Thermal acclimation of photosynthesis and respiration in *Pinus radiata* and *Populus deltoides* to changing environmental conditions

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Lai Fern, Genevieve Ow

UC
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This thesis is dedicated

to my parents who have been

so supportive all this time

The work presented in this thesis is, to the best of my knowledge and belief, original. The material has not been submitted, either in whole or in part, for a degree at this or any other University.

Lai Fern Ow

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LIST OF ABBREVIATIONS

A	photosynthesis
A_{max}	maximum photosynthetic rate at saturating light
ANOVA	analysis of variance
AOX	alternative oxidase
ATP	adenosine triphosphate
C_a	atmospheric CO_2 concentration
C_i	intercellular CO_2 concentration
CCCP	carbonylcyanide m-chlorophenylhydrazone
COX	cytochrome oxidase
CV	coefficient of variation
E_0	activation energy of respiration
GCM	global carbon cycle models
Gt	gigatons
HN	high nitrogen plants
J_{max}	maximum photosynthetic electron transport rate
K	kelvin
KCN	potassium cyanide
kPa	kilopascal
LN	low nitrogen plants
L_{stom}	relative stomatal limitation on photosynthesis

MAF	Ministry of Agriculture and Forestry
N	nitrogen
N_{area}	nitrogen on an area basis
N_{mass}	nitrogen on a mass basis
^{18}O	oxygen-18, stable isotope
Pa	pascal
PFD	photosynthetic photon flux density
Pg	petagrams
P_i	phosphate supply
PnET	net primary productivity ecosystem model
PNUE	photosynthetic nitrogen utilisation efficiency
Q	irradiance
Q_{10}	The temperature coefficient - a measure of the rate of change of a biological or chemical system as a consequence of increasing the temperature by 10 °C
R_{10}	respiration rate at a reference temperature of 10 ^{o}C
R_d	dark respiration rate
R/P	respiration/photosynthesis ratio
RuBP	ribulose-1,5-bisphosphate
SE	standard error of the mean
SHAM	salicylhydroxamic acid
SLA	specific leaf area
T	temperature
T_{avg}	temperature average

TCA	tricarboxylic acid cycle
T_{opt}	optimum temperature
V_{cmax}	maximum carboxylation rate of ribulose 1.5-bisphosphate

ABSTRACT

Although it has long been recognized that physiological acclimation of photosynthesis and respiration can occur in plants exposed to changing environmental conditions (e.g. light, temperature or stress), the extent of acclimation in different tissues (i.e. pre-existing and new foliage) however, has not received much attention until recently. Furthermore, few studies have investigated the extent of photosynthetic and respiratory acclimation under natural conditions, where air temperatures vary diurnally and seasonally. In this study, the effects of variations in temperature on respiratory CO_2 loss and photosynthetic carbon assimilation were examined under both controlled and natural environments. The purpose of the investigations described in this thesis was to identify the effects acclimation would have on two key metabolic processes in plants exposed to temperature change, with emphasis also placed on the role of nutrition (nitrogen) and respiratory enzymatic characteristics on the potential for acclimation in two contrasting tree species, *Pinus radiata* and *Populus deltoides*.

Controlled-environment studies (Chapter 2 and 3) established that rates of foliar respiration are sensitive to short-term changes in temperature (increasing exponentially with temperature) but in the longer-term (days to weeks), foliar respiration acclimates to temperature change. As a result, rates of dark respiration measured at any given temperature are higher in cold-acclimated and lower in warm-acclimated plants than

would be predicted from an instantaneous response. Acclimation in new foliage (formed under the new temperature environment) was found to result in respiratory homeostasis (i.e. constant rates of foliar respiration following long-term changes in temperature, when respiration is measured at the prevailing growth temperature). Available evidence suggests that substantial adjustments in foliar respiration tend to be developmentally dependent. This may in part explain why respiratory homeostasis was only observed in new but not in pre-existing tissues. Step changes in temperature (cold and warm transfers) resulted in significant changes in photosynthetic capacity. However, in stark contrast to the findings of respiration, there was little evidence for photosynthetic acclimation to temperature change.

The results obtained from field studies (Chapter 4) show that in the long-term over a full year, dark respiration rates in both tree species were insensitive to temperature but photosynthesis retained its sensitivity, increasing with increasing temperature. Respiration in both species showed a significant downregulation during spring and summer and increases in respiratory capacity were observed in autumn and winter. Thermal acclimation of respiration was associated with a change in the concentration of soluble sugars. Hence, acclimation of dark respiration under a naturally changing environment is characterized by changes in the temperature sensitivity and apparent capacity of the respiratory apparatus.

The results from controlled and natural-environment studies were used to drive a leaf-level model (which accounted for dark respiratory acclimation) with the aim of

forecasting the overall impact of responses of photosynthesis and respiration in the long term (Chapter 5). Modellers utilise the temperature responses of photosynthesis and respiration to parameterize carbon exchange models but often ignore acclimation and use only instantaneous responses to drive such models. The studies here have shown that this can result in erroneous estimates of carbon exchange as strong respiratory acclimation occurs over longer periods of temperature change. For example, it was found here that the failure to factor for dark respiratory acclimation resulted in the underestimation of carbon losses by foliar respiration during cooler months and an overestimation during warmer months - such discrepancies are likely to have an important impact on determinations of the carbon economy of forests and ecosystems.

The overall results substantiate the conclusion that understanding the effect of variations in temperature on rates of carbon loss by plant respiration is a prerequisite for predicting estimates of atmospheric CO_2 release in a changing global environment. It has been shown here that within a moderate range of temperatures, rate of carbon uptake by photosynthesis exceeds the rate of carbon loss by plant respiration in response to warming as a result of strong respiratory acclimation to temperature change. This has strong implications for models which fail to account for acclimation of respiration. At present, respiration is assumed to increase with increasing temperatures. This erroneous assumption supports conclusions linking warming to the reinforcement of the greenhouse effect.

Chapter 1

Introduction, review of literature and rationale

1.1 Introduction

The recent upward trend in global average temperatures began in the 1970s with increasingly pronounced warming occurring towards the end of the 20th century. The 1990s represented the warmest decade of the millennium, with 1998 the warmest year in that period (IPCC 2007). Hence, it is apparent that we live in a period of global warming. The underlying cause of this warming trend is generally accepted to be the anthropogenic emission of greenhouse gases which are responsible for the re-emission of long-wave radiation back to the earth's surface (the 'greenhouse effect'). The most prevalent greenhouse gas is CO_2, which is also the basic substrate for photosynthesis and thus the raw material for plant growth. Approximately half of all biomass consists of carbon. Therefore, plant growth and the global carbon cycle, which ultimately determines atmospheric concentration of CO_2, are inextricably linked.

Anthropogenic emissions of CO_2 are currently rising rapidly, with 7.0 Pg C year^{-1} coming from fossil fuel burning and cement-making (Morison and Morecroft 2006), and a further 1-2 Pg C year^{-1} from deforestation (IPCC 2007). Approximately 3 Pg of carbon remains

in the atmosphere as CO_2, where its concentration is increasing at a rate of 1-2 ppm by volume each year (Ainsworth and Long 2005). The remainder is dissolved in the ocean or taken up by terrestrial vegetation (Aber et al. 2001). Since plants are organisms whose body temperature varies with the temperature of their immediate environment, and since they commonly cannot move, except through reproduction, physiological acclimation of plant process (e.g. photosynthesis and respiration) are used to cope with changing environmental conditions. Although plants are unable to escape the different environmental perturbations they experience, they have evolved a variety of mechanisms that reduce the direct impact of changing temperatures on plant growth and development. Hence, extensive research has been devoted to the study of the effects of environmental factors on plant growth and metabolism. In terms of plant response to the environment three aspects are of importance: (1) short-term responses, (2) acclimation to new environmental conditions, and (3) survival. Each of these requires very different metabolic and regulatory responses.

It is often assumed that atmospheric warming enhances respiratory carbon losses, causing ecosystem or forest C-stocks to deplete (Walther et al. 2001). However, this is an oversimplistic assumption derived from the instantaneous temperature responses of active tissues. Long-term global warming should not be confused with short-term warming. Given sufficient time the initial effects of an increase in temperature may well be significantly reduced in magnitude, via processes of thermal acclimation. The purpose of the investigation described in this thesis was to assess the effects of changing ambient temperatures and variable levels of nutrition (nitrogen) on physiological and enzymatic

processes underpinning respiration and photosynthesis and their potential to acclimate in two contrasting tree species, *Pinus radiata* and *Populus deltoides*.

1.2 Review of literature

This review deals primarily with the acclimation potential of two main metabolic processes in plants, respiration and photosynthesis. The study of each individual component is not sufficient to comprehend the effects of temperature change on plant carbon balance as both processes are closely coupled. Emphasis is also given to the role of nitrogen and enzymatic processes associated with the process of respiratory and photosynthetic acclimation.

1.2.1 Photosynthesis

Photosynthesis is the primary physiological process responsible for plant carbon acquisition. Effects of environmental factors and stressful conditions on leaf photosynthesis (CO_2 assimilation) have been relatively well studied. Here I focus on the effects of temperature change on plant photosynthesis. Typically, photosynthesis is geared to an optimum temperature which not only varies with species but is different for different component processes (Berry and Bjorkman 1980). Above and below the optimum temperature, photosynthesis is less efficient. Traditionally, the decrease in plant growth at high temperatures is attributed to a higher optimum temperature for respiration than for photosynthesis, thus decreasing the daily net carbon gained. Research in recent years has confirmed this idea through observations of rates of photosynthesis that often decrease sharply at temperatures above the optimum value. At this point the balance

between CO_2 fixation and CO_2 release shifts in favour of release, because (1) the affinity of rubisco for CO_2 declines, (2) the capacity of rubisco activase required to maintain rubisco in an activated state declines to limiting levels (3) electron transport becomes limiting to photosynthesis (Yamori et al. 2005). In contrast, dark respiration steadily (exponentially) increases with temperature until the rate decreases rapidly near the lethal heat limit (Morison and Morecroft 2006). This difference in the temperature responses of photosynthesis and respiration is the basis for the notion that greenhouse warming will lead to a shift in the balance between photosynthesis and respiration in favour of carbon release. However, current environmental conditions appear more favourable for photosynthesis over respiration. Recent studies show that photosynthesis exceeds respiration on a global scale owing to the presence of green, growing plants exerting a positive carbon balance (Grace and Zhang 2006). Additionally, three forms of changes in the environment also exert a positive effect on photosynthesis (Lawlor 1987). These are (1) moderate increase in CO_2, which stimulates photosynthesis (2) warming in areas that are currently too cold for photosynthesis and (3) the deposition of anthropogenic nitrogen as ammonium or nitrate acting as a fertiliser, which enhances photosynthesis.

1.2.1.1 Photosynthesis in evergreen species

The leaf model of photosynthesis presented by Farquhar et al. (1980) assumes that photosynthesis is primarily limited by the slower of two processes (1) the maximum rate of rubisco-catalysed carboxylation and (2) or the regeneration of ribulose-1,5-bisphosphate (RuBP) controlled via the electron transport rate (RuBP-Limited). This model has been used extensively for scaling carbon uptake to canopies, ecosystems and

the biosphere. Furthermore, photosynthesis is temperature sensitive and this has been illustrated in recent studies (Monson et al. 2002; Monson et al. 2005; Zarter et al. 2006a) which have found that photosynthesis is highly responsive to day-to-day environmental changes as well as seasonal changes. However, evergreen species differ from deciduous species because they must respond to annual seasonal cycles that trigger metabolic events leading to acclimation, and thus must have the genetic potential to tolerate climatic extremes for their entire life cycle in order to survive. Therefore, evergreen leaves tend to exhibit lower rates of light-saturated photosynthesis and experience environmental/seasonal changes in photosynthetic capacity more gradually than do leaves of deciduous species. For example, recent reports (Makela et al. 2004; Misson et al. 2006) have shown that the rate of photosynthesis in evergreens varies with season. However, others have reported little seasonality in photosynthetic parameters in evergreens (Damesin et al. 1998; Eamus et al. 1999; Warren & Adams, 2004). Nonetheless, the reduction in photosynthetic rates observed during colder months has been attributed to low temperatures which limit photosynthesis by slowing the rate at which metabolic reactions occur. The cessation of growth in winter greatly reduces sink demand for the products of photosynthesis and thus induces photosynthetic down-regulation (Adams et al. 2002; Adams et al. 2004). In summer, increases in photosynthesis have been attributed to greater sink demand (Turnbull et al. 2002a).

1.2.1.2 Photosynthesis in deciduous species

Unlike evergreen species, phenological limitations in deciduous species enforce great seasonality in photosynthetic parameters (Wilson et al. 2000; Robakowski et al. 2002;

Kosugi et al. 2003; Xu and Baldocchi 2003; Misson et al. 2006). In some deciduous tree species, seasonal variation in photosynthetic response may be linked to seasonal variation in source activity and sink capacity (Sholtis et al. 2004). But generally, rapidly growing deciduous angiosperms tend to rapidly accelerate photosynthetic rates in spring as trees refoliate, photosynthesis remains high during summer, and declines rapidly in autumn as leaves senesce before abscising. As a result, deciduous trees, which will only have current-year leaves, must accomplish all carbon assimilation before the dormant season whereas evergreens are capable of continuing to accumulate dry matter throughout the year if environmental conditions allow. These differences in phenology may explain the significant differences in photosynthesis over seasons between these contrasting tree species.

1.2.1.3 The role of leaf age in photosynthesis

Age-dependent decreases in photosynthesis have been observed in a range of herbaceous (Hikosaka 1996), deciduous (Wilson et al. 2000; Onoda et al. 2005) and evergreen species (Escudero and Mediavilla 2003; Miyazawa et al. 2004; Niinemets et al. 2005). Such a decrease may be explained by: (1) selective degradation of rubisco; (2) inactivation of rubisco; or (3) decreased CO_2 diffusion in old senescing leaves as a result of increased mesophyll resistance (Hikosaka and Terashima 1995; Hikosaka et al. 2007). Another explanation for decreasing photosynthetic capacity in older leaves is that the fraction of nitrogen allocated to rubisco decreases with increasing leaf age (Wilson et al. 2000; Wilson et al. 2001). This finding is further supported by Rey and Jarvis (1998) who observed in their work with birch trees that the fractional allocation of nitrogen to rubisco

decreased with leaf age, and this decrease correlated with a down-regulation in photosynthesis. Several other studies have also shown that the relationship between nitrogen and photosynthetic capacity varies with leaf age. Leaf age driven changes in photosynthetic capacity was also observed by Wilson et al. (2000), where rapid increases in V_{cmax} were seen in spring and decreases in autumn. Similar trends were also observed in maple and oak trees in deciduous forests in Wisconsin (Reich et al. 1991), aspen and red oak trees in Michigan (Jurik 1986) and birch trees in Massachusetts (Bassow and Bazzaz 1998) where large reductions in V_{cmax} were evident alongside visible evidence of senescence.

1.2.1.4 Photosynthetic acclimation

The photosynthetic machinery in plants is subject to a range of environmental perturbations, and photosynthetic acclimation can occur in response to changing irradiance, water or nutrient supply (Turnbull et al. 1993; Frak et al. 2001; Noguchi et al. 2001b; Krause et al. 2004). However, since the focus of this thesis is on the effects of temperature change on acclimation, this review will attempt to provide an in-depth assessment into the current literature available on this subject area. Medium and long-term exposure (one to several days, up to a full season) to a new temperature regime results in adjustments in photosynthesis, causing the baseline responses to shift (Larcher 1969; Teskey and Will 1999; Gunderson et al. 2000; Makela et al. 2004). For instance, Douglas-fir seedlings were found to have adjusted their photosynthetic response to a new thermal regime within a 10-day period (Sorensen and Ferrell 1972). Numerous recent studies have also shown that photosynthesis can acclimate to changes in growth

temperature, with the result that rates of photosynthesis are similar in plants grown under contrasting thermal regimes (complete acclimation) (Berry and Bjorkman 1980; Hurry et al. 1995; Bunce 2000). Complete acclimation of photosynthesis was also observed some 40 years ago by Mooney and West (1964) who worked on the dessert shrub, *Larrea divaricata*, which was grown at three day/night temperature regimes ranging from 20/15 ^{o}C to 45/33 ^{o}C. All plants expressed similar photosynthetic rates when measured at the corresponding growth temperatures. Furthermore, Turnbull et al. (2002a) in their work with *Populus deltoides* exposed to day time warming also concluded that the optimum temperature of photosynthesis acclimated fully to a 6 ^{o}C range of temperature. However, it has been observed that different species have varying potential for photosynthetic acclimation (Gunderson et al. 2000; Weston and Bauerle 2007) where some species only display partial acclimation of photosynthesis whilst others exhibit complete acclimation (Berry and Bjorkman 1980; Rogers et al. 1998; Usami et al. 2001; Sholtis et al. 2004). This variability in acclimation is supported by reports from a number of tree species that have shown varying degrees of photosynthetic acclimation to temperature (Rook 1969; Smith and Hadley 1974; Strain et al. 1976; Slatyer 1977; Battaglia et al. 1996; Teskey and Will 1999; Medlyn et al. 2002b; Onoda et al. 2005; Misson et al. 2006).

Much effort has been placed on gaining a better understanding on the mechanisms underpinning the process of photosynthetic acclimation to higher as well as lower temperatures. For example, Berry and Bjorkman (1980), Stitt and Hurry (2002) and Yamori et al. (2005) have concluded that when first exposed to low temperatures, photosynthetic rates are typically strongly reduced. However, with subsequent

acclimation, increases in rates of photosynthesis occur as a result of a higher degree of unsaturation of membrane lipids and a greater concentration of proteins regulating photosynthetic capacity. But, prolonged exposure to low temperatures can result in photosynthetic photoinhibition (Lundmark et al. 1988; Alscher and Cumming 1990). The susceptibility to photoinhibition results from low temperatures causing (1) inhibition of photosynthesis thereby creating conditions for excessive excitation to occur, (2) inhibition of *de novo* protein synthesis necessary for repair of photodamage, and (3) inhibition of alternative ways of dissipating excessive excitation thus resulting in the accumulation of potentially harmful oxygen species (Schulze and Caldwell 1994; Morison and Morecroft 2006). However, plants often recover fully from cold-induced photoinhibition within hours to days, depending on species and the extent of inhibition (Lundmark et al. 1988). Though the underlying mechanisms involved in photosynthetic acclimation to changing environmental conditions play a key role in our understanding, the biochemical and molecular aspects of acclimation of photosynthesis to changing temperatures are out of the scope of this thesis, although they are currently under intense scrutiny elsewhere (see Sage and Kubien (2007)).

1.2.1.5 Leaf age and its effects on photosynthetic acclimation

The developmental state of leaves and its role in photosynthetic acclimation to temperature was investigated by Yelle et al. (1989), where young leaves apparently showed less acclimation to temperature than older leaves. Many studies have also observed photosynthetic acclimation to temperature to be greater in older foliage or occurring earlier in older and later in younger leaves (Turnbull et al. 1998; Griffin et al.

2000; Jach and Ceulemans 2000; Tissue et al. 2001; Luomala et al. 2003; Makela et al. 2004). However, other studies have found that photosynthetic acclimation to temperature may be greater in young leaves (Vu et al. 1997). This latter finding is further supported by Strand et al. (1999) who worked on *Arabidopsis* and found that new, young leaves developed at the new temperature acclimate more fully to changes in temperature than previously developed (older) leaves. There is considerable evidence in the literature suggesting that, although acclimation of photosynthesis to a new growth temperature can occur in pre-existing (older) leaves formed at the previous growth temperature, full acclimation to a new growth temperature requires leaves to be formed at the new growth temperature (Hurry et al. 1995; Strand et al. 1997; Loveys et al. 2002; Atkin et al. 2006a)**.** Clearly it is important to distinguish between the direct impacts of leaf developmental age versus the response of pre-existing and new leaves following temperature changes.

1.2.2 Respiration

The primary function of photosynthesis is to assimilate CO_2 into carbohydrates. A significant portion of the carbohydrate pool then becomes the main substrates of respiration. The function of respiration is to convert photoassimilates into products used in growth, maintenance, transport and nutrient assimilation processes. The energy conserved during photosynthesis is released in a biochemically regulated manner for the production of ATP via respiration. At the most fundamental level, respiration can be considered to have a dual nature: it is the source of metabolic intermediates (carbon

skeletons) used in the synthesis of cellular constituents as well as the source of ATP and reduced nucleotides.

Temperature is one of the most important environmental factors determining the rate of respiration. However, there is strong evidence suggesting that the short and long term response of respiration to temperature change is very different. The Q_{10} (the proportional increase in respiration for every 10 ^{o}C rise in temperature) of respiration describes the short-term sensitivity of respiration to temperature. There is, however, no consensus in the literature over the variability of this temperature coefficient. In a recent review, Atkin et al. (2005a) concluded that growth temperature had no consistent effect on Q_{10} values. This was supported by earlier work by Tjoelker et al. (1999a) who reported that the growing temperature environment had no significant effect on the Q_{10} of respiration. This has been further supported by numerous recent reports (Loveys et al. 2003; Armstrong et al. 2006a; Atkin et al. 2007; Atkinson et al. 2007) on a wide range of plant species. However, some studies have reported a changing Q_{10} (e.g. values of Q_{10} decrease with increasing temperature) (Slator 1906; Fukai and Silsbury 1977; Lawrence and Oechel 1983; Farrar and Williams 1991; Tjoelker et al. 2001; Ziska et al. 2004).

Despite the lack of consensus on the variability of Q_{10}, changes in respiration rates with changing environmental conditions are a widely accepted finding. Early reviews focusing on the effects of low temperature on plant respiration include those by Lyons and Raison (1970), Lyons (1973) and Long and Woodward (1988) – these concluded that respiratory capacity increased at low temperatures. This finding was further supported by work

conducted by Farrar and Williams (1991), Ryan (1995) and Reich et al. (1996). Similarly, some recent studies have also shown that plants grown or originating from cold climates tend to exhibit higher rates of dark respiration (Covey-Crump 2002; Lee et al. 2005). By contrast, Eagles (1967), Lambers (1985), Criddle et al. (1994) and Breymeyer et al. (1996) have reported that the effects of elevated temperature on respiration may sometimes vary but increases in respiration often correlate positively with increasing temperatures. Because this is likely to have a significant impact on global carbon budgets, many studies have since looked at the role of increasing atmospheric temperatures on respiration as global records reveal a warming trend. Recent reports by Teskey and Will (1999), Atkin and Tjoelker (2003), King et al. (2006), Rachmilevitch et al. (2006) and Wright et al. (2006) have further confirmed the finding that respiration generally increases with increasing temperature over a short time period. In the short-term, a change in temperature will result in an immediate alteration in the rate of respiration, with the extent of that alteration determined by the respiratory Q_{10}. However, long-term exposure to a change in temperature is likely to result in regulatory changes in respiratory metabolism which bring about either partial or full acclimation of respiration (Atkin et al. 2000b; Covey-Crump 2002; Griffin et al. 2002b; Bolstad et al. 2003; Atkin et al. 2005a; Atkin et al. 2006b; Armstrong et al. 2006a; Morison and Morecroft 2006; Wright et al. 2006).

1.2.2.1 Respiratory acclimation

Evidence of respiratory acclimation is extensive, but the effect varies from no acclimation (i.e. the instantaneous and the long-term temperature responses are identical) to complete

acclimation, where rates measured at the respective growth temperatures do not differ (Larigauderie and Korner 1995; Teskey and Will 1999; Tjoelker et al. 1999a; Atkin et al. 2000a; Gunderson et al. 2000; Atkin and Tjoelker 2003; Lee et al. 2005; Turnbull et al. 2005). Some preliminary evidence of respiratory acclimation was reported some 70 years ago, where the German ecophysiologist (Otto Stocker) noted that leaves of tropical trees in Java respired at about the same rate as leaves of willows in Greenland, when both were measured at temperatures in their natural habitats (reported by Hopkins 1998; Morison and Morecroft 2006). A more recent example is seen in wheat cultivars (Kurimoto et al. 2004a; Kurimoto et al. 2004b) which were found to exhibit almost complete acclimation so that their rate of respiration stayed constant over a 10 ^{o}C shift in growth temperature. Importantly, acclimation of respiration to temperature change can be significant and rapid (occurring in as quickly as 1-3 days after exposure to changed temperature regimes). Experiments on *Quercus* species conducted largely in the laboratory (Bolstad et al. 2003) have observed respiratory acclimation to temperature within two days. This finding is further supported by Rook (1969), Larigauderie and Korner (1995), Atkin et al. (2000b) and Yamori et al. (2005). Furthermore, Atkin et al. (2000b) and Gunderson et al. (2000) also observed significant thermal acclimation in field-grown maple seedlings such that warm-acclimated seedlings decreased respiration by 10% compared with cool-acclimated seedlings. In addition, Tjoelker et al. (1999b), in their work with five boreal tree species, also observed thermal acclimation of respiration, with acclimation greater in conifers than in broadleaved species.

1.2.2.2 Mechanisms of acclimation

In a recent review on the effects of temperature on respiration, Atkin et al. (2005a) proposed that there were at least two different modes of respiratory acclimation to temperature. "Type I" acclimation that is associated with a change in the rate of respiration primarily at moderate to higher temperatures, with little to no change in respiration occurring at low measuring temperatures (i.e. the Q_{10} value changes). Type I acclimation appears to reflect a change in the availability of respiratory substrates and/or the degree of adenylate restriction (Atkin and Tjoelker 2003). Moreover, Type I acclimation has been suggested to be rapid, occurring within 1 to 2 days following a change in ambient temperature (Rook 1969; Atkin et al. 2000; Covey-Crump 2002; Bolstad et al. 2003). "Type II" acclimation is associated with an increase in the rate of respiration across a wide range of measurement temperatures (no change in the Q_{10} of respiration is necessary in Type II acclimation). Type II acclimation is likely to be associated with temperature-mediated changes in respiratory capacity that can only be maximally realised through the formation of new tissues with altered foliar morphology and biochemistry (Atkin and Tjoelker 2003). Type II acclimation has been reported to be associated with changes in the relative amounts of enzymes (e.g. alternative oxidase (AOX)).

1.2.2.3 The role of leaf age in respiration

As with photosynthesis, the rate of respiration is also dependent upon the developmental state of tissues, with expanding immature tissues having higher rates of respiration than fully expanded, mature tissues (Azcon-Bieto and Osmond 1983b; McDonnell and Farrar

1993; Atkin and Cummins 1994; Millar et al. 1998; Armstrong et al. 2006a; Atkin et al. 2007). This decrease in respiration associated with tissue expansion reflects a decrease in demand for ATP required for growth. However, various other factors may also be responsible for the decrease in rates of respiration as tissues expand – these include changes in protein abundance and alterations in the density of mitochondria. Apart from changes in respiration rates, the developmental state of foliage has also been shown to determine the extent of respiratory acclimation to temperature in different tissue types (e.g. pre-existing *versus* new leaves). For example, newly emerged, young leaves (formed at the new temperature environment) have been shown to be able to exhibit complete acclimation to changing environmental conditions (Loveys et al. 2003; Talts et al. 2004; Armstrong et al. 2006a; Atkin et al. 2006a; Atkin et al. 2006b). Complete acclimation in these tissues are possible because new tissues have the ability to alter their structure (i.e. anatomy/morphology), chemical composition, and enzymatic capacity to the degree required for complete acclimation of respiration. However, this finding is by no means universal and is likely to be especially variable in species with long-lived foliage, where complete acclimation can occur in pre-existing, mature leaves if the tissues are sufficiently long-lived. In short-lived species, such changes cannot occur due to senescence of mature leaves (Bruhn et al. 2007; Zaragoza-Castells et al. 2007).

1.2.2.4 Emerging role of the alternative oxidase (AOX) pathway in respiration

Higher plant mitochondria have two respiratory electron transport pathways. One is the cytochrome (COX) pathway, which is similar to that in animals (hence much is known about it), and the other is the alternative-cyanide-resistant pathway (AOX). Both

pathways are involved in the consumption of substrate and in CO_2 emission and O_2 uptake, but the alternative pathway is nonphosphorylating and therefore largely uncoupled from energy production (lacking in proton translocation). The basis of this pathway is a short branch in the mitochondrial electron transport chain prior to the cytochrome *c* oxidase (Lambers 1985). The alternative pathway takes electrons from the ubiquinone pool and passes the electrons to O_2 through an oxidase (AOX) (Amthor 2000). Compared to the cytochrome pathway, conservation of energy for the production of ATP is substantially reduced, and free energy initially generated is lost as heat. For most plants, the precise role of this pathway has not been defined, except that the heat produced has been found to be used by some species (e.g. the spadix of the arum lilies) to volatilise attractants for insect pollination (Breymeyer et al. 1996). High levels of carbohydrates have been found to activate the AOX pathway (Steingrover 1981; Azcon-Bieto and Osmond 1983b). The latter finding has led to the suggestion that AOX may be responsible for consuming excess levels of carbohydrates within a plant via rapid glycolysis or greater activity of the Krebs cycle (Lambers 1982).

There is emerging evidence in the literature suggesting that at low temperatures or in cold climates, a higher proportion of mitochondrial electron transport occurs via the alternative (AOX) pathway (Lambers 1985; Stewart et al. 1990; Vanlerberghe and McIntosh 1992; Gonzalez-Meler et al. 1999; Kurimoto et al. 2004b). For example, in the early 1980s, Kiener and Bramiage (1981) observed that chilling hypocotyls of cucumbers resulted in an increase in respiration rate which was partly due to increased activity of AOX. This finding was further supported by Gonzalez-Meler et al. (1999) and Purvis and

Shewfelt (1993) who also observed increased electron partitioning to AOX at lower temperatures. Moreover, several reports (Vanlerberghe and McIntosh 1992; Gonzalez-Meler et al. 1999; Ribas-Carbo et al. 2000; Atkin et al. 2002; Kurimoto et al. 2004b; Fiorani et al. 2005; Atkin et al. 2007) have also observed the absence of AOX activity at higher temperatures but engagement of the AOX pathway under cooler conditions. Overall, these findings suggest that AOX may be temperature sensitive and at low temperatures has a major role in plant respiration. The significance of this may be to prevent the formation of toxic oxygen species that may result from an over-reduction of the ubiquinone pool following inhibition of COX at low temperatures (Purvis and Shewfelt 1993; Wagner and Krab 1995; Maxwell et al. 1999; Wright et al. 2006). Additionally, reports investigating the responses of Q_{10} in these two pathways came to the conclusion that the Q_{10} of AOX was lower than that of COX (McNulty and Cummins 1987; Stewart et al. 1990). However, Gonzalez-Meler et al. (1999) found that the Q_{10} of the AOX and COX pathways in mung bean leaves and soybean hypocotyls were similar. These conflicting findings in the literature are good reasons to suggest that more research is required before we can gain an in-depth understanding of the role AOX plays in plant respiration.

The classical approach used to measure the activity of the AOX and COX pathways in isolated as well as in intact tissues involves the use of inhibitors (e.g. potassium cyanide (KCN) and salicylhydroxamic acid (SHAM)). AOX activity is determined from the rate of respiration in the presence of KCN, which blocks the COX pathway, and corrected for residual respiration in the presence of both KCN and SHAM. COX activity is determined

from the decrease in the rate of respiration when SHAM is added in the absence of KCN. (Hoefnagel et al. 1995; Ribas-Carbo et al. 1995; Noguchi et al. 2001a). However, this technique only provide estimates of maximum rates of *in vivo* AOX and COX activity which does not necessarily reflect true *in vivo* capacity of both pathways. Furthermore, increased capacity of either pathway does not necessarily indicate an increase in the actual electron flow through both pathways in the absence of inhibitors (Day et al. 1996; Lennon et al. 1997). The only reliable methodology presently available to study electron partitioning between AOX and COX in the absence of inhibitors is the use of the oxygen-isotope fractionation (differences in the isotopic fractionation of ^{18}O between the two terminal oxidases) method (Gonzalez-Meler et al. 1999; Nagel et al. 2001; Noguchi et al. 2001a). This technique can be used on intact tissues or isolated mitochondria and enzymes (Ribas-Carbo et al. 2000).

1.2.2.5 Respiration / photosynthetic (*R*/*P*) ratio

From the point of view of plant carbon balance, studying photosynthesis or respiration in isolation is problematic. Interactions between these plant processes must be taken into account. Plant growth should be proportional to the balance between carbon gains and carbon losses. Hence, it is important to identify the major components of this balance, since it is only when all the gains and losses of carbon by a plant are considered that a full account of the carbon balance, and growth, can be made.

Because photosynthesis and respiration are temperature sensitive, a change in temperature results in immediate alteration in the rate of photosynthesis and respiration,

with the extent being determined by the temperature coefficient of each process (Atkin et al. 2007). Many authors have reported that dark leaf respiration and photosynthesis are interdependent, with respiration relying on photosynthesis for substrate, whilst photosynthesis depends on respiration for a range of compounds, such as carbon skeletons for protein synthesis and ATP for sucrose synthesis (Raghavendra et al. 1994; Hoefnagel et al. 1995; Hopkins 1998; Thornley and Cannell 2000; Atkin et al. 2005a; DeLucia et al. 2007). Furthermore, the temperature sensitivity of photosynthesis differs from that of respiration and as a result, *R*/*P* is altered following short-term (i.e. minutes to hours) changes in measuring temperature (Atkin et al. 2006b). However, in many species, homeostasis of *R*/*P* is re-established for plants experiencing contrasting temperatures over sustained periods (i.e. as a result of thermal acclimation of specific rates of respiration and photosynthesis) (Dewar et al. 1999; Tjoelker et al. 1999a; Gifford 2003; Loveys et al. 2003), although this is not a universal finding and it is likely to vary between species. Nonetheless, a large amount of support for a homeostatic *R*/*P* ratio is available in the literature. For example, Gifford (1995) found that when a diverse range of species was grown at constant temperatures ranging between 15 to 30 ^{o}C, the *R*/*P* ratio was constant. Likewise, soybean (*Glycine max*) grown at a range of growth temperatures between 20 and 35 ^{o}C showed no difference in their *R*/*P* values, owing to acclimation of respiration to temperature (Ziska and Bunce 1997). This conclusion is further supported by Loveys et al. (2002), who also observed no difference in *R*/*P* values of plants grown at 18 and 23 ^{o}C, but four out of the six species they investigated exhibited higher *R*/*P* values at 28 ^{o}C. They concluded that the response of *R*/*P* to growth temperature varies with species. Therefore, the general conclusion is that *R*/*P* is relatively homeostatic at

moderate growth temperatures (Gifford 1995; Dewar et al. 1999; Loveys et al. 2003; Atkin et al. 2006b; Atkin et al. 2007) but increases often occur when plants are exposed to unfavourably high temperatures. Conversely, the absence of a homeostatic *R*/*P* ratio is also likely to occur when plants are exposed to very low temperatures, because respiration and photosynthesis do not have identical temperature responses to cooler conditions (Atkin et al. 2005a; Atkin et al. 2006b). Furthermore, respiration and photosynthesis may differ in their ability to acclimate to low temperatures (Atkin et al. 2006b). In addition, many recent reports have suggested that, because respiration is more temperature sensitive in the short-term than photosynthesis, the *R*/*P* ratio is likely to increase under growth conditions of elevated temperatures (Tjoelker et al. 1999a; Atkin *et al.*, 2000b; Loveys et al. 2002; Atkin et al. 2006b; Rachmilevitch et al. 2006; Atkin et al. 2007). Nonetheless, the evidence from numerous studies support the presence of a constant *R*/*P* ratio over contrasting growth temperatures, and global carbon cycle models often assume homeostasis of this ratio.

1.2.2.6 Plant respiration in a warmer world

Warming could potentially increase the biological release of carbon to the atmosphere via plant respiration, which at the global scale currently accounts for at least 60 gigatons (Gt) of carbon released into the atmosphere each year. This is a massive flux compared with the relatively small release of CO_2 from the combustion of fossil fuels (<6 Gt C year $^{-1}$) (Houghton et al. 2001; Schimel et al. 2001; Gifford 2003). Both short- and long-term variation in ambient air temperature could have profound effects on the carbon balance of forests. Acclimation of respiration to elevated temperatures has clear implications for

predictions and expectations of higher plant respiration in a warmer world. For example, reduced sensitivity of respiration to temperature increase (as discussed earlier) could reduce the magnitude of the positive feedback between climate and the carbon cycle. With strong acclimation, actual leaf respiration at higher temperatures predicted for the end of the 21st century may well be significantly reduced, and more carbon will be stored in forests (King et al. 2006). This would correspond to less carbon released back into the atmosphere and a weaker amplification of additional greenhouse-effect warming.

1.2.3 The importance of nitrogen

Tissue N concentration is an important determinant of the rate of key physiological processes in plants, such as photosynthesis and respiration (Lewis et al. 2004; Takashima et al. 2004). Like photosynthesis and respiration, nitrogen content in leaves is temperature sensitive (Rachmilevitch et al. 2006). Leaf N originates from the soil, hence the concentration and total pools of N within the leaf is dependent upon the capacity of the soil to provide N to the roots and the capacity of the root to supply the whole plant with N (Schulze and Caldwell 1994; Reich et al. 2006). This distribution of nutrients can often be affected by environmental constraints such as temperature, vapour pressure deficit and irradiance (Foster and Aber 1997; Wang et al. 2002; Lewis et al. 2004).

