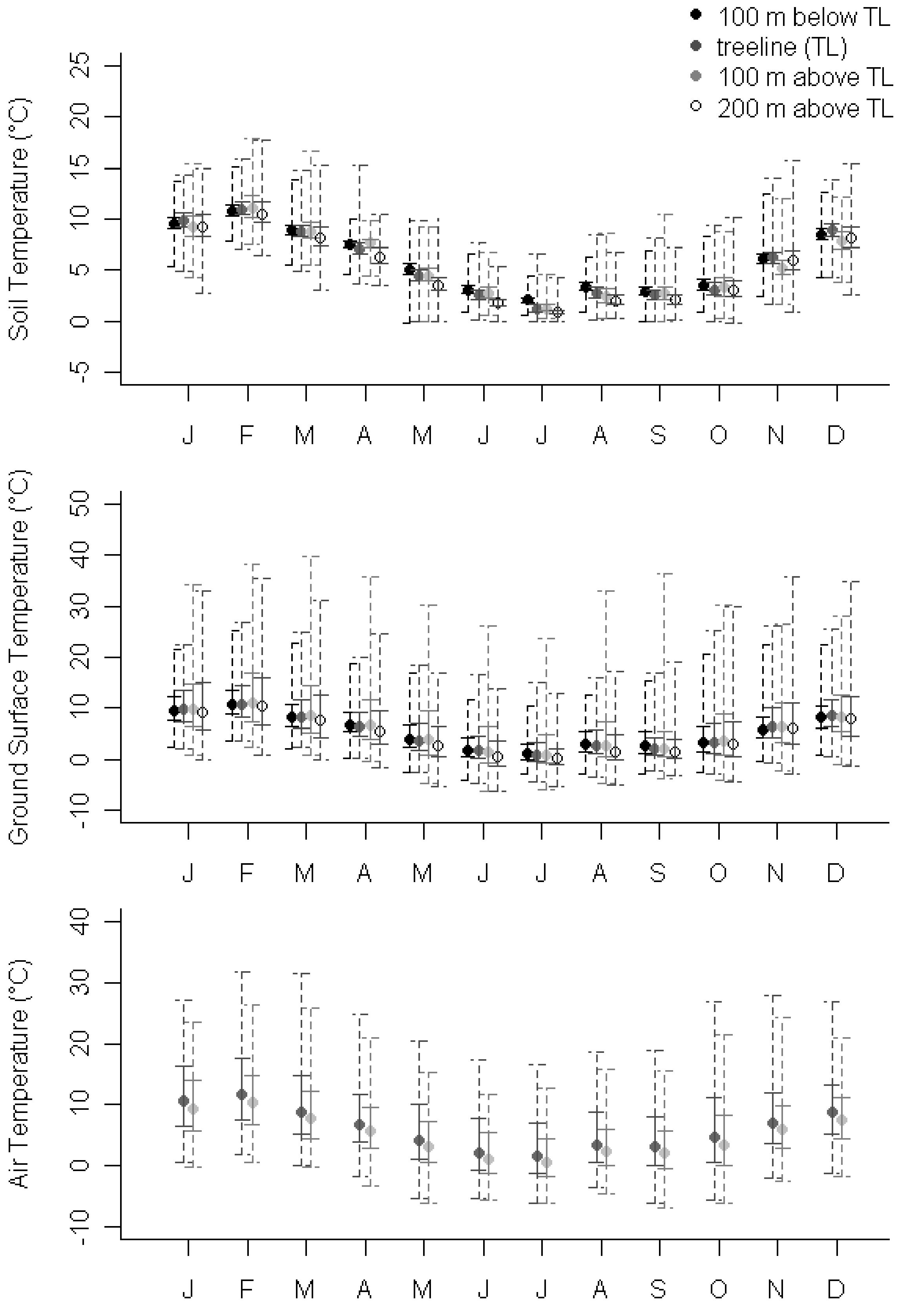


Figure A1.2 continued; (b) Temperatures across the Kelly Creek treeline ecotone.

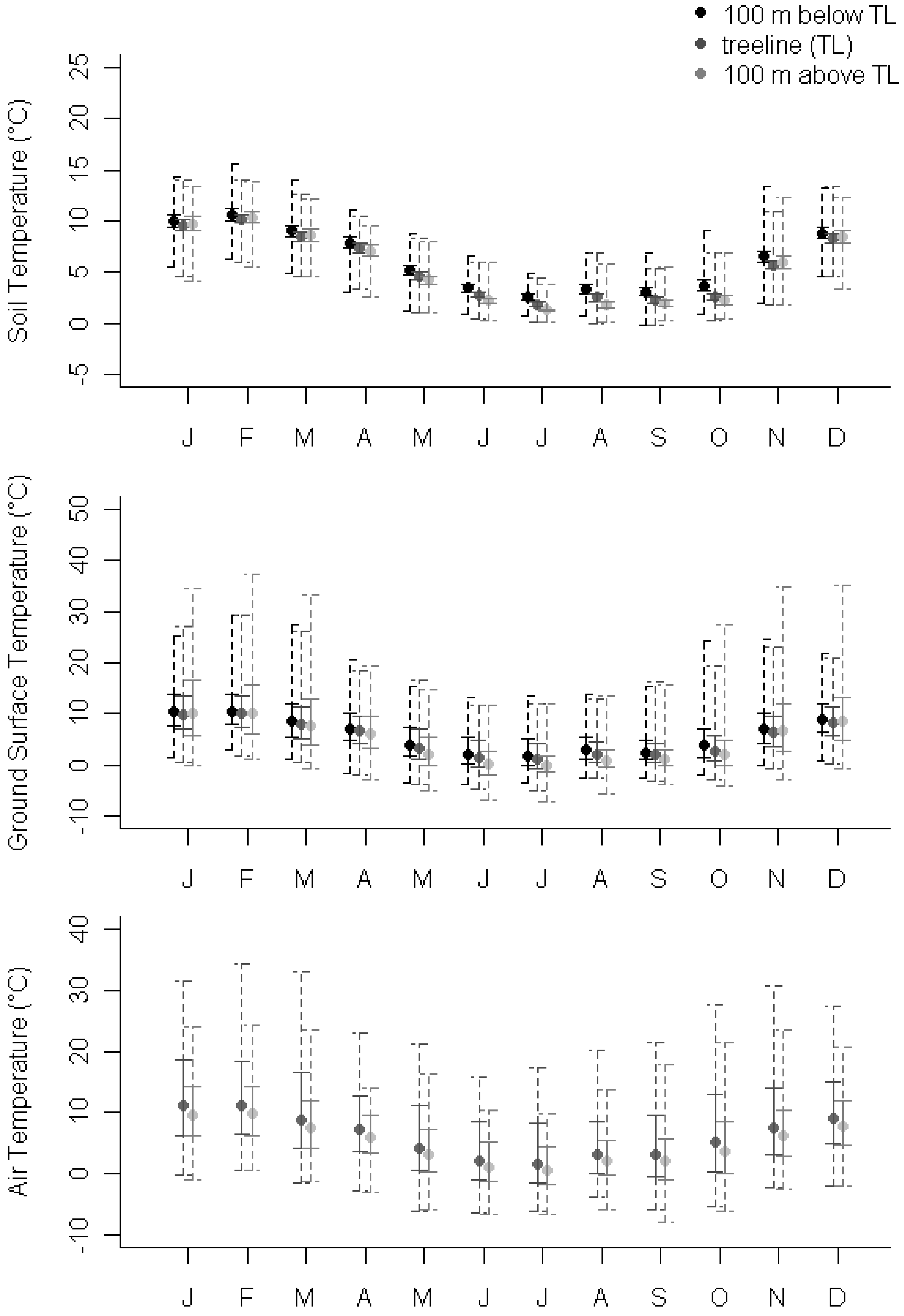


Figure A1.2 continued; (c) Temperatures across the Mikonui treeline ecotone.