1.2.3.1 Nitrogen (N) and photosynthetic acclimation

There is overwhelming evidence of a strong correlation between the rate of photosynthesis and foliar N concentration (Field and Mooney 1983; Evans 1989; Reich et al. 1991; Sullivan et al. 1996; Walcroft et al. 1997; Reich et al. 1998a; Carswell et al.

2000; Dreyer et al. 2001; Frak et al. 2001; Turnbull et al. 2002b; Takashima et al. 2004; Diaz-Espejo et al. 2006; Morison and Morecroft 2006; Yasumura et al. 2006), although some studies show mixed/inconclusive results (Lawlor 1987; Machler et al. 1988; Cheng and Fuchigami 2000; Wilson et al. 2001). The positive correlation between N and photosynthesis may be explained by the proportion of photosynthetic N allocated to rubisco (the nitrogen-rich carbon-fixing enzyme), chlorophyll and the electron transport in the thylakoid membranes – all are important in determining rates of photosynthesis and, subsequently, photosynthetic nitrogen use efficiency (PNUE) (Evans 1989; Foster and Aber 1997; Frak et al. 2001; Adams 2004; Takashima et al. 2004; Atkinson et al. 2007; Bown et al. 2007). Increased leaf N is required to support increased rates of rubisco carboxylation and RuBP regeneration via electron transport which are associated with increased rates of photosynthesis (Wullschleger 1993). Though N is usually considered to be the foliar nutrient that most affects photosynthesis, conifers in particular appear to be less responsive to increase N concentrations than most other plant species (Field and Mooney 1983; Evans 1989; Boyce et al. 2006). Interestingly, the relationship between photosynthesis and N concentration is believed to also vary with the developmental stage of leaves (Field 1983; Field and Mooney 1983; Kull et al. 1998; Wilson et al. 2000; Wilson et al. 2001; Dungan 2003). For example, Schoettle and Smith (1999) found a relationship between photosynthesis and nitrogen concentration in young foliage of *Pinus contorta* (lodgepole pine) but no relationship was found with middle-aged or old foliage. This lack of a relationship in older foliage was further confirmed by Rey and Jarvis (1998), who observed that the fractional allocation of N to rubisco in birch trees

decreased with leaf age, and more importantly, this decease correlated with a down-regulation in photosynthesis.

The association between N and photosynthetic acclimation to environmental conditions has received some attention. There is evidence in the literature suggesting that photosynthetic acclimation to elevated CO_2 tends to be more pronounced under N limitation (Bazzaz 1990; Gunderson et al. 2002). Nutrient limitation has been suggested as the driving force for acclimation in general, since acclimation tends to increase PNUE (Curtis 1996). However, experimental evidence supporting this hypothesis has so far been inconclusive (Bunce 1992; Schulze and Caldwell 1994; Gunderson et al. 2002). Additionally, attention has also been placed on investigation of the role of foliar N in photosynthetic acclimation to temperature. For example, Martindale and Leegood (1997) concluded that N supply can affect the extent of cold acclimation of the photosynthetic apparatus, a phenomenon that requires additional N investment in chloroplastic and cytosolic proteins (Stitt and Hurry 2002). By contrast, photosynthetic acclimation to elevated temperatures has been explained by the reallocation of N away from the photosynthetic apparatus to other parts of a plant resulting in reduced photosynthetic rates (Field et al. 1992; Krapp and Stitt 1995; Sholtis et al. 2004; Dwyer et al. 2007). It is noteworthy however, that this down regulation of photosynthesis may not be solely associated with the transport of N away from the photosynthetic apparatus, but may be a result of a dilution effect brought about by starch and sugar accumulation in leaves. Furthermore, Misson et al. (2006), in their study on forest ecosystems, suggested that acclimation of photosynthetic parameters to seasonal changes in temperature are

controlled by a range of factors (e.g. light, soil water content and leaf developmental stage). Nonetheless, N content is considered one of the key factors determining the plant's ability to acclimate to seasonal or temperature changes. This finding is further supported by Field and Mooney (1983), Breymeyer et al. (1996), Dang et al. (1998), Wilson et al. (2000), Xu and Baldocchi (2003) and Grassi et al. (2005). Therefore, we can conclude that photosynthetic acclimation to temperature involves adjustments of photosynthetic capacity and the reallocation of N between photosynthetic components.

1.2.3.2 Nitrogen (N) and respiratory acclimation

Although the impact of N supply on respiratory acclimation is not well understood, there is *a priori* evidence suggesting that N availability could influence respiratory acclimation. For example, linear relationships between leaf N and specific rates of respiration have been found in trees, shrubs and herbaceous species (Ryan 1995; Reich et al. 1996; Reich et al. 1998b; Tjoelker et al. 1999b; Dreyer et al. 2001; Griffin et al. 2001; Griffin et al. 2002a; Loveys et al. 2003; Turnbull et al. 2003; Lee et al. 2005; Morison and Morecroft 2006; Noguchi and Terashima 2006; Reich et al. 2006; Atkinson et al. 2007). However, there are also studies which have failed to find a relationship between leaf N and respiration (Pavlik 1983; Byrd et al. 1992; Poorter et al. 1995; Wang et al. 2002). Interestingly, Tjoelker et al. (1999b) observed that thermal acclimation of respiration to higher temperatures in five boreal tree species was larger for conifers than broad-leaved species and this was associated with pronounced reductions in leaf N concentrations in conifers at higher temperatures. This difference in responses between species may be attributed to differences in leaf structure and chemistry whereby, unlike the broad-leaved

species, the needle-leaved conifers simply have a greater capacity to bring about reduction in leaf N concentration in response to higher growth temperatures. Similarly, Bolstad et al. (2003), working on *Quercus* tree species, also concluded that the downward acclimation of respiration at higher temperatures is likely to be attributed to pronounced reductions in leaf N concentrations. On the other hand, Ryan (1995), Tjoelker et al. (1999b) and Atkin et al. (2006b) have concluded that growth in the cold often results in the accumulation of N in the leaf and this has been found to be positively correlated to leaf respiration. This conclusion is further supported by the work of Korner (1989) and Atkin et al. (2006a). Cold acclimation of respiration is dependent on increases in the capacity for mitochondrial respiration (Armstrong et al. 2006b), which in turn must be supported by an increase in N investment in respiratory protein. Hence, limitations in N supply could restrict the extent to which respiration acclimates to low growth temperatures. By contrast, a recent report by Atkinson et al. (2007), who worked on several herbaceous plant species grown at both high and low N availability (2000 and 25 μM, respectively) which were transferred from warm to cooler conditions (25 / 20 ^{o}C (day/night) to 15 / 10 ^{o}C), showed that the accumulation of N in leaves is not essential for cold acclimation. Despite the conflicting findings present in the literature, there is strong evidence suggesting that the degree of cold and warm acclimation may be greatest in leaves that are able to exhibit large changes in the availability of respiratory substrates (i.e. soluble sugars) and/or nitrogen (i.e. protein) concentrations (Loveys et al. 2003).

1.2.4 Modelling of plant carbon fluxes

Models are an important tool for understanding forest and ecosystem function as well as predicting responses to global change. Models help summarise the results of many individual experiments by incorporating hypotheses and conclusions into a quantitative framework. Models can provide estimates as well as be used in the simulation of long-term experiments. More importantly, models help with the prediction of future rates of photosynthesis and respiration at a range of scales from the leaf level to global carbon cycle models (GCMs) (Atkin et al. 2005b). At present, most models assume that respiration and photosynthesis will increase with temperature in a fixed response (i.e. both processes will not acclimate to future changes in temperature) (Loveys et al. 2003; Armstrong et al. 2006a; Atkin et al. 2006b). So far, a range of model simulations have given rise to large differences between estimates of storage and fluxes under current and future climates (Breymeyer et al. 1996; Aber et al. 2001; Jarvis et al. 2004), so uncertainties still exist about the responses of forests and ecosystems to changes in air temperature and atmospheric CO_2. For example, model estimates differ in (1) the amount of carbon sequestered over a forest stand's lifetime; (2) the response of productivity and carbon release to increased atmospheric temperature and CO_2. The differences in estimates generated from various models suggests that we are currently still unable to provide accurate predictions on how carbon sequestration in various forest stands will respond to future climatic conditions and much work is still required before this goal can be achieved.

Although our understanding of the ecophysiology of plant carbon exchange has come a long way, it is still far from being predictive at a global scale. The challenges of scaling-

up from plants to canopies, landscapes, and even the global level is the driving force behind many efforts in modelling and innovative approaches to validations of various models. Furthermore, only few models until recently have simulated how photosynthesis and respiration rates might acclimate to increased temperature as climate warming occurs (Korner 1995). Acclimation is a process that could affect the response of plant or forest productivity and more importantly, influence estimates of carbon release into the atmosphere over long periods (Bergh et al. 1998; Luo et al. 2001; Loveys et al. 2003; Makela et al. 2004). Even small fractional changes (as a result of acclimation) in respiration can have large impacts on calculations of carbon sequestration (Turnbull et al. 2005). For example, some early as well as recent (1987 – 2003) carbon balance models often use a static Q_{10}, a static R_d parameter, or both, to describe the short-term response of respiration and virtually ignore temperature acclimation (a list of these models can be found in Wythers et al. (2005)) . The estimates from these models are often an over prediction of respiration and an under prediction of productivity which is especially evident over long periods. To avoid the above inconsistencies, Tjoelker et al. (2001) suggested that models should incorporate respiratory acclimation and a temperature-driven Q_{10}. The inclusion of temperature-variable parameters is of even greater significance if models are to be applied across a large spatial extent where a broad range of climates is expected. This suggestion is supported by Gifford (1994), Gifford (1995), Arnone and Korner (1997), Dewar et al. (1999) and Gifford (2003) who went further to suggest that carbon cycle models using temperature-driven algorithms may result in homeostasis of whole-plant respiration / photosynthesis (R / P) in cool to moderate temperature environments (i.e. R / P is insensitive to growth temperature). Therefore, the

above findings suggest that current modelling of climate change and carbon exchange, which generally ignores thermal acclimation, may be flawed. For example, Wythers et al. (2005) observed differences (reductions) in foliar respiration and increases in net primary productivity using temperature-driven parameters in their PnET ecosystem model. By contrast, Law et al. (2000) used static parameters to drive an ecosystem model and came to the conclusion that carbon release from a *Pinus ponderosa* forest was enhanced at higher atmospheric temperatures and productivity negatively affected. Additionally, Wythers et al. (2005) also observed that predicted foliar respiration using static respiratory parameters increased by 8% and 11% under two other climate warming scenarios (KONZ and COWET respectively). However, when modified algorithms were incorporated into the above models, predicted foliar respiration only increased by 2 and 1% respectively. Similarly, King et al. (2006) reported that the incorporation of acclimation of leaf respiration into a global ecosystem model (GTEC 2.0) resulted in lower predicted rates of leaf respiration at higher temperatures and more carbon stored in both plants and soils. These are emerging evidence supporting the use of temperature-driven parameters in models. Simply adhering to the use of static parameters may contribute to incorrect predictions of future conditions. The influence of acclimation to temperature has been found (as seen in earlier sections of this review) to be of a significant magnitude and there is increasing evidence that it should be incorporated into plant, forest or ecosystem climate-carbon simulations.

1.3 Rationale of the present study

From the literature review presented above, it is clear that acclimation of photosynthesis and respiration to temperature can occur in plants but may differ in the degree and speed in which the process takes place. Of particular focus in this present study are the effects of changing temperature on the potential and extent of photosynthetic and respiratory acclimation. It is well documented in the literature that photosynthesis and respiration tend to exhibit a quasi-exponential instantaneous response to increasing temperature. However, beyond a temperature optimum, rates of both processes decline. In the case of respiration, the temperature optimum is often much higher than that of photosynthesis and is only reached just below lethal temperatures. This results in rates of respiration rising over a greater range of temperatures than photosynthesis. This can potentially result in increase emissions of CO_2 at higher atmospheric temperatures, ultimately acting as a positive feedback to the greenhouse effect. It is with this in mind that I investigated the *potential of acclimation of both photosynthesis and respiration in both an evergreen and deciduous tree species to temperature change under both controlled and field conditions.*

The well-established hypothesis of a positive relationship between photosynthesis, respiration and nitrogen in the literature led to the investigation of the *role of nutrition (focused solely on N) in the process of acclimation*. Furthermore, since respiration was the parameter of key focus here and because of previous speculation regarding the role of COX and AOX pathways in responses to changing or stressful conditions, I also studied *changes in the activity of COX and AOX to temperature change*. More importantly, the

data collected during the course of this research were used to develop an existing *leaf-level model* driven by meteorological data to simulate the effects of temperature on rates of photosynthesis and respiration over long periods and to generate annual estimates of both processes. This is important as presently only few studies have accounted for acclimation of both photosynthesis and respiration in their models.

1.4 Simplified schematic illustration

Figure 1 describes the overarching design for this research. As previously mentioned, acclimation of photosynthesis and respiration can occur in a range of plant species. However, the extent to which acclimation occurs in different tissue types (e.g. pre-existing and new tissues) has not been widely studied. Pre-existing and new leaves can possess significant differences in anatomy, biochemistry or morphology as a result of a change in the environment and these differences may determine the extent to which a leaf acclimates to temperature change. In addition, the importance of nutrition (especially nitrogen) in photosynthesis and respiration is well documented in the literature. Therefore, the role of nitrogen in acclimation to temperature change is likely to be important. In this study the role of N was investigated by comparing young trees grown with high and low levels of N availability. Furthermore, to date there have been few field studies undertaken over an entire year with data for both photosynthesis and respiration (over different seasons). This lack of data over long periods has resulted in models typically being driven by a single fixed respiration rate and then adjusted by a fixed Q_{10} – this gives rise to the potential for erroneous estimates. Field analysis conducted for an entire year provides data to ensure that annual estimates of photosynthesis and respiration

in an evergreen and deciduous species is more robust, especially as the process of temperature acclimation is taken into account.

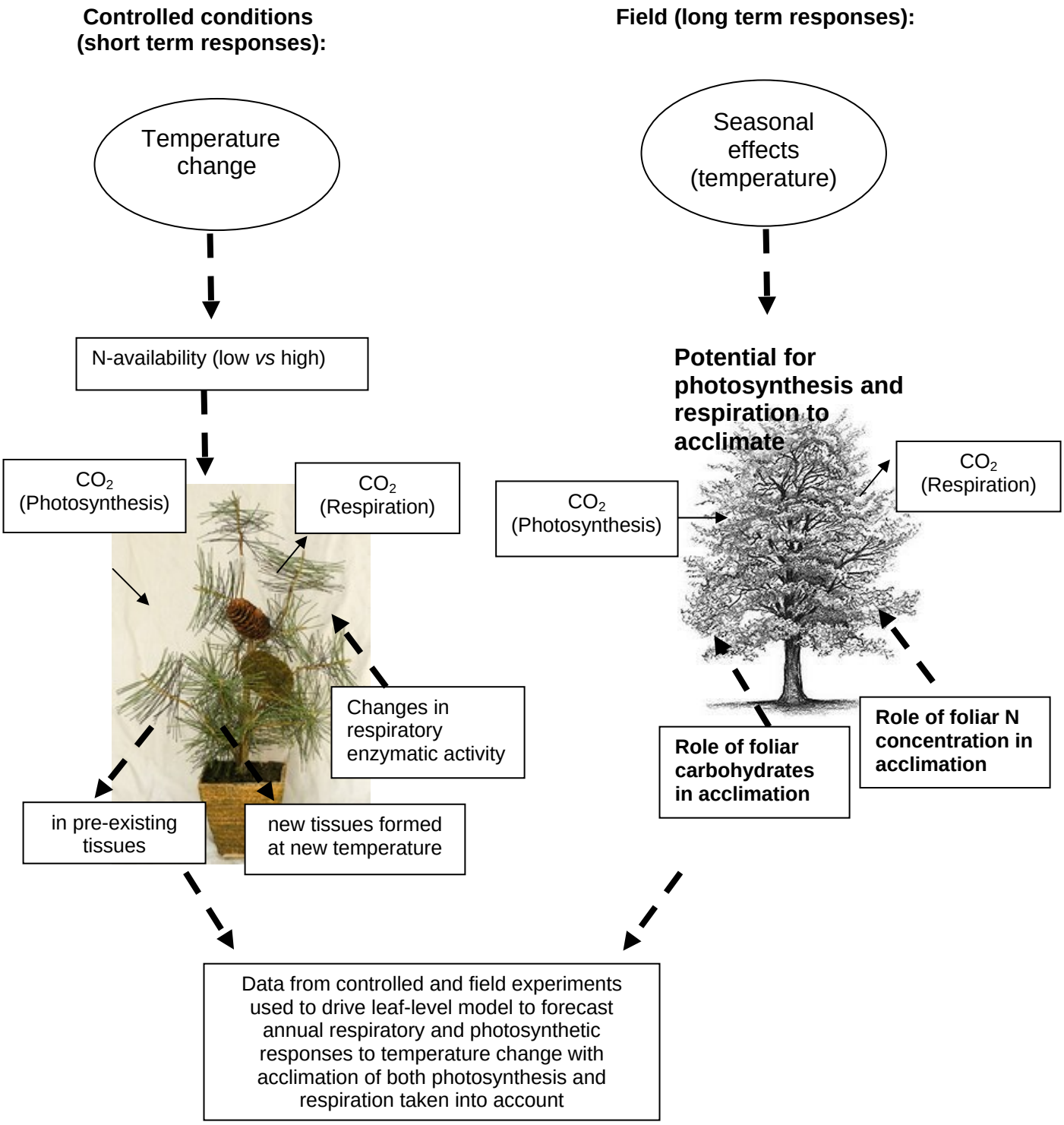

Figure 1.1 Simplified schematic illustration of an investigation into the potential for photosynthetic and respiratory acclimation under field and controlled environmental conditions.

1.5 Botanical description of study species

1.5.1 *Pinus radiata*

Pinus radiata is a native of California, USA. This conifer was introduced into New Zealand where it has become the basis of the plantation timber industry. *Pinus radiata* was first planted on Mount Peel station, Canterbury in the early 1850s. Since then it has been planted to form extensive forests throughout New Zealand.

Pinus radiata is an evergreen conifer belonging to the family Pinaceae (Salmon 2000). The tree is cylindrical in shape and can grow 40-60 m in height. The branches are upward-outward spreading and the bark is dark grey-brown and deeply fissured. The needles commonly occur in clusters of three held together at the base by tiny scales and persist on the tree for approximately three years. The male and female flowers are separate but on the same tree. The males form cylindrical catkins and the females form cones. Pollination occurs from August to September in the Southern Hemisphere.

Pinus radiata is now the most important plantation species in the southern hemisphere. Major advantages of this species are that it is hardy, fast growing and can adapt to a range of soil types, altitudes and climatic conditions. In New Zealand, *Pinus radiata* outgrows a large number of tree species on nearly every site, from Northland to Southland. It produces 20 to 25 cubic metres of wood per hectare each year and is ready to harvest in 25 to 30 years.

Pinus radiata timber is highly versatile. The light coloured, even textured wood is suitable for furniture, joinery, mouldings, construction, packaging, poles, pulp and paper. It can also be sliced or peeled for veneers and plywoods and used in areas such as boat building. *Pinus radiata* made up 85.3% of planting stock sold in 2007 whilst *Pseudotsuga menziesii* (Douglas fir) remains the second most prominent species, making up 8.1% of the sales in 2007. Hence, the economical value of this species has led to the development of extensive pine forest estates. Surveys conducted by the Ministry of Agriculture and Forestry (MAF) estimates that 3000 hectares of new pine forests were planted in 2007 and approximately 25 000 hectares were replanted on harvested areas.

Figure 1.2 Foliage of *Pinus radiata* (left) and *Populus deltoides* (right) – species used in this research.

1.5.2 *Populus deltoides*

Poplars are native to Europe, Asia, Africa as well as North and South America. The genus *Populus* belongs to the family Salicaceae (Salmon 1999). Next to pines, poplars are

probably the commonest introduced trees in New Zealand which is one reason why both species were selected to be used in this research. The hybrid variety (*Populus nigra*) used in this research was introduced into New Zealand from Europe. This tree grows in tight groupings and is straight-stemmed and has a narrow crown. The leaves are broadly triangular with pointed apices and serrated margins. The bark of young trees are generally smooth and pale grey whilst on older trees, the bark has a darker colour and is deeply furrowed. Male and female flowers are on separate trees and it is wind-pollinated.

Like pine, poplar trees have also been planted extensively. Poplars are ideal for soil conservation. Therefore, wide spaced planting of poplar often occur on hill slopes whilst closed space planting tend to occur along gullies and streams. Poplar trees are wind tolerant and upright in form thus, it is widely used as shelter belts. Apart for its use against soil erosion, poplars also have a potential as a timber resource and stock fodder supply. The establishment of poplar is fast and easy hence, poplar trees have become an integral part of many rural landscapes.

1.6 Overview

To address the questions raised, this thesis is arranged into six chapters covering the following topics:

Chapter 1

General introduction concerning the acclimation potential of photosynthesis and respiration in a range of species currently presented in the literature. This chapter also outlines the aims and overview of this research.

Chapter 2

An investigation was carried out to understand the extent of photosynthetic and respiratory acclimation that can occur in different tissue types as a result of step changes in temperature (short term) in *Populus deltoides.* A key aim was to investigate acclimation of both processes in pre-existing and new leaves. Measurements described in this chapter were carried out on potted poplar trees placed in growth chambers with and without N fertiliser for the duration of the experiment. The key objectives of this chapter were to determine (1) if greater foliar N concentration would enhance the potential for photosynthetic and respiratory acclimation (2) examine the extent of photosynthetic and respiratory acclimation in pre-existing leaves and new leaves which had developed under new temperature conditions.

The findings of this chapter have been published in *New Phytologist* (2008, Vol. 178: 123-134). A copy of the paper can be found in Appendix 1.

Chapter 3

An investigation was carried out to determine the extent of photosynthetic and respiratory acclimation in different tissue types as a result of step changes in temperature (short term)

in *Pinus radiata*. A key aim was to investigate the acclimation potential of both processes in pre-existing and new needles. The objectives and measurements described in this chapter were similar to those carried out in Chapter 2. The overall objective here was to establish an understanding of the acclimation response of photosynthesis and respiration in two contrasting tree species (deciduous *vs* evergreen).

The contents of this chapter have been accepted for publication in *Functional Plant Biology* (in press).

Chapter 4

The extent of foliar CO_2 assimilation and release is often studied under controlled–environment using constant temperature conditions which may differ from natural conditions where air temperature varies diurnally and seasonally. Hence, the key aims here were to determine (1) the effects of seasonal variation on leaf respiration and photosynthesis in mature trees of *Pinus radiata* and *Populus deltoides* growing under field conditions (2) establish the effects of natural variation in temperature on the potential for photosynthetic and respiratory acclimation over the course of a year (long term) and (3) assess if respiratory carbon efflux would be significantly stimulated in both species under warmer conditions through a change in the balance between respiration and photosynthesis.

The research in this chapter has been submitted for publication in *Global Change Biology*.

Chapter 5

Having achieved the results reported in Chapters 2, 3 and 4, the aim of the analysis described in this chapter was to incorporate the findings into a coupled photosynthesis-stomatal conductance model for leaves which integrates the effects of photosynthesis and dark respiration and incorporates the presence of respiratory acclimation to temperature, using variable respiratory parameters (e.g. variable R_{10} and Q_{10} values). The key objective of this chapter was to determine the consequences of using alternative temperature response functions equipped with acclimation algorithms on modelled carbon budgets. Some of the findings of this chapter were incorporated into the Chapter 4 manuscript submitted to *Global Change Biology*.

Chapter 6

The final chapter consists of a general discussion and synthesis of the results presented in the body of this thesis. The effects of acclimation of photosynthesis and respiration to temperature change in both species (pine and poplar), and areas of possible future research, are discussed.

Chapter 2

Thermal acclimation of leaf respiration but not photosynthesis in *Populus deltoides* x *nigra*

2.1 Introduction

Photosynthesis and respiration are the two major biological components regulating the exchange of carbon between the atmosphere and the terrestrial biosphere. A small difference between these two fluxes is the defining aspect of the carbon balance of an ecosystem (Schimel et al. 2001). A change in temperature can result in an immediate change in the rates of photosynthesis and respiration, with the magnitudes being determined by the short-term sensitivity of each process to temperature. The temperature sensitivity of foliar photosynthesis differs from that of respiration (Morison and Morecroft 2006), hence the balance between the two processes may be altered following a short-term change in temperature (Dewar et al. 1999; Loveys et al. 2002; Atkin et al. 2006b). This balance in turn has a major impact on net CO_2 emissions and carbon storage in terrestrial ecosystems.

In contrast to instantaneous responses, the effect of long-term changes in growth temperatures on rates of photosynthesis and respiration depends on the degree to which these processes acclimate. Acclimation is defined as adjustments in physiological processes to allow plant performance or fitness to remain the same at a new growth

temperature (Berry and Bjorkman 1980; Hopkins 1998). The degrees to which photosynthesis and respiration acclimate are clearly important determinants of long-term plant responses to environmental change, but they are poorly understood. Our limited understanding indicates that the degree of acclimation differs between species (i.e. higher in fast and slower in slow growing species); some species acclimate strongly whilst others are incapable of even partial acclimation (Berry and Bjorkman 1980; Larigauderie and Korner 1995; Loveys et al. 2003).

Rates of respiration typically increase exponentially with short-term increases in temperature (minutes to hours). The precise shape of this exponential relationship is dependent on both the antecedent temperature conditions and the degree of acclimation, which can be quite rapid, occurring within hours to days (Atkin et al. 2000b). In turn, the extent of acclimation depends on the environmental conditions to which the plant is genetically adapted (Larigauderie and Korner 1995; Tjoelker et al. 2001). Hence, predicting rates of respiration as a function of temperature requires knowledge of both previous temperature conditions and the rate (and extent) of acclimation.

While the instantaneous temperature response of respiration may be exponential over a range of temperatures, the long-term (days to weeks) response is rarely so. Instead, the long-term response usually results in an upward shift of the respiratory temperature response curve with plants grown at cooler temperatures and a downward shift of the response curve as plants are grown in warmer temperatures (Lee et al. 2005). Atkin and Tjoelker (2003) proposed mechanisms for two acclimation scenarios, which they term

Type I and Type II acclimation, to explain both the short and long-term changes in respiration in response to changes in temperature. For type I acclimation, changes in growth temperature may result in relatively rapid changes in Q_{10} with no change in respiration rates at low temperatures. This form of acclimation occurs predominantly among mature tissues (in this case, pre-existing leaves). In contrast, type II acclimation leads to changes in respiration at both low and high temperatures (i.e., an overall shift of the temperature response curve) and is considered to occur over longer periods (Zaragoza-Castells et al. 2007) or in leaves that have developed at the new temperature. In this chapter, temperature-treated (for weeks to months) pre-existing and newly developed leaves were used to test for the occurrence of Type I and II acclimation, respectively.

Photosynthesis is also strongly affected by temperature and, in the short-term, rates of photosynthesis typically increase in response to temperature, reach a maximum value at an optimum temperature, then decline at higher temperatures (Sage and Kubien 2007). At temperatures above the optimum value, rates of photosynthesis often decrease sharply (rates of respiration are typically found to increase beyond this point). Photosynthetic acclimation to a long-term change in temperature may result in a change in the shape of the response curve or a shift of the entire curve, thus changing the absolute rate and/or the temperature optimum (Berry and Bjorkman 1980; Turnbull et al. 2002a; Sage and Kubien 2007). For example, rates of photosynthesis in plants exposed to low temperatures may be reduced initially but, with subsequent acclimation, photosynthetic rates may increase as a result of a higher degree of unsaturation of membrane lipids and a greater

concentration of proteins regulating photosynthetic capacity (Berry and Bjorkman 1980; Stitt and Hurry 2002; Yamori et al. 2005; Sage and Kubien 2007). In contrast, long-term exposure of plants to temperatures above those normally experienced in their growing conditions may result in reduced rates of photosynthesis resulting from the inactivation of Rubisco (Salvucci et al. 2001) and changes in photosynthetic membrane composition (Sharkey et al. 2001).

Tissue N is an important determinant of the rate of key physiological processes in plants (Lewis et al. 2004; Takashima et al. 2004) and is also temperature sensitive, varying with changes in growth temperature (Rachmilevitch et al. 2006). There is strong evidence for a correlation between the rate of photosynthesis, respiration and foliar N concentration (photosynthesis: [Field and Mooney 1983; Reich et al. 1991; Turnbull et al. 2002b; Morison and Morecroft 2006] respiration: [Tjoelker et al. 1999b; Griffin et al. 2002a; Loveys et al. 2003; Atkinson et al. 2007]). However to date, very few studies (Field et al. 1992; Krapp and Stitt 1995; Martindale and Leegood 1997; Atkinson et al. 2007) have focused on the role of leaf N in photosynthetic and respiratory acclimation, particularly in tree species. Many previous studies (references above) have confirmed earlier findings of a strong relationship between N and rates of photosynthesis and respiration, and have not extended their investigation to the role of N in thermal acclimation.

This chapter reports the findings of an experiment which demonstrates the effects of step temperature changes on the rate of both photosynthesis and respiration of *Populus deltoides* x *nigra* saplings. During the course of this three month experiment, day and

night growth temperatures were adjusted by 5 to10 ^{o}C, well within the growing range experienced by this species in the field. The objectives of this study were to (1) examine the extent of photosynthetic and respiratory acclimation in pre-existing leaves and leaves developed under new temperature conditions and (2) establish the impact of leaf nitrogen status (manipulated using low and high levels of nitrogen availability) on the potential for thermal acclimation. The hypothesis was that both photosynthetic and respiratory acclimation to temperature change would be apparent in this fast-growing deciduous species, but that it would be limited in pre-existing leaves and leaves with low N status. A key objective was to determine the extent of error involved when short-term temperature responses of respiration are used to predict long-term responses.

2.2 Materials and Methods

2.2.1 Growth conditions and experimental design

This experiment was conducted using growth chambers at the University of Canterbury, Christchurch, New Zealand from October to December 2005. The chambers (Contherm 630, climate simulator) were set at a constant photosynthetically active irradiance of 700 μmol m^{-2} s^{-1} and 70% relative humidity. The photoperiod was maintained at 10/14 hours dark/light using 400 W metal halide lamps. The temperature treatments were night/day 10 ^{o}C / 15 ^{o}C, 15 ^{o}C / 20 ^{o}C and 20 ^{o}C / 25 ^{o}C and the three treatments were allocated randomly across the chambers.

Populus deltoides x *nigra* ('Veronese') stem cuttings were obtained from Oxford (50 km northwest of Christchurch). Eight plants growing in 9 L pots were widely spaced in each

chamber, allowing good air flow and light penetration during the experiment. The growing medium consisted of a mixture of bark and chip (coarse organic material) with a ratio of 8:2 supplemented with a base level of slow release fertiliser (8.8 N: 5.5 P: 10.6 K plus trace elements) and the plants were watered to saturation and allowed to drain daily. Half the plants (high nitrogen, HN) were supplied with 7 g of compacted 1-2 mm granules of a nitrogen- rich (38% N) fertiliser (Enduro, Rosenlew RKW, Finland) that was well mixed with the potting mix. The remainder was designated as low nitrogen (LN).

Prior to the experiment, plants were established in a well lit glasshouse for 1.5 months at ambient daytime temperatures of 15-20 ^{o}C, after which they were transferred to growth cabinets. The initial temperature treatment regimes as described above were imposed for one week after which the first physiological measurements were made. This allowed for the construction of 'acclimated' temperature response curves (at the growth temperatures) before the shift of plants to new temperature regimes. Following these initial measurements, plants from the 10 ^{o}C / 15 ^{o}C (night / day) temperature treatment were transferred to the 15 ^{o}C / 20 ^{o}C treatment, plants from the initial 15 ^{o}C / 20 ^{o}C treatment were moved to the 20 ^{o}C / 25 ^{o}C treatment, and plants from the 20 ^{o}C / 25 ^{o}C treatment were transferred to the 10 ^{o}C / 15 ^{o}C treatment. The three temperature transfers will hereafter be denoted in the text by the respective day or night temperatures (15-20 and 20-25 and 25-15 for photosynthesis or 10-15 and 15-20 and 20-10 for respiration). Following a week in the new conditions, a second set of physiological measurements were made on leaves that were present prior to the temperature changes. After five

additional weeks in the new growing conditions, a final set of measurements was made on all plants using new leaves that had emerged and developed in the new temperature conditions. Space constraints did not allow for a constant temperature control ("control" plants) but the 3-way transfer regime provided strong evidence to suggest that temperature change was the driving factor in plant responses. Leaves used for measurements were 3 to 4 weeks-old and leaf areas were within the range of 40 -90 cm^2 (obtained using a Li-3100C area meter; Li-Cor Inc., Lincoln NE, USA). Measurements were always conducted on leaves that were close to the apex and receiving full illumination. A large-leaved poplar variety was used, hence the leaf area may be higher than that normally reported in studies working on poplar.

2.2.2 Measurements of respiration, photosynthesis and leaf characteristics

Leaf-level gas exchange measurements were made with two cross-calibrated, portable open-path gas-exchange systems with CO_2 control (Li-6400, Li-Cor Inc., Lincoln NE, USA) with the standard 2 by 3 cm chamber equipped with blue-red light-emitting diodes mounted on the top of the cuvette. Environmental controls within the cuvette were maintained to match the growing conditions, unless otherwise specified. Curves of the response of photosynthesis, A, to intercellular CO_2 concentration, C_i, were generated by changing the external CO_2 concentration, C_a, in 14 steps from 150 to 0 Pa at a constant photosynthetically active irradiance, Q, of 1500 μmol m^{-2} s^{-1}. Measurements were made at each C_a set point when photosynthesis reached a constant value (when the coefficient of variation for the CO_2 concentration differential between the sample and reference analysers was below 1% and visibly stable). This condition was typically achieved within

1-2 minutes after a stable set point had been reached. Curves of the response of photosynthesis to irradiance were generated by changing incident *Q* in 10 steps from 2000 μmol m^{-2} s^{-1} to darkness. During the light response curves, the external CO_2 partial pressure was held constant at 37 Pa. Measurements of photosynthesis in pre existing and new leaves were made at the day-time growth temperatures of 15 ^{o}C, 20 ^{o}C and 25 ^{o}C. Temperatures were maintained using thermoelectric coolers (of the Li 6400) and air saturation deficit in the cuvette was maintained between 1.0 to 1.5 kPa. Measurements from the A/C_i curves were used to determine values for the maximum rate of carboxylation (V_{cmax}) and the apparent maximum rate of electron transport at saturating irradiance (J_{max}). The A/C_i response data were analysed using the biochemical model of photosynthesis as described by Farquhar et al. (1980) and fitted using the Marquardt-Levenberg algorithm (Sigma Plot, Software version 8.0 SPSS Inc. Chicago, Illinois). Measurements from the *A*/*Q* curves were fitted using a rectangular hyperbola (Thornley and Johnson 2000) and used to determine values of maximum photosynthesis (A_{max}) at saturating irradiance and at ambient CO_2 concentration. Unlike the analysis of respiration, calculations of photosynthetic parameters from A/C_i and A/Q curves did not include a correction factor for the gasket effect (Pons and Welschen 2002). Although it was assumed that CO_2 exchange processes only occurred in the part of the leaf enclosed in the leaf chamber, it was unlikely to result in the underestimation of photosynthetic rates because the gasket effect was found to be absent when measurements were made at saturating light (present study at 1500 μmol m^{-2} s^{-1}) and the error was negligible in comparison to the high photosynthetic rate (photosynthetic parameters in this present

study ranged between, A_{max}: 8.1 to 24.4, J_{max}: 37.1 to 167.4 and V_{cmax}: 11.2 to 80.2) (Pons and Welschen 2002).

Measurements of the rate of dark respiration, R_d, were made at the end of the dark period on excised leaves collected prior to onset of illuminated chamber conditions. Measurements were made on fully expanded leaves (with intact petioles) in a separate growth chamber. Upon excision, the ends of the petiole were wrapped in a moist paper towel, sealed in a small plastic bag and placed in the dark - measurements were completed within 2-3 hours. Previous measurements have shown that leaf respiration remains stable under these conditions for several hours (Turnbull et al. 2005) and this was confirmed here. The leaf area used to calculate respiration rate included a correction factor to account for darkened leaf material under the cuvette gasket following Pons and Welschen (2002). As the poplar leaves almost always extended beyond the gasket, it was assumed that half the leaf area under the gasket (approximately $3cm^2$) contributed respired CO_2 into the chamber which was added to all total leaf area calculations to avoid an overestimation of dark respiration rate. To avoid disturbance, the portable gas-exchange system was kept in the dark growth chamber during the measurements and controlled externally from a lap-top computer. The instantaneous response of dark respiration was determined by making measurements of R_d at temperatures of 10 ^oC, 15 ^oC, 20 ^oC and 25 ^oC in pre existing and new leaves before and after transfer in all 3 temperature treatments. The growth chamber and leaf chamber temperature conditions were controlled similarly and the leaves were allowed to equilibrate to the new temperature conditions for 30-45 minutes prior to the onset of the respiration

measurements. Values of R_d at each temperature were calculated from the mean of five measurements made over 3 minutes. Curves of the response of R_d to temperature, T_l (K), were analysed as described in Atkin et al. (2005b), where:

$$R_d = R_{10} Q_{10}^{[(Tl-T0)/10]}$$

where R_{10} is the respiration rate at the base temperature, T_0 (here 10 ^oC or 283 K) and Q_{10} is a parameter describing the change in respiration with a 10 ^oC increase in temperature. The curves were fitted using the Marquardt-Levenberg algorithm (Sigma Plot, Software version 8.0 SPSS Inc. Chicago, Illinois).

The relative enzymatic activities for dark respiration via the cytochrome (COX) and alternative (AOX) oxidase pathways were measured using oxygen electrodes with dual digital controllers (Model 20, Rank Brothers Ltd.). Rates of oxygen uptake were measured for leaf samples placed in buffer solution (Delieu and Walker 1981; McCutchan and Monson 2001) for 5-10 minutes using data acquisition software (TracerDAQ, version 1.7; Measurement Computing Corporation, Massachusetts USA). Leaf discs measuring 26 mm^2 were incubated in running buffer containing 30mM salicylhydroxamic acid (SHAM, an inhibitor of AOX activity) for 1.5 hours in darkness for the measurement of COX activity. In addition, AOX activity was measured following 1.5-hour incubation of leaf discs in 3mM potassium cyanide (KCN, an inhibitor of COX activity). Appropriate controls (without inhibitors) were also incubated in a similar way before measurements were made. Residual respiratory activity was determined following

incubation of separate discs in both inhibitors (average 11.6% (±0.004) of total respiration; this was subtracted from each value before calculating activity as a percentage of total uninhibited respiration). Leaf discs incubated in 50 μM carbonylcyanide m-chlorophenylhydrazone (CCCP) were used to assess the fully uncoupled rate of oxygen consumption. The activity of each pathway was calculated as a percentage of the fully uncoupled rate.

Total nitrogen concentration of leaves on an area and mass basis(N_a and N_m) were determined on leaves dried at 70 ^oC, weighed, finely ground in a ball mill and analysed using a CNS analyzer (Carlo Erba Na 1500, Milan, Italy). Specific leaf area, S (m^2 kg^{-1}), was calculated from measurements of leaf area and dry mass.

2.2.3 Statistical analysis

Two-way analyses of variance (ANOVA) were used to test for the effects of temperature and nitrogen supply on S, A, R_d and N (SAS Institute, software version 8.2, Cary, North Carolina, USA). Differences were considered significant if $P \leq 0.05$. Treatment means were separated by least significant difference tests at $P \leq 0.05$. Differences between means in Figure 2.4 were evaluated with a one-way ANOVA.

2.3 Results

2.3.1 Foliar characteristics

There were significant differences in nitrogen concentration, on both a mass and area basis, N_m and N_a respectively between high (HN) and low (LN) nitrogen plants (Figs.

2.1A and B). N_m was significantly greater in HN plants than in LN plants. New leaves in the HN and LN treatments showed a significant increase in N_a in response to cold transfer (25 to 15 ^oC; Fig. 2.1B). There was no significant change in N_a in pre-existing and newly emerged leaves in either the HN or LN treatments in response to warm transfer (15 to 20 ^oC and 20 to 25 ^oC; Fig. 2.1B). A significant decrease in *S* was observed in response to cold-transfer in newly emerged leaves in the HN treatment only (25 to15 ^oC; Fig. 2.1C). Following warm-transfer (15 to 20 ^oC and 20 to 25 ^oC; Fig. 2.1C) a significant increase was found in newly emerged leaves only but also in LN pre-existing leaves shifted from 15 to 20 ^oC.

2.3.2 Photosynthesis

A_{max} at saturating irradiance and ambient CO_2 concentration decreased significantly in pre-existing and newly emerged leaves in response to cold transfer (25 to 15 ^oC; Fig. 2.2A). A 60 and 50% reduction in A_{max} was observed in newly emerged leaves from HN and LN plants, respectively. There was no significant change in A_{max} values of warm-transferred (15 to 20 ^oC and 20 to 25 ^oC; Fig. 2.2A) plants in the HN treatment but a significant increase was exhibited in pre-existing and newly emerged leaves of LN plants. Quantum yield was insensitive to temperature transfer (data not shown). V_{cmax} decreased significantly in both HN and LN treatments in response to cold transfer (25 to 15 ^oC; Fig. 2.2B), with pre-existing leaves displaying a similar response to newly emerged leaves. V_{cmax} increased initially in response to warm transfer (15 to 20 ^oC and 20 to 25 ^oC; Fig.

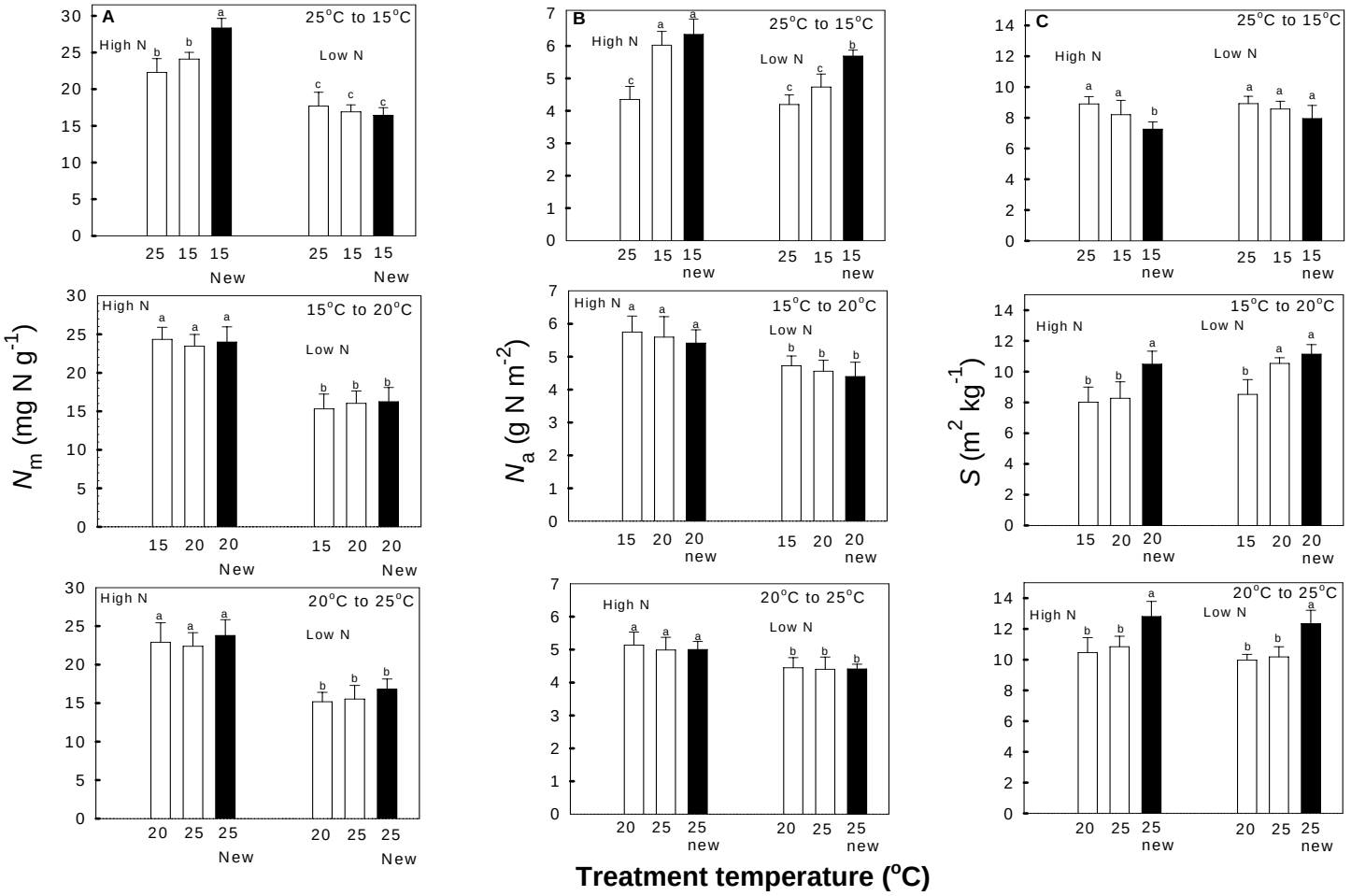

Fig. 2.1 Variation in foliar nitrogen concentration on mass (*N*m) (a), area (*N*a) basis (b) and specific leaf area, (*S*) (c), in *Populus deltoides* × *nigra* ('Veronese') plants under three temperature transfer regimes (25–15°C, 15–20°C and 20–25°C). Each panel displays the response of pre-existing leaves at the initial growth temperature and 1 week after plants were transferred to the new temperature (open bars). The closed bar represents the response of new leaves that expanded in the new temperature. Data are presented for plants grown under high and low nitrogen availability. All values are means ± SE; $n = 8$. Different letters indicate means are significantly different at the $P < 0.05$ level.

2.2B), in pre-existing leaves but was followed by subsequent partial re-adjustment in newly emerged leaves. J_{max} in cold-transferred plants generally displayed a similar response to that of V_{cmax}; values did not change significantly in response to the 20 to 25 ^{o}C transfer (Fig. 2.2C). The J_{max} / V_{cmax} ratio increased significantly following cold-transfer (25 to 15 ^{o}C; Fig. 2.2D) and generally decreased in response to warm-transfer (15 to 20 ^{o}C and 20 to 25 ^{o}C; Fig. 2.2D). The long-term response of A_{max} and V_{cmax} to temperature (determined from values at the three growth temperatures before and after transfer) did not differ greatly between the initial (plants grown in 3 different temperature regimes for 1 week) and temperature responses after transfer (Figs 2.3A and B). Overall the R_d/ A_{max} ratio of pre-existing and new leaves declined with increasing temperature both before and after transfer but this was more accentuated in new leaves (Fig. 2.3C).

2.3.3 Respiration

The instantaneous temperature response of R_d displayed a characteristic exponential increase with increasing temperature (Figs. 2.4A, B & C). Newly emerged leaves after cold transfer (20 to 10 ºC; Fig. 2.5A) displayed a significant increase in R_{10} values in both HN and LN plants, whilst no significant change was seen in pre-existing leaves. Newly emerged leaves in warm-transferred plants (10 to 15 ºC and 15 to 20 ºC; Fig. 2.5A) exhibited a significant decrease in R_{10} in both HN and LN treatments. Temperature transfer had no significant effect on Q_{10} (within the range of 10-20 ^{o}C) in either cold- and warm-transferred plants (Fig. 2.5B). The long-term (acclimated) respiratory response to temperature, as determined by actual values of R_d at the respective growth temperatures

(solid lines, Fig. 2.4) was less pronounced than the instantaneous responses. The degree of acclimation in newly emerged leaves (Fig. 2.4C) was greater than that for pre-existing leaves (Figs. 2.4A & B), such that R_d in newly emerged leaves did not differ across the 10 ºC range ("thermal homeostasis"). Accordingly, rates of leaf respiration were predicted at the new temperatures from the response obtained at the initial temperature (before the plants were transferred) and compared these predicted values to actual values determined for pre-existing and newly emerged leaves at the new growth temperatures (Table 2.1). The initial response over-estimated the acclimated R_d of new leaves following an increase in temperature from 15 to 20 ºC by as much as 69% and under-estimated the response to a decrease in temperature from 20 to 10 ºC by up to 44% (Table 2.1).

Table 2.1. Rate of leaf respiration in *Populus deltoides* x *nigra* plants transferred between various growth temperatures. Rates are quoted as values predicted based upon the temperature response curves of leaves before transfer (Fig. 2.4A) and actual rates determined in pre-existing leaves after transfer (Fig. 2.4B) and new leaves formed after transfer to the appropriate growth temperatures (Fig. 2.4C). Using the predicted and actual rates derived from Fig. 2.4, a value of % difference was obtained. Values with negative signs indicate an under-estimation of respiration (Data averaged across N treatments).

Temperature transfer treatments	**Respiration (μmol $m^{-2}s^{-1}$)**		
	Predicted rates	Actual rates	% difference
Pre-existing leaves			
10 ^{o}C to 15 ^{o}C	1.24	1.06	17.0
15 ^{o}C to 20 ^{o}C	1.72	1.29	33.3
20 ^{o}C to 10 ^{o}C	0.63	0.82	-23.2
Newly emerged leaves			
10 ^{o}C to 15 ^{o}C	1.24	0.99	25.3
15 ^{o}C to 20 ^{o}C	1.72	1.02	68.6
20 ^{o}C to 10 ^{o}C	0.63	1.13	-44.2

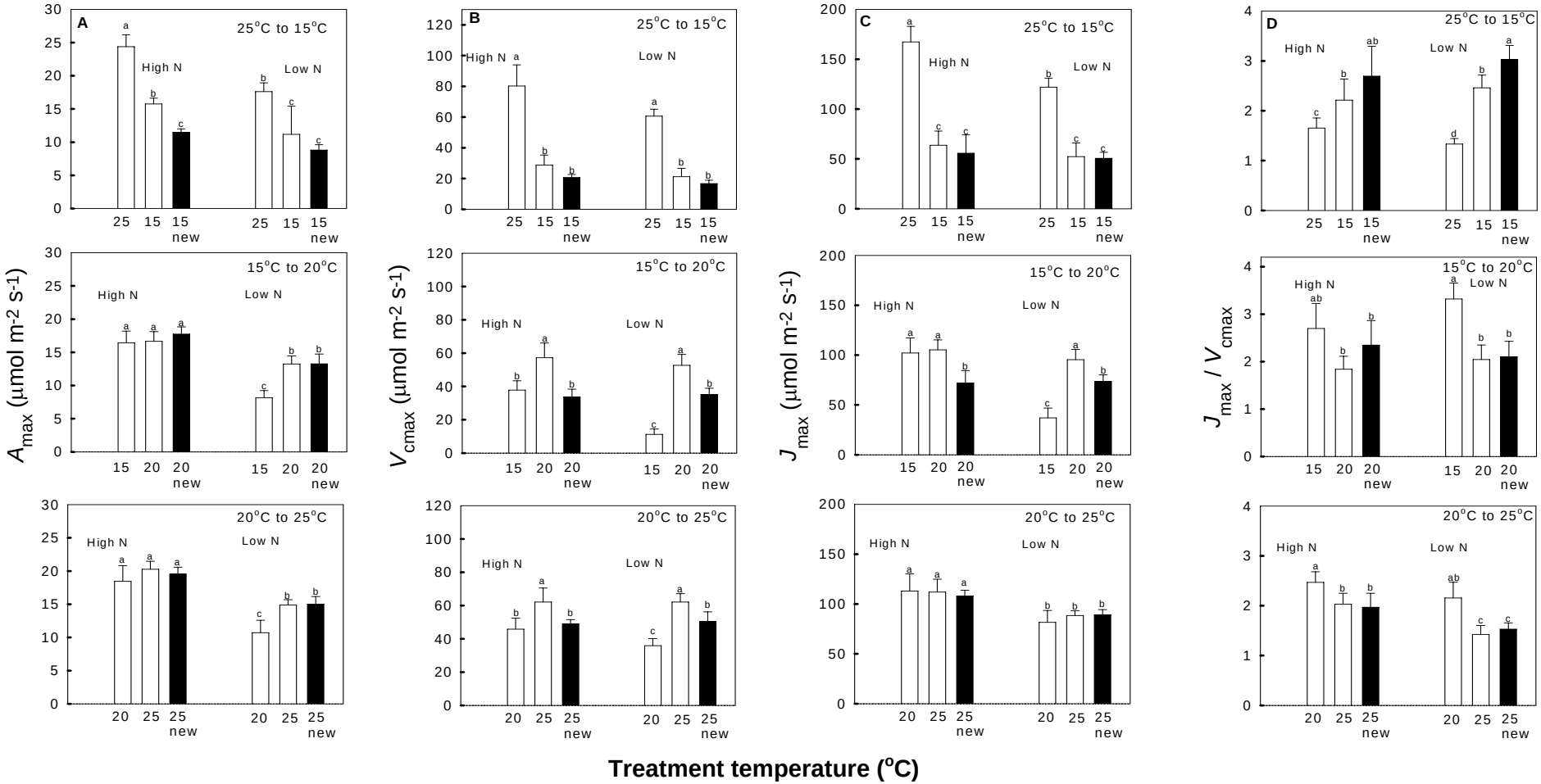

Fig. 2.2 Photosynthetic parameters calculated from *A/Q* (curves of the response of photosynthesis to irradiance) responses *A*max (a) and from *A/C*i (curves of the response of photosynthesis, *A*, to intercellular CO2 concentration, *C*i) responses *V*cmax (b), *J*max (c) and *J*max/*V*cmax (d) in *Populus deltoides* × *nigra* ('Veronese') plants under three temperature transfer regimes (25 to 15°C, 15 to 20°C and 20 to 25°C). Each panel displays the response of pre-existing leaves at the initial growth temperature and 1 week after plants were transferred to the new temperature (open bars).The closed bar represents the response of new leaves that expanded in the new temperature. Data are presented for plants grown under high and low nitrogen availability. All values are means ± SE; $n = 8$. Different letters indicate means are significantly different at the $P < 0.05$ level.

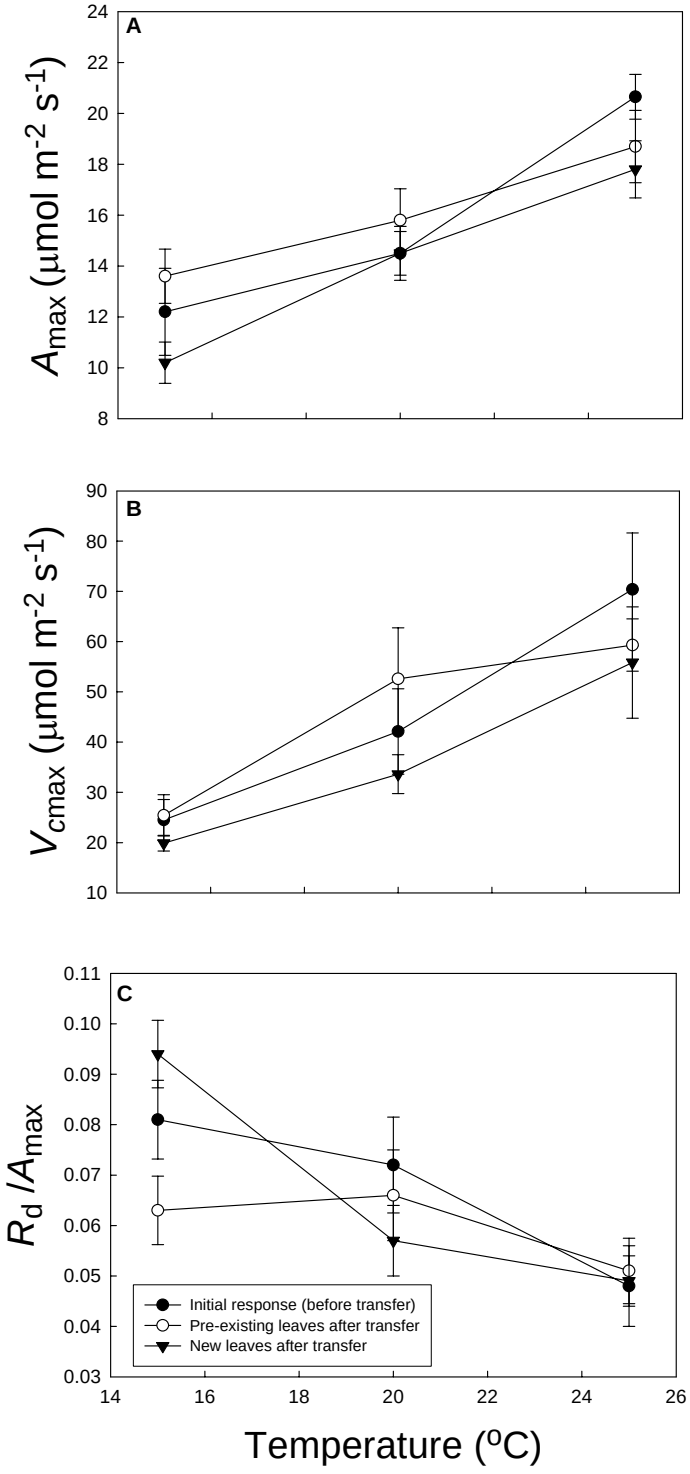

Fig. 2.3 Temperature response of *A*max (a), *V*cmax (b) and *R*d/*A*max (c) at three daytime growth temperatures (15, 20 and 25°C) for *Populus deltoides* × *nigra* ('Veronese') plants before and following transfer between three temperature regimes (means are averaged (*n* = 8) across nitrogen (N) treatments).

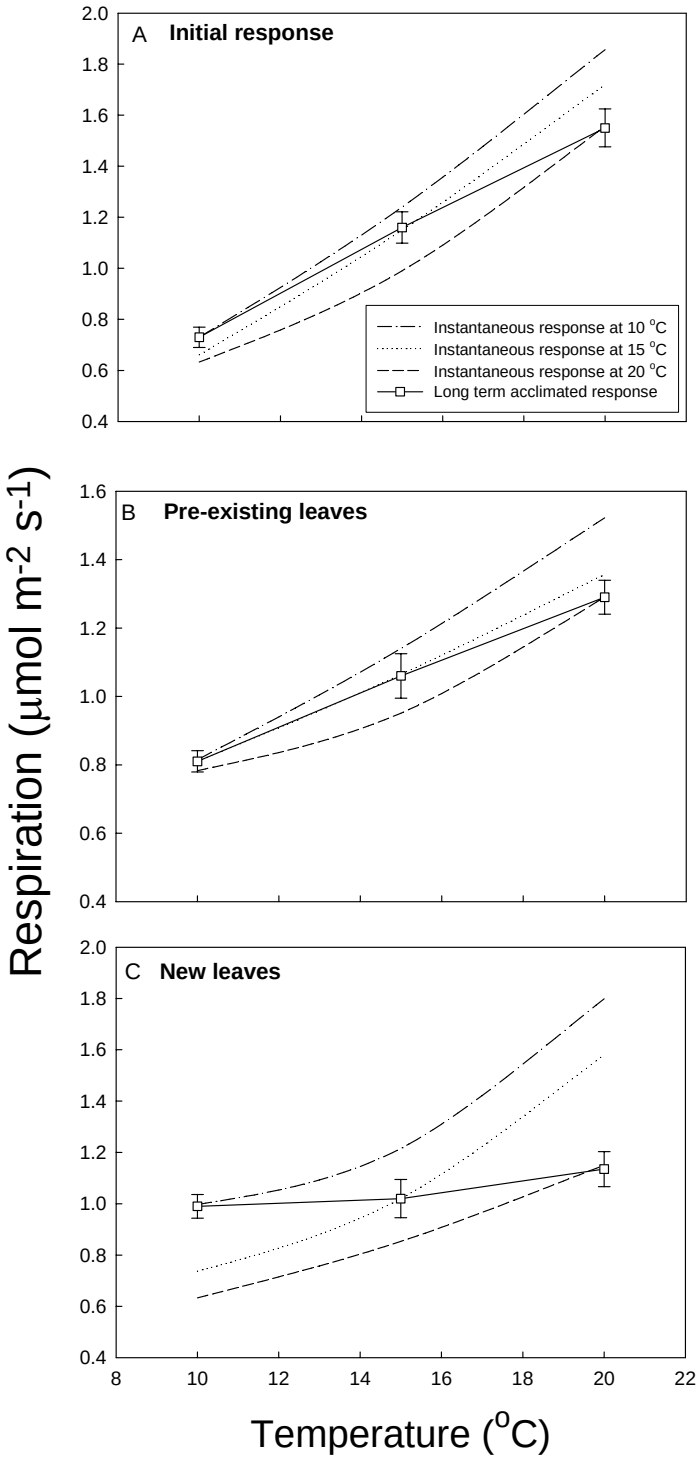

Fig. 2.4 Instantaneous (determined over a range of temperatures within a 3 h period) and 'acclimated' (actual values of *R*d at the respective night-time growth temperatures) responses of respiration to temperature for: (a) pre-existing leaves at the initial night-time temperature (before transfer of plants to new temperature regimes); (b) pre-existing leaves at the new night-time temperature; and (c) newly expanded leaves at the new night-time temperature. Values at the night-time growth temperatures are means ± SE. One-way ANOVA tests indicated significant differences in *R*d at each growth temperature ($P = 0.001$ for 10°C, $P = 0.05$ for 15°C and $P = 0.001$for 20°C) (data presented are averaged ($n = 8$) across nitrogen (N) treatments).

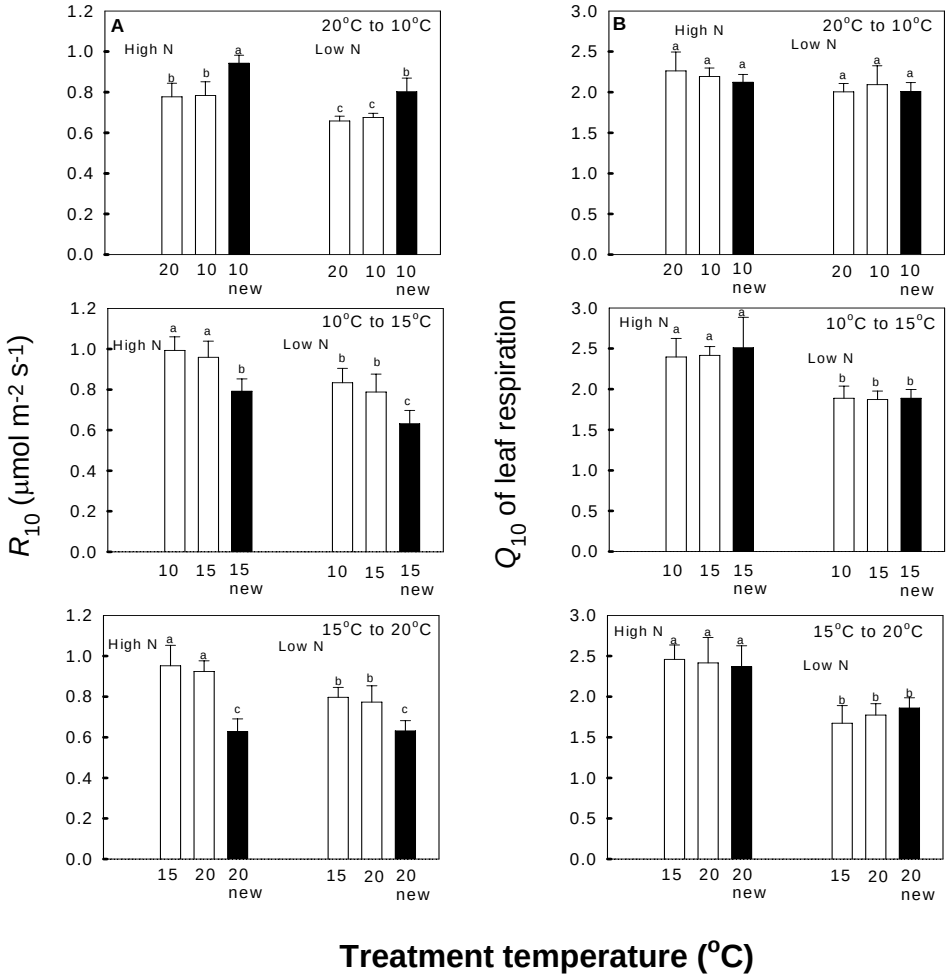

Fig. 2.5 Temperature response parameters of dark respiration (*R*10 – modelled respiration at a base temperature of 10°C (a) and *Q*10 (b) under three temperature transfer regimes (20 to 10°C, 10 to 15°C and 15 to 20°C). For detailed explanation on how values were derived, refer to the Materials and Methods section. Each panel displays the response of pre-existing leaves at the initial growth temperature and 1 week after plants were transferred to the new temperature (open bars). The closed bar represents the response of new leaves that expanded in the new temperature. Data are presented for plants grown under high and low nitrogen availabilities. All values are means ± SE; $n = 8$. Different letters indicate means are significantly different at the $P < 0.05$ level.

The enzymatic activity of the COX pathway generally did not change significantly in response to warm transfer (10 to 15 ºC or 15 to 20 ºC) but was reduced significantly in newly emerged leaves of cold-transferred plants (20 to 10 ºC; Table 2.2). AOX activity in

Table 2.2 Activity of the cytochrome (COX) and alternative oxidase (AOX) pathways as a percentage of total respiratory activity in leaf discs of *Populus deltoides* x *nigra* plants exposed to both increasing and decreasing temperatures. Measurements were made in *pre-existing leaves* at the initial temperature and 7-days after transfer to the new growth temperature and in *new leaves* at the new growth temperature. Plants were grown under low, LN, and high, HN, nitrogen supply.

Treatment	Cytochrome pathway activity (% of total respiratory activity)			Alternative oxidase pathway activity (% of total respiratory activity)		
	Pre-existing leaves	Pre-existing leaves after transfer	Newly emerged leaves	Pre-existing leaves	Pre-existing leaves after transfer	Newly emerged leaves
20 to 10 ºC						
LN	101.5 (4.0)^{b}	76.0 (10.5)^{ab}	48.8 (8.5)^{a}	16.3 (5.9)^{a}	38.9 (9.0)^{b}	56.1 (11.7)^{c}
HN	100.5 (19.0)^{b}	98.1 (5.8)^{b}	67.5 (19.0)^{a}	16.0 (5.5)^{a}	52.8 (5.0)^{b}	47.7 (6.3)^{b}
10 to 15 ºC						
LN	100.5 (4.0)^{a}	98.3 (5.0)^{a}	91.5 (13.7)^{a}	3.7 (5.5)^{a}	59.1 (8.1)^{c}	40.1 (16.4)^{b}
HN	64.4 (6.7)^{a}	110.2 (7.3)^{b}	111.4 (17.5)^{b}	9.8 (2.9)^{a}	66.1 (14.6)^{c}	30.1 (9.4)^{b}
15 to 20 ºC						
LN	84.2 (1.8)^{a}	87.6 (9.1)^{a}	69.1 (5.5)^{a}	19.5 (7.8)^{a}	48.6 (15.5)^{b}	49.1 (2.3)^{b}
HN	101.1 (6.4)^{a}	72.3 (11.6)^{a}	71.9 (10.8)^{a}	29.4 (8.0)^{a}	34.6 (13.1)^{a}	28.4 (4.9)^{a}

Values shown are means (± standard error of the mean, SEM) where n=4. Different letters within rows indicate statistically different values at $P<0.05$ using least significant difference test of treatment means.

cold-transferred plants (20 to 10 ºC) increased significantly in pre-existing leaves and remained high in newly emerged leaves (Table 2.2). In warm-transferred plants (10 to 15 ºC and 15 to 20 ºC), AOX activity increased in pre-existing and new leaves, but no change was found in HN 15 to 20 ºC plants (Table 2.2).

2.4 Discussion

The results clearly show strong acclimation of respiration to growth temperature in *Populus deltoides*, regardless of the nitrogen status of the leaves. However, the degree of acclimation in pre-existing leaves was limited compared with newly emerged leaves which displayed the capacity to acclimate fully (thermal homeostasis). The comparison between predicted and actual rates of respiration indicates a distinct limitation in the use of instantaneous temperature response curves to predict long-term respiratory responses in pre-existing and new leaves. In contrast to respiration, there was little evidence for acclimation of photosynthetic parameters to growth temperature (in the range 15 to 25 ºC). Taken together, these findings have important implications for the carbon balance of deciduous species in the face of temperature variation.

2.4.1 Photosynthetic response to changes in growth temperature

Previous work suggests high variability in the degree of photosynthetic and respiratory acclimation among species, with some species exhibiting full acclimation whilst others appear incapable of even partial acclimation (Atkin et al. 2005a; Atkin et al. 2006b). The results (Fig. 2.2A) show that 10 °C cold transfer resulted in a reduced photosynthetic

capacity despite increasing tissue nitrogen content (Fig. 2.1B), whilst 5 °C increases in temperature led to small increases in photosynthetic capacity in low N plants only. These results are consistent with previous work by Rook (1969) who found a 25% decrease in photosynthetic rates of *Pinus radiata* seedlings after transfer to cooler temperatures. But in a recent study, Yamori et al. (2006) found that cold-grown spinach leaves typically exhibit greater photosynthetic capacities than their warm grown counterparts and suggested that acclimation of rubisco kinetics to the lower growth temperature should result in increased photosynthetic rates. Clearly, the response of photosynthesis to cooler temperatures is not universal and may depend on species. In the present study, the long-term temperature response of photosynthesis over the range 15 to 25 ºC remained remarkably constant despite changes in growth temperature (Figs. 2.3A and B). This suggests that, in stark contrast to respiration (Fig. 2.4), photosynthetic processes in poplar are incapable of significant acclimation to temperature changes. Although the measurements carried out in this study do not allow for the identification of an optimum temperature for this species, we should not rule out the possibility of changes in T_{opt}. Nonetheless, the absence of photosynthetic acclimation is an important finding and is consistent with the notion that photosynthesis can generally operate effectively between 0 to 30 ºC (Sage and Kubien 2007).

Rates of photosynthesis are limited either by ribulose-1,5-bisphosphate (RuBP) carboxylation or by RuBP regeneration (Berry and Bjorkman 1980). As RuBP carboxylation-limited photosynthesis does not have the same temperature dependence as RuBP regeneration-limited photosynthesis, temperature change may result in an

imbalance between the two processes and a change in the J_{max} / V_{cmax} ratio (Farquhar and von Caemmerer 1982; Hikosaka et al. 1999; Onoda et al. 2005). Here, the ratio of the capacities of RuBP regeneration to carboxylation (J_{max} / V_{cmax}) increased in cold transferred plants whilst it decreased in warm transferred plants (Fig. 2.2D). This is consistent with previous work by Dreyer et al. (2001) and Onoda et al. (2005), although in the latter study this response was not universal among species. The increase in the J_{max} / V_{cmax} ratio at low growth temperatures is considered to relieve the limitation of RuBP regeneration on photosynthetic rate whilst a decrease in the ratio with increasing temperature is a result of a greater activation energy for V_{cmax} than for J_{max} (Onoda et al. 2005). The decrease in the J_{max} / V_{cmax} ratio in response to increased temperatures is highly conserved in a wide range of species but the interspecific differences in the response of J_{max} / V_{cmax} to growth temperature are not known.

2.4.2 Respiratory responses to changes in growth temperature

The results show that respiration in poplar is strongly sensitive to short-term changes in temperature but acclimation reduces the magnitude of the response over longer periods (days, weeks or months). While this has been previously demonstrated, the extent to which it occurs in different tissues and species is still poorly understood (Larigauderie and Korner 1995; Atkin et al. 2000; Atkin and Tjoelker 2003; Wright et al. 2006). Cold-transferred poplar displayed a significant increase in R_d whilst warm transferred plants (15 to 20 ºC and 20 to 25 ºC; Fig. 2.5A) exhibited a significant decrease. As a result, the acclimated response of R_d at the actual growth temperature in new leaves was much less

pronounced than the instantaneous response with nearly identical rates of respiration at contrasting temperatures, indicating full respiratory homeostasis over a 10 ºC temperature range (Fig. 2.4C).

The results also show that acclimation potential for respiration in both warm- and cold-transferred plants is limited in pre-existing leaves and that full acclimation is only possible in new leaves emerged after the change in temperature. This supports work by others (Hurry et al. 1995; Loveys et al. 2002; Atkin et al. 2005a; Atkin et al. 2006a) showing that limited acclimation of respiration can occur in pre-existing leaves, but full acclimation is restricted to leaves emerging after the change in temperature. However, this finding is by no means universal, and is likely to be especially variable in species with long-lived leaves (Bruhn et al. 2007; Zaragoza-Castells et al. 2007) which, if given more time (e.g. 60 days), may express a greater degree of acclimation in pre-existing leaves. In the present study, respiratory acclimation was achieved, in part, through changes in *S* (i.e. the amount of metabolizing tissue per unit area). This highlights the importance of leaf structural changes in acclimation to changing growth temperature, and provides a strong explanation for the limited response in pre-existing leaves. Whilst limited changes in *S* are possible following a change in environment, this parameter is largely determined at the time of leaf development and expansion. Importantly for our understanding of the likely potential for respiratory acclimation under field conditions, It was found that, while tissue N content clearly regulates rates of respiration, it has little or

no impact on the extent of thermal acclimation (at least within the range of N concentrations experienced here).

The responses above may be placed in the context of the acclimation mechanisms suggested by Atkin et al. (2005a). Pre-existing leaves displayed a response which was partly consistent with the Type I scenario proposed by Atkin et al. (2005a), although Q_{10} was rather insensitive. Previous studies have shown that the Q_{10} often decreases with increasing growth temperature. The results here are, however, consistent with those of Tjoelker et al. (1999a), showing very little change in Q_{10} after cold or warm transfer (Fig. 2.5B). In contrast, new leaves (Fig. 2.4C) clearly displayed characteristics of Type II acclimation, which is often associated with temperature–mediated changes in respiratory capacity achieved through the development of new tissues (Atkin and Tjoelker 2003). Type II acclimation may also be associated with enzymatic changes (e.g. changes in the relative amounts of enzymes or proteins invested into the respiratory chain) resulting in the relative change of AOX and COX activity (Atkin et al. 2005a). This was especially evident in cold transferred plants, where COX activity declined whilst AOX activity increased (increase in AOX activity was consistent in both cold and warm transferred plants). This switching between the AOX and COX pathways has been shown in spinach, beans and maize grown at different levels of irradiance and different chilling conditions (Ribas-Carbo et al. 2000; Noguchi et al. 2001a). AOX is highly sensitive to changes in temperature and engagement of the non-phosphorylating AOX pathway may be advantageous to plant acclimation to cold temperature where energy that

would otherwise be conserved as ATP is converted to heat (Ribas-Carbo et al. 2000). For example, Atkin et al. (2005b) showed that changes in growth temperature can alter the partitioning of electrons between different respiratory pathways and the engagement of AOX may vary with age, growth temperature, irradiance or phosphate supply (Gonzalez-Meler et al. 1999; Ribas-Carbo et al. 2000) . It is also worth noting that this study provides estimates of maximum rates of *in vivo* AOX and COX activity only, and the results should not be taken as true *in vivo* capacity of the two pathways or *in vivo* switching between these pathways. The more reliable methodology presently available to study electron partitioning between AOX and COX without the use of inhibitors is the use of the oxygen-isotope fractionation which measures the differences in the isotopic fractionation of ^{18}O between the two terminal oxidases (Ribas-Carbo et al. 2005). Clearly the findings here suggest directions for future work in this area.