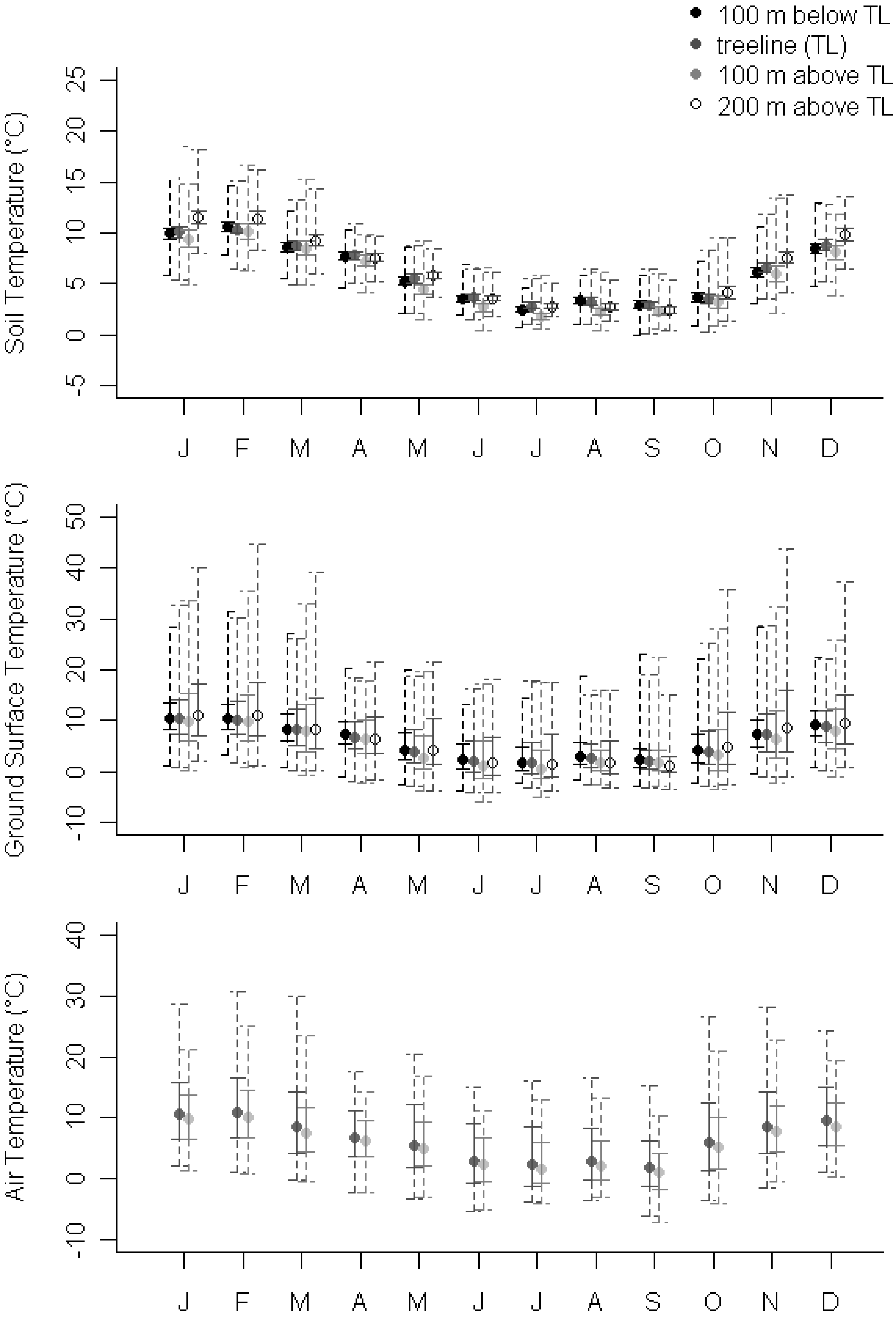


Figure A1.2 continued; (d) Temperatures across the Mt Fox treeline ecotone.

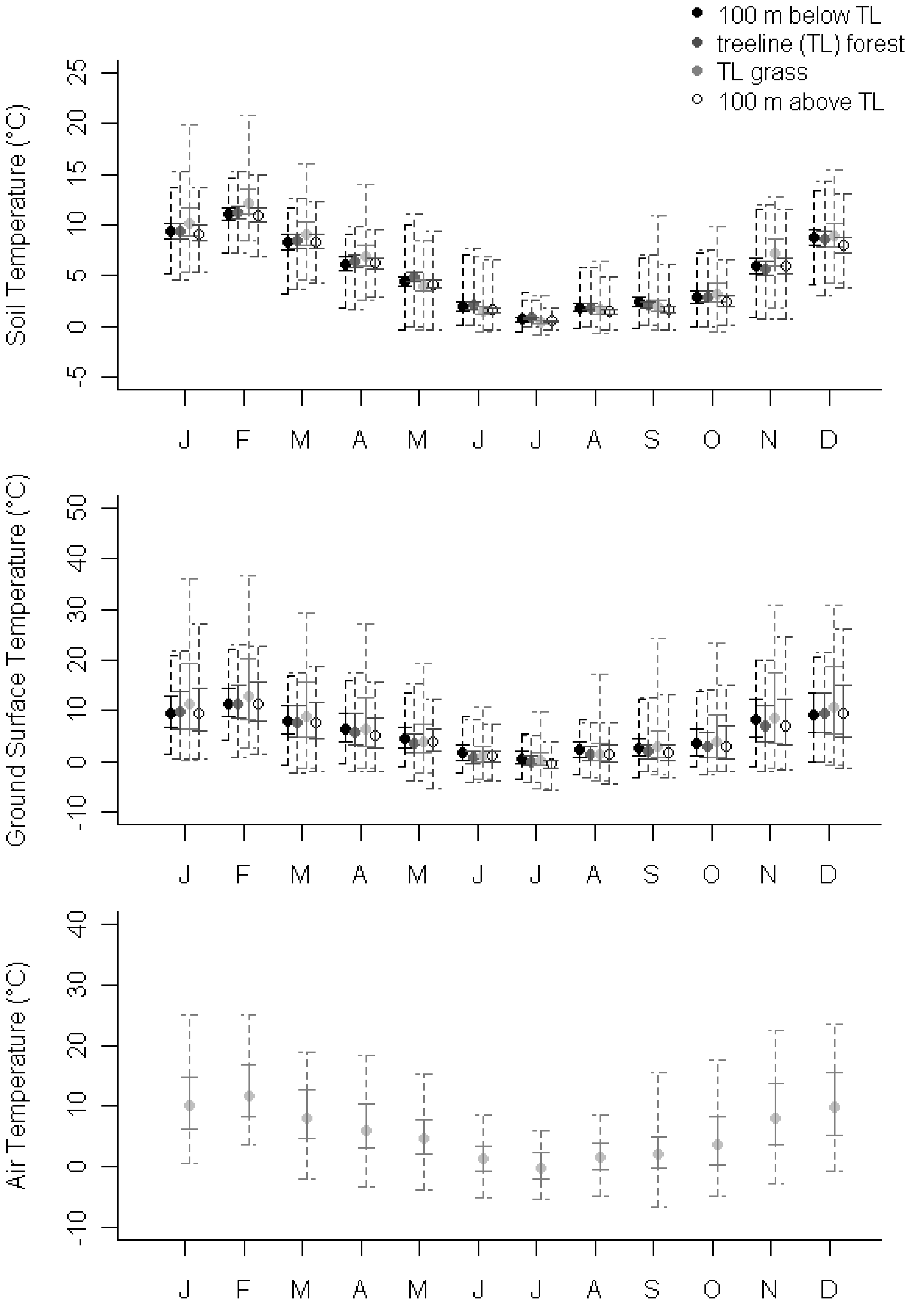


Figure A1.2 continued; (e) Temperatures across the Kaweka treeline ecotone.

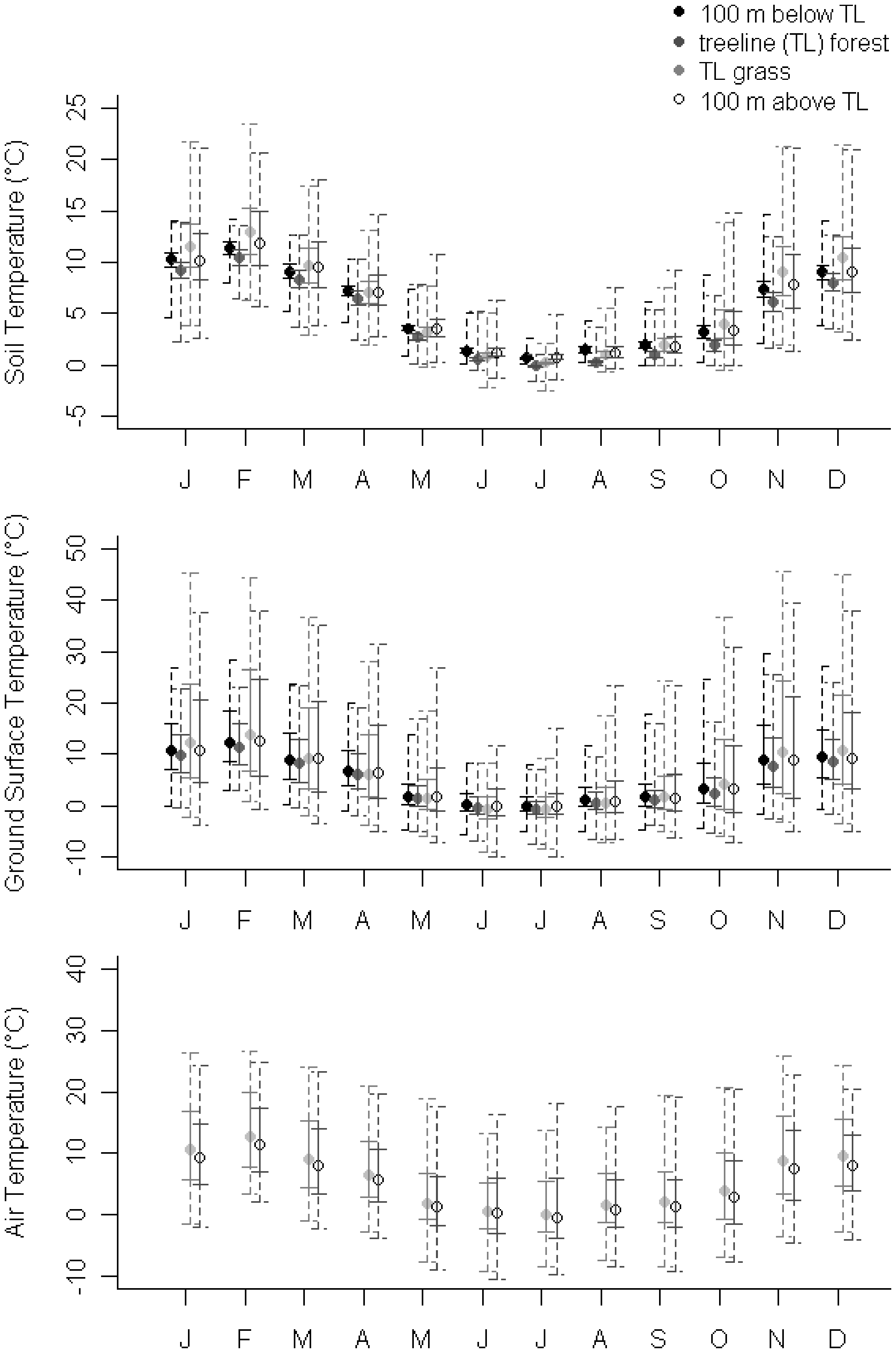


Figure A1.2 continued; (f) Temperatures across the Rainbow treeline ecotone.

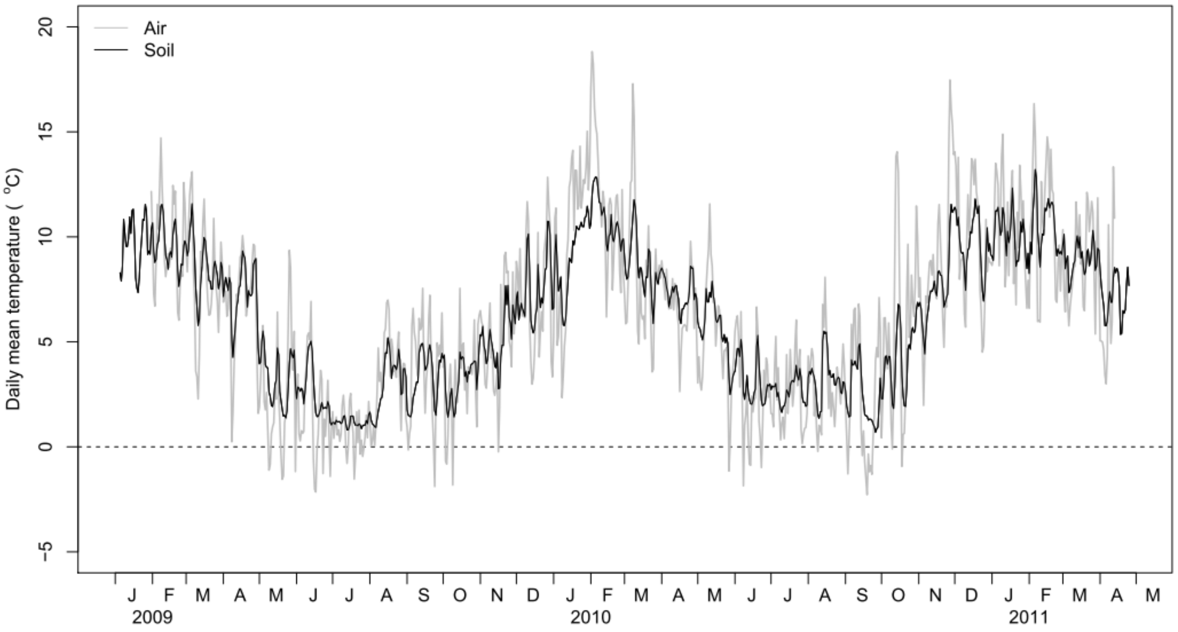


Figure A1.3 (a) Mean daily soil and air temperatures at the Camp Creek treeline.

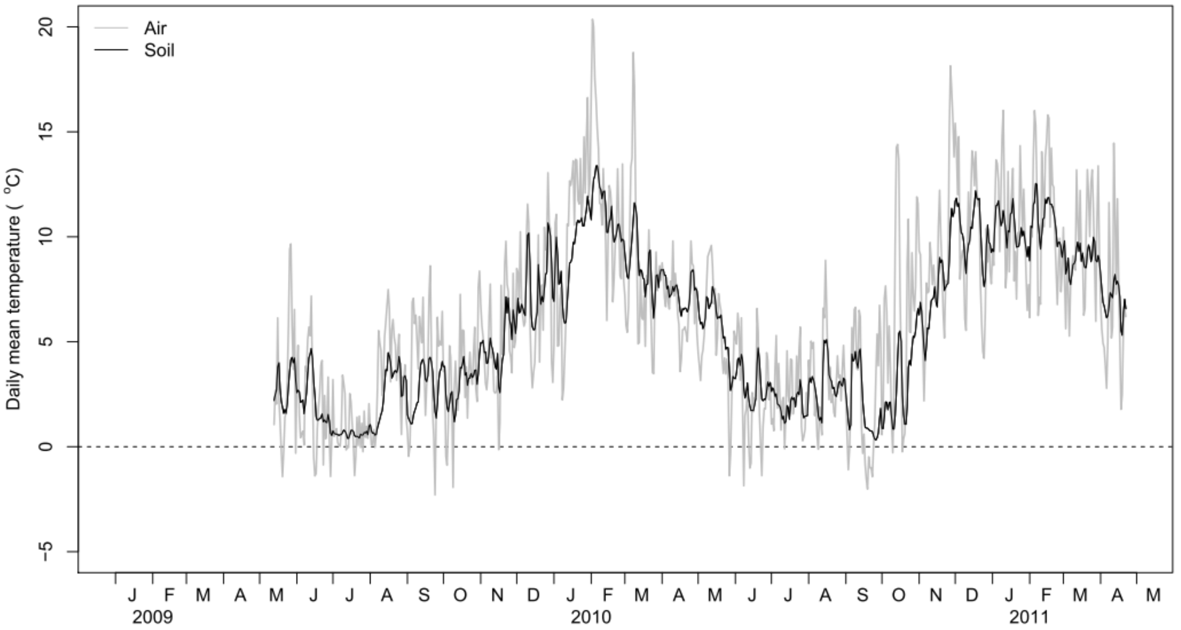


Figure A1.3 (b) Mean daily soil and air temperatures at the Kelly Creek treeline.