2.4.3 Balance between photosynthesis and respiration

Inclusion of partial and full acclimation of respiration into models may significantly affect calculations of long-term plant and ecosystem carbon balance. The importance of acclimation to calculations of respiration is demonstrated in Table 2.1, where predicted rates of respiration in pre-existing and newly emerged leaves at new temperatures were very different from the actual rates observed, showing that the instantaneous response is a poor predictor of long-term acclimated responses of respiration to changes in temperature. In the absence of respiratory acclimation, models of the impacts of climate

warming would predict greater increases in respiration than photosynthesis (Dewar et al. 1999; Atkin et al. 2000b; Gifford 2003). The results here show that R_d / A_{max} at the leaf level decreased in response to increasing temperature (Fig. 2.3C). Although these results deviate from those of Loveys et al. (2003) who proposed that the R_d / A_{max} ratio is insensitive to environmental conditions, these findings are however, consistent with those of Xu et al. (2006) showing significant seasonal variation in the ratio for three shrub species. Such responses in the R_d / A_{max} ratio suggest that increases in temperature over a moderate range may favour the uptake of carbon in trees rather than its loss to the atmosphere.

2.5 Summary

This chapter has highlighted that poplar, a deciduous species with a high level of developmental plasticity, has considerable potential for respiratory acclimation to changes in growth temperature. In contrast, there was little evidence of temperature acclimation in photosynthetic characteristics. Nitrogen status of leaves had little, if any, impact on the degree of acclimation. This is important for our understanding of respiratory responses of trees under conditions of varying soil nutrition. The presence of respiratory acclimation in the face of increasing atmospheric temperatures would serve to reduce the potential for positive feedback of respiration in the carbon cycle. Hence, the next chapter will focus on the acclimation potential of photosynthesis and respiration in an evergreen species - with different potentials for generating new tissues. This should

help us gain a better understanding of the extent of thermal acclimation in a range of plant species.

Chapter 3

Thermal acclimation of respiration but not photosynthesis in *Pinus radiata*

3.1 Introduction

With the concern for global warming has come a heightened interest in the effects of elevated temperatures on physiological processes in trees and the productivity of forests (Teskey and Will 1999). The carbon balance of forest ecosystems is defined by the difference between photosynthetic carbon gain and respiratory carbon loss. On an annual basis, respiration can consume between 30 and 70% of photosynthetic carbon fixation (Ryan et al. 1994; Ryan et al. 1996; Turnbull et al. 2005). In addition, given that plant respiration results in the release of 60 Gt of carbon annually (King et al. 2006), 80% which is from forest trees (i.e. pines) (Ryan et al. 1994), it is essential that respiratory responses of forest trees to environmental variables be better understood. With global warming, higher respiratory costs may cause lower productivity in forest and may enhance the release of stored carbon into the atmosphere, potentially adding to the greenhouse effect.

Temperature has a large influence on the processes of photosynthesis and respiration. In the short-term (minutes to hours), photosynthesis increases exponentially with increasing

temperature, eventually displaying a declining rate of increase such that an optimum temperature can be identified, after which rates start to decline. By contrast, short-term responses of dark respiration tend to remain exponential until close to lethal temperatures. Despite being highly sensitive to short-term changes in temperature, photosynthetic and respiratory responses to longer-term (days to weeks) temperature change is commonly reduced in magnitude.

The degree to which respiration and photosynthesis change with temperature is highly variable, and is influenced by a range of external and internal factors (Larigauderie and Korner 1995; Atkin et al. 2000a; Atkin et al. 2000b; Tjoelker et al. 2001). Acclimation to temperature occurs when the rate of a physiological process adjusts in response to a change in temperature over the course of hours to weeks. Rates of photosynthesis have long been known to acclimate to prevailing temperature (Berry and Bjorkman 1980) but the degree of photosynthetic acclimation is highly variable between species as well as within genera (Atkin et al. 2006b). Acclimation can change the shape of the photosynthetic temperature response curve or result in a shift in the curve such that the absolute rate and/or the optimum temperature changes. Similarly, rates of respiration have also been found to acclimate rapidly (in hours to days) following a change in prevailing temperature (Rook 1969; Atkin and Tjoelker 2003). As with photosynthesis, respiratory acclimation potential varies between species (Atkin et al. 2005a; Atkin et al. 2005b; Loveys et al. 2003). The short-term temperature response of respiration is often exponential but in the longer-term, it is seldom so (Gifford 2003). Respiratory

acclimation to higher and lower temperatures often results in the downward and upward shift of the temperature response curve of respiration, respectively.

Based on the background above, it is clear that the ratio of respiration to photosynthesis is also likely to be sensitive to temperature. Over a short-time period, this ratio is generally considered to increase with increasing temperature. By contrast, it has been suggested that the longer-term response of the respiration to photosynthesis ratio tends to be homeostatic but with some exceptions across a range of temperatures and species (Gifford 1995; Gifford 2003). While the specific responses of photosynthesis and respiration are thought to be independent, both respiration and photosynthesis are physiologically linked (Turnbull et al. 2004; Whitehead et al. 2004a) and are also known to respond to a range of environmental variables. If the balance between photosynthesis and respiration were to be altered as a result of increasing temperature, there is a risk that respiration would be stimulated, releasing stored carbon into the atmosphere and acting as a positive feedback to the greenhouse effect. Therefore, an in-depth understanding of how respiratory and photosynthetic processes will respond to temperature in the short and long-term is important if models of forests or ecosystem carbon exchange are to accurately predict plant responses to climate change.

Rates of dark respiration and photosynthesis often correlate with tissue *N* content across diverse taxa and environments (respiration: [Ryan 1995; Reich et al. 1998b; Reich et al. 2006; Wright et al. 2006] photosynthesis: [Field 1983; Reich et al. 1991; Turnbull et al. 2002b; Morison and Morecroft 2006]). However to date, few studies (Field et al. 1992;

Krapp and Stitt 1995; Martindale and Leegood 1997; Atkinson et al. 2007) have focused on the role of leaf N in photosynthetic and respiratory acclimation, particularly in tree species. Many previous studies (reference above) have confirmed earlier findings of a strong relationship between N and rates of photosynthesis and respiration, but have not extended their investigation into the role of N in thermal acclimation.

In this chapter the extent of photosynthetic and respiratory acclimation to changing temperature in pre-existing and new needles of *Pinus radiata* were examined. The previous chapter investigated the responses of photosynthesis and respiration in poplar in response to step changes in temperature under controlled conditions (Chapter 2; Ow et al. 2008). The previous investigation showed significant thermal acclimation in respiration but not photosynthesis. In this present study, day and night-time growth temperatures were adjusted up or down by 5-10^{o}C. The objectives of this study were to: (1) examine the extent of photosynthetic and respiratory acclimation in pre-existing and new needles – the hypothesis was that a high degree of both photosynthetic and respiratory acclimation would not be expressed in pre-existing needles (at the new temperature) as pines (and other evergreen species) are slow-growing, with a low potential for phenotypic plasticity; (2) establish whether leaf nutritional status (manipulated using low and high levels of N availability) influences the potential for acclimation – the hypothesis was that low leaf N would limit acclimation potential. A key objective was to determine the extent to which short-term predictions of the temperature responses of respiration can be used to accurately predict long-term responses to changing temperature.

3.2 Materials and methods

3.2.1 Plant material and environmental conditions

This experiment was conducted at the University of Canterbury in Christchurch, New Zealand (Latitude 43^{o} 25' E, longitude 172^{o} 58' N, at sea level) from August to November 2006. Small saplings of *Pinus radiata* were grown from cuttings (New Zealand Forest Research Institute, Rotorua, New Zealand) in 9 L pots which contained a mixture of bark and chip (coarse organic material) with a ratio of 8:2, respectively. The potting mix contained a base level of slow release fertilizer (NPK in the proportions 8.8: 5.5: 10.6 + trace elements). The plants were initially maintained in a glasshouse (photon flux density (PFD) at approximately 1000-1100 μmol m^{-2} s^{-1}) before the experiment at temperatures in the middle of the experimental range (i.e. 15-20 °C) and watered to capacity every four to five days. Half the saplings were supplemented with 7g of compacted 1-2 mm grain nitrogen- rich (38% N) fertilizer (Enduro, Rosenlew RKW, Finland). The experiment was conducted in three growth chambers (Contherm 630, climate simulator) set at a PFD of 700 μmol m^{-2} s^{-1} and 70% relative humidity. The lighting regime was maintained at a 10/14 h dark/light period, using 400 watt metal halide lamps. This experiment consisted of a total of three treatments, randomly allocated amongst the chambers, which each contained eight well spaced plants to allow for thorough air flow and light penetration. The temperature treatments were 10^{o}C / 15^{o}C (night / day), 15^{o}C / 20^{o}C and 20^{o}C / 25^{o}C.

The initial temperature treatment regimes described above were imposed for one week after which the first gas exchange measurements were made. This allowed for the

construction of 'acclimated' temperature response curves (at the growth temperatures) before the transfer of plants to new temperature regimes. Following the initial gas exchange measurement, the plants were transferred between the three temperature treatments whereby plants from the $10^{o}C$ / $15^{o}C$ (night / day) temperature regime were transferred to the $15^{o}C$ / $20^{o}C$ temperature regime, plants from the initial $15^{o}C$ / $20^{o}C$ temperature regime were moved to the $20^{o}C$ / $25^{o}C$ temperature regime, and plants from the $20^{o}C$ / $25^{o}C$ temperature regime were transferred to the $10^{o}C$ / $15^{o}C$ growth environment. The three temperature transfers will hereafter be denoted in the text by the respective day or night temperatures (15-20 and 20-25 and 25-15 for photosynthesis or 10-15 and 15-20 and 20-10 for respiration). Following a week in the new environment a second set of gas exchange measurements were made. While the timing of transfers was clearly artificial, it is consistent with previous rates of acclimation (e.g. Atkin et al. 2000b). Findings of chapter 4 (measurements of seasonal variation in photosynthesis and respiration in this species in the field) also confirm that the timing is appropriate. After eight additional weeks in these growth conditions, a final round of gas exchange measurements was made on new needles which developed under the new temperature regime. Space constraints did not allow for constant temperature ("control") plants, but in any case, the three-way transfer regime provided strong evidence to suggest that temperature change was the driving factor in plant responses.

3.2.2 Gas exchange measurements

Leaf-level gas exchange measurements were made on current year needles (two to six months old) with two cross-calibrated, portable open-path gas-exchange systems with

CO_2 control (Li-6400, Li-Cor Inc., Lincoln NE, USA) using the standard 2 by 3 cm chamber equipped with blue-red light-emitting diodes mounted on the top of the cuvette. Six needles from two fascicles on secondary branches were used for each measurement. Environmental controls within the cuvette were maintained to match the chamber conditions, unless otherwise specified. Measurements of the responses of photosynthesis (A) to intercellular CO_2 partial pressure (C_i) response curves were generated by altering the external CO_2 concentration (C_a) in 14 steps from 150 to 0 Pa at a constant PFD of 1500 μmol m^{-2} s^{-1}. Measurements were made at each C_a set point when photosynthetic gas exchange had equilibrated (taken to be when the coefficient of variation (CV) for the CO_2 concentration differential between the sample and reference analysers was below 1% and visibly stable). This condition was typically achieved within 1-2 min after a stable set point had been reached. The response of photosynthesis (A) to irradiance (Q) were generated by altering the incident PFD in 10 steps from 2000 to 0 μmol m^{-2} s^{-1} using a light source consisting of blue-red light-emitting diodes mounted on the top of the cuvette. During the light response curves, the external CO_2 partial pressure was held constant at 37 Pa. The desired needle temperature for the measurement of photosynthesis (the respective day-time growth temperatures of 15°C, 20°C and 25°C) was maintained using thermoelectric coolers (of the Li 6400) and water vapour pressure deficit was maintained between 1.0 to 1.5 kPa. A/C_i response curves were used to determine values for the maximum rate of carboxylation (V_{cmax}) and the apparent maximum rate of electron transport at saturating irradiance (J_{max}). The A/C_i response data were analysed using the biochemical model of photosynthesis as described by Farquhar et al. (1980) with appropriate parameter corrections for temperature (Bernacchi et al. 2003). *AQ* - light

response curves was used to determine values of maximum assimilation (A_{max}) at saturating light and at ambient CO_2. The *AQ* response is described by a rectangular hyperbola as in Thornley and Johnson (2000). Needle surface area was determined from measurements of fascicle diameter and needle length according to the equation in Turnbull et al. (1998). Surface areas were within the range of 20 - 45 cm^2 and measurements were always conducted on needles that were close to the apex, receiving full illumination. Calculations of photosynthetic parameters from A/C_i and A/Q curves did not include a gasket correction factor (Pons and Welschen 2002). It was assumed that CO_2 exchange processes only occurred in the part of the sample enclosed in the chamber hence unlikely to result in the underestimation of photosynthetic rates. Moreover, the gasket effect was found to be absent when measurements were made at saturating light (present study at 1500 μmol m^{-2} s^{-1}) because the error was negligible in comparison to the high photosynthetic rate (photosynthetic parameters in this present study ranged between, A_{max}: 8.1 to 18.9, J_{max}: 62.3 to 147.5 and V_{cmax}: 31.2 to 77.2) (Pons and Welschen 2002).

Measurements of leaf dark respiration (R_d) were made at the end of the dark period on excised needles removed prior to onset of daytime (illuminated) chamber conditions. Measurements were made on current-year needles (two to six months old) in a separate growth chamber to control measurement temperature. Upon excision, the ends of the fascicles were wrapped in moist paper towel and sealed in a plastic bag and placed in the darkened growth chamber. Previous measurements have shown that leaf respiration remains stable under these conditions for many hours (Turnbull et al. 2005) and this was confirmed here. Measurements were completed within 2-3 h of removal of needles. The

surface area used to calculate respiration rate included a correction factor to account for darkened leaf material under the cuvette gasket. It was assumed that approximately 2cm^2 of sample under the gasket contributed respired CO_2 into the chamber which was added to all total surface area calculations to avoid an overestimation of dark respiration rate (Lambers and Ribas-Carbo 2005). The Li-6400 portable gas-exchange system was itself kept in the darkened growth chamber during the measurements and controlled externally from a lap-top computer. The instantaneous response of dark respiration was determined by making measurements of R_d at temperatures of 10^{o}C, 15^{o}C, 20^{o}C and 25^{o}C in pre-existing and new needles before and after transfer in all three temperature treatments. The growth chamber and leaf chamber temperature conditions were similarly controlled and the needles were allowed to equilibrate to the new temperature conditions for 30-45 min prior to the onset of the respiration measurements. The rate of dark respiration was calculated from the mean of five measurements made over 3 min. Respiratory temperature response curves were analyzed as previously described (Atkin et al. 2005b), where dark respiration rate at a given temperature is given by:

$$R = R_{10} Q_{10}^{[(Tl-T0)/10]}$$

where R_{10} is the respiration rate at the base temperature, T_0 (here 10^{o}C or 283 K), T_1 is foliage temperature (K) and Q_{10} - the proportional change in respiration with a 10^{o}C increase in temperature. Non-linear curve fitting was performed using the Marquardt-Levenberg algorithm (Sigma Plot, Software version 8.0 SPSS Inc. Chicago, Illinois). Nitrogen was determined on needles dried at 70^{o}C, ground to powder and measured with

a CNS analyzer (Carlo Erba Na 1500, Milan, Italy). Specific leaf area (*S*) was calculated following the measurement of individual surface area and dry mass.

3.2.3 Oxygen electrode measurements

The relative enzymatic activities for dark respiration via the cytochrome (COX) and alternative (AOX) oxidase pathways were measured using oxygen electrodes with dual digital controllers (Model 20, Rank Brothers Ltd.). Rates of oxygen consumption were monitored in needle samples in buffer (Delieu and Walker 1981; McCutchan and Monson 2001) for 5 to 10 min using data acquisition software (TracerDAQ, version 1.7; Measurement Computing Corporation, Massachusetts USA). Needle segments measuring 1 cm were incubated in running buffer containing 30mM salicylhydroxamic acid (SHAM, an inhibitor of AOX activity) for 1.5 h in darkness for the measurement of COX activity. In addition, AOX activity was measured following 1.5-hour incubation of needles in 3mM potassium cyanide (KCN, an inhibitor of COX activity). Appropriate controls (without inhibitors) were also incubated in a similar way before measurements were made. Residual respiratory activity was determined following incubation of separate needles in both inhibitors (average 11.6% (±0.008) of total respiration was subtracted from each value before calculating activity as a percentage of total uninhibited respiration). Needles incubated in 50 μM carbonylcyanide m- chlorophenylhydrazone (CCCP) were used to assess the fully uncoupled rate of oxygen consumption. The activity of each pathway was calculated as a percentage of the fully uncoupled rate.

3.2.4 Statistical analysis

Two-way analyses of variance (ANOVA) were used to test for the main effects and interactions of temperature and nutritional content (High–N and Low-N) on photosynthetic parameters, needle N, respiratory parameters, *S*, AOX and COX activity (SAS Institute, software version 9, Cary, North Carolina, USA). Differences were considered significant if $P < 0.05$. Treatment means were compared by least significant difference to determine whether means of the dependent variable were significantly different at $P = 0.05$. Differences between means in Figure 3.3 were evaluated with a one-way ANOVA.

3.3 Results

3.3.1 Needle characteristics

The values of *S* recorded over the range of temperatures varied from 15 to 23.5 m^2 kg^{-1}. Analysis of variance revealed significant differences in *S* of pre-existing and new needles at the new temperature in response to both cold and warm transfer (Table 3.1). Cold-transferred pre-existing and newly developed needles (Table 3.1; 25-15) had significantly lower *S*. Newly developed needles, following transfer to the cooler environment, had lower *S* values than their pre-existing counterparts at the same temperature. By contrast, warm-transferred pre-existing and new needles (Table 3.1; 15-20 & 20-25) had significantly higher *S* values relative to the values observed at the initial temperature. The values of nitrogen recorded over the range of temperatures varied from 2.24 to 3.95 g m^{-2}. A nitrogen-treatment effect was evident between HN and LN plants on both a mass and area basis (N_a: Table 3.1). Cold-transferred pre-existing and new needles

(Table 3.1; 25-15) showed a significant increase in leaf N whilst warm-transferred pre-existing and new needles (Table 3.1; 15-20 & 20-25) displayed a significant decrease in leaf N, with the exception of warm-transferred HN plants (Table 3.1; 20-25) where no significant change was observed at the new temperature.

3.3.2 Photosynthesis

A_{max} recorded over the range of temperatures varied from 8 to 19 μmol m^{-2} s^{-1}. Cold-transfer of plants resulted in no significant change in A_{max} for HN plants but a significant decrease was found in pre-existing and new needles of LN plants after transfer (Fig 3.1a; 25-15). Warm-transferred plants displayed no significant change in A_{max} values in either pre-existing or new needles (Fig 3.1a; 15-20 & 20-25). Values of V_{cmax} over the range of temperatures varied from 31 to 78 μmol m^{-2} s^{-1}. Cold transfer resulted in a large decrease in V_{cmax} in both HN and LN plants (Fig 3.1b; 25-15). By contrast, following warm transfer, a large (approximately two-fold) increase was observed in pre-existing and new needles (Fig 3.1b; 15-20 & 20-25). J_{max} ranged from 60 to 148 μmol m^{-2} s^{-1}. J_{max} declined significantly following cold transfer (Fig 3.1c; 25-15), although pre-existing needles were limited in their ability to achieve values obtained by new needles. J_{max} displayed a significant increase in response to warm transfer (Fig 3.1c; 15-20 & 20-25). As was found for V_{cmax}, pre-existing needles at the new temperature achieved values very close to those observed in new needles. Generally, changes in the response of the J_{max}/ V_{cmax} ratio to temperature were very limited (in both HN and LN plants) (Fig 3.1d; 25-15, 15-20 & 20-25). The long-term responses of V_{cmax} and J_{max} as a function of temperature

Table 3.1. Specific leaf area (S m^2 kg^{-1}) and nitrogen content per unit leaf area (N_a g m^{-2}) in pre-existing and new needles for *Pinus radiata* plants exposed to both increasing and decreasing temperatures. Measurements were made in pre-existing needles at the initial temperature, 7-days after transfer to the new temperature and in new needles at the new temperature. Plants were grown under low (LN) and high N (HN) availability.

Temperature transfer	***S*** **(m^2 kg^{-1})**				***N*****a (g m^{-2})**			
	Pre-existing leaves at initial temperature	Pre-existing leaves at new temperature	New leaves at new temperature	ANOVA Statistics (P)	Pre-existing leaves at initial temperature	Pre-existing leaves at new temperature	New leaves at new temperature	ANOVA Statistics (P)
25 to 15 ºC								
High N	22.9 (0.5)^{a}	19.3 (0.3)^{c}	15.9 (0.4)^{d}	<0.05	2.53 (0.1)^{b}	3.27 (0.1)^{a}	3.20 (0.1)^{a}	<0.05
Low N	21.7 (0.4)^{b}	19.2 (0.2)^{c}	15.1 (0.2)^{d}		2.24 (0.1)^{c}	2.60 (0.1)^{b}	2.68 (0.1)^{b}	
15 to 20 ºC								
High N	19.5 (0.2)^{d}	22.1 (0.3)^{b}	23.0 (0.3)^{a}	<0.05	3.95 (0.1)^{a}	2.88 (0.1)^{b}	2.93 (0.1)^{b}	<0.05
Low N	19.3 (0.3)^{d}	20.6 (0.2)^{c}	21.9 (0.2)^{b}		2.85 (0.1)^{b}	2.40 (0.1)^{c}	2.33 (0.1)^{c}	
20 to 25 ºC								
High N	22.2 (0.2)^{c}	23.3 (0.4)^{ab}	23.5 (0.3)^{a}	<0.05	2.75 (0.04)^{a}	2.62 (0.1)^{a}	2.70 (0.04)^{a}	<0.05
Low N	20.7 (0.2)^{d}	22.6 (0.4)^{bc}	22.3 (0.3)^{c}		2.59 (0.1)^{a}	2.28 (0.1)^{b}	2.25 (0.04)^{b}	

Values shown are means (± standard error of the mean, SEM) where n=8. Different superscripts within a given temperature transfer indicate statistically different values at P<0.05 using least significant difference test of treatment means.

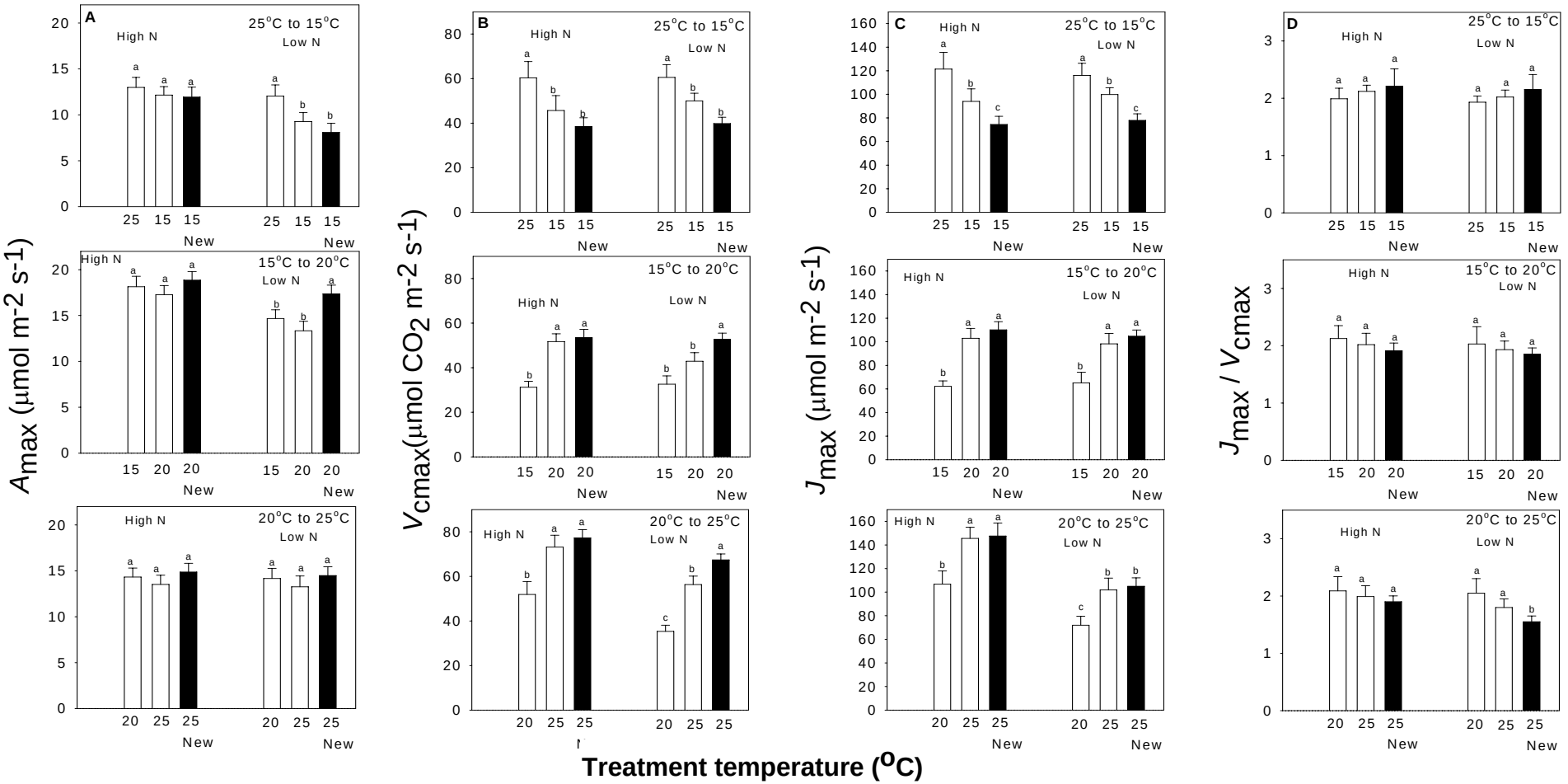

Fig 3.1. Photosynthetic parameters calculated from the *A/c*$_i$ *and A/Q* response in *Pinus radiata* plants (A_{max}, V_{cmax}, J_{max} and J_{max}/ V_{cmax}) under three temperature transfer regimes (25-15°C, 15-20°C and 20-25°C). Each frame displays the photosynthetic response of pre-existing needles at the initial growth temperature and one week after the plants were transferred to the new temperature (open bars). The closed bar represents the responses of newly expanded needles in the new temperature. Data are presented for plants grown under HN and LN availability. All values are means ± standard error; n= 8. Different letters indicate means are significantly different at the P<0.05 level.

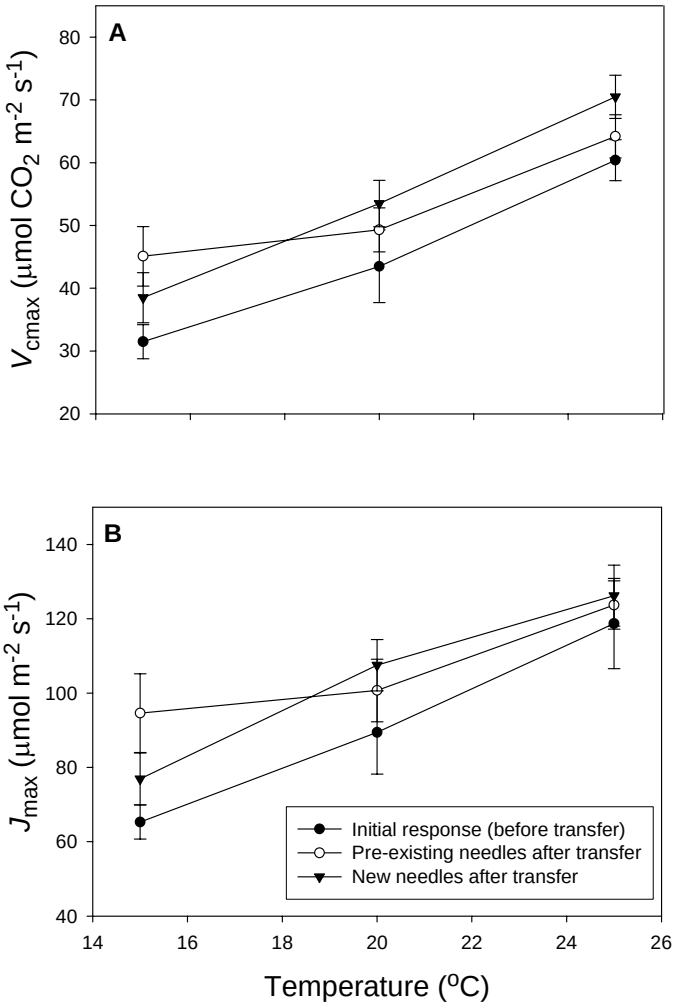

Fig 3.2. Photosynthetic temperature response of (a) V_{cmax} (b) J_{max} at three day-time growth temperatures (15, 20 and 25°C) for *Pinus radiata* plants (means are averaged across N treatments)(n=8).

(determined by taking J_{max} and V_{cmax} at the three growth temperatures before and after transfer) did not change following transfer (Figs 3.2a & b).

3.3.3 Respiration

The instantaneous response of respiration in the dark (R_d) to temperature was found to be sensitive to temperature change (Figs 3.3a, b & c). Following transfer in pre-existing needles, little to no difference in the values of respiration at low temperatures was

observed between treatments (Fig 3.3b). Differences between treatments were only found at moderate to higher temperatures, with the highest values of respiration observed in the 10°C treatment. In new needles R_d was greater in cold-grown plants at both low and high measurement temperatures (Fig 3.3c). The long-term response of respiration (determined by actual R_d at the respective growth temperatures – solid line Figs 3.3a, b & c) was less pronounced than that of the instantaneous response in pre-existing needles after transfer (Fig 3.3b). However, in newly-formed needles, the long-term response of R_d was virtually insensitive to temperature (thermal homeostasis; Fig 3.3c).

Changes in the temperature response characteristics of R_d following growth temperature transfer (calculated from the instantaneous curves) show that leaf respiration increased significantly in response to cold transfer (R_{10} values lower at 20ºC than at 10ºC in new needles (Fig 3.4a; 20-10). Whilst the shift of plants to a cool temperature increased rates of respiration, the shift of plants to warmer temperatures resulted in a decline in R_{10} in new needles (Fig 3.4a; 10-15 & 15-20). The acclimation potential of pre-existing and new needles was clearly different. Significant changes in values of R_{10} in response to either cold and warm transfer (Fig 3.4a; 20-10, 10-15 & 15-20) were only achieved in new needles formed at the new temperature, whilst pre-existing needles at the new temperature were found to show little to no change in R_{10} when compared to the initial response. The values of Q_{10} over the 10-20ºC range varied from 2 to 3.2. Q_{10} was sensitive to temperature transfer, although the magnitude of change varied between treatments. Following cold transfer, no significant response was observed in Q_{10} (Fig 3.4b; 20-10). By contrast, with warm transfer, Q_{10} values generally declined with

increasing temperature (Fig 3.4b; 10-15 & 15-20), although this was most pronounced in plants that had the lowest initial growth temperature (10-15) and significantly so only in new needles.

The respiration/photosynthesis ratio (R_d/A_{max}) generally declined with warm transfer and increased following cold transfer. Following cold transfer, there was very little response in pre-existing needles, but new needles displayed a significant increase in the ratio relative to the initial response (Fig 3.4c; 20-10). Similarly, warm transfers led to no change in the ratio in pre-existing needles at the new temperature but a significant decrease in the ratio was seen in new needles (Fig 3.4c; 10-15 & 15-20). Changes in the long-term (determined by taking R_d/A_{max} at the three growth temperatures before and after transfer) response of R_d/A_{max} to growth temperature were absent before transfer and in pre-existing leaves after transfer (Fig 3.5). Changes to R_d/A_{max} were limited to new needles, in which a greater than two-fold increase was observed at 10ºC relative to the ratio observed at 15 and 20ºC (Fig 3.5).

3.3.4 Enzymatic activity of the cytochrome and alternative oxidase pathways

The enzymatic activity of the COX and AOX pathways (as a percentage of total respiratory activity in leaves) is shown in Table 3.2. COX pathway activity remained remarkably consistent in response to warm transfer (Table 3.2; 10-15 & 15-20) but declined significantly in cold-transferred plants (Table 3.2; 20-10). This was especially evident in new needles. AOX activity in cold-transferred plants increased significantly in pre-existing needles and was sustained in new needles (Table 3.2; 20-10). In warm-

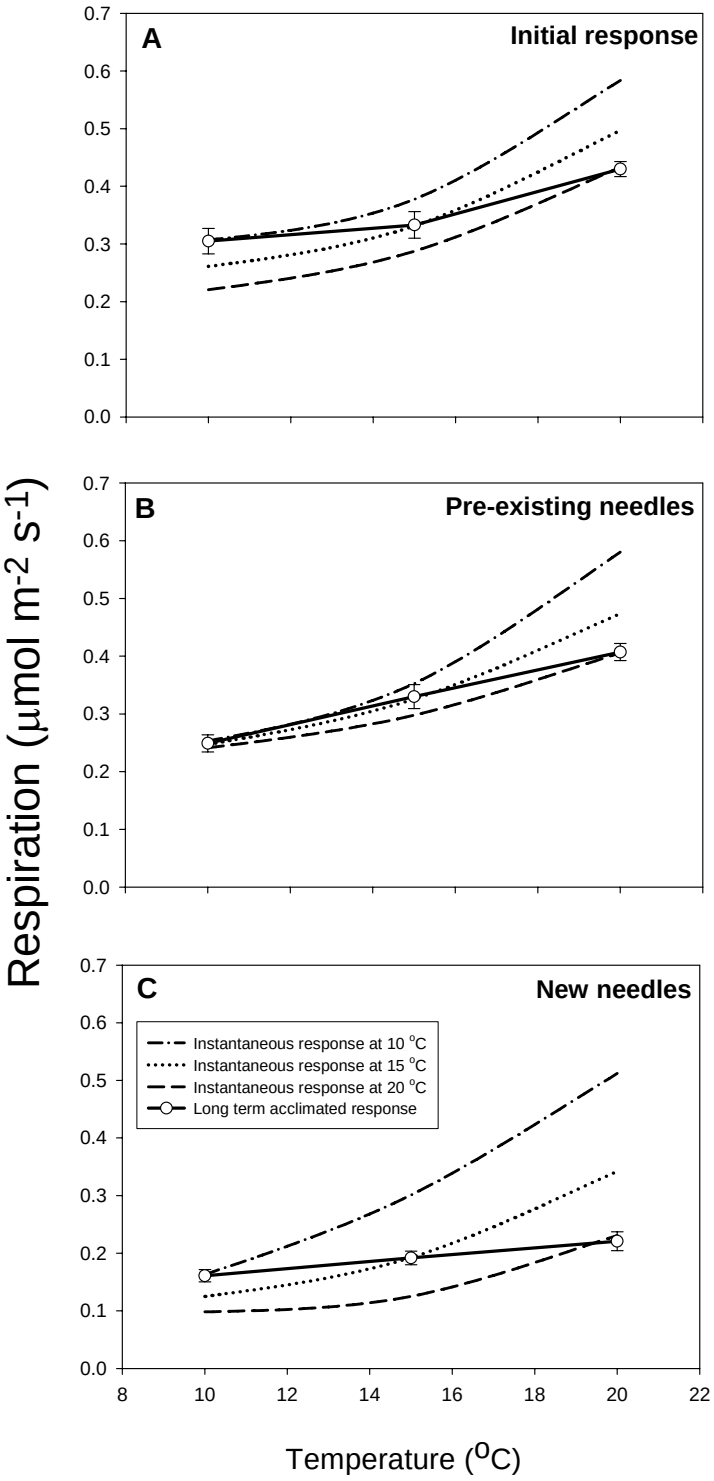

Fig 3.3. The instantaneous respiratory temperature response of (a) pre-existing needles at the initial night-time temperature (before transfer of plants to new temperature regimes) (b) pre-existing needles at the new night-time temperature and (c) new needles at new night-time temperature. The acclimated response (dark solid line) is determined by taking actual values of R_d at the respective night-time growth temperatures. Values at the night-time growth temperatures are means ± SE. One-way ANOVA tests indicated significant differences in R_d at each growth temperature (P= 0.0005 for 10°C, P= 0.00051 for 15°C and P= 0.0005 for 20°C) (data presented are averaged across N treatments).