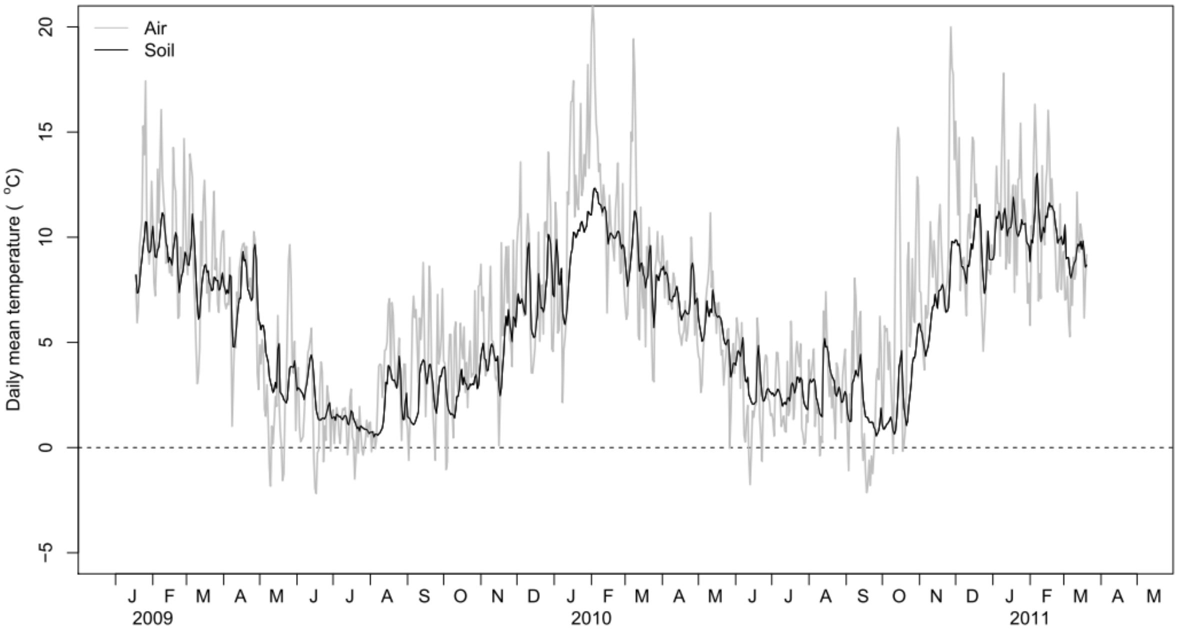


Figure A1.3 continued; (c) Mean daily soil and air temperatures at the Mikonui treeline.

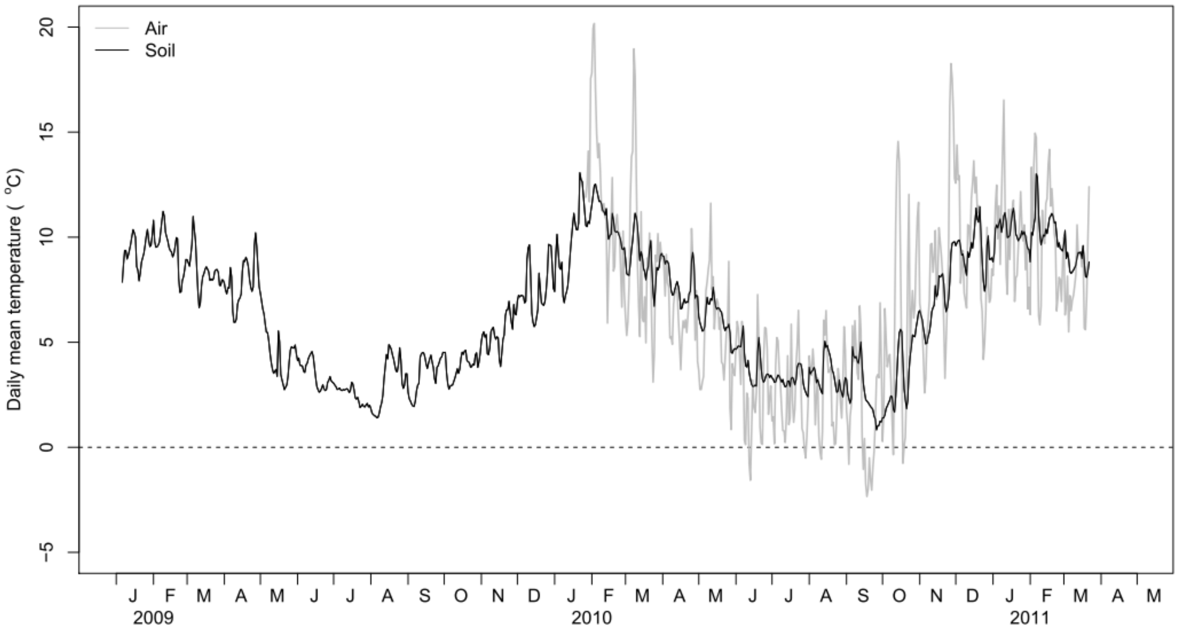


Figure A1.3 continued; (d) Mean daily soil and air temperatures at the Mt Fox treeline.

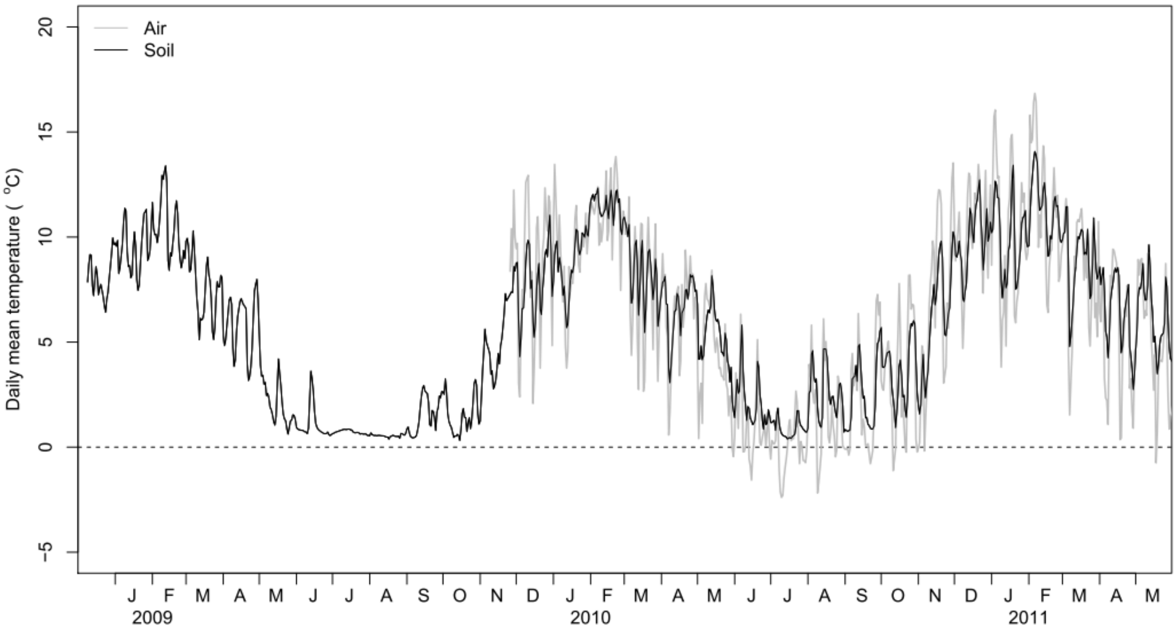


Figure A1.3 continued; (e) Mean daily soil and air temperatures at the Kaweka treeline.

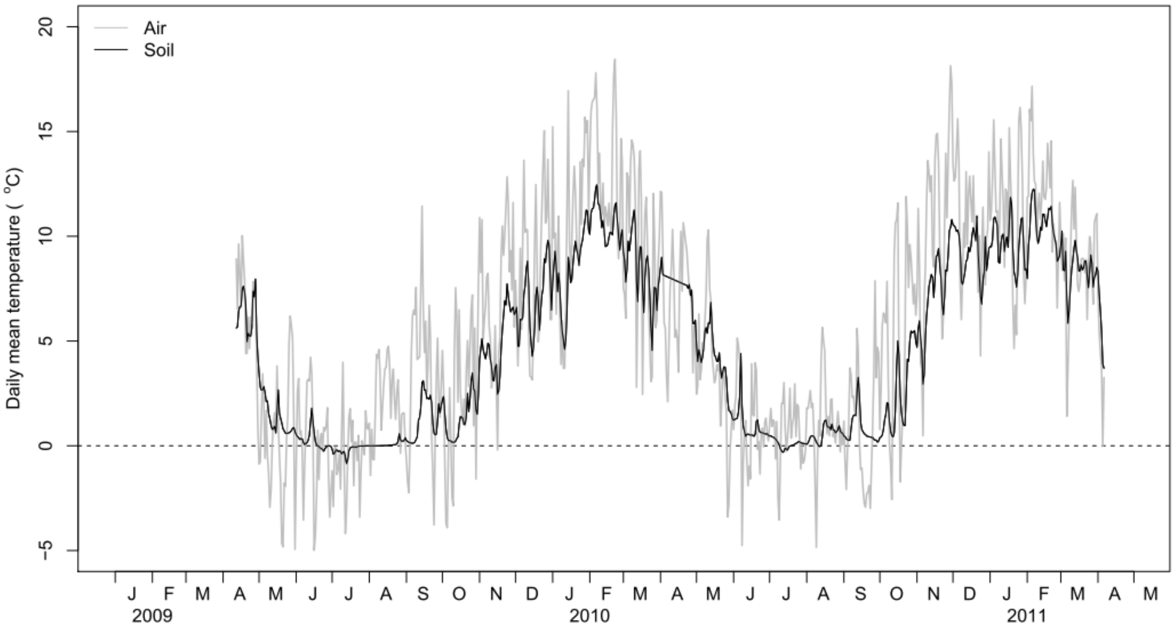


Figure A1.3 continued; (f) Mean daily soil and air temperatures at the Rainbow treeline.

Table A1.1. Temperature summaries for West Alps gradual treeline (TL) sites.

		Annual			Warmest Months					Extremes		Growing Degree Days		
Altitude from TL (m)	**Logger Position**	**Avg. T_{mean}**	**Avg. T_{min}**	**Avg. T_{max}**	**1 Month T_{mean}**	**3 Months T_{mean}**	**6 Months T_{mean}**	**Coldest Month T_{mean}**	**Frost Days**	**Extr. T_{min}**	**Extr. T_{max}**	**GDD 0**	**GDD 5**	**GDD 10**
Camp Creek														
-100	Soil	6.3	5.8	6.8	10.8	10.1	9.0	2.3	0	0.2	15.4	2285	754	70
-100	Ground	6.1	4.2	9.2	11.0	10.2	9.0	1.9	35	-3.0	30.9	2240	806	135
TL	Soil	5.8	5.2	6.5	10.3	9.5	8.5	1.9	0	0.1	14.8	2106	655	49
TL	Ground	5.6	3.4	9.1	10.5	9.8	8.6	1.3	55	-4.0	33.2	2068	719	113
TL	Air	5.8	2.5	10.7	10.7	9.9	8.6	1.6	103	-6.4	27.6	2139	769	146
+100	Soil	5.4	4.9	6.0	9.9	9.3	8.1	1.7	0	0.1	14.1	1984	593	21
+100	Ground	4.8	2.1	8.5	9.5	8.8	7.6	0.3	114	-7.7	30.1	1776	575	75
+100	Air	5.2	1.7	10.0	10.2	9.4	8.1	0.8	138	-8.2	26.8	1934	665	120
+200	Soil	4.7	4.2	5.2	9.7	8.9	7.6	0.6	3	-0.1	14.7	1704	507	27
+200	Ground	4.1	1.7	7.3	9.2	8.4	7.2	0.0	126	-6.6	27.0	1537	484	58
Kelly Creek														
-100	Soil	6.0	5.6	6.4	10.8	9.8	8.6	2.1	0	-0.1	15.1	2194	694	44
-100	Ground	5.6	3.9	8.1	10.9	9.6	8.3	1.1	46	-4.0	25.4	2070	681	83
TL	Soil	5.7	5.2	6.3	11.0	9.9	8.6	1.2	1	0.0	15.3	2090	695	66
TL	Ground	5.5	3.5	8.6	10.9	9.8	8.5	0.9	57	-4.7	26.7	2018	691	97
TL	Air	6.1	2.7	11.5	11.6	10.3	8.9	1.7	96	-6.0	31.8	2242	833	186
+100	Soil	5.4	4.8	6.1	11.1	9.7	8.3	1.3	0	0.0	17.8	1972	599	47
+100	Ground	5.5	2.8	10.3	10.9	9.7	8.5	1.0	67	-6.3	39.6	2028	709	109
+100	Air	5.0	2.1	9.1	10.3	9.1	7.8	0.6	112	-6.9	26.3	1874	618	114
+200	Soil	5.1	4.5	6.0	10.5	9.3	8.0	0.9	1	-0.1	17.6	1891	578	50
+200	Ground	4.7	2.2	8.7	10.3	9.1	7.7	0.1	95	-6.2	35.6	1748	578	100
Mikonui														
-100	Soil	6.2	5.7	6.7	10.6	9.9	8.8	2.6	0	-0.2	15.6	2244	718	48
-100	Ground	5.8	3.5	8.9	10.6	9.9	8.8	1.7	50	-3.9	29.2	2108	729	97
TL	Soil	5.5	5.1	5.9	10.2	9.4	8.3	1.9	0	-0.2	14.0	2002	610	30
TL	Ground	5.1	2.9	8.2	10.1	9.4	8.2	1.1	73	-4.9	29.1	1876	616	72
TL	Air	6.2	2.2	12.8	11.2	10.5	9.2	1.6	119	-6.3	34.2	2260	857	189
+100	Soil	5.3	4.9	5.7	10.4	9.6	8.4	1.3	0	0.1	13.8	1929	617	29
+100	Ground	4.6	1.9	8.3	10.3	9.7	8.3	-0.1	122	-7.3	37.3	1727	620	96
+100	Air	5.0	2.0	9.1	9.9	9.2	7.9	0.6	125	-7.8	24.2	1856	611	95
Mt Fox														
-100	Soil	6.1	5.7	6.5	10.6	9.7	8.6	2.5	0	0.0	18.5	2202	676	40
-100	Ground	5.9	3.8	8.9	10.6	10.1	8.8	1.9	46	-3.7	31.5	2138	740	99
TL	Soil	6.1	5.8	6.6	10.4	9.8	8.7	2.8	0	0.2	15.1	2235	692	41
TL	Ground	5.6	3.2	9.3	10.3	9.8	8.6	1.7	62	-4.2	32.7	2039	687	88
TL	Air	6.3	2.6	12.0	10.9	10.4	9.2	1.9	108	-6.1	30.6	2334	892	190
+100	Soil	5.5	5.0	6.1	10.1	9.3	8.2	1.8	0	0.4	16.7	1993	600	31
+100	Ground	5.0	2.1	9.3	10.0	9.4	8.1	0.6	100	-6.0	35.4	1828	628	92
+100	Air	5.6	2.7	9.7	10.2	9.6	8.4	1.0	102	-7.0	25.0	2096	734	133
+200	Soil	6.4	6.0	7.0	11.5	10.9	9.5	2.4	0	0.5	18.2	2345	820	83
+200	Ground	5.8	2.7	11.2	11.0	10.5	9.1	1.2	93	-4.1	44.6	2108	807	163

Table A1.2. Temperature summaries for East Alps abrupt treeline (TL) sites.