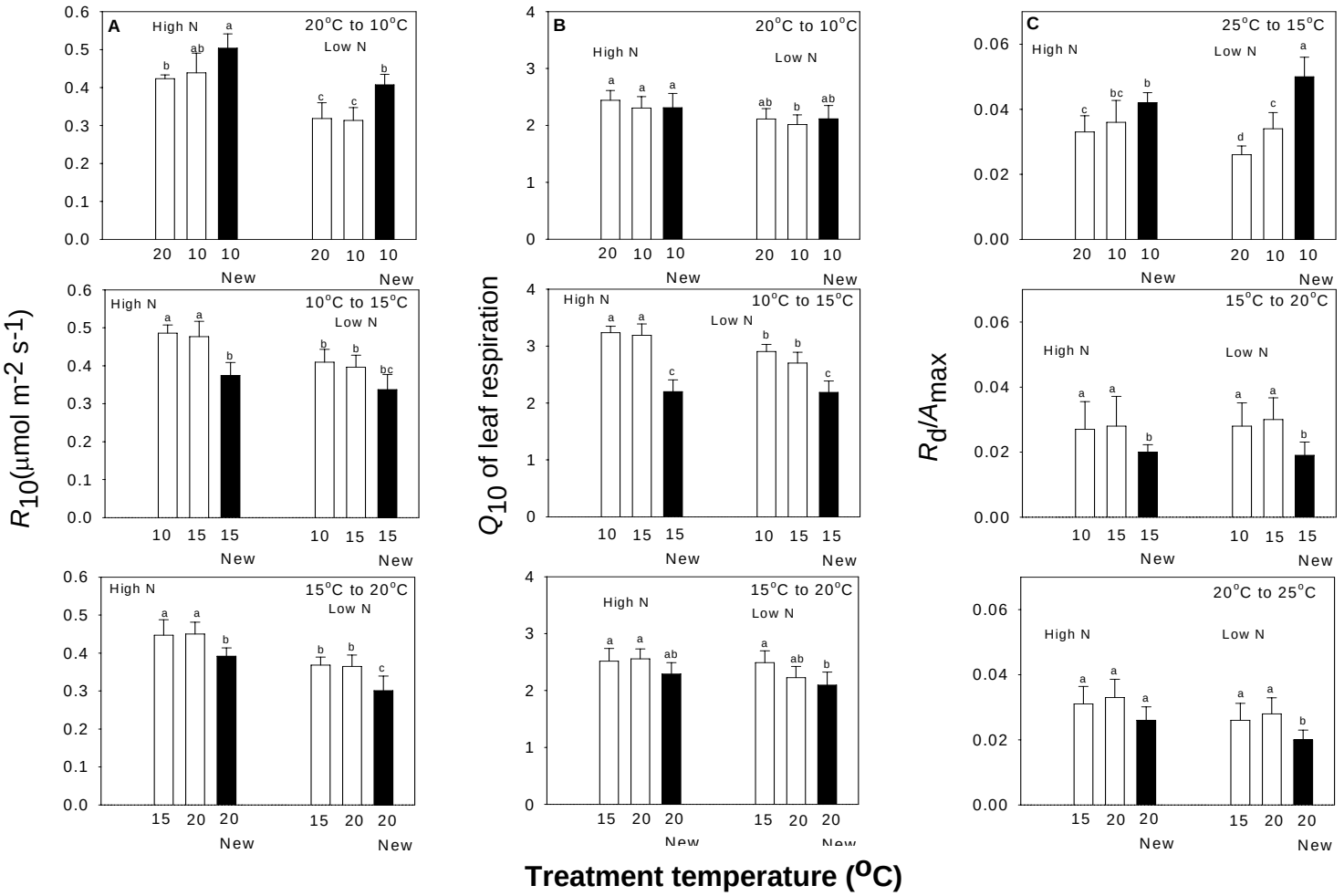

Fig 3.4. Temperature response parameters of dark respiration (R_{10}, Q_{10} and R_d/A_{max}) under three temperature transfer regimes (20-10°C, 10-15°C and 15-20°C). Each frame displays the respiratory response of pre-existing needles at the initial growth temperature and one week after the plants were transferred to the new temperature (open bars). The closed bar represents the response of newly expanded needles in the new temperature. Data are presented for plants grown under HN and LN availability. All values are means ± standard error; n= 8. Different letters indicate means are significantly different at the P<0.05 level.

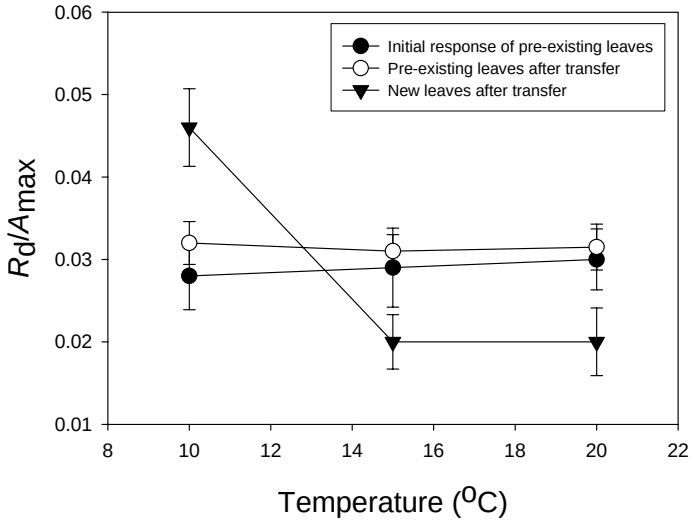

Fig 3.5. Ratios of needle *R* in darkness (R_{dark}) to light saturated photosynthesis at ambient CO_2 (A_{max}) at three night-time growth temperatures (10, 15 and 20°C) for *Pinus radiata* plants (means are averaged across N treatments) (n=8).

transferred plants, there was a less pronounced and transient increase in AOX activity in pre-existing needles of LN plants - this response was sustained in new needles of HN plants only (Table 3.2; 10-15 & 15-20).

3.4 Discussion

This study has provided an analysis of the components of needle characteristics and gas exchange that undergo change when *Pinus radiata* acclimates to a range of contrasting growth temperatures. The findings of this study clearly show that: (1) photosynthetic parameters tend to retain a relatively fixed response to temperature and do not acclimate following changes in growth temperature; (2) acclimation of R_d does occur, but the degree of acclimation exhibited by pre-existing and new needles differs - in new needles, near perfect thermal homeostasis of respiration was observed over a 10ºC temperature range –

Table 3.2. Enzymatic capacity of the cytochrome (COX) and alternative oxidase (AOX) pathways as a percentage of total respiratory capacity in needles of *Pinus radiata* plants exposed to both increasing and decreasing temperatures. Measurements were made in pre-existing needles at the initial temperature and 7-days after transfer to the new temperature and in new needles at the new temperature. Plants were grown under low and high N availability (see Table 3.1 for leaf N contents).

Temperature transfer	Cytochrome pathway capacity (% of total respiratory capacity)			Alternative oxidase pathway capacity (% of total respiratory capacity)		
	Existing leaves	Existing leaves after transfer	New leaves	Existing leaves	Existing leaves after transfer	New leaves
20 to 10 ºC						
Low N	102 (12)^{a}	75 (13)^{ab}	57 (8)^{b}	22 (6)^{b}	45 (10)^{a}	44 (11)^{a}
High N	98 (16)^{a}	60 (12)^{b}	50 (11)^{b}	27 (7)^{b}	48 (10)^{a}	53 (13)^{a}
10 to 15 ºC						
Low N	91(12)^{a}	91 (18)^{a}	102 (19)^{a}	16 (3)^{b}	40 (8)^{a}	16 (4)^{b}
High N	85 (18)^{a}	81 (16)^{a}	79 (19)^{a}	19 (5)^{b}	41 (8)^{a}	38 (6)^{a}
15 to 20 ºC						
Low N	89 (20)^{a}	81 (15)^{a}	86 (17)^{a}	17 (5)^{b}	29 (7)^{a}	15 (4)^{b}
High N	95 (19)^{a}	93 (11)^{a}	91 (14)^{a}	19 (5)^{a}	31 (6)^{a}	27 (5)^{a}

Values shown are means (± standard error of the mean, SEM) where n=4. Different letters within rows indicate statistically different values at P<0.05 using least significant difference test of treatment means.

this was absent from pre-existing needles at the new temperature; (3) thermal acclimation displayed in new needles results in a change in the R_d/A ratio which favours respiration at low temperatures and photosynthesis at high temperatures. These results are important to our understanding of tree function in response to changing environmental conditions and to modelling efforts to predict vegetation responses to global climate change.

3.4.1 Photosynthetic response to temperature transfer

Studies investigating the temperature response of photosynthesis report great variability in the degree of photosynthetic acclimation among species, with some species exhibiting full acclimation whilst others are incapable of even partial acclimation (Berry and Bjorkman 1980; Atkin et al. 2005a). The photosynthetic data suggest that cold transfer resulted in a reduced photosynthetic capacity in *Pinus radiata* (despite increasing tissue nitrogen content) whilst 5°C increases in temperature led to significant increases in photosynthetic capacity. The observations of decreased photosynthetic rates in response to cold transfer are consistent with previous work by Rook (1969) who found a 25% decrease in photosynthetic rates of *Pinus radiata* seedlings following transfer to cooler temperatures. Overall, the long-term temperature response of photosynthesis over the range of 15-25ºC (Figs 3.2a & b) shows that photosynthesis retains its instantaneous sensitivity to temperature, as evidenced by an increase in values for V_{cmax} and J_{max} with increasing growth temperature. The shift to a new temperature did not elicit significant change in the shape or position of the long-term photosynthetic temperature response curve. These observations indicate that only a very limited degree of photosynthetic

acclimation occurs in *Pinus radiata* in the face of changes in temperature. This confirms the findings of the previous chapter for poplar grown under controlled conditions (Chapter2; Ow et al. 2008).

It was also observed that the ratio of the capacities of ribulose-1,5-bisphosphate (RuBP) regeneration to RuBP carboxylation (J_{max} / V_{cmax}) was insensitive to temperature change (Fig 3.1d). This is consistent with previous work by Onoda et al. (2005). Similarly, Medlyn et al. (2002) also observed that the J_{max} / V_{cmax} ratio did not change with changes in growth temperature in *Pinus taeda* and *Pinus pinaster*. In a previous study, Hikosaka (1997) posed the hypothesis that temperature acclimation is a result of a re-allocation of nitrogen between the electron transport and carboxylation processes so as to ensure that both processes are co-limiting at ambient conditions. Under this hypothesis, it is predicted that the J_{max} / V_{cmax} ratio at a given temperature should be lower for plants exposed to low temperatures. In this study however, the transfer to cooler conditions (25 to 15ºC) led to very little change in this ratio. Other studies investigating acclimation to growth temperature have similarly failed to find any evidence of such a change (Bunce 2000; Medlyn et al. 2002a). Across a range of temperatures (10-35^{o}C), rates of photosynthesis are limited either by RuBP carboxylation or by RuBP regeneration (Berry and Bjorkman 1980). An increase in the J_{max} / V_{cmax} ratio at low growth temperatures is considered to relieve the limitation of RuBP regeneration on photosynthetic rate, whilst a decrease in the ratio with increasing temperature is a result of a greater activation energy

for V_{cmax} than for J_{max} (Onoda et al. 2005). J_{max} has been found to possess a lower optimum temperature than V_{cmax} (Dreyer et al. 2001), and this may explain the imbalance between the two processes when growth temperature changes, resulting in changes in the J_{max} / V_{cmax} ratio (Farquhar and von Caemmerer 1982; Onoda et al. 2005). However, a change in the J_{max} / V_{cmax} ratio does not always indicate an acclimation response as it may be simply due to the differences in the short-term responses of J_{max} and V_{cmax} to temperature. Regardless of the factors involved in temperature acclimation of photosynthesis, Onoda et al. (2005) posed the hypothesis that some species (i.e. pines) may have photosynthesis that is limited by RuBP carboxylation over a wide range of temperatures, but does not have plasticity in the J_{max} / V_{cmax} ratio. This may have the advantage of incurring less cost in reconfiguration of the photosynthetic apparatus with temperature acclimation.

3.4.2 Respiratory responses to changes in temperature

In the present study, the findings show that respiration in *Pinus* is strongly temperature sensitive in the short-term (increasing approximately exponentially with temperature), but generally shows compensatory adjustments (acclimation) over a longer period (weeks and months). These findings are consistent with previous findings for a range of largely non-tree species (Larigauderie and Korner 1995; Atkin et al. 2000; Atkin and Tjoelker 2003; Wright et al. 2006). Cold transferred plants (Fig 3.4a; 20-10) displayed a significant increase in rates of R_d in new needles only, whilst warm transferred plants (Fig

3.4a; 15-20 & 20-25) exhibited a significant decrease in rates in new needles. Rates of respiration at growth temperatures were greater than predicted by the instantaneous response in cold acclimated needles, and less than predicted by the instantaneous response in warm acclimated needles. As a result, the long-term (acclimated) response of R_d in pre-existing and new needles was much less pronounced than the instantaneous response (Figs 3.3a, b & c). This was especially evident in new needles, with near identical rates of respiration at contrasting temperatures, indicating full respiratory homeostasis over a 10ºC temperature range (Fig 3.3c). Similar to the conclusions of chapter 2; Ow et al. (2008), data presented in Figure 3.3 shows that the instantaneous response is a poor predictor of long-term acclimated responses of respiration to changes in temperature. The findings here are consistent with Atkin and Tjoelker (2003) and Atkin et al. (2005a) where the short-term temperature response curves appear to be poor indicators of long-term respiratory CO_2 release and therefore should only be used with caution.

These findings clearly suggest that acclimation potential may be limited in pre-existing needles in *Pinus*, and that full acclimation may only be achieved in new needles grown under the new temperature. Moreover, this limitation is exhibited in both warm- and cold-transferred plants. The majority of studies investigating acclimation of leaf respiration have investigated plants with short-lived leaves (Loveys et al. 2003; Armstrong et al. 2006a; Armstrong et al. 2006b). These studies have concluded that acclimation is developmentally dependent (i.e. new leaves are required to develop at the new growth

temperature for full acclimation of dark respiration). There are, however, remarkably few supporting studies involving tree species, and even fewer involving conifers. Interestingly, the findings here differ from those of Bolstad et al. (2003), who found a high degree of thermal acclimation in dark respiration in fully expanded, pre-existing leaves of deciduous species such as *Quercus alba* and *Quercus rubra*. Other studies have also reported evidence of substantial acclimation in leaves of other long-lived species (Bolstad et al. 1999; Atkin et al. 2000b; Bolstad et al. 2001). Such studies have proposed that full acclimation can occur in pre-existing leaves, but only if the tissues are sufficiently long-lived to allow for sufficient time for cellular and biochemical changes to take place within pre-existing leaves. This is often absent from short-lived species as the changes cannot take place due to senescence.

While there is strong evidence from previous studies showing that tissue N content clearly regulates rates of respiration, it had little or no impact on the extent of thermal acclimation in this present study (at least within the range of N concentrations experienced here). This was further confirmed in a recent study conducted on poplar (Chapter 2; Ow et al. 2008). Here, respiratory acclimation was achieved, in part, through changes in *S* (i.e. the amount of metabolizing tissue per unit area). This highlights the importance of leaf structural changes to thermal acclimation. However, despite significant changes in *S* following transfer to a new temperature, the response of R_d in pre-existing leaves was limited - suggesting that physiological changes are largely

determined at the time of leaf development and expansion, and involve alteration of structure (anatomy/morphology), enzymatic capacity and chemical composition (Atkin et al. 2006a). This is important for our understanding of respiratory responses of trees under conditions of varying soil nutrition.

Although respiration responds to temperature on both short-and long-term time scales, the Q_{10} of respiration describes the short-term sensitivity of respiration to temperature (i.e. seconds to hours). In this present study, despite changes in R_d with cold transfer, the Q_{10} of leaf respiration did not change significantly. However, warm transfers led to a significant decrease in Q_{10} values (Fig 3.4b; 10-15 & 15-20). This is consistent with previous studies which have shown that the Q_{10} of respiration often decreases with increasing temperature (Tjoelker et al. 2001). The relative responses of R_{10} and Q_{10} in pre-existing and new needles in this study allow for the assessment of the extent to which acclimation involves type I and type II mechanisms (Atkin and Tjoelker 2003). Type I acclimation (changes in respiration at moderate to higher temperatures) corresponds well with the findings for pre-existing needles. By contrast, new needles clearly displayed characteristics of Type II acclimation (change in respiration at high and low temperatures), which is often associated with temperature–mediated changes in respiratory capacity achieved through the development of new tissues with altered anatomy and morphology (Atkin and Tjoelker 2003). Analysis of enzymatic activity of the COX and AOX pathways (Table 3.2) supports the notion that Type II acclimation may result in changes in the relative amounts of enzymes or proteins invested into the

respiratory chain (Atkin et al. 2005a). This was especially evident in cold transferred plants, where COX activity declined whilst AOX activity increased significantly. This switching between the AOX and COX pathways is well documented in a range of species such as *Alocasia odora, Spinacia oleracea, Zea mays* and *Phaseolus vulgaris* (Ribas-Carbo et al. 2000; Nagel et al. 2001; Nogochi et al. 2001) and have been confirmed in the previous chapter involving poplar (Chapter 2; Ow et al. 2008). Furthermore, the AOX pathway is highly sensitive to changes in temperature and engagement of this pathway may have advantages for plant acclimation to colder temperature, whereby energy that should otherwise be conserved as ATP is converted to heat (Ribas-Carbo et al. 2000). The nature of these measurements (using inhibitors of COX and AOX) only help with our understanding of enzymatic activity and not actual *in vivo* capacity. However, the findings here should still prove a useful directive to future work aimed at understanding enzymatic changes that accompany respiratory acclimation in changing environments.

3.4.3 Balance between photosynthesis and respiration

Given the tight coupling that exists between photosynthetic and respiratory metabolism (Atkin et al. 2006b) but the different temperature sensitivities of the two processes, a change in the respiration/photosynthesis ratio can occur following a change in growth temperature. Although the R_d/A ratio varied with cold and warm transfers (Fig 3.4c), in the long-term a strong negative relationship with temperature in new needles was observed i.e. the ratio was greater at low temperature and decreased at high temperatures.

The ratio increased at low temperature due to increasing dark respiration (a result of acclimation) and simultaneously decreasing photosynthesis, whilst the lower ratio resulted from lower R_d (a result of acclimation) but greater photosynthesis at higher temperatures.

3.5 Summary

The present study has highlighted that pine has considerable potential for respiratory acclimation to changes in growth temperature but there was little evidence of temperature acclimation in photosynthetic characteristics. Similar to the findings of the previous chapter, nitrogen status of needles had little, if any, impact on the degree of acclimation. These results suggest that as temperatures increase over the moderate range, CO_2 uptake in *Pinus* will be favoured over CO_2 release. The overall impact of this would be a reduction in the potential for positive feedback of respiration in the carbon cycle as temperature increases, in contrast to previous suggestions that the opposite might be the case (Cox et al. 2000; Houghton et al. 2001; Luo et al. 2001). At present, most climate-C cycle models assume that rates of photosynthesis and respiration will increase with increasing temperature in a predictable way that will remain constant over time (i.e. they will not acclimate to changes in temperature (Rustad 2001)). The result of this may be an over-estimate of the effects of global warming on respiratory CO_2 release over long periods.

So far, analyses into the effects of photosynthetic and respiratory acclimation in different tissue types and species have been conducted under controlled conditions (Chapters 2 and 3). In order to understand the extent of photosynthetic and respiratory acclimation to diurnal and seasonal changes, the next chapter studies the effects of seasonal variation in temperature on photosynthesis and respiration on both pine and poplar in their natural environment. Therefore, the next chapter will focus on the potential for acclimation to diurnal and seasonal changes in temperature and the role of foliar properties (e.g. nitrogen and carbohydrates) as potential drivers to the process of acclimation.

Chapter 4

Seasonal variation in foliar carbon exchange in *Pinus radiata* and *Populus deltoides*: respiration acclimates fully to changes in temperature but photosynthesis does not

4.1 Introduction

Understanding the processes and factors influencing seasonal variation in respiratory and photosynthetic parameters is critical for accurate modelling of CO_2 exchange between vegetation and the atmosphere (Armstrong et al. 2006a; Misson et al. 2006). Leaf respiration plays a key role in determining the growth and survival of plants (Hurry et al.1995) but also has a strong influence on net ecosystem exchange and atmospheric CO_2 concentrations (Gifford 2003). Plant respiration is associated with the production of energy and carbon skeletons essential for cellular maintenance and biosynthesis. Approximately 30 to 80% of daily carbon fixed by photosynthesis is respired back into the atmosphere, with almost half of whole plant respiration attributable to foliage (Loveys et al. 2002). In fact, plant respiration generally contributes between 60 and 70% of total CO_2 released into the atmosphere from terrestrial ecosystems (Xu et al. 2001), with the remaining CO_2 respired from below-ground processes.

The extent of leaf respiratory CO_2 release is often studied under controlled–environments using constant temperature conditions (Atkin et al. 2000b) - these may differ from natural conditions which vary diurnally and seasonally. Respiration is highly sensitive to short-term changes in temperature (Atkin et al. 2005a), and the relationship is described by the parameter Q_{10} (the increase in respiration rate for a 10 °C increase in temperature). In the long term, changes in temperature may result in acclimation (Covey-Crump 2002; Atkin et al. 2003; Bolstad et al. 2003; Armstrong et al. 2006a), where an adjustment in the rate of respiration occurs that compensates for the initial change in temperature (Atkin et al. 2003). Leaf respiration has been found to acclimate to changes in growth temperature, such that cold-grown plants (e.g. during winter) display higher rates of respiration at a set temperature than their warm-grown counterparts (Reich et al. 1996; Atkin et al. 2005a; Ow et al. 2008). Full acclimation, or perfect homeostasis, of respiration, is said to occur when plants display identical rates of respiration when grown at different temperatures and measured at the respective growth temperatures. The degree of respiratory acclimation varies greatly between species (Larigauderie et al. 1995) but the speed of respiratory acclimation to changes in temperature is also critical in determining the rate of respiration at any given time. This variation in respiration must be accounted for in ecosystem CO_2 exchange models and in coupled climate and carbon-cycle models if future atmospheric CO_2 concentrations are to be predicted accurately (Wythers et al. 2005; King et al. 2006).

Given that the rate of respiration is linked to photosynthesis through its dependence on substrate supply and also via the demands for ATP (Whitehead et al. 2004a; Atkin et al.

2006), the temperature response of photosynthesis has become an important focus of recent study (Medlyn et al. 2002). Many studies have assessed the seasonality of photosynthesis to better understand photosynthetic contribution to net carbon exchange and to improve estimates in global carbon budgets. Based on the biochemical model of Farquhar et al. (1980), photosynthetic rate is limited either by RuBP (ribulose-1,5-bisphosphate) carboxylation or by the rate of RuBP regeneration. Hence, it is important that seasonal variation in maximum carboxylation velocity (V_{cmax}) and maximum rate of electron transport (J_{max}) are well established. Rates of photosynthesis have long been known to acclimate to prevailing temperature (Berry et al. 1980) but the degree of photosynthetic acclimation is highly variable between species as well as within genera (Atkin et al. 2006). Acclimation can change the shape of the photosynthetic temperature response curve or result in a shift in the curve such that the absolute rate and/or the optimum temperature changes. Furthermore, photosynthetic parameters have been observed to differ seasonally (Eamus et al. 1999; Makela et al. 2004; Misson et al. 2006; Zarter et al. 2006b) and this may be the result of variations in leaf phenology (evergreen versus deciduous), light or temperature acclimation, or nitrogen and water status. The seasonality in photosynthetic parameters in temperate evergreen species is generally regarded to be less than that in deciduous species (Damesin et al. 1998, Warren et al. 2004; Zarter et al. 2006a).

In this study the effect of seasonal variation in temperature on leaf respiration and photosynthesis was investigated in mature trees growing in field conditions for two contrasting species: a broadleaved, deciduous (*Populus deltoides*) and a coniferous,

evergreen (*Pinus radiata*). The earlier chapters (chapters 2 and 3) looked at the responses of photosynthesis and respiration in these species to step changes in temperature under controlled conditions (Ow et al. 2008a, 2008b). These investigations showed significant thermal acclimation in respiration but not with photosynthesis for both species. Based on the findings of chapters 2 and 3, the hypothesis was that a greater magnitude of acclimation would occur with respiration and less so with photosynthesis, and foliar carbohydrate and nitrogen content will serve as potential drivers in the process of acclimation. A key objective in the present study was to use natural variation in ambient temperature to establish whether variation in respiration and photosynthesis over the course of a year was an age-dependent or a temperature-dependent (acclimation) response. Another objective was to assess if foliage respiration would be stimulated significantly in the trees during the warmer months resulting from a change in the balance between respiration and photosynthesis.

4.2 Materials and methods

4.2.1 Site description

This study was carried out on mature pine (*Pinus radiata* D. Don) and poplar (*Populus deltoides* x *nigra var. italica*) trees growing at a flat, free-draining site at Lincoln, south of Christchurch, New Zealand (Latitude 43^{o} 25' S, longitude 172^{o} 58' E, at sea level). Annual precipitation at the site is approximately 648 mm, most of which occurs in winter and spring. Gas exchange measurements were made on trees exposed to natural seasonal variation at a monthly interval between July 2005 and June 2006. Maximum and minimum temperatures during the period of observation were in the range of 5.4 to 31.5

^{o}C and -2 to 14.8 ^{o}C, respectively. The highest temperature occurred in November whilst the lowest temperature was observed in July. Measurements were conducted on fully expanded leaves/needles. Tree height was 5-6 m for the pine trees and 7-8 m for poplar. Scaffolds (providing access to 4-5 m above ground) were erected on the north and east side of the pine and poplar trees respectively, to maximize the exposure of leaves/needles to sunlight throughout the day.

4.2.2 Gas exchange measurements and tissue analysis

Photosynthesis and respiration measurements were made using two cross-calibrated, portable open- flow gas-exchange systems with CO_2 control (Li-6400, Li-Cor Inc., Lincoln NE, USA). Six current-year pine needles from two fascicles (same needle age class followed) and poplar leaves that had an area of 600 mm^2 or larger, growing on secondary branches (n=6, two shoots from 3 trees/species), were used for each measurement. Environmental controls within the cuvette were maintained to match the atmospheric conditions, unless otherwise specified. Measurements of the responses of photosynthesis (A) to intercellular CO_2 partial pressure (C_i) were generated by altering the external CO_2 concentration (C_a) in 14 steps from 150 to 0 Pa at a constant photon flux density (Q) of 1500 μmol m^{-2} s^{-1}. Measurements were made at each C_a set point when photosynthetic gas exchange had equilibrated (taken to be when the coefficient of variation (CV) for the CO_2 concentration differential between the sample and reference analysers was below 1% and visibly stable). This condition was typically achieved within 1-2 minutes after a stable set point had been reached. Light response curves were generated by altering the incident Q in 10 steps from 2000 to 0 μmol m^{-2} s^{-1} using a light

source consisting of blue-red light-emitting diodes mounted on the top of the cuvette. During the light response curves, the external CO_2 partial pressure was held constant at 37 Pa. The desired needle/leaf temperature for the measurement of photosynthesis (set at the forecast maximum temperature for the day of measurement) was maintained using thermoelectric coolers of the Li-6400 and water vapour pressure deficit was maintained between 1.0 to 1.5 kPa. A/C_i response curves were used to determine values for the maximum rate of carboxylation (V_{cmax}), the apparent maximum rate of electron transport at saturating irradiance (J_{max}) and values of the limitation imposed by the stomata on the rate of photosynthesis (L_{stom}). The A/C_i response data were analysed using the biochemical model of photosynthesis as described by Farquhar et al. (1980). The dependence of leaf photosynthesis (J_{max} and V_{cmax}) to temperature was determined based on the ambient temperature range experienced by leaves in the field, using the temperature response function of Leuning (2002). This allowed a comparison to be made with published short-term photosynthetic temperature responses. The response of photosynthesis (A) to irradiance (Q) was used to determine values of maximum photosynthesis (A_{max}) at saturating irradiance and at ambient CO_2 concentration. The A/Q response is described by a rectangular hyperbola as described by Thornley et al. (2000). Needle surface area was determined from measurements of fascicle diameter and needle length following Turnbull et al. (1998). For consistency with previous studies, surface areas are presented on a one-sided basis for poplar and total surface area for pine.

Measurements of respiration were made at the end of the night-period on excised needles/leaves collected prior to sunrise. Needles that began growth in the spring of the

previous year as well as fully expanded poplar leaves with an area of 600 mm^2 or larger were collected. Upon excision, the ends of the fascicles/petioles were wrapped in moist paper towel in a plastic bag and placed in a darkened growth chamber. Previous measurements have shown that leaf respiration remains stable under these conditions for several hours (Turnbull et al. 2005). The leaf/needle area used to calculate respiration rate included a correction factor to account for darkened leaf material under the cuvette gasket (following Lambers and Ribas-Carbo (2005)). Measurements were completed within 2-3 hours of removal of needles/leaves. The Li-6400 portable gas-exchange system was itself kept in the darkened growth chamber during the measurements and controlled externally from a lap-top computer. The instantaneous response of dark respiration was determined by making measurements of R_d at five temperature points over the range 5 to 22 ^oC. The growth chamber and leaf chamber temperature conditions were controlled similarly and the needles/leaves were allowed to equilibrate to the new temperature conditions for 30 to 45 minutes prior to respiration measurements. The rate of dark respiration was calculated from the mean of 5 measurements made over 3 minutes.

Respiratory parameters were determined as described in Atkin et al. (2005b), where dark respiration rate at a given temperature is given by:

$$R_d = R_{10} Q_{10}^{[(T_1 - T_0)/10]}$$

where R_{10} is the respiration rate at the base temperature, T_0 (here 10 ^oC or 283 K), T_1 is foliage temperature (K) and Q_{10} - proportional change in respiration with a 10 ^oC increase

in temperature. Non-linear curve fitting was performed using the Marquardt-Levenberg algorithm (Sigma Plot, Software version 8.0 SPSS Inc. Chicago, Illinois).

Nitrogen concentration on an area basis was determined on needles/leaves dried at 70 ^{o}C, ground to powder and measured with a CNS analyzer (Carlo Erba Na 1500, Milan, Italy). Total non structural carbohydrate and starch content were determined on dried and ground material using the methanol: chloroform: distilled water method of Tissue et al. (1995). Plant material was dried in an oven at 70 ^{o}C for 2 days, ground into a fine powder and then extracted 3 times with 2 ml of a methanol:chloroform:water (12:5:3) solution to separate the soluble sugars from the pellet containing starch. The homogenate was centrifuged at 9000g for 5 minutes, and the combined supernatant was transferred to a fresh 10-ml test tube where a further 2 ml of distilled water was added, then the solution was mixed thoroughly and left overnight in a refrigerator. The following day, the chilled supernatant was transferred to a fresh test tube which contained 5% phenol, distilled water and sulfuric acid (H_2SO_4) (details of chemical concentration is given in Guo et al. (2002)). The soluble sugars were determined at the absorbance of 490 nm against distilled water, using glucose as a standard. The pallet for starch determination was left to dry in a fume hood overnight, the pallet was later treated with 5 ml of 35% perchloric acid for an hour for starch hydrolysis. The mixture was later filtered and the filtered solution was transferred on to a fresh test tube. This solution was assayed as mentioned above. Specific leaf area (*S*) was calculated following the measurement of individual surface area and dry mass.

4.2.3 Data analysis

Repeated measures analysis of variance was used to examine the effects of seasonal temperature variations on foliar properties, photosynthesis and respiration, using month as the time variable (SAS Institute, software version 9, Cary, North Carolina, USA). Regressions were used to analyse the relationships between gas exchange characteristics (V_{cmax}, J_{max}, R_{10}, Q_{10}) and temperature, leaf N on an area basis, sugar and starch concentrations in leaves (Sigma Plot, Software version 8.0 SPSS Inc. Chicago, Illinois).

4.3 Results

4.3.1 Foliar characteristics

Values of specific leaf area, S, measured over the period of observation for pine and poplar varied between 12.0 to 16.8 $m^2\ kg^{-1}$ and 31.1 to 39.6 $m^2\ kg^{-1}$ respectively (Fig. 4.1A). The values of S for pine were not significantly different from July 2005 to June 2006 although small decreases were observed during winter (Fig. 4.1A). Similarly, there was also no significant seasonal differences in S for poplar (Fig. 4.1A) but small increases were observed from March to April 2006 during a period of senescence. The values of N_a recorded over the period of observation for pines varied from 1.53 to 2.26 g m^{-2} whilst values for poplar varied between 2.11 to 3.45 g m^{-2} (Fig. 4.1B). There was no significant change in N_a throughout the 12-month period of observation for pine but a significant difference was observed in poplar in October 2005 and April 2006 (Fig. 4.1B). Seasonal patterns of leaf sugar and starch concentrations are shown in Figs. 4.1C and D. Both sugar and starch displayed a similar pattern of change - an increase throughout autumn and winter and a decline in the warmer months. The difference in sugar content

of pine needles between the maximum which occurred in winter (July 2005) and the minimum which occurred in summer (January 2006) was approximately 50%. Though smaller in magnitude, the difference between the maximum and minimum starch

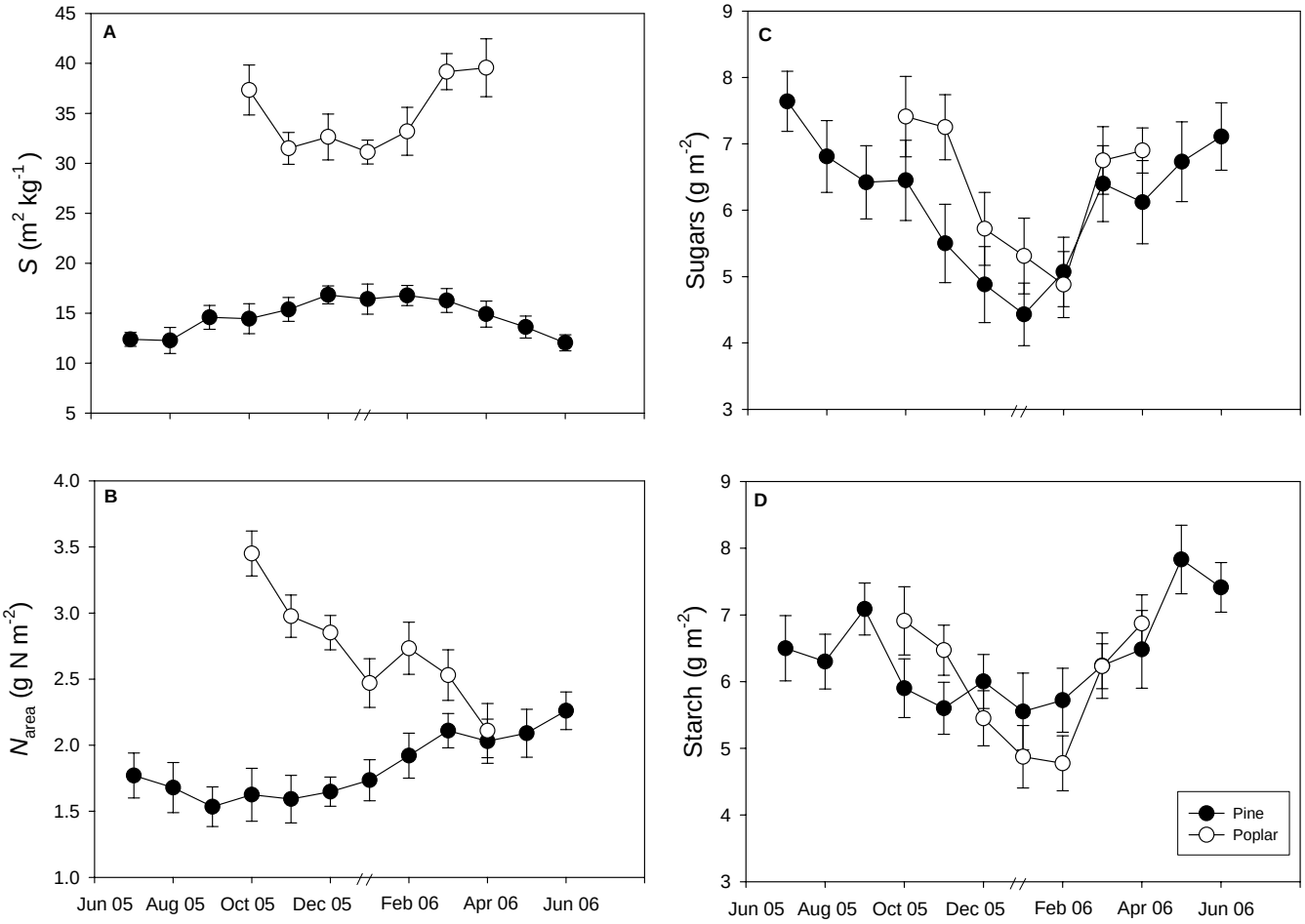

Figure 4.1. Seasonal variation in (A) specific leaf area, *S*, (B) nitrogen on an area basis (N_{area}), (C) soluble sugar and (D) starch concentration in field-grown trees of *Pinus radiata* and *Populus deltoides* as a function of time. Values shown are means (± SEM) where n=6.

concentration in pine needles was at least 20%. The low levels during summer are consistent with leaf expansion during this period.