		Annual			Warmest Months					Extremes		Growing Degree Days		
Altitude from TL (m)	**Logger Position**	**Avg. T_{mean}**	**Avg. T_{min}**	**Avg. T_{max}**	**1 Month T_{mean}**	**3 Months T_{mean}**	**6 Months T_{mean}**	**Coldest Month T_{mean}**	**Frost Days**	**Extr. T_{min}**	**Extr. T_{max}**	**GDD 0**	**GDD 5**	**GDD 10**
Kaweka														
-100	Soil	5.3	4.8	5.9	11.0	9.7	8.2	0.7	4	-0.5	14.7	1931	642	53
-100	Ground	5.7	3.5	8.4	11.3	10.1	8.8	0.5	82	-3.6	22.2	2094	791	129
TL Forest	Soil	5.3	4.8	5.8	11.2	9.7	8.3	0.8	0	0.0	15.2	1929	656	64
TL Forest	Ground	5.1	2.9	7.8	11.4	10.2	8.6	0.1	114	-4.0	23.2	1895	745	131
TL Grass	Soil	5.5	2.5	9.4	11.8	10.6	9.0	-0.2	126	-6.5	25.1	2077	844	174
TL Grass	Ground	5.7	4.9	6.7	12.2	10.4	9.0	0.5	25	-0.8	20.8	2062	784	117
TL Grass	Air	6.0	2.8	11.3	12.8	11.6	9.8	0.2	93	-5.1	36.6	2198	956	232
+100	Soil	5.3	3.0	8.1	11.5	10.1	8.6	0.0	103	-3.8	22.2	1979	754	127
+100	Ground	5.1	4.7	5.7	11.0	9.5	8.1	0.6	5	-0.3	15.0	1883	641	53
Rainbow														
-100	Soil	5.5	5.0	6.0	11.4	10.3	9.1	0.7	0	0.0	14.6	1808	688	80
-100	Ground	5.4	2.7	9.5	12.2	10.8	9.4	0.0	107	-5.7	29.4	2012	852	179
TL Forest	Soil	4.5	4.0	5.1	10.5	9.4	8.1	0.0	34	-1.5	13.9	1488	543	32
TL Forest	Ground	4.7	2.2	8.0	11.4	9.9	8.6	-0.5	140	-7.4	25.6	1772	715	120
TL Grass	Soil	5.9	4.8	7.2	13.0	11.7	10.1	0.2	16	-2.5	23.4	1973	888	219
TL Grass	Ground	5.7	1.3	13.3	13.7	12.3	10.4	-0.6	151	-8.9	45.7	2165	1049	334
TL Grass	Air	5.6	1.7	11.4	12.7	11.0	9.6	0.1	145	-9.1	26.6	2145	925	233
+100	Soil	5.6	4.3	7.2	11.8	10.5	9.2	0.7	19	-1.5	21.2	1825	721	107
+100	Ground	5.3	0.8	13.0	12.6	10.9	9.5	-0.1	152	-9.9	39.4	2003	881	215
+100	Air	4.7	0.8	10.1	11.3	9.6	8.4	-0.4	163	-10.3	24.6	1849	717	127

Appendix 2. Growing season temperature

(Supplement to Chapter 3)

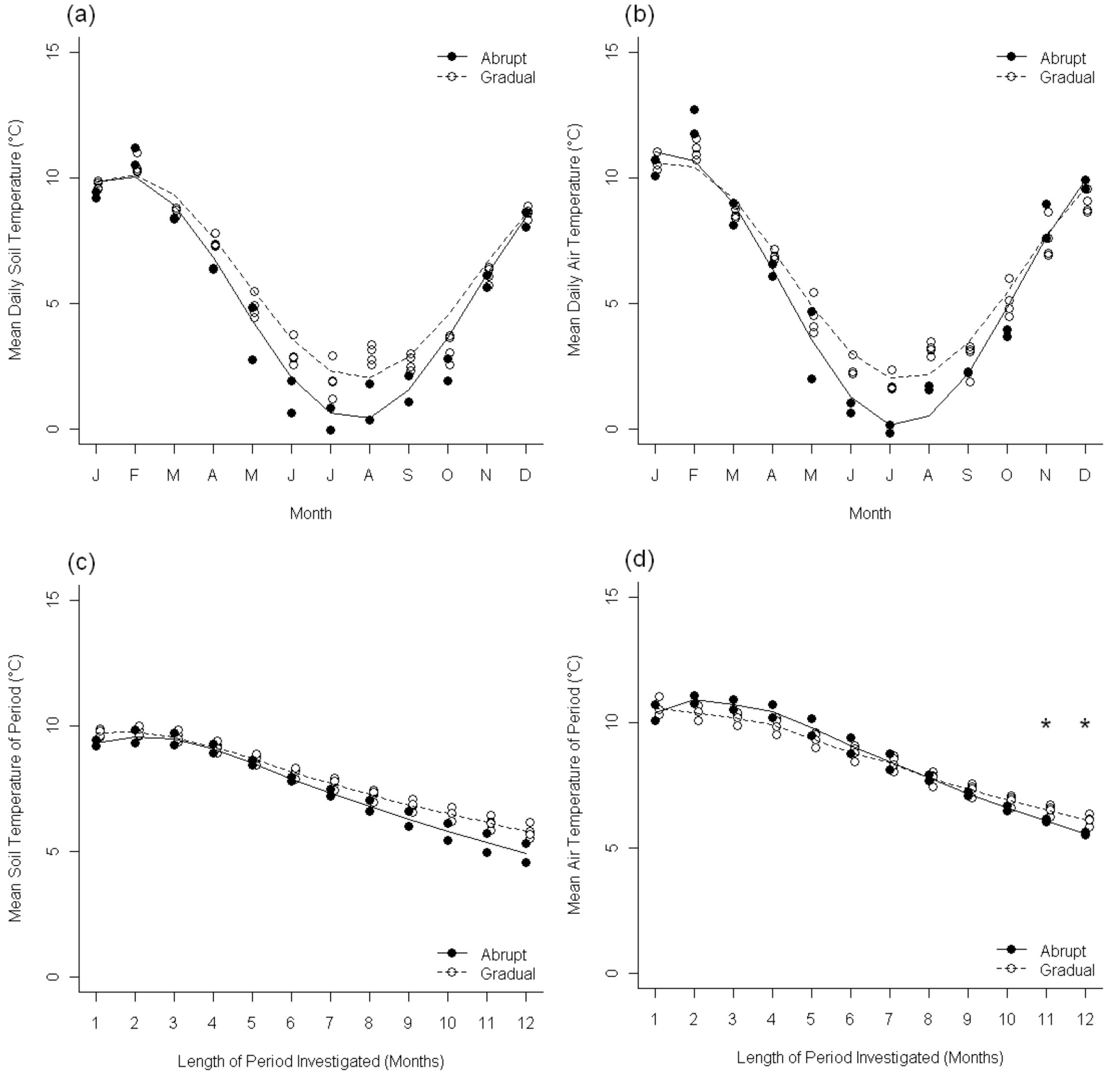


Figure A2.1. Mean monthly soil (a) and air (b) temperatures at the six treeline sites measured in this study (as presented in Figure 2.4). Soil and air temperatures differ between abrupt and gradual treelines in the colder months. When temperatures of increasingly long periods centered around January (mid summer) are investigated (c, d), soil temperatures are never significantly different between the two treeline types (c), but much colder mid-winter air temperatures at abrupt treelines create significantly different means (*) when periods of 11 months or a full year are considered (d). Lines in (a, b) are predicted values from linear mixed-effect models (see Section 2.3.2); lines in (c, d) are drawn through the means for the two different treeline types.

Table A2.1. Air temperature summary statistics for 20 treeline sites. Temperature at sites indicated with an asterisk (*) was measured exactly at treeline as part of this study. Sites are ordered by decreasing latitude (for site details, see Table 3.2).

Site No.	Annual	Warmest Month	3Warmest Months	Coldest Month		Frost Days	Growing Degree Days		Growing Season MINAIR2				Days with Min ≥ 5°C
	Mean Daily T_{mean}	Mean Daily T_{mean}	Mean Daily T_{mean}	Mean Daily T_{mean}	Extr. T_{min}	Sum (d)	GDD0	GDD5	Length (d)	Mean Daily T_{mean}	Mean Daily T_{min}	Mean Daily T_{max}	Sum (d)
1	6.97	9.49	9.12	4.82	-4.94	25	2545	840	365	6.97	4.75	9.19	186
11	7.29	9.52	9.29	5.28	-4.52	9	2644	904	365	7.29	5.25	9.34	204
12	5.6	10.84	10.08	0.44	-10.04	108	1890	755	180	8.93	5.35	12.44	104
13	5.84	12.48	11.06	-1	-10.7	130	2230	912	180	9.41	4.99	13.84	109
14	4.98	9.77	9.11	0.55	-7.96	143	1747	575	159	8.16	4.29	12.03	93
15	4.71	9.04	8.53	1.03	-8.07	126	1777	536	148	7.71	4.37	11.04	70
16	5.67	10.82	10.23	0.58	-12.16	152	2075	791	158	9.27	4.59	14	90
17	6.21	11.47	10.84	1.1	-9.18	113	2295	922	181	9.42	4.74	14.1	91
18	5.92	11.4	10.81	0.51	-11.62	119	2208	886	182	9.3	5.13	13.46	101
*3	6.34	10.93	10.37	1.87	-6.12	108	2334	892	200	8.91	4.95	14.41	92
19	5.18	10.81	10.04	-0.23	-10.29	169	1981	762	129	9.52	4.19	14.85	66
*4	6.16	11.16	10.47	1.62	-6.33	119	2260	857	176	9.12	4.82	15.87	84
*5	6.09	11.56	10.33	1.66	-6.03	96	2242	833	201	8.46	4.96	13.73	111
*6	5.83	10.73	9.91	1.59	-6.38	103	2139	769	192	8.29	4.83	13.07	84
*8	5.63	12.73	11	0.13	-9.07	145	2145	925	165	9.73	5.03	15.95	114
20	6.35	11.56	10.72	1.05	-9.63	101	2377	1038	206	9.24	5.54	12.93	116
21	6.93	11.71	11.39	2.47	-6.27	78	2517	1005	205	9.49	5.25	13.71	118
*9	5.51	11.78	10.58	-0.16	-6.52	126	2077	844	192	8.65	5.09	13.3	105
22	6.02	10.84	10.64	0.6	-7.09	97	2025	845	215	8.76	5.21	12.3	108
23	5.76	10.81	10.04	1.28	-9.2	131	2121	740	159	8.91	4.39	13.43	83

Appendix 3. Responses to growing season temperature (Supplement to Chapter 6)

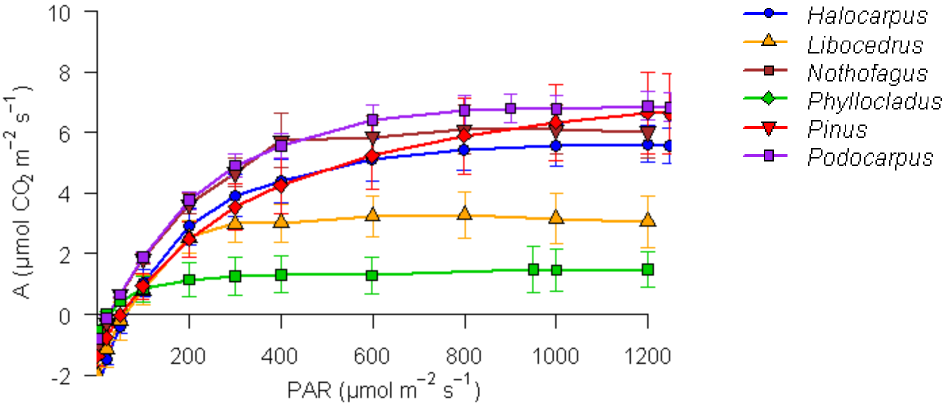


Figure A3.1. Response of net photoysnthesis (A) to photosynthetically active radiation (PAR) (light response curves) of the six species at 20°C. Plotted points are averages of three plants (error bars are 1 SE).

Table A3.1. Summary results for the regression models of net photosynthesis in response to growing temperature for six species. Models fitted included a linear and quadratic (optimum) response (*Linear* and *Quad*), as well as a null model with only an intercept (*Null*). Models were compared using AIC*c* (Aikaike Information Criterion corrected for small sample size, see Section 2.2.2 for details). $\Delta_{i:}$ indicates the difference in AICc between i^{th} model and the top-ranked model; $w_{i:}$ Model probability given the data and candidate set; and LogLik: Maximised log likelihood. The number of parameters fitted (K) is 2, 3 and 4, for the null, linear and quadratic models, respectively. The most parsimonious (best) KL model for each species is indicated in bold, and the corresponding variance explained is presented (R^2). The best KL models are displayed in Figure 6.2.

Species		**Net photosynthesis**			
	Model	**ΔAIC*c***	**w_i**	**LogLik**	**R^2**
Halocarpus	**Linear**	**0.00**	**0.84**	**-20.00**	**0.45**
	Quad	4.17	0.10	-19.92	
	Null	5.38	0.06	-24.42	
Libocedrus	**Linear**	**0.00**	**0.86**	**-13.37**	**0.64**
	Quad	3.64	0.14	-13.02	
	Null	10.82	0.00	-20.51	
Nothofagus	**Null**	**0.00**	**0.48**	**-20.97**	**0.00**
	Linear	0.71	0.34	-19.59	
	Quad	1.97	0.18	-18.05	
Phyllocladus	**Linear**	**0.00**	**0.72**	**-7.60**	**0.92**
	Quad	1.90	0.28	-6.20	
	Null	27.58	0.00	-23.23	
Pinus	**Quad**	**0.00**	**0.97**	**-15.69**	**0.84**
	Linear	7.15	0.03	-21.62	
	Null	16.39	0.00	-28.08	
Podocarpus	**Quad**	**0.00**	**1.00**	**-9.58**	**0.81**
	Linear	12.37	0.00	-17.93	
	Null	15.88	0.00	-21.42	

Table A3.2. Summary of the linear mixed effects models of growing and measurement temperature (*GrowT* and *MeasT*, respectively) on net photosynthesis. A null model with only an intercept was also fitted (*Null*). For model specification, A*B is short-hand for A + B + interaction between A and B. The most parsimonious KL model for each species is in bold font. See Table A3.1 and Section 2.2.2 for details about table content. See Figure 6.3.

Species	Model	K	ΔAICc	w_i	LogLik	R^2
Halocarpus	**GrowT+MeasT**	**6**	**0.00**	**0.53**	**-35.60**	**0.84**
	GrowT	5	1.72	0.22	-38.13	
	MeasT	4	2.50	0.15	-40.04	
	GrowT*MeasT	8	4.28	0.06	-33.84	
	Null	3	5.69	0.03	-43.02	
Libocedrus	**MeasT**	**4**	**0.00**	**0.93**	**-45.96**	**0.79**
	GrowT+MeasT	6	5.88	0.05	-45.92	
	Null	3	8.65	0.01	-51.61	
	GrowT*MeasT	8	9.82	0.01	-44.37	
	GrowT	5	14.16	0.00	-51.61	
Nothofagus	**MeasT**	**4**	**0.00**	**0.54**	**-42.37**	**0.83**
	GrowT*MeasT	8	1.13	0.31	-36.43	
	GrowT+MeasT	6	2.51	0.15	-40.64	
	Null	3	12.46	0.00	-49.93	
	GrowT	5	12.96	0.00	-47.42	
Phyllocladus	**GrowT*MeasT**	**8**	**0.00**	**1.00**	**-20.41**	**0.94**
	GrowT+MeasT	6	11.41	0.00	-29.80	
	GrowT	5	14.76	0.00	-33.08	
	MeasT	4	28.95	0.00	-41.65	
	Null	3	35.17	0.00	-46.11	
Pinus	**GrowT*MeasT**	**8**	**0.00**	**1.00**	**-27.21**	**0.98**
	GrowT+MeasT	6	24.57	0.00	-43.19	
	GrowT	5	29.78	0.00	-47.40	
	MeasT	4	33.44	0.00	-50.70	
	Null	3	41.52	0.00	-56.09	
Podocarpus	**GrowT**	**5**	**0.00**	**0.62**	**-32.79**	**0.71**
	GrowT*MeasT	8	2.01	0.23	-28.50	
	GrowT+MeasT	6	2.73	0.16	-32.55	
	Null	3	16.52	0.00	-43.87	
	MeasT	4	18.05	0.00	-43.29	

Table A3.3. Summary results for the regression models of diameter and height growth in response to mean growing season temperature for six species. See Table A3.1 and Section 2.2.2 for details about table content. The best KL models (in bold) are displayed in Figure 6.4.