4.3.2 Photosynthesis

Values of V_{cmax} ranged between 16.2 and 30.6 μmol m^{-2} s^{-1} in pine and between 48.8 and 87.4 μmol m^{-2} s^{-1} for poplar (Fig. 4.2A). V_{cmax} in pine increased gradually during the warmer seasons (spring and summer) and exhibited a small decline in autumn and remained low throughout winter. By contrast, the response in poplar was much more variable, with V_{cmax} low in spring but then displaying an approximately 2-fold increase in summer followed by a rapid decline in autumn. The seasonal response of J_{max} (Fig. 4.2B) was found to be similar to that of V_{cmax}. The ratio of J_{max} to V_{cmax}, an indicator of electron transport capacity (Fig. 4.2C), varied between 1.92 and 2.69 in pine and between 1.76 and 2.73 in poplar. There was very little change in the ratio for poplar from December 2005 to April the next year but the ratio was relatively high in spring and a rapid increase was observed between October and November 2005 where the ratio increased from 2.17 to 2.73. Some variation was observed in pine where a lower ratio was observed in December 2005 and January 2006 but with the exception of August 2005 and April 2006 which exhibited exceptionally high values, a relatively consistent ratio was observed in the remaining months. The lower ratio observed in summer may be partly explained by a greater sensitivity of V_{cmax} relative to J_{max} to increasing leaf temperature. A_{max} in pine and poplar varied between 7.3 to 14.3 and 9.8 to 21.0 μmol m^{-2} s^{-1}, respectively (Fig. 4.2D). Values of A_{max} for pine remained relatively constant for most of the annual period of observation with gradual increases in rates occurring from November 2005 through to February 2006. This was subsequently followed by a decrease in rates observed in autumn and winter of the following year. Values of A_{max} for poplar were much more variable - an approximately 50% increase was observed between the period of November

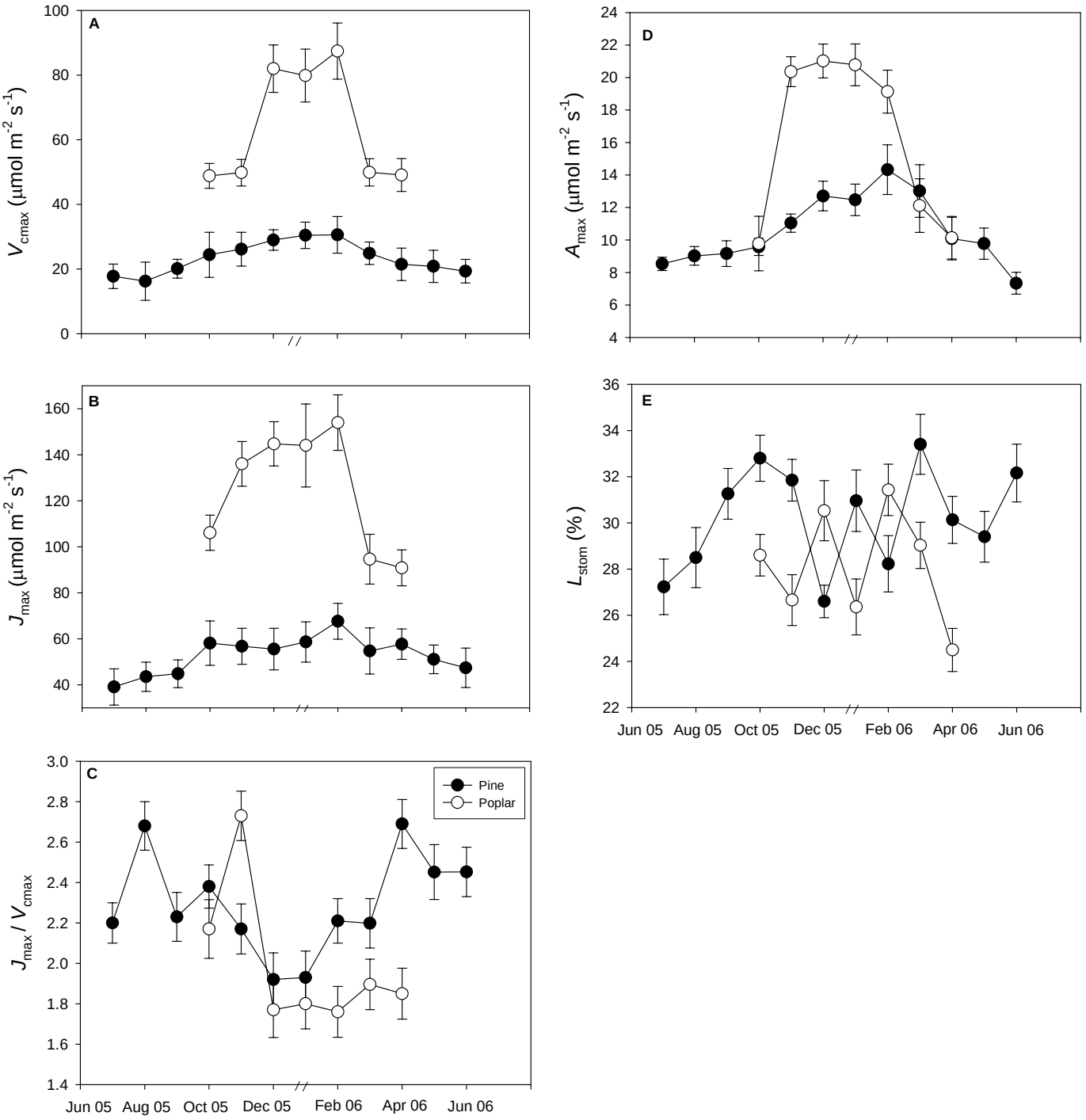

Figure 4.2. Photosynthetic parameters calculated from A/C_i and A/Q responses in *Pinus radiata* and *Populus deltoides* grown in the field (A) V_{cmax}: maximum rate of ribulose-1,5-bisphosphate (RuBP) carboxylation, (B) J_{max}: maximum rate of RuBP regeneration, (C) J_{max}/ V_{cmax} ratio, (D) A_{max}: net CO_2 assimilation measured at saturating irradiance and ambient CO_2 and (E) L_{stom}: limitation imposed by the stomata on the rate of photosynthesis as a function of time. Values represent the mean of six replicates (± SEM).

2005 through to February 2006. This increase was then followed by a decline in March and April 2006 (autumn). L_{stom} varied over a narrow range from 26.6 to 33.4% and 24.5 to 31.4% in pine and poplar, respectively (Fig. 4.2E). L_{stom} was generally low in both pine and poplar (<33%) and was not significantly different between seasons in both species. Photosynthetic parameters in the long term in both pine and poplar were found to be strongly dependent on temperature (Fig.4.3). To determine if seasonal variation in photosynthetic parameters can be explained by foliar properties (e.g. nitrogen and carbohydrate concentrations), regressions were applied (Fig. 4.4). These show that foliar N status did not influence V_{cmax} and J_{max} significantly but a negative relationship was observed with carbohydrate concentration.

4.3.3 Respiration

Short-term temperature response curves were generated for leaf tissue throughout the year and values for R_{10} and Q_{10}, were calculated. R_{10} ranged between 0.13 - 0.36 and 0.55 - 0.71 μmol m^{-2} s^{-1} for pine and poplar, respectively (Fig. 4.5A). Values of R_{10} in poplar were relatively constant except for a decreasing trend observed from October through to December (late spring). R_{10} of pine was found to be highest in winter and spring but rapidly declined in summer and gradually increased again in autumn. A difference in rate of approximately 30% was observed between winter and summer. Q_{10} varied between 2.10 - 2.28 in pine and 2.15 - 2.30 in poplar across a temperature range of 5-22 ^{o}C (Fig. 4.5B). Q_{10} of poplar was found to decrease from October but remained relatively consistent throughout summer (December 2005 – February 2006) with an increase observed in autumn. A similar trend is seen in pine (Q_{10}: 2.25 in July to 2.10 in the

summer). Regression analysis (Fig. 4.4) confirmed positive relationships between Q_{10} and foliar sugar and starch concentrations. R_{10} was positively related to soluble sugar content in pine only. Foliar N content was significantly related only to Q_{10} in pine.

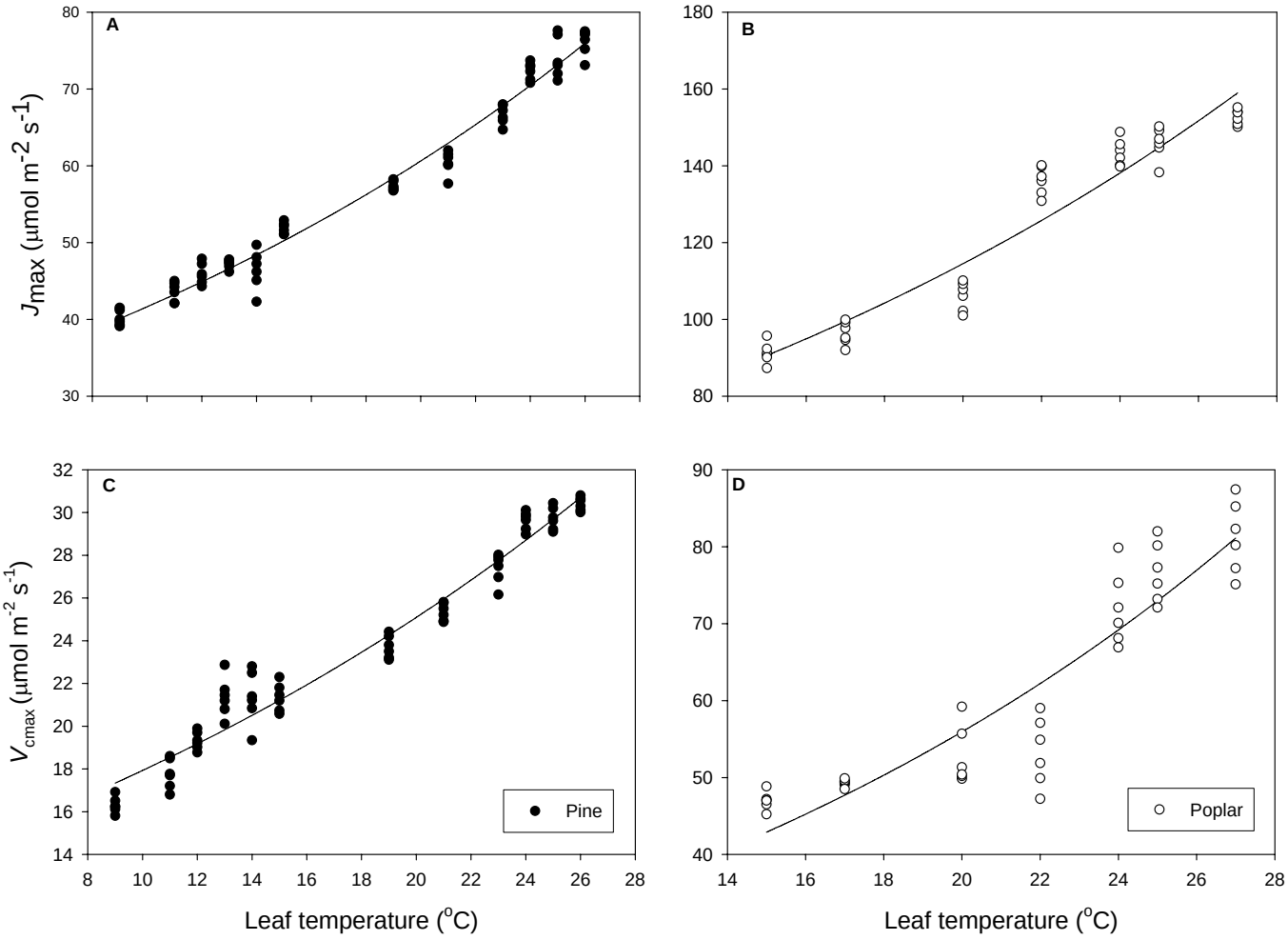

Figure 4.3. Temperature responses of (A) maximum rate of RuBP regeneration (J_{max}) and (B) maximum rate of rubisco carboxylation (V_{cmax}) at prevailing ambient temperatures for *Pinus radiata* and *Populus deltoides.* Each plot is represented by the following equations: (a) y= 2.1095*x* +19.582 (r^2=0.9649); (b) y= 5.7447*x* +1.4558 (r^2=0.9277); (c) y= 0. 7953*x* +9.5847 (r^2=0.9604); (d) y= 3.0481*x* +3.5534 (r^2=0.8036)

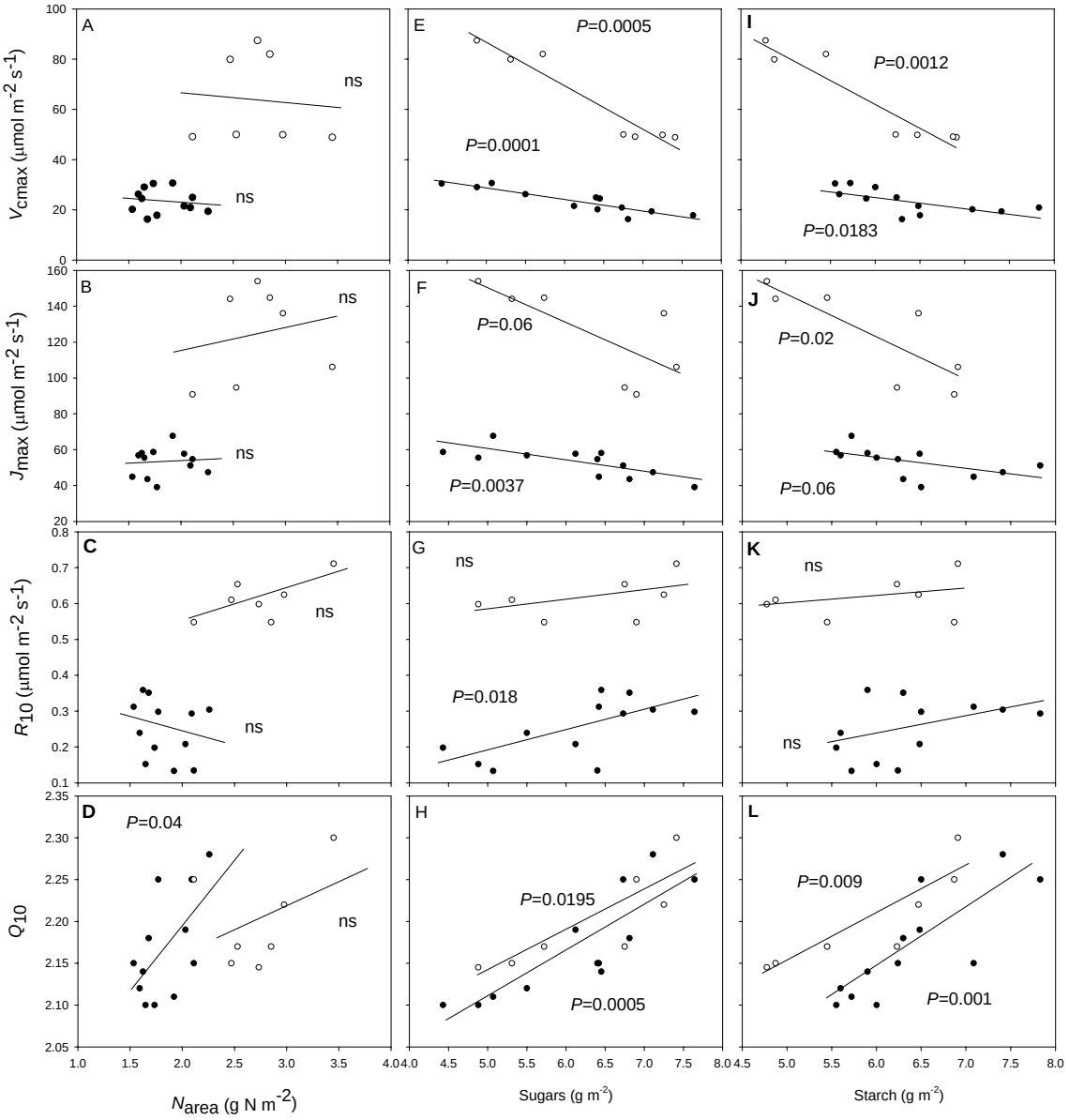

Figure 4.4. The relationship between (a) V_{cmax} (b) J_{max} (c) R_{10} (d) Q_{10} and area-based leaf nitrogen, sugar and starch concentrations in leaves of *Pinus radiata* and *Populus deltoides* sampled over a 12-month period. The closed and open circles represent the responses of pine and poplar respectively (each point represents the mean for each sampling date).

Table 4.1. Significant regressions in figure 4.4 (above) are described by the following equations

Figure	*Pinus radiata*	r^2	*Populus deltoides*	r^2
	y=0.1548*x* + 1.8846	0.3537	ns	
4.4E	y=-4.604*x* + 51.655	0.8275	y=-17.412*x* + 173.85	0.9252
4.4F	y=-6.3153*x* + 91.632	0.5854	y=-19.249*x* + 245.93	0.5374
4.4G	y=0.0567*x* - 0.0991	0.4454	ns	
4.4H	y=0.0548*x* + 1.8325	0.7191	y=0.0483*x* + 1.8954	0.6966
4.4I	y=-4.4784*x* + 52.029	0.4424	y=-18.998*x* + 176.69	0.8961
4.4J	y=-6.1226*x* + 92.015	0.3109	y=-23.812*x* + 265.77	0.669
4.4L	y=0.0699*x* + 1.7223	0.6608	y=0.0566*x* + 1.8648	0.7763

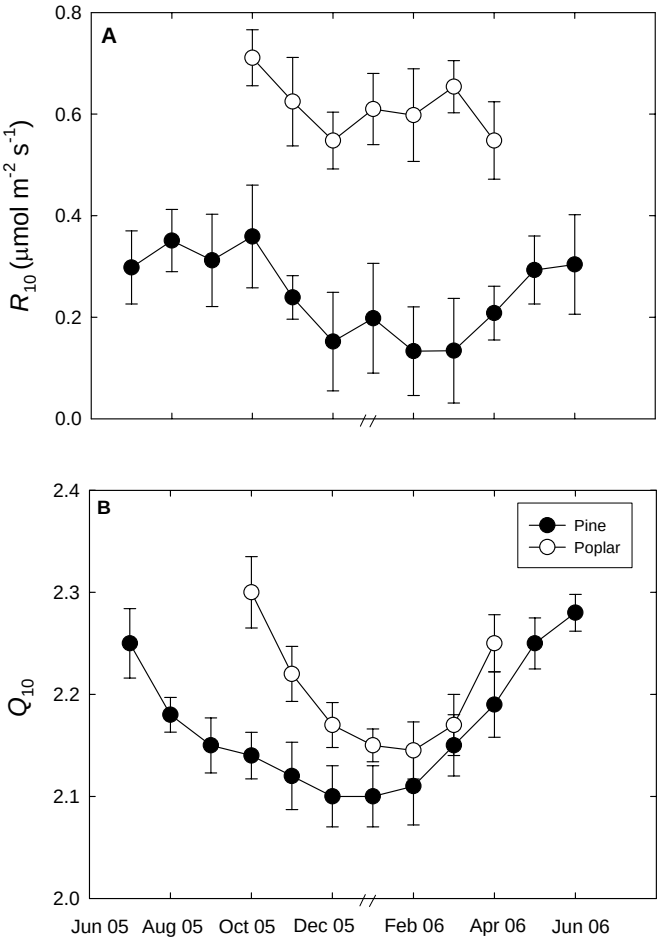

Figure 4.5. Parameters for the temperature response of respiration (R_{10}, and Q_{10}) in *Pinus radiata* and *Populus deltoides* from seasonal measurements- across a temperature range of 5-22 ^{o}C. All values are means ± SEM; n= 9.

As the temperature response of respiration varied throughout the year, the relationship between R_{10}, Q_{10} and air temperature averaged across a varying number of preceding days (1-day, 3-day, 7-day, 10-day, 20-day and monthly minimum temperature averages) were examined. A strong negative relationship between R_{10} and Q_{10} and preceding temperature was found (Fig. 4.6), with the relationships being strongest with a preceding 3-day minimum temperature average (Fig. 4.7). The data for the long term response of R_d to temperature showed strong respiratory acclimation to a wide range of minimum temperature (Fig. 4.8), in marked contrast to the long-term response of photosynthesis (Fig. 4.3). In fact overcompensation occurred – i.e. a slight *downward* adjustment in respiration rates at higher temperatures was observed in both species. The R_d/A_{max} ratio for pines was highly variable, exhibiting a decline from July 2005 through to February 2006 (Fig. 4.9). An increase in the ratio was only observed in autumn. The R_d/A_{max} ratio of poplar decreased by approximately 30% in spring (October and November 2005) and this decline continued throughout summer with an increase observed only in autumn (between February and March 2006).

4.4 Discussion

To my knowledge, few, if any, previous studies have involved the combined investigation of seasonal changes in photosynthetic (J_{max} and V_{cmax}), respiratory (R_{10} and Q_{10}) and leaf parameters (N_a, S, carbohydrate concentrations) in both evergreen and deciduous mature trees over a 12 month period in the field. This combination of measurements has allowed for the observation into the dynamics of both photosynthetic and respiratory responses to

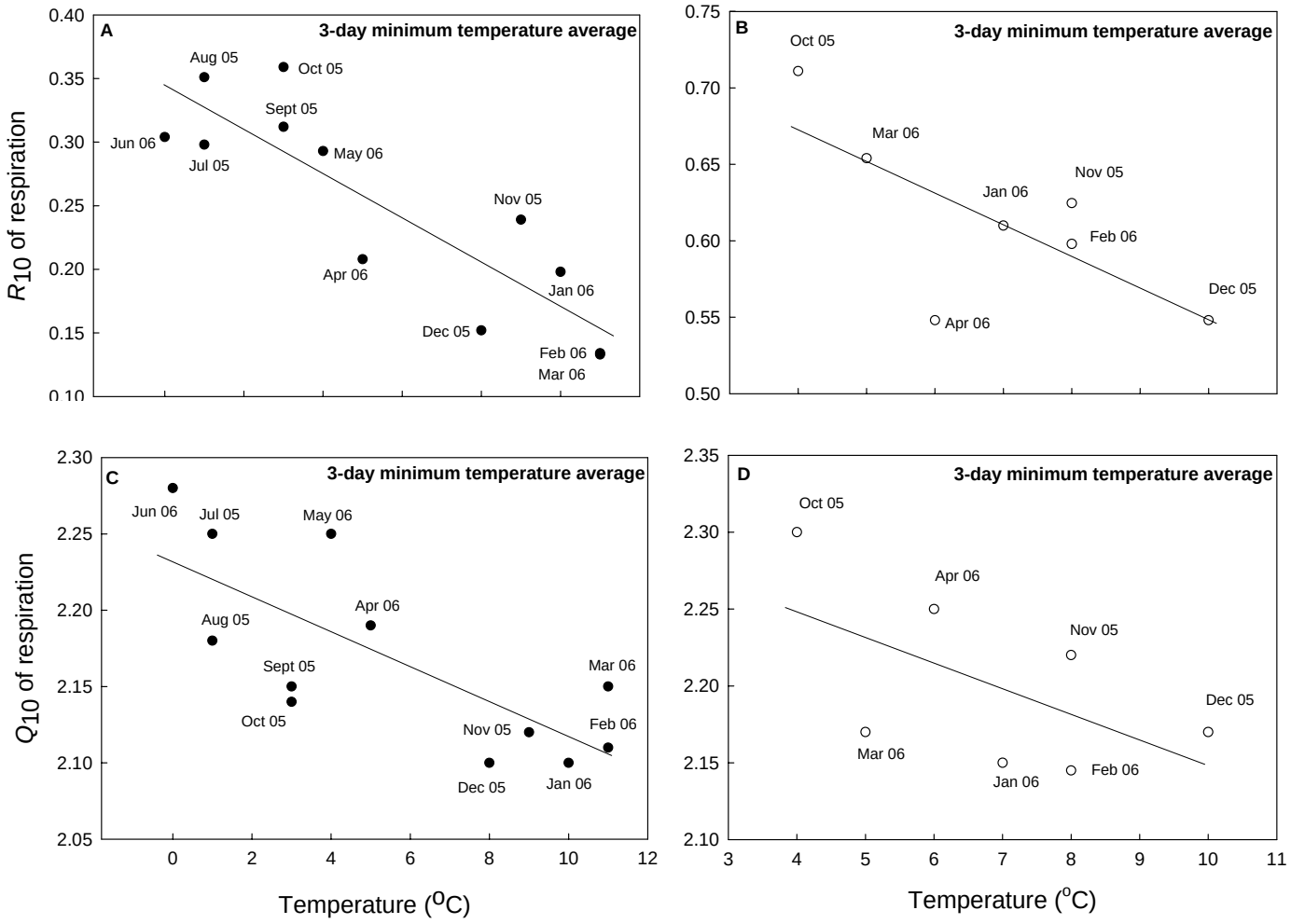

Figure 4.6. Area-based dark respiration rate **(**μmol m^{-2} s^{-1}) and the Q_{10} of leaf respiration in relation to the 3-day minimum temperature average (minimum temperature of the measuring day and the two days preceding the measuring day) in *Pinus radiata* and *Populus deltoides* sampled over different seasons. The linear regressions shown are described by the following equations: pine (R_{10}), y= -0.0175*x*+0.3447 (r^2= 0.8625), *P*=0.0265; pine (Q_{10}), y= -0.0115*x*+2.2314 (r^2= 0.5654), *P*=0.0003; poplar (R_{10}), y= -0.0205*x*+0.7538 (r^2=0.5185), *P*=0.014; poplar (Q_{10}), y= -0.0167*x*+2.315 (r^2=0.3415), *P*=0.0003.

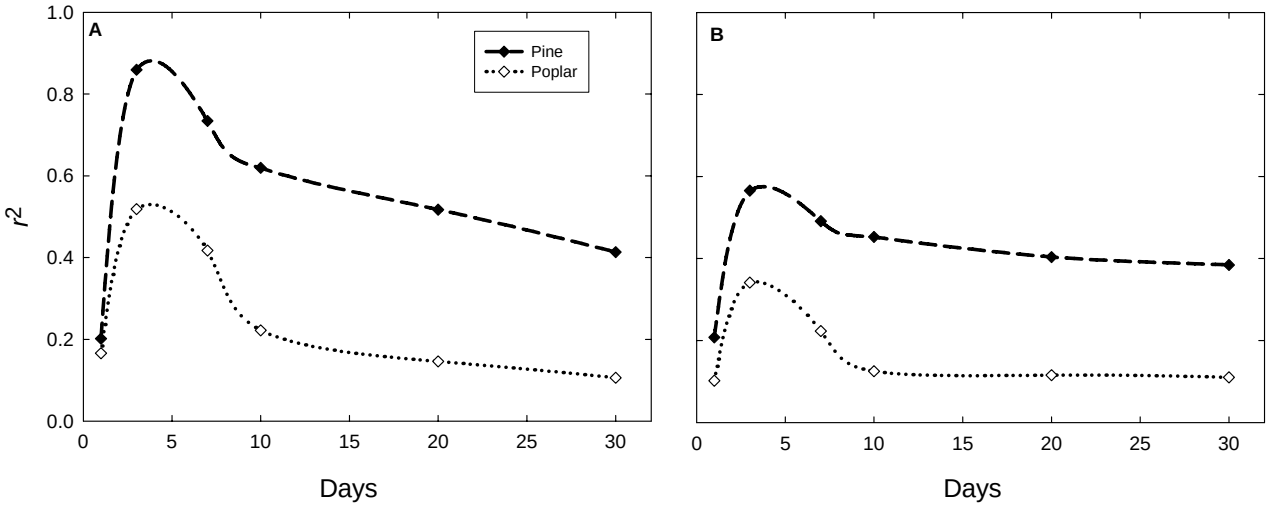

Figure 4.7. Correlation coefficients of (a) R_{10} and (b) Q_{10} of *Pinus radiata* and *Populus deltoides* plotted as a function of the time window (days) used to calculate preceding average ambient temperature experienced by the trees.

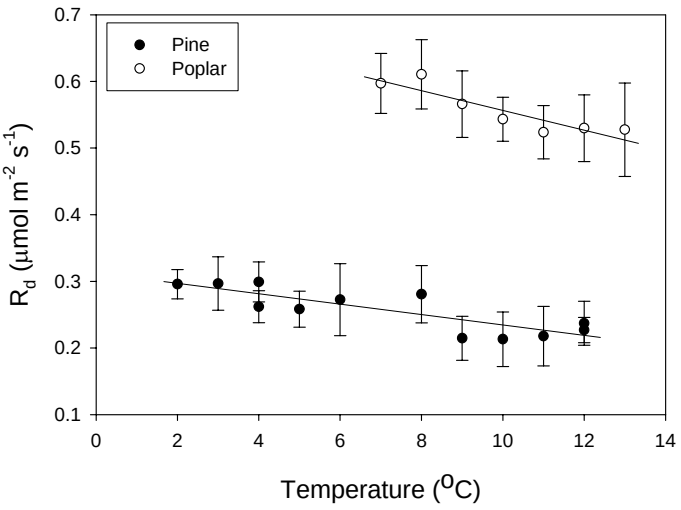

Figure 4.8. Long term (acclimated) respiration rate of pine and poplar as a function of temperature. Values plotted are respiration rate at the minimum temperature experienced by the trees for the night preceding the measurement. The regressions shown have the following equations: pine: y= -0.0077*x*+0.3116 (r^2=0. 7007), *P*= 0.0007; poplar: y= -0.0147*x*+0.7041 (r^2=0.8139), *P*= 0.0055.

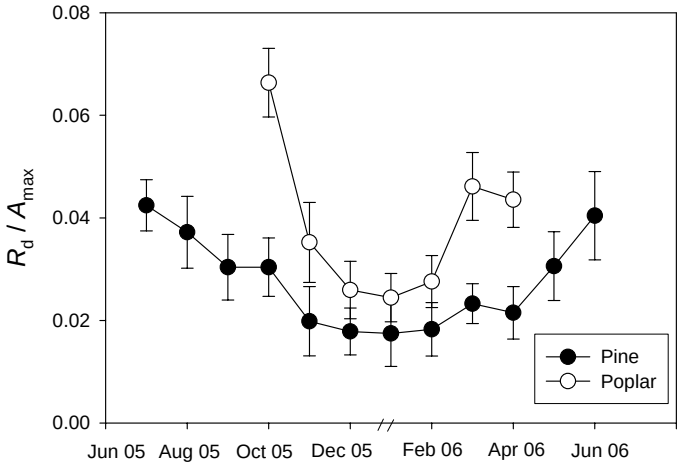

Figure 4.9. Ratio of respiration in darkness (R_{dark}) to light saturated photosynthesis at ambient CO_2 (A_{max}) in *Pinus radiata* and *Populus deltoides* sampled over different seasons. Values represent the mean of six replicates (± SE).

a wide range of natural temperature variation (minimum night-time temperature range -2 to 15 °C, maximum day-time temperature range 5 to 32 °C). The findings here show that the two processes comprising canopy net carbon exchange differ markedly in their long-term (acclimation) responses to temperature - photosynthesis does not acclimate but respiration does very strongly. These responses are discussed in terms of the context they provide for our understanding of plant carbon balance in response to environmental change.

4.4.1 Response of photosynthesis to temperature

The results here show that seasonal variation in photosynthetic parameters is explained well by changes in air temperature over different seasons. This is consistent with earlier studies (Medlyn et al. 2002; Dungan 2003; Xu et al. 2003) that have shown that photosynthetic parameters are strongly dependent on leaf temperature. Importantly, there

was a consistent long-term relationship between photosynthesis and temperature in both species, with no evidence of photosynthetic acclimation over a wide range of temperatures (Fig. 4.3). In these two tree species the temperature response of photosynthesis tends to retain a relatively fixed curvilinear response to temperature over a wide range of growth temperatures (5 to 32 °C). Importantly, when the long-term temperature responses are fitted (Leuning 2002) it was found that the parameters do not differ from previously published instantaneous responses (e.g. Leuning 2002; Medlyn et al. 2002) (Table 2.1). This provides field confirmation of previous findings (chapters 2 and 3) for these two species in controlled environment experiments (Ow et al. 2008a, 2008b). Although we should not preclude the likelihood that changes in growth temperature might elicit shifts in the temperature optimum of photosynthesis or result in partial temperature acclimation (Campbell et al. 2007; Kattge and Knorr 2007; Sage & Kubien 2007), it is quite clear that photosynthesis does not acclimate in the way that respiration does (see next section).

The results found that the ratio of the capacities of ribulose-1,5-bisphosphate (RuBP) regeneration to RuBP carboxylation (J_{max} / V_{cmax}) (Fig. 4.2C) to be seasonally variable, especially in pine, where the ratio was lower in spring and summer and higher during the cooler seasons. This finding is consistent with previous work by Hikosaka et al. (1999), Onoda et al. (2005), Misson et al. (2006) and Hikosaka et al. (2007) who also observed seasonal changes in this ratio and concluded that the changes observed are a result of a temperature-dependent J_{max} / V_{cmax} ratio. A similar finding was shown for poplar grown under controlled conditions where the J_{max} / V_{cmax} ratio increases with decreasing temperature and vice versa (Ow et al. 2008a). By contrast, Medlyn et al. (2002) and

Table 4.2. Parameters describing the temperature response of V_{cmax} and J_{max} in this study (see Fig. 3) and in previous studies. H_{av}, H_{dv} and S_{vv} are the activation and deactivation energies and an entropy term for V_{cmax}. H_{aj}, H_{dj} and S_{vj} are the corresponding terms for J_{max}.

H_{av} (J mol $^{-1}$)	H_{dv} (J mol $^{-1}$)	S_{vv} (J mol^{-1} K^{-1})	H_{aj} (J mol $^{-1}$)	H_{dj} (J mol $^{-1}$)	S_{vj} (J mol^{-1} K^{-1})	Species	Source
43700	194000	610	48977	207199	638	*Pinus radiata*	Current study
68230	188400	649	74420	200000	653	*Populus deltoides*	Current study
75400	175000	559	65300	129000	420	*Fagus sylvatica*	Dreyer *et al.* (2001)
67600	144000	451	46100	280000	888	*Quercus petraea*	Dreyer *et al.* (2001)
45027	203594	650	45977	199354	650	*Pinus radiata*	Walcroft *et al.* (1997)
52750	202600	669	61750	185600	621	*Pinus sylvestris*	Wang *et al.* (1996)

Hollinger (1992) in their studies of mature maritime pine (*Pinus pinaster*) and a deciduous and evergreen oak (*Quercus lobata* and *Quercus agrifolia*), concluded that seasonal changes in the J_{max} / V_{cmax} ratio were absent. Across a range of temperatures (10 to 35 ^{o}C), rates of photosynthesis are limited either by RuBP carboxylation or by RuBP regeneration (Berry et al., 1980). Therefore, the increase in the J_{max} / V_{cmax} ratio at low growth temperatures may relieve the limitation of RuBP regeneration on photosynthetic rate whilst a decrease in the ratio with increasing temperature is a result of a greater activation energy for V_{cmax} than for J_{max} (Onoda et al. 2005). RuBP carboxylation-limited photosynthesis does not have the same temperature dependence as RuBP regeneration-limited photosynthesis. J_{max} has been found to possess a lower optimum temperature than V_{cmax} (Dreyer et al. 2001) which should explain the imbalance between the two processes when growth temperature changes, resulting in variation in the J_{max} / V_{cmax} ratio (Farquhar et al. 1982; Onoda et al. 2005).

The proportion of nitrogen allocated to rubisco and electron transport in the thylakoid membranes is particularly important in determining rates of photosynthesis (Atkinson et al. 2007). Therefore, variations in photosynthetic parameters are highly correlated with leaf nitrogen concentration on an area basis (Dang et al. 1998; Wilson et al. 2000; Xu et al. 2003; Misson et al. 2006). In contrast with previous findings of a strong correlation between N_a and photosynthesis, the results here showed no clear relationship between *seasonal* changes in N_a and photosynthesis in either pine or poplar. Rather, variation in photosynthetic parameters is better explained by changes in air temperature over different seasons. Seasonal changes in photosynthetic capacity (as described by V_{cmax} and J_{max}) were also negatively correlated with leaf carbohydrate status (soluble sugars and starch, Fig.4.4). This finding is consistent with Turnbull et al. (2002) but not with other studies (Paul et al. 2003; Davey et al. 2006) showing a positive relationship between photosynthesis and carbohydrates.

4.4.2 Response of respiration to temperature

Following the controlled-environment studies with seedlings of these species (Chapters 2 and 3; Ow et al. 2008a, 2008b), the present study provides strong field support that temperature acclimation of respiration occurs in field-grown trees including evergreen and deciduous species (Gunderson et al. 2000; Lee et al. 2005; Xu & Griffin 2006; Tjoelker et al. 2008). Also, this study has demonstrated that leaf respiration of pine and poplar varies seasonally in response to changes in temperature. The data collected suggest higher respiratory rates in the broad-leaved deciduous poplar whist the needle-leaved pine had lower rates of respiration (Fig. 4.5A). In the short term, changes in temperature will

cause rates of respiration to change, but as the length of exposure to a new growth temperature increases, the effect that temperature has on respiration will depend on the acclimation potential of the respiratory process (i.e. the ability of the species to adjust rates of R_d in response to changing environmental conditions) (Atkin et al. 2003; Atkin et al. 2005a; Atkin et al. 2005b). In a study investigating temperature acclimation of R_d in five boreal tree species, Tjoelker et al. (1999b) concluded that although the degree of acclimation varied widely between species, conifers tend to exhibit a higher acclimation potential to temperature than deciduous species. In the present study, strong respiratory acclimation to seasonal changes in temperature was observed in both pine and poplar (i.e. reduction in the rate of respiration at higher temperatures (Fig. 4.8) as well as a decrease in the Q_{10} of respiration). In fact, in this field study and in recent work under controlled conditions (Chapter2; Ow et al. (2008a) for poplar and Chapter 3; Ow et al. (2008b) for pine), it was shown that long-term exposure to changes in temperature result in complete acclimation or respiratory homeostasis, in which plants growing at contrasting temperatures have identical rates of respiration when measured at their respective growth temperatures (Atkin et al. 2005a; Atkin et al. 2007; Armstrong et al. 2006a; Armstrong et al. 2006b). Intriguingly, there is evidence here to suggest that R_d may overcompensate for increases in temperature (Fig. 4.8). These results support the notion (Tjoelker et al. 2001; Atkin et al. 2005a; Wythers et al. 2005; King et al. 2006) that the use of a static R_d and Q_{10} is inappropriate over large temperature ranges.

Since respiration is closely coupled to photosynthesis, rates of respiration should most likely vary in a substrate-dependent manner. Furthermore, correlations between leaf

respiration and soluble sugars (or total non-structural carbohydrates) have been observed in earlier studies (Ryan 1991; Noguchi et al. 1997; Atkin et al. 2000b; Griffin et al. 2001; Tissue 2002; Turnbull et al. 2003; Xu et al. 2006). A significant correlation between respiration and soluble sugars was also observed in this study. Furthermore, foliar carbohydrates changed across seasons, with an increase in carbohydrate concentrations at lower temperatures when up-regulation of R_d was observed. This is consistent with previous findings (Azcon-Bieto et al. 1983; Tissue 2002; Turnbull et al. 2003). Cold temperatures often have a greater effect on carbohydrate translocation and carbon use than on carbon assimilation, resulting in an altered balance between substrate supply and use which may lead to higher carbohydrate concentrations (Farrar et al. 1991). However, it is noteworthy that the positive correlation between respiration and soluble sugar content is not universal (Loveys et al. 2003). For example, Atkin et al. (2000b) concluded that soluble sugar concentration in field-grown *Eucalyptus pauciflora* leaves remained high throughout the year of study. Additionally, a strong relationship was found between carbohydrate content and Q_{10} but less so for R_{10}. The substrate dependence of the Q_{10} of respiration in plant tissues is consistent with Atkin & Tjoelker (2003). In most cases, substrate availability regulates respiratory flux at moderate temperatures. However, apart from substrate-dependence, changes in respiratory capacity is also likely to be coupled to enzymatic and adenylate restrictions. Hence, further studies in diverse species under a range of environmental conditions are warranted to develop our mechanistic understanding of these relationships.

4.4.3 Balance between respiration and photosynthesis

Given the tight coupling that exists between photosynthetic and respiratory metabolism (Whitehead et al. 2004a; Atkin et al. 2006) and because both processes are temperature sensitive, a change in temperature results in an immediate alteration in the rate of respiration and photosynthesis, with the extent of the alteration being determined by the temperature coefficient of each process (Atkin et al. 2007). The temperature sensitivity of respiration differs from that of photosynthesis, resulting in the alteration of the respiration/photosynthesis ratio following a short-term (minutes to hours) change in temperature (Dewar et al. 1999; Gifford 2003; Atkin et al. 2006). However, in many species, homeostasis of the respiration/photosynthesis ratio is achieved when plants are exposed to contrasting temperatures for sustained periods (a result of thermal acclimation of specific rates of respiration and photosynthesis) (Tjoelker et al. 1999a; Loveys et al. 2003). Unlike recent studies (Atkin et al. 2006; Atkin et al. 2007), the results here do not show homeostasis in the respiration/photosynthesis ratio over the course of an annual seasonal cycle (Fig. 4.9). Rather, the ratio in both pine and poplar was found to be consistently higher in the cooler months and lower in spring and summer due to lower ratio of respiration to photosynthesis. This finding is consistent with recent work conducted on similar species under controlled conditions (Chapters 2 and 3; Ow et al. 2008a; Ow et al. 2008b), where the respiration/photosynthesis ratio was found to be consistently higher under colder relative to warmer temperatures. These results suggest that, as temperatures increase over a moderate range, the rate of photosynthesis in both pine and poplar will increase more rapidly than the rate of respiration. The overall impact

of this would be a reduction in the potential for positive feedback of respiration in the carbon cycle, in contrast to previous suggestions that the opposite might be the case (Cox et al. 2000; Houghton et al. 2001; Luo et al. 2001).

4.5 Summary

In conclusion, these results demonstrate that leaf respiration and photosynthesis are sensitive to natural changes in temperature over the course of a year. However, the long-term temperature responses of respiration and photosynthesis were found to be fundamentally different. Whilst respiration rate acclimates fully to a wide range of minimum temperatures, photosynthetic rate maintains a relatively fixed curvilinear response to a wide range of daytime temperatures. Thus it was found that thermal acclimation resulted in lower respiration relative to photosynthesis in the warmer months. These findings can have implications for carbon cycle models predicting the effects of rising temperatures on future CO_2 fluxes between plants and the atmosphere. There is now considerable evidence from recent studies suggesting that it is essential that temperature/seasonal–mediated changes in metabolism be taken into account in models predicting carbon fluxes. Thus, the next chapter will cover the use of a modified leaf-level model to quantify the effects of thermal acclimation of respiration in both *Pinus radiata* and *Populus deltoides* under controlled and field conditions. In this model, a linear temperature-dependent basal respiration and Q_{10} as a function of running mean temperature was introduced and photosynthetic carbon fixation was quantified using a static and a variable approach.

Chapter 5

Modelling the responses of foliar carbon exchange in *Pinus radiata* and *Populus deltoides*

5.1 Introduction

Concern over climate warming has intensified interest in the flux of carbon between the terrestrial biosphere and the atmosphere. Carbon dioxide flux is of particular concern because it is a greenhouse gas. Over the past 200 years, atmospheric CO_2 concentration has risen by 30% (Houghton et al. 2001) and a likely outcome of such a change is an alteration in global temperature patterns. For example, it is now widely accepted that global temperatures will be 1-6 ^{o}C warmer by 2100 (Hansen et al. 1999; IPCC 2007) and these changes are likely to vary among regions and exhibit seasonal and diurnal variations. Atmospheric carbon is fixed into plant biomass through photosynthesis and returned to the atmosphere via respiration. The difference between these fluxes determines the carbon balance in forests. Although carbon is released back to the atmosphere via both autotrophic and heterotrophic pathways, the former accounts for almost half of total respiratory carbon flux (Houghton et al. 2001; Wythers et al. 2005). Hence, autotrophic respiration plays a key role in governing forest carbon balance and accurate modelling of the response of autotrophic respiration to changing climate will be

critical so as to effectively predict carbon uptake and storage and consequently identify the range of environmental factors that regulate carbon sequestration.

Models that operate at the organ level (i.e. root, stem and leaf) are often used to examine the effects of environmental change on plant productivity. Models typically use a network of interactive algorithms that estimate carbon assimilation, respiration and allocation. For an in-depth review of process models see Ryan et al. (1996), Makela et al. (2000) and Sitch et al. (2003). Many biological processes, including respiration, are dependent upon temperature, hence most models incorporate temperature into their respiratory and photosynthetic calculations but to varying degrees of complexity (including a range of assumptions and generalizations).

Often models simulating respiration assume that dark respiration (R_d) increases exponentially with temperature with the Q_{10} value being assumed to be 2.0. However, the response of R_d to short-and long term changes in temperature is highly dynamic and the instantaneous exponential respiratory function has been shown to poorly describe observations made on various plant species (Gifford 2003; Wythers et al. 2005; King et al. 2006). Furthermore, Q_{10} values are also highly variable in response to diurnal changes in temperature (Atkin and Tjoelker 2003). Q_{10} values typically range from 1.2 to 4.0, with variability occurring even within individual plants (Ryan 1991; Cramer et al. 1999; Kimball et al. 2000; Clark et al. 2001; Potter et al. 2001; Sitch et al. 2003). Moreover, in their recent work, Tjoelker et al. (2001) concluded that Q_{10} of respiration declined with increasing measuring temperature. Therefore, observed responses of respiration using a

static Q_{10} of 2 may be inappropriate especially when large temperature ranges are involved.

Additionally, rates of respiration are known to acclimate to thermal environments over longer time periods (days to weeks) through adjustments in the temperature response function. Many previous studies have demonstrated that R_d acclimates with time to prevailing temperatures (Larigauderie and Korner 1995; Tjoelker et al. 1999a; Atkin et al. 2000b; Noguchi et al. 2001b; Bolstad et al. 2003; Atkin et al. 2005a; Armstrong et al. 2006a; Xu and Griffin 2006; Xu et al. 2006). Respiratory acclimation can be substantial and rapid and can have a significant impact on carbon budgets. Typically, temperature acclimation to warmer conditions results in the downward shift of the short-term temperature response function but longer periods have been shown to bring about respiratory homeostasis (Bolstad 1999; Armstrong et al. 2006a; Ow et al. 2008 – see Chapters 2 and 3**)**. Consequently, it is likely that the response of R_d to variation in days or seasons may differ from predictions based on short term temperature responses.

In spite of the evidence showing that temperature responses of respiration often cannot adequately fit a simple static Q_{10} exponential relationship and a static R_d parameter, it is still widely used in models (see Wythers et al. (2005) for a list of 19 published models using either a static Q_{10} parameter, static R_d parameter, or both) as there has been no clear and generalisable alternative made available. Furthermore, most of the current models do not take into account the process of acclimation.

In this chapter a modelling approach was used to investigate the response of two contrasting species which have been the focus of this thesis (pine and poplar) to variation in temperature under both field and controlled conditions, and together with meteorological data, model annual leaf carbon exchange. The purpose of this study was to test the efficacy of a coupled photosynthesis-stomatal conductance model for leaves (Leuning 1995) which integrates the effects of photosynthesis and dark respiration and takes into account the presence of acclimation to temperature and a variable Q_{10} value (which were determined in Chapters 2, 3 and 4). The objective was to examine the consequences of the alternative temperature response and acclimation algorithms on modelled carbon budgets.

5.2 Materials and Methods

5.2.1 Modelling acclimation of photosynthesis and respiration

The relative contribution of seasonal changes in photosynthetic carbon fixation and acclimation of respiration was quantified for both *Pinus radiata* and *Populus deltoides* using a leaf-level model. Acclimation of dark respiration was modelled based on the assumption that over longer periods, rates of respiration at a reference temperature of 10 ^{o}C should decline linearly with increasing growth temperature whilst in the short term, respiration should exhibit an exponential response to temperature with a Q_{10} value of approximately 2. Rather than using a single temperature response, the model holds a limitless number of short-term responses of respiration to temperature that will shift with longer–term changes in temperature (Fig. 5.1). With acclimation, a leaf can operate within the space of the short term responses depending on the ambient temperature or

recent previous temperature history. For example, if the leaf acclimates, respiration declines as a function of temperature averaged over a certain time period. Leaves exhibiting respiration rate above the acclimated response in the long term will show reduction in rates of respiration, moving towards the acclimated (homeostatic) response if the temperature is kept high (Fig. 5.1). By contrast, leaves exhibiting respiration rates below the acclimated response will increase in the long term when exposed to prolonged periods of low temperatures (Fig. 5.1). If there is no acclimation, the leaf moves along a single response dependent on ambient temperature (Fig. 5.1).

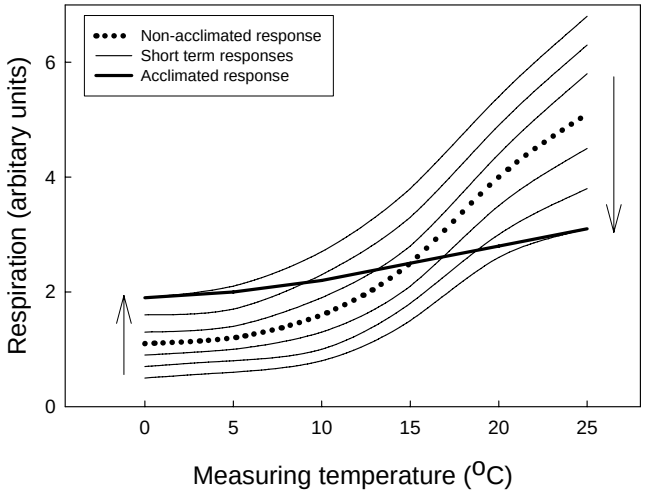

Figure 5.1. Model of leaf dark respiration as a function of temperature in the short-and longer –term.

A tight relationship exists between stomatal conductance and rate of photosynthesis and this forms the basis for many studies modelling whole-leaf carbon assimilation. In the calculations performed here, photosynthesis was quantified using the widely-known biochemical model of Farquhar et al. (1980), which determines that rate of photosynthesis as the minimum of either carboxylation or electron transport-limited rates

of CO_2 assimilation, less R_d. This was coupled with the gas-exchange model of Leuning (1995) integrated with an energy budget equation. This coupled-model approach describes the photosynthetic behaviour of leaves by taking into account the biochemical limitation for CO_2 (demand) as well as stomatal limitations to supply CO_2. In order to assess annual net carbon exchange, photosynthesis was quantified using a static (e.g. mid-summer parameters) and a variable (parameters following seasonal observations - see chapter 4) approach similar to that described by Whitehead et al. (2004b). Values of J_{max} (maximum rate of ribulose 1-5, bisphosphate (RuBP) regeneration) and V_{cmax} (maximum rate of RuBP carboxylation) which were required to drive this model was determined from A/C_i response curves (see chapters 2, 3 and 4) at a range of temperatures. This coupled model is ideal for determining seasonal changes in carbon exchange as it is largely driven by meteorological variables which are often readily available.

In its original form, the leaf-level model uses a fixed value for the parameter R_d set at 10% of foliar photosynthetic capacity at 20 ^oC and a Q_{10} of respiration fixed at 2 ("static or non-acclimated response"). To test the effects of thermal acclimation on dark respiration and the effects of a temperature-variable Q_{10} (Tjoelker et al. 2001) ("variable or acclimated response"), a linear temperature-dependent basal respiration and Q_{10} as a function of running mean temperature was introduced such that

$$R_{d0} = R_{d0} + (R_{da} * (T_{mn} - T_{mo}))$$

$$Q_0 = Q_0 + (Q_a * (T_{mn} - T_{mo}))$$

where R_{d0} is respiration rate and Q_0 the Q_{10} value at a base temperature of 10 ^{o}C, R_{da} is the acclimation slope for R_{10} and Q_a is the acclimation slope for Q_{10} based on measurements of acclimated dark respiration at different growth temperatures (see chapters 2, 3 and 4), T_{mn} is the modified running mean temperature (mean temperature of the current hour) and T_{mo} is the original running mean temperature (mean temperature of the previous hour). The models ran on an hourly time-step and were driven by meteorological data (e.g. air temperature, photon flux density, wind speed, relative humidity and atmospheric CO_2 concentrations). The respiration model integrated hourly rates of respiration over a 24-hour period to provide daily values of R_d which were then summed over a whole year. Respiration during the light period was assumed to be 50% of R_d (Atkin et al. 2000a). The running mean temperature was determined from measured daily temperature climatic data using a 3-day period (3-day running mean determined based on data derived from chapter 4 and recent published work by Tjoelker et al. (2008)). This time window is also supported by findings that suggest that a shift in the temperature response curve can occur over a period as short as one to several days (Atkin et al. 2000; Bolstad et al. 2003; Atkin et al. 2005a). Generally, annual dark respiration was quantified for two scenarios: no acclimation of R_d (a single temperature response curve), and acclimation of R_d using the 3-day running mean temperature as a driving variable for data derived from controlled experiments (Chapters 2 and 3). Data derived from seasonal field measurements (Chapter 4) were also driven with a 3-day running mean temperature and analysed using static respiration parameters (Q_{10} and R_d) and an alternative respiration algorithm where changes in the curve (i.e. elevation) of respiration as a function of temperature together with a variable Q_{10} that has been found to decline

with increasing temperature (Tjoelker et al. 2001) were taken into account. Meteorological data to run the model (period of study: 2005-2006) were obtained from the New Zealand national climate centre (NIWA). Climatic data used for modelling came from a weather station at Broadfield Ews in the town of Lincoln approximately 25 km south of Christchurch (Latitude 43^{o} 25' N, longitude 172^{o} 58' E, a.s.l.) – the station nearest the experimental site where the field data were gathered (see chapter 4).

5.3 Results

5.3.1 Modelling of net carbon exchange

Controlled environment experiments conducted on both pine and poplar (Chapters 2 and 3) generally show no evidence of a change in the temperature response of photosynthesis on leaves grown at different temperatures. Modelled net carbon exchange responses of pine and poplar based on data generated from growth chamber studies show a slight increase in annual values when temperature acclimation of respiration is taken into account (Table 5.1). Values of annual net carbon exchange for pine using an acclimated and non acclimated scenario is 142.7 and 139.7 mol m^{-2} respectively (Table 5.1). A similar response was found for poplar, where the acclimated scenario exhibited a greater annual value, but with a difference of only 3.9% between scenarios (Table 5.1).

Modelled net carbon exchange responses of both species derived from seasonal field measurements are shown in Table 5.2. Over a 12-month period the variable response in pine resulted in lower net carbon exchange values throughout the cooler months (winter, autumn and spring). By contrast, higher values were observed from December 2005

through to February 2006, which is consistent with the data for poplar. The overall (annual) response differed between the two scenarios (static *vs* variable) by 9 and 9.4% for pine and poplar, respectively.

Table 5.1. Annual simulation of dark respiration and net carbon exchange of *Pinus radiata* and *Populus deltoides* (per m^2 leaf area) based on data generated from growth chamber experiments. Dark respiration and net carbon exchange were simulated assuming no acclimation and acclimation to 3-day running mean temperatures

	Annual R_d (mol m^{-2})		**% difference**
	Non acclimated response	Acclimated response	
Species:			
pine	59.36	51.80	-12.74
poplar	65.15	57.23	-12.16
	Annual net carbon exchange (mol m^{-2})		**% difference**
	Non acclimated response	Acclimated response	
Species:			
pine	139.66	142.66	+2.15
poplar	121.92	126.68	+3.90

5.3.2 The impact of acclimation on annual leaf respiration

Based on data collected from the response of small trees in growth chamber experiments, daily leaf respiration was found to vary over the year between 0.1 to 0.6 mol C m^{-2} day^{-1} and 0.2 to 1.0 mol C m^{-2} day^{-1} for pine and poplar, respectively (Figs. 5.2A and B). On an annual basis, acclimation tended to reduce the total respiratory flux (Table 5.1). Acclimation to a 3-day running mean temperature reduced respiration by around 13% compared to non acclimated respiration for pine. A slightly lower reduction was observed for poplar (12%).

Table 5.2. Simulated monthly net carbon exchange of *Pinus radiata* and *Populus deltoides* over a 12 and 7-month period respectively. Net carbon exchange was simulated assuming no acclimation (static), and acclimation to 3-day running mean temperatures (variable). Percent differences between responses are shown for each month.

	Net carbon exchange (mol $m^{-2}month^{-1}$)		
	Static response	Variable response	% difference
Species: pine			
July 2005	7.06	5.15	-27
August	8.76	6.52	-26
September	9.25	7.13	-23
October	11.57	10.69	-8
November	12.32	11.51	-7
December	13.33	14.11	+6
January 2006	12.73	13.52	+6
February	11.63	12.43	+7
March	10.82	9.75	-10
April	9.48	7.87	-17
May	7.72	6.52	-16
June	6.39	5.01	-22
12-month response	121.06	110.21	-9
Species: poplar			
October 2005	22.13	15.12	-32
November	23.03	16.63	-28
December	22.41	24.97	+11
January 2006	21.66	23.37	+8
February	19.95	21.66	+9
March	19.03	16.08	-16
April	15.39	12.30	-20
7-month response	143.6	130.13	-9.4

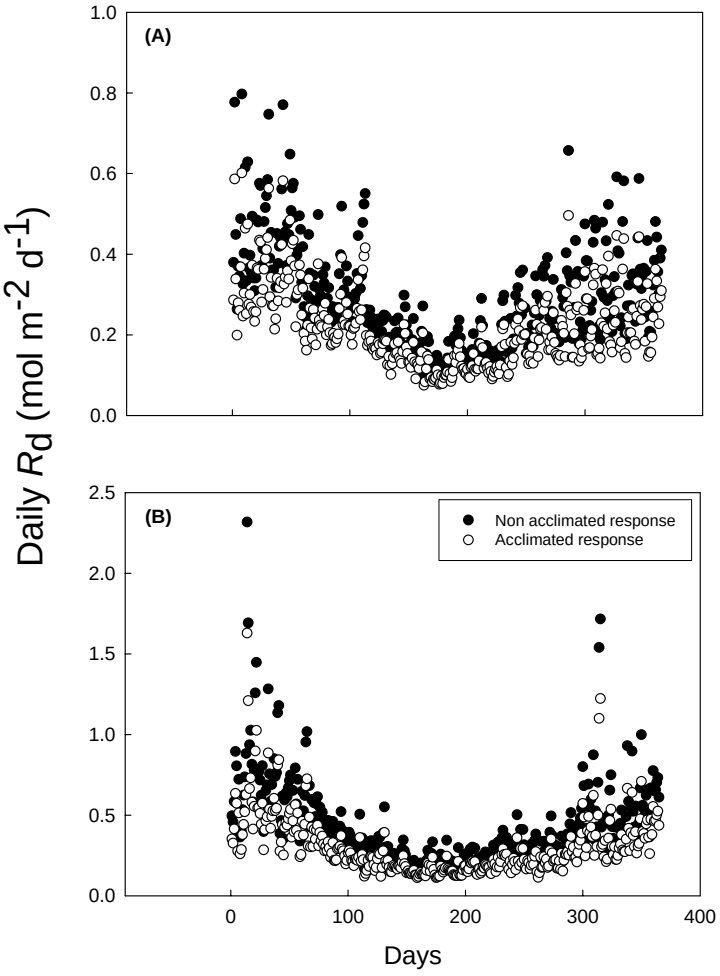

Figure 5.2. Model simulation of leaf dark respiration of pine (A) and poplar (B) over a one-year period. Simulations were analysed for leaves where dark respiration did not acclimate (closed circles), and where dark respiration acclimated to a 3-day running mean temperature (open circles). Data used in simulations were obtained from growth chamber experiments (Chapters 2 and 3).

Modelling based on field data show that predicted respiration rates using the variable (dynamic) respiratory response differed from respiration estimates based on the fixed (static) response. The static model consistently under-estimated respiration throughout the cooler months and overestimated during warmer months (Fig 5.3). The use of respiratory parameters that varied with temperature resulted in higher values of R_d throughout autumn, winter and for the most part of spring in pine (Fig. 5.3A). Values of R_d differed between the two responses (static *vs* variable) during this period by between 6 and 37% on a monthly basis. Conversely, modelled results showed that in the warmer months (November – February), monthly rates of respiration were lower using the dynamic modelling scenario, with the greatest difference between responses occurring in December 2005 (-16%; Fig.5.3A). The net effect of these differences on an annual basis was approximately 11% (annual R_d for pine: 30.61 and 33.87 mol m^{-2} for the static and dynamic scenarios, respectively). In poplar, the dynamic scenario also resulted in higher values of R_d in spring and autumn (Fig. 5.3B) but lower R_d in the warmer months (December – February). In poplar the effect of these differences in calculated R_d on a growing-season basis was a 3.6% underestimation by the static algorithm (34.01 and 35.25 mol m^{-2} for the static and dynamic scenarios, respectively).

5.4 Discussion

5.4.1 Temperature acclimation of dark respiration

There is no single curve that can effectively explain the response of respiratory acclimation when ambient temperature changes (Fig.5.1). Instead, the temperature response of R_d is made up of a family of curves where R_d is dependent on recent or

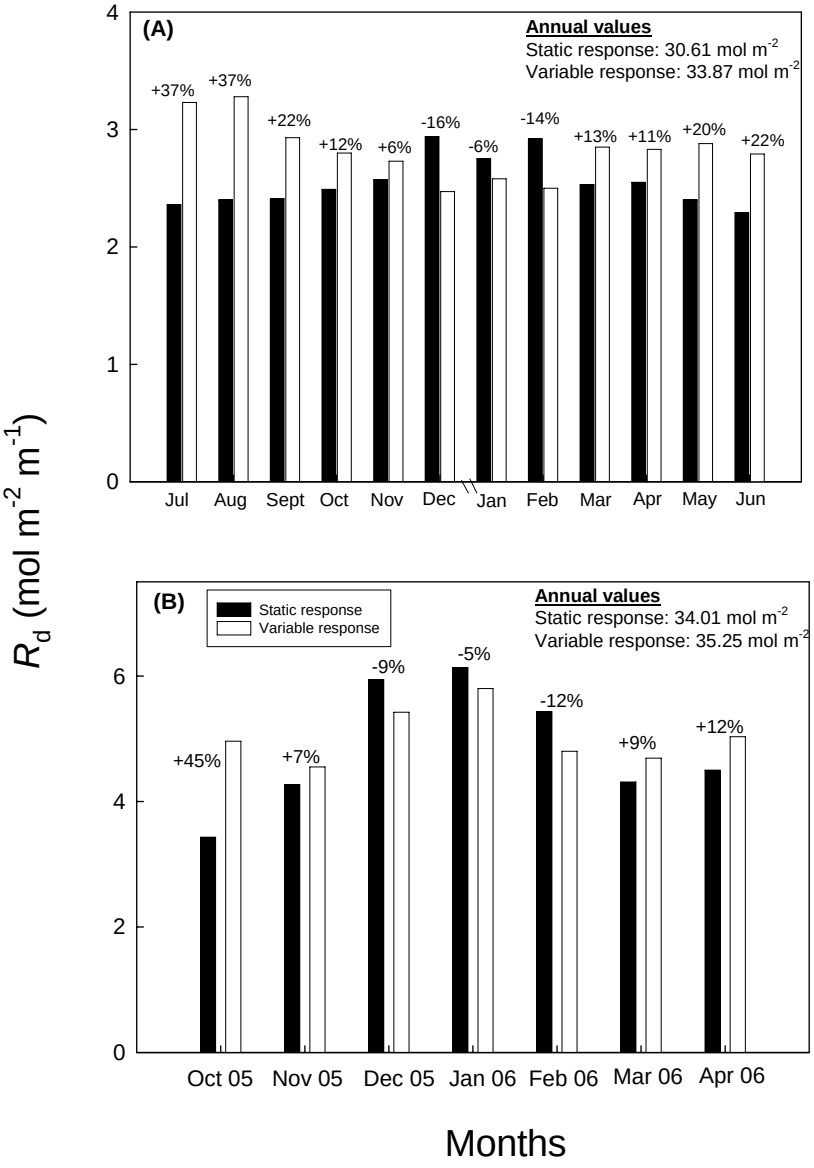

Figure 5.3. Simulated monthly total leaf dark respiration over a 12 and 7-month period between 2005 to 2006 for (A) pine and (B) poplar. Respiration was simulated assuming no acclimation (static; closed bars) and taking into account acclimation using a 3-day running mean temperature (variable; open bars). Percent differences between responses are shown for each month (% difference between scenarios were calculated based on variable/static).

longer-term physiological history. This notion has important implications for the interpretation and modelling of R_d measurements. The present study, and other recent work (Wythers et al. 2005; King et al. 2006) have shown that respiratory acclimation can have a substantial effect on annual calculations of R_d. When acclimation is taken into account, it generally reduces estimates of annual R_d. Although the present study involved only the use of a leaf-level model, it is clear that acclimation will result in a dampening of predicted increases in respiration as global temperatures increase (King et al. 2006).

Furthermore, there is strong evidence that leaf respiration can acclimate to changes in ambient temperature over a short period of time (hours to days) (Larigauderie and Korner 1995; Teskey and Will 1999; Tjoelker et al. 1999a; Atkin et al. 2000b; Bolstad et al. 2003; Atkin et al. 2005a; Lee et al. 2005). The above findings indicate that a temperature response measured one day will clearly differ from measurements made a week later or even a month later (depending on changes in ambient temperature). To my knowledge, limited information is available in the literature pertaining to seasonal changes in photosynthetic and respiratory parameters (e.g. Photosynthesis: J_{max} and V_{cmax}; Respiration: R_{10} and Q_{10}) in pine and poplar (an evergreen and deciduous species). Hence, incorporating the seasonal data into a model not only accounts for the rapid process of acclimation but also helps increase the validity of the modelled responses.

As previously mentioned, the present study and other recent work (Wythers et al. 2005; King et al. 2006) have shown that respiratory acclimation can have a substantial effect on annual calculations of R_d, and in turn, on values of carbon uptake and storage.

Importantly, this may vary with species, with the timing of measurements used to parameterize models and with the period of calculation. For example, the underestimation of R_d in pine over cooler months and the overestimation over warmer months (Fig 5.3A) resulted in an annual R_d value that differed by 11% between the dynamic and static scenarios. By contrast, for poplar, where leaves are only present during warmer months, the static calculation underestimated annual R_d by only 3.6% over the variable calculation (Fig 5.3B). Model modification also resulted in changes in annual total respiration estimates for the controlled environmental experiments - total respiration was generally lower in simulations taking into account respiratory acclimation compared with simulations that ignored respiratory acclimation. Differences in annual respiration between models for both species were between 12 to 13% (Table 5.1). Clearly the failure to account for acclimation of R_d may have varying impacts on the modelled annual respiratory response of evergreen and deciduous species. Importantly, it may also result in an overestimate of CO_2 release under warmer conditions. This is particularly pertinent to considerations under global warming scenarios. Furthermore, since there is now strong evidence that leaf respiration can acclimate to changes in ambient temperature over a short period of time (days) (Larigauderie and Korner 1995; Teskey and Will 1999; Tjoelker et al. 1999a; Atkin et al. 2000b; Bolstad et al. 2003; Lee et al. 2005), it seems apparent that we should incorporate seasonally dynamic responses of respiration into carbon exchange models in order to help improve the validity of modelled responses. The findings here should have some implications for the majority of commonly used forest and ecosystem models (Tjoelker et al. 2001; Wythers et al. 2005). It has been shown here that thermal acclimation modulates the direct effects of temperature on

carbon exchange rates in plants (Tjoelker et al. 1999a; Tjoelker et al. 1999b; Atkin et al. 2000b; Luo et al. 2001; Atkin and Tjoelker 2003; Bolstad et al. 2003; Atkin et al. 2005a).

5.5 Summary

The key findings of this chapter are (1) taking into account temperature acclimation of R_d will allow models to account for respiratory response to temperature in a more biologically realistic way (2) The use of variable Q_{10} and R_d values can alter modelled estimates of carbon exchange in plants (3) current models may over-predict respiration and may result in underestimated productivity estimates, especially under warmer conditions.

Until recently, there has generally been much more emphasis on measurements for obtaining parameter values of photosynthesis than those needed for respiration (Whitehead and Walcroft 2005). Hence, little is known about the process of carbon release and its response to environmental variables. However, forests are the principal site of storage and release of terrestrial carbon and minor changes in carbon fluxes can have important implications for national and global carbon balance. To quantify the potential amount of carbon release via forests and to predict future changes in relation to environmental factors, it is necessary to determine the rates of carbon uptake and release by different forest types and to identify factors that regulate carbon fluxes. The general consensus is that no model alone will provide the answers needed to predict long term forest or ecosystem responses to future changes in climatic and environmental factors, but integrating our understanding from individual process studies (such as the findings

presented in chapters 2, 3 and 4) will help in forecasting future long term changes in forest carbon fluxes. Future work should use the results from empirical investigations and scale-up from leaf-level responses to forests and ecosystem studies to provide a broader picture.

Chapter 6

General discussion and conclusions

6.1 Diagrammatic illustration of principal findings

This study set out to investigate the effects of temperature change on the potential of photosynthetic and respiratory acclimation in two contrasting tree species, *Populus deltoides and Pinus radiata*. The controlled-environment study allowed for the investigation of the effects of short-term temperature change (the transfer to cooler and warmer temperatures) on the potential for acclimation of both processes in pre-existing and new tissues (developed under the new temperature environment). By contrast, the field observations facilitated the study of continuous long-term temperature change on the acclimation of photosynthesis and respiration in foliage exposed to natural diurnal and seasonal changes.

The figures below are a synthesis of the experimental results reported in Chapters 2, 3, 4 and 5. An explanation of Figure 6.1 (controlled environment experiments) is as follows:

(1) Changing environmental conditions (temperature change - cold and warm transfers) had a strong influence on rates of photosynthesis and respiration. The

Controlled-environment studies:
Short term responses of *Pinus radiata* and *Populus deltoides*:

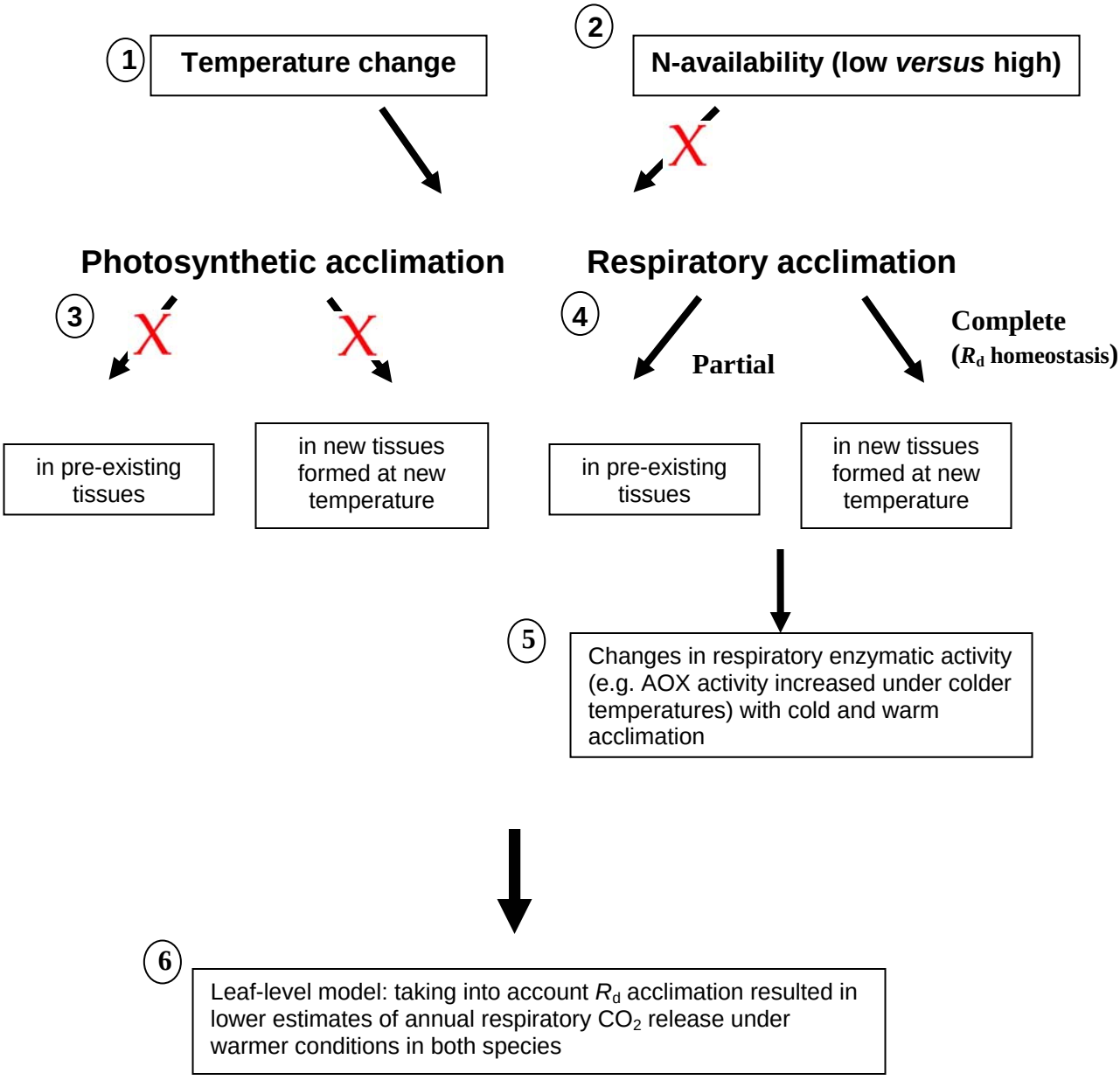

Figure 6.1 Diagram showing the acclimation potential of photosynthesis and respiration in pre-existing and new tissues as well as changes in respiratory enzymatic properties under changing temperature conditions. Details for each numbered response can be found in the text and crosses indicate no response.

transfer to lower temperatures generally led to reduced rates of photosynthesis whilst 5 ^{o}C increases in temperature generally resulted in enhanced photosynthetic rates. Rates of dark respiration, on the other hand, exhibited an approximately exponential function to temperature in the short term.

(2) A weak relationship between foliar N concentrations, photosynthesis and respiration was observed. Plants with lower N content had no less capacity for acclimation than plants with high N content.

(3) Photosynthesis: there was no evidence of photosynthetic acclimation in both pre-existing and new tissues (formed at the new growth temperature). The long term temperature responses of photosynthesis (determined from values at the respective growth temperatures) exhibited a consistent increase with increasing temperature and did not differ greatly between the responses before and after transfer to warmer and cooler temperatures. This is in stark contrast with the observations made for respiration, which was clearly insensitive to a 10 ^{o}C range of temperature in the long term.