Species	Diameter Growth					Height Growth				
	Model	ΔAIC*c*	w_i	LogLik	R^2	Model	ΔAIC*c*	w_i	LogLik	R^2
Halocarpus	**Quad**	**0.00**	**0.48**	**-7.10**	**0.24**	**Linear**	**0.00**	**0.79**	**-15.30**	**0.07**
	Null	0.92	0.30	-10.70		Quad	2.67	0.21	-14.72	
	Linear	1.54	0.22	-9.55		Null	10.07	0.01	-21.92	
Libocedrus	**Linear**	**0.00**	**0.55**	**-22.56**	**0.13**	**Linear**	**0.00**	**0.79**	**-47.13**	**0.13**
	Null	1.78	0.23	-24.76		Quad	2.77	0.20	-46.97	
	Quad	1.87	0.22	-22.04		Null	7.75	0.02	-52.38	
Nothofagus	**Quad**	**0.00**	**0.77**	**-15.92**	**0.31**	**Null**	**0.00**	**0.83**	**-10.92**	**0.00**
	Linear	3.60	0.13	-19.26		Linear	3.24	0.16	-10.40	
	Null	4.05	0.10	-20.86		Quad	9.24	0.01	-10.40	
Phyllocladus	**Quad**	**0.00**	**0.86**	**-9.50**	**0.55**	**Linear**	**0.00**	**0.75**	**-11.20**	**0.26**
	Linear	4.17	0.11	-13.60		Quad	2.69	0.19	-10.52	
	Null	6.26	0.04	-16.31		Null	5.09	0.06	-15.40	
Pinus	**Null**	**0.00**	**0.48**	**-19.62**	**0.00**	**Quad**	**0.00**	**0.91**	**-5.01**	**0.49**
	Linear	1.14	0.27	-18.74		Linear	4.88	0.08	-9.13	
	Quad	1.29	0.25	-17.13		Null	8.20	0.01	-12.25	
Podocarpus	**Quad**	**0.00**	**0.54**	**-16.32**	**0.55**	**Quad**	**0.00**	**0.82**	**-40.50**	**0.41**
	Linear	0.30	0.46	-18.16		Linear	3.73	0.13	-44.04	
	Null	10.45	0.00	-24.69		Null	5.50	0.05	-46.39	

Table A3.4. Summary results for the regression models of specific leaf area (SLA) in response to growing temperature for six species. See Table A3.1 and Section 2.2.2 for details about table content.

Species			SLA		
	Model	ΔAICc	wi	LogLik	R^2
Halocarpus	**Null**	**0.00**	**0.44**	**-93.11**	**0.04**
	Linear	*0.17*	*0.04*	*-91.97*	
	Quad	2.03	0.16	-91.57	
Libocedrus	**Linear**	**0.00**	**0.74**	**-64.78**	**0.28**
	Quad	2.14	0.25	-64.53	
	Null	8.72	0.01	-70.37	
Nothofagus	**Null**	**0.00**	**0.65**	**-129.07**	**-0.01**
	Linear	1.76	0.27	-128.72	
	Quad	4.19	0.08	-128.61	
Phyllocladus	**Linear**	**0.00**	**0.54**	**-109.61**	**0.09**
	Null	1.28	0.29	-111.49	
	Quad	2.33	0.17	-109.44	
Pinus	**Quad**	**0.00**	**0.99**	**-97.60**	**-0.03**
	Null	10.25	0.01	-105.28	
	Linear	12.64	0.00	-105.25	
Podocarpus	**Linear**	**0.00**	**0.62**	**-141.20**	**0.11**
	Null	2.14	0.21	-143.50	
	Quad	2.66	0.16	-141.19	

Table A3.5. Summary results for the regression models of above-ground biomass and the proportion of this biomass allocated to foliage in response to growing temperature for six species. See Table A3.1 and Section 2.2.2 for details about table content. The best KL models (in bold) are displayed in Figure 6.5.

Species	Above-ground biomass					Proportion Foliage				
	Model	ΔAICc	wi	LogLik	R^2	Model	ΔAICc	wi	LogLik	R^2
Halocarpus	**Null**	**0.00**	**0.78**	**-55.23**	**0.00**	**Quad**	**0.00**	**0.80**	**-56.49**	**0.33**
	Linear	2.90	0.18	-55.23		Null	3.26	0.16	-61.26	
	Quad	6.25	0.03	-55.22		Linear	5.93	0.04	-61.14	
Libocedrus	**Linear**	**0.00**	**0.67**	**-74.51**	**0.17**	**Quad**	**0.00**	**0.67**	**-63.72**	**0.21**
	Quad	2.71	0.17	-74.41		Null	2.31	0.21	-67.65	
	Null	2.93	0.16	-77.29		Linear	3.45	0.12	-66.90	
Nothofagus	**Linear**	**0.00**	**0.55**	**-54.86**	**0.13**	**Quad**	**0.00**	**0.86**	**-62.53**	**0.39**
	Null	1.39	0.27	-56.93		Linear	4.13	0.11	-66.14	
	Quad	2.23	0.18	-54.43		Null	6.71	0.03	-68.80	
Phyllocladus	**Null**	**0.00**	**0.72**	**-38.89**	**0.00**	**Null**	**0.00**	**0.80**	**-44.77**	**0.00**
	Linear	2.21	0.24	-38.34		Linear	3.22	0.16	-44.72	
	Quad	5.61	0.04	-38.02		Quad	6.28	0.03	-44.23	
Pinus	**Linear**	**0.00**	**0.83**	**-66.23**	**0.41**	**Linear**	**0.00**	**0.46**	**-57.31**	**0.10**
	Quad	3.35	0.15	-66.23		Null	0.07	0.44	-58.81	
	Null	7.78	0.02	-71.58		Quad	3.15	0.10	-57.20	
Podocarpus	**Quad**	**0.00**	**0.98**	**-48.91**	**0.56**	**Quad**	**0.00**	**0.56**	**-48.55**	**0.33**
	Linear	8.21	0.02	-54.70		Linear	1.11	0.32	-50.79	
	Null	10.56	0.00	-57.33		Null	3.16	0.12	-53.27	

Table A3.6. Summary of best KL models of relationship between net photosynthesis and (left) height growth, (middle) height growth and (right) biomass for each species. A model with a linear response was compared to a null model (intercept only). The best KL models are displayed in Figure 6.6.

	Height growth			Diameter growth			Biomass		
Species	**Trend**[1]	**Evidence Ratio**[2]	**R^2**[3]	**Trend**[1]	**Evidence Ratio**[2]	**R^2**[3]	**Trend**[1]	**Evidence Ratio**[2]	**R^2**[3]
Halocarpus	x	3.35	-0.02	-	10.11	0.39	x	4.88	-0.07
Libocedrus	+	4.00	0.33	x	2.85	0.02	x	2.70	0.03
Nothofagus	x	4.88	-0.07	x	3.35	-0.01	x	3.00	0.01
Phyllocladus	+	4.00	0.36	+	8.09	0.43	x	3.17	0.02
Pinus	-	15.67	0.48	x	2.57	0.05	-	1.94	0.28
Podocarpus	+	49.00	0.55	+	8.09	0.40	+	19.00	0.46

[1] An ‘x’ indicates that the best KL model was the Null model (no trend), whereas a ‘+’ indicates an increasing linear trend, and ‘-’ a decreasing linear trend

[2] Evidence ratio provides a measure of the strength of the evidence for the different models and is calculated as the ratio of w_i (model probability) of the best model to the w_i of the other model

[3] Adjusted R^2 for the model with the linear relationship