(4) Respiration: differing degrees of respiratory acclimation were observed in pre-existing and new tissues. In both species, complete acclimation or respiratory homeostasis (near identical rates of respiration across a 10 ^{o}C temperature range) was found in new tissues but not in pre-existing tissues (partial acclimation) at the new growth temperature. Clearly new tissues benefited from the ability to modify

cellular responses, morphology and biochemistry which appear to be critical aspects in the process of acclimation.

(5) Changes in respiratory enzymatic activity occurred in response to cold and warm transfer of plants. The shift of plants to colder temperatures resulted in significantly greater alternative pathway activity, but contrary to expectations the shift to warmer conditions generally led to no significant change in the activity of the cytochrome pathway.

(6) The use of a modified leaf-level model to simulate responses of dark respiration and net carbon exchange over longer periods suggests the need to account for respiratory acclimation. In the absence of acclimation, models are likely to produce erroneous estimates of atmospheric CO_2 release via plant respiration. Failure to incorporate acclimation may result in the over-estimation of dark respiration under warmer conditions and this in turn may lead to an under-estimation of productivity. This also has implications for the role of plant respiration in the carbon cycle.

An explanation of Figure 6.2 (field observations) is as follows:

(1) Seasonal changes in temperature and foliar carbohydrates had a strong influence on the process of acclimation. Leaf N content generally exerted a positive influence on respiration but no relationship was found between N and photosynthesis

(2) Photosynthesis: The seasonal variation observed in *Pinus radiata* occurred gradually. Photosynthetic parameters increased from spring through to summer whilst lower rates were observed in autumn and winter. Poplar on the other hand, was highly active throughout spring and summer but a loss in activity was observed in autumn.

Respiration: Seasonal variation was consistent in both species - an increase in rates of R_d was observed in autumn and winter whilst decreases occurred during spring and summer.

(3) Strong acclimation to temperature was observed with R_d in the long term with slight over-compensation occurring at higher temperatures. This was absent in photosynthesis which continued to increase with increasing maximum daytime ambient temperatures. This was consistent in both species.

(4) The modified leaf-level model driven by static (fixed mid summer) R_d parameters resulted in the underestimation of respiration during cooler months and an

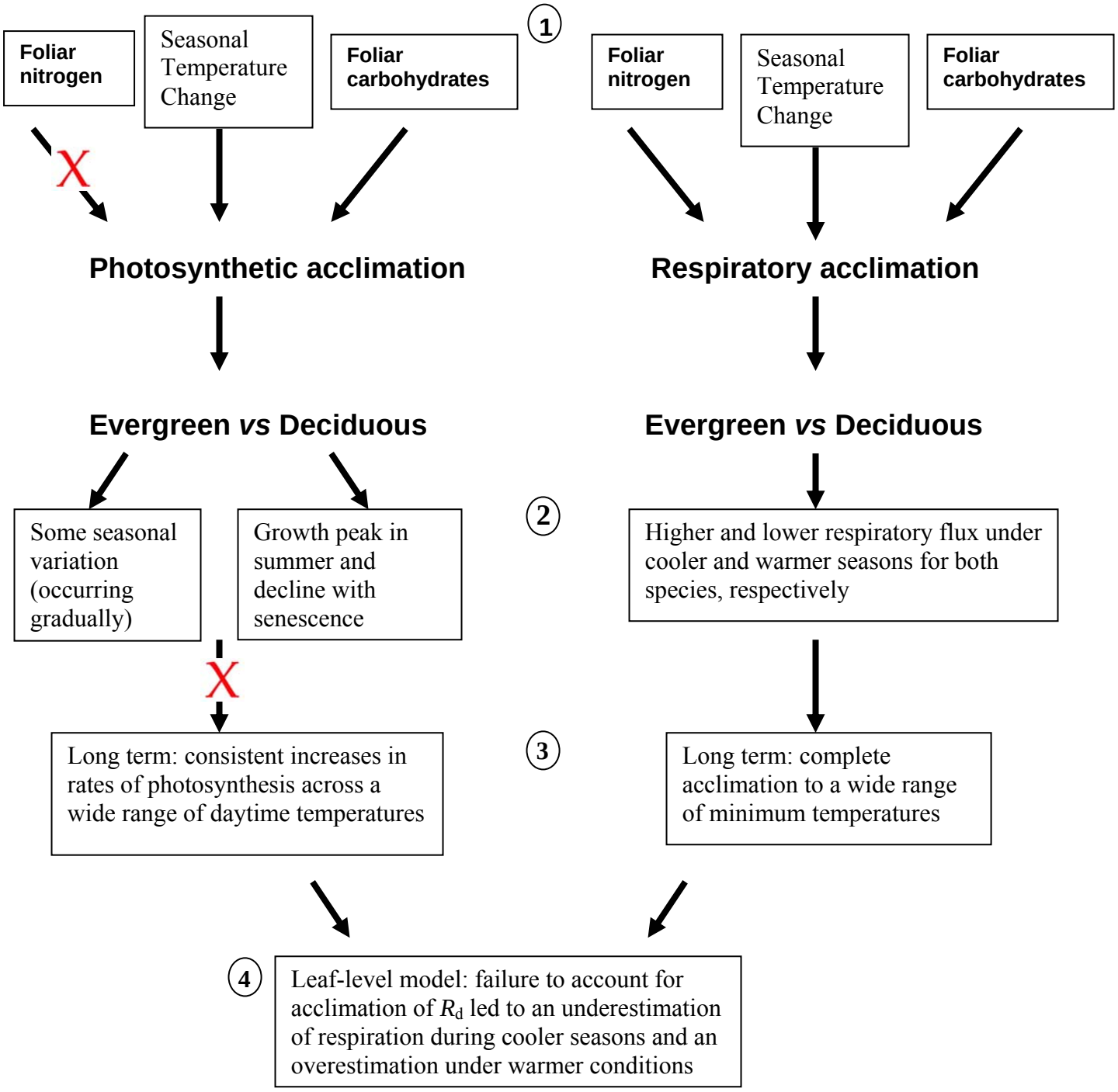

Figure 6.2 Diagram showing the acclimation potential of photosynthesis and respiration in two contrasting tree species as well as the regulation of foliar properties on key metabolic processes exposed to seasonal changes. Details for each numbered response can be found in the text and crosses indicate no response.

overestimation of respiration under warmer conditions (compared to a model incorporating acclimation of respiration (driven by temperature-variable R_d parameters)). Such differences may have a significant impact on calculations of forest carbon budgets and highlights the importance of using temperature-variable parameters when running process-based models.

6.2 Discussion of principal findings

The results of this research show strong respiratory acclimation to temperature under both natural and controlled conditions but no strong evidence of photosynthetic acclimation to similar temperature changes. This is in contrast to other reports (involving a range of plant species) in the literature which have observed that the temperature dependence of photosynthesis is affected by growth temperatures and more importantly, photosynthetic rates can acclimate to shifts in growth temperature which in turn improves leaf carbon balance (Teskey and Will 1999; Gunderson et al. 2002; Sage and Kubien 2007). With respect to respiration, the present study consistently showed a downward adjustment (in the medium term) in the temperature response of respiration at higher growth temperatures whilst respiratory homeostasis (complete acclimation) across a 10 ^{o}C temperature range was confirmed over a longer period in the controlled-environment studies (i.e. limited to new leaves formed under the new temperature environment). This finding concurs with previous reports which have also shown that leaves developed at a new temperature acclimates more fully to changes in temperature than previously developed (pre-existing) leaves (Talts et al. 2004; Armstrong et al. 2006a; Atkinson et al. 2007) . However, it is also noteworthy that acclimation may occur in previously

developed leaves, if given sufficient time under the new thermal regime. Previous studies (Bolstad 1999; Bolstad et al. 2001; Bruhn et al. 2007) have suggested the possibility of complete acclimation in pre-existing leaves, but only if the leaves are sufficiently long-lived to allow time for changes in cellular structure and biochemistry to occur within these leaves. Hence, in species with short-lived leaves, such changes cannot occur due to senescence.

The study of tree species under field conditions showed that in the long-term, near identical rates of dark respiration were displayed across a wide range of minimum temperatures, with a slight decline observed at greater temperatures. This provided strong evidence supporting the presence of respiratory acclimation to diurnal and seasonal temperature change. Furthermore, this has confirmed that the process of dark respiratory acclimation to temperature is rapid, in this case, occurring within three days. More importantly, the novel aspect of this study lies in the fact that there has been limited information in the literature pertaining to seasonal values of R_{10} and Q_{10} which are required, together with meteorological data, to accurately drive models measuring plant carbon fluxes. Until now, most models have been using a single R_{10} value and working on the assumption that the Q_{10} would remain static at 2.0. Therefore, the published R_{10} and Q_{10} parameters from this study should inform various carbon models which simulate plant responses to temperature change.

The results from both controlled and field studies support the notion that increases in temperature over a moderate range of temperatures (e.g. 10 to 25 ^{o}C) will be in favour of

carbon uptake over carbon release in *Pinus radiata* and *Populus deltoides*. Apart from respiratory acclimation, high photosynthetic rates maintained at elevated temperatures gave rise to a lower R_d/ A_{max} ratio with increasing temperature. This contrasts with predictions suggesting that respiration will increase exponentially with temperature and more rapidly than photosynthesis. Rather, the findings here strongly support the presence of respiratory acclimation to temperature change and this is likely to result in a strong insensitivity of rates of respiration to increasing temperatures. Hence, the present study, alongside previous research (Strand et al. 1999; Tjoelker et al. 1999a; Atkin and Tjoelker 2003; Atkin et al. 2005a) reinforces the need to take into account acclimation of respiration when predicting carbon fluxes of forests and ecosystems to avoid inaccurate or erroneous estimates.

.6.2.1 Respiratory acclimation to temperature

Several earlier as well as recent studies have shown that species differ in their capacity for respiratory acclimation to temperature; while some species acclimate strongly others only managed with partial acclimation (Berry and Bjorkman 1980; Teskey and Will 1999; Tjoelker et al. 1999a; Xiong et al. 2000). Apart from differences between species, the potential for temperature acclimation has also been found to differ between tissue types (e.g. pre-existing *versus* newly developed tissues). For example, temperature acclimation in previously developed tissues often involves changes in existing cellular machinery (e.g. changes in protein abundance within existing organelles). By contrast, acclimation that occurs in newly developed tissues often involves physical as well as enzymatic changes (i.e. tissues developed under colder temperatures tend to be thicker,

with lower specific leaf area and higher nitrogen and sugar concentrations than tissues developed under warmer temperatures (Hurry et al. 1995; Ryan 1995; Tjoelker et al. 1999a; Atkin et al. 2006b). The greater degree of plasticity of new tissues may help explain the consistent presence of respiratory homeostasis (observed here) in these tissue types. Interestingly, with respect to the controlled- environment studies, complete acclimation (respiratory homeostasis) was absent from pre-existing leaves whilst results from the field analysis also involving pre-existing foliage showed complete homeostasis. The difference in the degree of acclimation observed may be explained by the subtle day-to-day changes in temperature occurring in the field which is clearly different from the step-changes of 5-10 °C simulated by the controlled cabinet analysis. Additionally, the use of a deciduous and an evergreen tree species here allowed for a comparison to be made on the relative effects of leaf age on respiratory acclimation. Here, I reached a conclusion confirming that a similar magnitude of acclimation of respiration to temperature occurred in both species. Such findings confirm that in the long term, temperature acclimation is likely to constrain autotrophic respiratory carbon losses in a manner that differs from predictions based on short term respiratory response functions (e.g. an approximate exponential function).

6.2.2 Mechanisms underpinning respiratory acclimation

At present, photosynthesis exceeds respiration on a global basis and this response has been attributed to three forms of environmental changes that have exerted a positive effect on photosynthesis (see section 1.2.1). However, as mean global surface temperature is expected to rise further within the next few decades (IPCC 2007), it is not

clear if this increase in temperature will begin to enhance rates of respiration that cannot be compensated for by photosynthesis. Furthermore, respiration generally increases exponentially over the range of temperatures commonly experienced by plants and exhibits a sharp reduction only when lethal temperatures have been reached (Atkin et al. 2005b). Here however, it has been confirmed that the exponential rise in dark respiration rate occurs only in the short-term. This instantaneous response should not be confused with the long-term response of respiration, which exhibits varying degrees of acclimation (from partial to complete acclimation). My controlled-environment and field studies suggest that long term acclimation of respiration to fixed differences in growth temperature and seasonal acclimation to prevailing ambient temperatures were of similar magnitude in both species. However, this was not necessarily expected given that temperature effects on tissue development (morphological plasticity) are thought to have larger effects on gas exchange physiology than shorter term temperature effects mediated by physiological plasticity (Tjoelker et al. 2008).

The findings of this research identifies well with current thought on the temperature response of respiration. First, the shape of the exponential function flexibly adjusts to altered environmental conditions. This mechanism has been referred to as 'Type I' acclimation, and can occur within a matter of hours to days (Atkin and Tjoelker 2003; Atkin et al. 2005a). In the longer term, plants can alter their respiratory capacity, leading to lower rates of respiration at high measurement and vice versa - such a response has been termed 'Type II' acclimation (Atkin and Tjoelker 2003). Review of the literature suggests that earlier reports support a static Q_{10} of 2.0 but recent findings suggest that Q_{10}

decreases as the measurement temperature increases (Tjoelker et al. 2001). Furthermore, there is emerging evidence for the role of enzymes in respiratory acclimation to temperature where at low temperatures, respiration is thought to be restricted by the activity of AOX and COX, whereas respiratory flux at higher temperatures becomes increasingly limited by adenylates (ADP) or substrates (Atkin et al. 2005a; Atkin et al. 2005b).

Results here reveal that not only can respiratory acclimation be substantial, but it can also occur rapidly in response to changing environmental conditions. The field data provides an average temperature window of three days but previous reports by Rook (1969) and Atkin et al. (2000b) have in fact shown that acclimation of R_d can occur in as quickly as a day or two. To my knowledge, very few other studies have investigated the time taken for leaf respiration to acclimate to shifts in ambient temperature (Bolstad et al. 2003; Lee et al. 2005). The ability to acclimate rapidly to medium-term (i.e. days) shifts in temperature suggests that respiration rates at a set temperature and the temperature sensitivity of leaf respiration is constantly changing in response to changes in the environment.

6.2.3 Temperature response of photosynthesis

The temperature dependence of photosynthesis for a given plant as observed in its natural habitat is the result of a complex interaction between the prevailing environment and the characteristics inherent in the species. The term ‘acclimation’ is used to describe an observed shift in the temperature optimum for photosynthesis and modifications of the peak photosynthetic rate at the optimum temperature, with changing growth temperature

(Kattge and Knorr 2007). Temperature acclimation to low growth temperatures is considered to result from increased quantities of rate-limiting enzymes and particularly from reduced rubisco activity (Ferrar et al. 1989; Sharkey et al. 2007). By contrast, acclimation to higher temperature is a result of greater activity and thermal stability of the photosynthetic machinery (Berry and Bjorkman 1980; Kirschbaum and Farquhar 1984). Light saturated rates of photosynthesis are low at extreme low/high temperatures and have an optimum at an intermediate temperature (Ishikawa et al. 2007). With changes in growth temperature many plants show considerable phenotypic plasticity in their photosynthetic characteristics. In many species, V_{cmax} increases in a curvilinear fashion from 15 to 30 ^{o}C, with deactivation often only substantial at very high temperatures (Leuning 1995; Medlyn et al. 2002a; Hikosaka et al. 2006; Ishikawa et al. 2007). Similarly, the deactivation of J_{max} occurs at high temperatures and shifts in the optimal temperature for apparent J_{max} with growth temperature have been observed in some species but not in others (Campbell et al. 2007; Sage and Kubien 2007). It is apparent that there are large interspecific differences in the temperature optima for photosynthesis and it seems that most, if not all, plants have a considerable ability to modify their photosynthetic machinery in such a way as to function more efficiently in conditions in which they actually grow. Similar shifts in J_{max} and V_{cmax} were observed in my controlled-environmental experiments when plants were exposed to step changes in temperature (cold and warm transfer). Due to logistical limitations (time constraints), instantaneous photosynthetic response curves were not measured in the experiments described here - rather measurements were only made at the respective growth temperatures. However, when these data are fitted using a photosynthetic temperature

response function (Leuning 2002) they show remarkable conformity with published short-term photosynthetic temperature responses (Leuning 2002; Medlyn et al. 2002a) (Table 6.1).

Furthermore, the absence of a long-term temperature dependent photosynthetic response (observed here) may be explained by the moderate temperature range used in these experiments. In general, photosynthesis can function effectively between 7 to 40 ^{o}C in most plant species (Campbell et al.2007; Sage and Kubien 2007). For example, as mentioned earlier, V_{cmax} and J_{max} do not show signs of decline until well beyond 30 ^{o}C, suggesting that the optimum temperature for photosynthesis is unlikely to be within the range of ambient temperatures used in the study. Taken together, these may in part explain the inability to establish a temperature optimum for photosynthesis. The use of moderate temperatures in the controlled environment experiments (Chapters 2 and 3) may have been partially responsible for the absence of significant shifts in the temperature responses of photosynthesis. However, the finding that no acclimation was displayed in the field (Chapter 4) despite observations made over a wide range of ambient day time growth temperatures provides strong evidence that unlike respiration, photosynthesis appears less sensitive to variation in temperature.

Table 6.1. Parameters describing the temperature response of V_{cmax} and J_{max} in the current study as well as in previous studies. H_{av}, H_{dv} and S_{vv} are the activation and deactivation energies and an entropy term for V_{cmax}. H_{aj}, H_{dj} and S_{vj} are the corresponding terms for J_{max}.

H_{av} (J mol^{-1})	H_{dv} (J mol^{-1})	S_{vv} (J mol^{-1} K^{-1})	H_{aj} (J mol^{-1})	H_{dj} (J mol^{-1})	S_{vj} (J mol^{-1} K^{-1})	Species	Source
43307	210101	670	48977	207199	643	*Pinus radiata* (HN)	Chapter 3
53700	212300	665	55124	215003	640	*Pinus radiata* (LN)	Chapter 3
63149	189793	709	67601	224265	722	*Populus deltoides* (HN)	Chapter 2
70014	190314	711	69337	193754	699	*Populus deltoides* (LN)	Chapter 2
75400	175000	559	65300	129000	420	*Fagus sylvatica*	Dreyer et al. (2001)
67600	144000	451	46100	280000	888	*Quercus petraea*	Dreyer et al. (2001)
69700	404000	1285	50400	154000	494	*Betula pendula*	Dreyer et al. (2001)
45027	203594	650	45977	199345	650	*Pinus radiata* (fert.)	Walcroft et al. (1997)
52672	206083	650	46337	198924	650	*Pinus radiata* (unfert.)	Walcroft et al. (1997)
52750	202600	669	61750	185600	621	*Pinus sylvestris*	Wang et al. (1996)

6.3 The impact of foliar properties

6.3.1 Nitrogen

As nitrogen is often a limiting resource for plant growth, efficient use of N is believed to contribute to plant fitness and there is considerable evidence in the literature supporting a correlation between leaf N, respiration and photosynthesis (Hikosaka and Terashima 1995; Ryan 1995; Reich et al. 1998b; Tjoelker et al. 1999b; Carswell et al. 2000; Wilson et al. 2000; Griffin et al. 2002b; Lewis et al. 2004; Misson et al. 2006; Reich et al. 2006; Xu and Griffin 2006; Bown et al. 2007; Campbell et al. 2007; Hikosaka et al. 2006; Ishikawa et al. 2007). However this finding is by no means universal - field observations presented in Chapter 4 showed no clear relationship between seasonal changes in leaf N and photosynthesis in either pine or poplar. Rather, variation in photosynthetic parameters was much better explained by changes in air temperature over the year. However, a positive relationship was found with respiration. Nonetheless, leaf N is known to be temperature sensitive - it is often significantly greater in tissues grown in cooler conditions (Makino et al. 1994; Hikosaka 1997; Rachmilevitch et al. 2006; Dwyer et al. 2007). This provides support for the role of leaf N in the process of thermal acclimation and nitrogen deficiency provides a plausible explanation for any delayed physiological responses to changes in temperature. In fact, it has been proposed that the degree of acclimation may be greatest in tissues that exhibit large changes in nitrogen concentration (Tjoelker et al. 1999b; Tjoelker et al. 2001; Dungan 2003; Lewis et al. 2004; Sholtis et al. 2004; Xu and Griffin 2006). Though it seems apparent that leaf N concentration should play a key role in the process of photosynthetic and respiratory acclimation to temperature, the controlled and field studies here indicated otherwise.

Similar to a recent report by Atkinson et al. (2007), the controlled-environmental experiments here showed that the differences in leaf N concentration between plants did not greatly influence the responses of photosynthesis and respiration to temperature change. Plants grown at low levels of N did not have a lesser capacity for respiratory acclimation to temperature. These findings are consistent with the prediction that short-term thermal acclimation is not primarily related to adjustments in N concentration. Temperature appeared to directly influence the responses of photosynthesis and respiration rather than acting via changes in tissue N content.

6.3.2 Carbohydrates

Many of the complex interactions between photosynthesis, respiration and temperature are still relatively uncertain, although it is probable that the interaction is mediated by leaf carbohydrate levels. Several lines of evidence suggest that the linear relationship between the rate of photosynthesis and the subsequent rate of dark CO_2 efflux can be explained in terms of quantitative changes in carbohydrates, the common metabolites to both processes. In addition, the role of carbohydrates in respiratory metabolism has recently received increased attention as a potential mechanism underpinning thermal acclimation of dark respiration (Atkin and Tjoelker 2003), particularly in constraining the ratio of respiration to photosynthesis in leaves and plants grown under various environmental conditions (Amthor 2000; Gifford 2003). One explanation suggests that the short- and long-term effects of temperature on respiration may be mediated through effects on the rate of metabolism of the sucrose pool (Gunn and Farrar 1999; Rachmilevitch et al. 2006). In the short term an increase in temperature will increase the rate of sucrose

metabolism, transport and utilization whilst carbohydrate-dependent changes in gene expression may be accountable for the acclimation of respiration observed in tissues exposed to long-term temperature change (Guy et al. 1992; Volder et al. 2004). This was further supported by Atkin et al. (2000b) who confirmed that acclimation of dark respiration was associated with a change in the concentration of soluble sugars. Furthermore, my study involving field-grown pine and poplar alongside recent work by Campbell et al. (2007) have confirmed that acclimation to low temperatures is associated with an increase in the concentration of soluble carbohydrates (Guy et al. 1992; Turnbull et al. 2004) which may in part explain the increase in respiration rates consistently observed with respiratory acclimation to low growth temperatures (Farrar and Williams 1991; Lewis et al. 2004; Xu and Griffin 2006). Cold temperatures often have a greater relative effect on carbohydrate translocation and carbon use than on carbon assimilation, resulting in an altered balance between substrate supply and use and leading to increased carbohydrate concentration. Additionally, there is strong support in the literature of a direct regulation of respiration by substrate availability (Azcon-Bieto et al. 1983; Azcon-Bieto and Osmond 1983a; Tjoelker et al. 1999b; Bolstad et al. 2003; Turnbull et al. 2003; Atkin and Tjoelker 2003; Atkin et al. 2005a; Lee et al. 2005; DeLucia et al. 2007). Azcon-Bieto et al. (1983) confirmed that when free sugar levels were high, substrate supply to the mitochondria increased and this in turn stimulated respiration, but this was found to occur via the alternative pathway.

The above findings should not result in the exclusion of the possibility that respiration may be regulated by other factors. It is also noteworthy that, despite numerous reports

supporting the correlation between carbohydrates and respiration, there have been other reports that have shown otherwise (Gunn and Farrar 1999; Noguchi et al. 2001b; Atkin et al. 2000b; Loveys et al. 2003; Luomala et al. 2003). For example, Luomala et al. (2003), in their 3-year study of Scots pine, observed no change in carbohydrate concentration despite changes in ambient temperature. This was surprising as it was expected that greater temperatures would enhance the use of carbohydrates and prevent the accumulation of starch to prevent photosynthetic feedback inhibition by increasing the rate of metabolic processes (Azcon-Bieto 1983; Makela et al. 2004). The absence of a correlation here suggests that variation in the degree of acclimation is not always associated with the extent to which soluble sugars accumulate at different temperatures. Moreover, it has been proposed that a small rise in mean temperature is unlikely to be met by a programmed response in carbon partitioning (Guy et al. 1992). Evidently there is still much uncertainty in relation to the regulation of leaf properties on photosynthetic and respiratory acclimation. A better understanding of the relationships between metabolic processes and leaf properties will improve our ability to predict physiological adjustments of respiration and photosynthetic rates and the temperature responses of both processes under future environmental conditions.

6.3.3 Respiratory enzymatic characteristics

Mitochondrial oxygen consumption is catalysed by two enzymes (AOX and COX), with different catalytic properties (see section 1.2.2.4 for details). The alternative oxidase pathway has sometimes been termed a ‘wasteful’ component of plant respiration. However, different lines of investigation have suggested a number of possible roles for

AOX (Azcon-Bieto et al. 1983; Siedow and Umbach 1995; Day et al. 1996; Cannell and Thornley 2000). It is now well established that the cytochrome pathway is primarily controlled by the availability of ATP and P_i but low cellular demand for ATP eventually slows the TCA cycle down. By contrast, AOX is not prone to regulation by adenylates and operates during periods of large demand for carbon skeleton intermediates – it can maintain a high carbon flux through glycolysis and the TCA cycle and thus plays an important role in the regulation of plant metabolism. Additionally, it can also act as a safety valve to prevent the overreduction of the ubiquinone pool and formation of reactive oxygen species (ROS) under conditions of environmental stress (Ribas-Carbo et al. 1995; Wright et al. 2006).

The partitioning of electrons between AOX and COX is strongly influenced by growth temperature. Available evidence suggests that relative engagement of AOX increases at low temperatures (Vanlerberghe and McIntosh 1992; Gonzalez-Meler et al. 1999; Ribas-Carbo et al. 2000; Kurimoto et al. 2004b; Fiorani et al. 2005; Atkin et al. 2007; Campbell et al. 2007). These conclusions are however, not universal (Stewart et al. 1990; Gonzalez-Meler et al. 1999; Kurimoto et al. 2004a; Hachiya et al. 2007). Nonetheless, the consensus is that constraints on the respiratory electron transport chain as a result of low temperature have been found to inhibit the cytochrome pathway (Wagner and Krab 1995; Atkin et al. 2005a) and this may result in an overreduction of the ubiquinone pool and an increase in ROS production. This in turn, serves as a signal for the increased synthesis of AOX that is required to help reduce ubiquinone and slow down the production of ROS. Temperature-mediated increases in ROS could be alleviated, in part, by increases in

respiratory capacity, particularly through increases in flux via the non-phosphorylating pathway (Purvis and Shewfelt 1993; Maxwell et al. 1999; Rachmilevitch et al. 2006). This may explain why AOX activity was found to be consistently higher in the cold-acclimated foliage of both species examined here. The enhancement of AOX activity in cold-grown plants may, in part, represent a mechanism to increase respiration via the non-phosphorylating pathway because of its significance in maintaining higher respiratory flux at lower growth temperatures. By contrast, available evidence suggest that under warmer conditions, the respiratory flux is regulated by the supply of substrates and adenylates, catalysed by COX (Azcon-Bieto et al. 1983; Ribas-Carbo et al. 2000; Noguchi et al. 2001a; Atkin et al. 2005b). But results from this study for pine and poplar under controlled-environmental conditions generally showed no significant change in COX activity despite a 5 ^{o}C step increase in temperature. This finding is consistent with a recent report by Campbell et al. (2007), involving a wide range of plant functional groups (forbs, grasses and evergreen trees/shrubs). The absence of any significant change in activity may be explained by the moderate shifts in growth temperatures which may not have warranted a change (e.g. increase) in enzymatic activity.

It is noteworthy that here the relative contributions of AOX and COX to foliar respiration was estimated using inhibitors (potassium cyanide (KCN) and salicylhydroxamic acid (SHAM)). The use of inhibitors tends to result in the under-estimation of activity in both pathways. So far, the most reliable and currently available method for the assessment of *in vivo* activity is the use of the differential fractionation of ^{18}O between the two terminal oxidases (Nagel et al. 2001). However, widespread use of the oxygen isotopic technique

is still limited, partly because the set-up (e.g. measuring systems) is often too costly. Putting aside cost, conclusive results concerning the degree of engagement of the different pathways await measurements based upon oxygen-isotope discrimination (see below: section 6.4.2).

6.3.4 Modelling of plant carbon fluxes

Models that operate at the organ level (i.e. root, stem and leaf) are often used to examine the effects of environmental change on plant carbon fluxes. A modified leaf-level model was used in this thesis with the aim of examining the consequences of the alternative temperature response and acclimation algorithms on modelled carbon budgets. The model integrates the effects of photosynthesis and dark respiration and takes into account the presence of respiratory acclimation to temperature and a variable Q_{10} value. Estimates based on data from the controlled environment experiments show that acclimation tended to reduce the total respiratory flux on an annual basis. Acclimation to a 3-day running mean temperature reduced respiration by approximately 13% compared to non-acclimated respiration for pine, while a slightly lower reduction was observed for poplar (12%).

Available evidence suggest that temperature responses of respiration does not adequately fit to a simple static Q_{10} exponential relationship and a static R_d parameter, hence modelling based on field data were analysed using a static and variable/dynamic (using seasonal parameters) scenario. Estimates derived from variable parameters fit well with expectations that temperature acclimation should reduce foliar respiratory carbon loss

during warm periods and increase CO_2 release during cool periods. More importantly, the present research has shown that respiratory acclimation can have a substantial effect on annual calculations of R_d, and in turn, on values of carbon uptake and storage. However, this may vary with species, with the timing of measurements used to parameterize models and with the period of calculation. For example, the underestimation of R_d in pine over cooler months and the overestimation over warmer months (Fig 5.3) resulted in an annual R_d value that was 10.7% higher in the dynamic compared to the static scenario. By contrast, for poplar, where leaves are only present during warmer months, the static algorithm underestimated R_d by 3.6% between both scenarios. Clearly the failure to account for acclimation of R_d may have varying impacts on the modelled annual respiratory response of evergreen and deciduous species, and incorporating the seasonally dynamic response of respiration into carbon exchange models could help improve the validity of modelled responses. This is particularly pertinent to considerations under global warming scenarios.

6.4 Further studies

A number of further studies following on from the results reported in this thesis can be suggested. These are discussed below.

6.4.1 Challenge of testing plant responses to temperature

At least four empirical means have been widely used and have been proven to be successful in assessing plant responses to temperature: (1) studying current growth (key plant metabolism) processes across a range of thermal gradients, (2) field-warming

experiments (Bruhn et al., 2007), (3) studying current growth in response to natural temporal variation in temperature and (4) manipulating temperatures around plants and testing their responses under controlled conditions. However, each of these approaches has some advantages and disadvantages. For example, type (4) tests are often limited in time and space and are commonly confined to relatively artificial growth conditions and are, in most cases, restricted to younger tissues if work is carried out on trees. The other three options are often less precise in the sense of isolating temperature effects from other effects, and good replication may often be absent. But such studies are closer to real world conditions. Therefore, a challenge for future research is to maximize the combined use of all four options. Importantly, the use of controlled experiments which mimic "natural" environmental observations is a major challenge.

6.4.2 Process-based models and mechanistic changes underpinning the acclimation response of respiration

To determine how respiration might affect plant carbon balance and productivity requires estimation of annual respiration and photosynthesis. A range of process based models offer promise for computing these annual budgets but these models have not been widely applied in forestry. However, these approaches must be widely tested using temperature-variable parameters assembled in several locations and in various species before we can gain full confidence in these models. More importantly, as shown here and in previous studies, the use of the fixed exponential function for respiration is no longer a suitable model with which to predict long term temperature responses. This is because while short term temperature responses of respiration usually conform to an exponential model, in the

long term this is rarely so. Acclimation to temperature often results in shifts in the temperature response curves and in the long term, respiration has been shown to lose its sensitivity to temperature. Despite evidence of thermal acclimation of respiration, many models still do not account for acclimation in their estimates. In fact, to circumvent this problem, some models do not calculate respiration altogether and assume that net primary productivity is a fixed proportion of gross primary productivity. In order to stimulate the effects of temperature acclimation of respiration at the leaf-level, I introduced a linear temperature-dependent basal respiration and Q_{10} as a function of running mean temperature as shown below.

$$R_{d0} = R_{d0} + (R_{da} * (T_{mn} - T_{mo}))$$

$$Q_0 = Q_0 + (Q_a * (T_{mn} - T_{mo}))$$

Details of the above equations are available in Chapter 5. The incorporation of the dynamic response of respiration (acclimation) into carbon exchange models is likely to improve the validity of modelled responses.

Furthermore, little is known about the mechanistic changes responsible for the processes of acclimation to temperature – this should be a challenge taken on by future research. For example, as mentioned earlier, plant mitochondria have two ubiquinol-oxidizing pathways, the cytochrome and the alternative pathway. The precise role of AOX is not well defined in plants but it is thought to prevent the over-production of reactive oxygen species in the respiratory chain, especially under stressful conditions, through prevention

of an over-reduced ubiquinone pool (Kurimoto et al. 2004b; Lambers and Ribas-Carbo 2005). Many abiotic stresses, including sudden exposure to low temperature, have been found to induce AOX synthesis and this is often also associated with *de novo* synthesis of the AOX protein. Given the differential role of the cytochrome and the alternative pathways in mitochondrial electron transport, it is conceivable that differences in the responses of these two pathways are central to warm and cold acclimation response of R_d. Therefore, a challenge for future research could lie in the investigation of the actual engagement of these components of the respiratory chain (using the oxygen discrimination technique) under field conditions.

6.4.3 Response of photosynthesis to temperature

Unlike respiration, photosynthetic studies have received much attention. There is already good understanding of the mechanisms controlling the temperature response of photosynthesis in plants. While there is a good general knowledge of the potential limitations at and below the thermal optimum, major areas of uncertainty remain, particularly with respect to thermal limitations to photosynthesis beyond the thermal optimum. In the immediate future, efforts should be made to understand the limitations of photosynthesis above the temperature optimum. Another area of importance that future efforts could be directed towards is that of the lability of the rubisco activase enzyme. For example, increases in the heat stability of rubisco activase have been observed during acclimation to warmer temperatures and this corresponded to enhancements in photosynthesis above the thermal optimum. Hence, it appears that the heat tolerance of photosynthesis may be improved via enhancements to the thermal tolerance of the

activase. Over the longer term, there is a need to develop a better understanding of the acclimation potential of photosynthesis in natural populations. For example, the greatest warming is likely to affect the more thermally stressed environments, which are inhabited mostly by highly specialized species. Hence, the question to ask is: do these species have the potential for acclimation to allow them to persist under a warmer climate?

6.4.4 Concluding statement

While this study focused on the leaf-level responses of two key metabolic processes in two contrasting tree species to changing temperature conditions, it has also highlighted the importance of large-scale analysis of forests and ecosystems. This study has successfully raised the issue of the importance of dark respiratory acclimation and reached the conclusion that under moderate increases in atmospheric temperatures, increases in photosynthesis will likely exceed increases in respiration. Furthermore, the findings here reinforce the need to not confuse the short term response of respiration (exponential rise in respiration with temperature) with the long term response (a reversible shift in the shape or elevation of the temperature respiration response curve). Thermal acclimation can lead to rates of respiration that differ very little across a range of temperatures. This relative insensitivity of respiration to temperature in the medium to long term has important implications for plant carbon balance and particularly for concerns surrounding the potential for respiratory exacerbation of future climate warming.

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References

Aber, J., R.P. Neilson, S. McNulty, J.M. Lenihan, D. Bachelet and R.J. Drapek 2001. Forest Processes and Global Environmental Change: Predicting the Effects of Individual and Multiple Stressors. BioScience. 51:735-751.

Adams, C.R.Warren, M.A. 2004. What determines rates of photosynthesis per unit nitrogen in Eucalyptus seedlings? Functional Plant Biology. 31:1169-1178.

Adams, W.W., C.R. Zarter, V. Ebbert and B. Demmig-Adams 2004. Photoprotective strategies of overwintering evergreens. Bioscience. 54:41-49.

Ainsworth, E.A. and S.P. Long 2005. What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. New Phytologist. 165:351-372.

Alscher, R.G. and J.R. Cumming 1990. Stress responses in plants: adaptation and acclimation mechanisms. Ed. N.S. Allen. John Wiley & Sons Ltd., New York.

Amthor, J.S. 2000. The McCree-de Wit-Penning de Vries-Thornley respiration paradigms: 30 years later. Annals of Botany. 86:1-20.

Armstrong, A.F., D.C. Logan and O.K. Atkin 2006a. On the developmental dependence of leaf respiration: responses to short- and long-term changes in growth temperature. American Journal of Botany. 93:1633-1639.

Armstrong, A.F., D.C. Logan, A.K. Tobin, P. O'Toole and O.K. Atkin 2006b. Heterogeneity of plant mitochondrial responses underpinning respiratory acclimation to the cold in *Arabidopsis thaliana* leaves. Plant Cell & Environment. 29:940-949.

Arnone, J.A. and C. Korner 1997. Temperature adaptation and acclimation potential of leaf dark respiration in two species of *Ranunculus* from warm and cold habitats. Arctic and Alpine Research. 29:122-125.

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

Atkin, O.K., D. Bruhn and M.G. Tjoelker 2005b. Response of Plant Respiration to changes in temperature: Mechanisms and consequences of variations in Q_{10} values and acclimation. *In* Plant Respiration Eds. H. Lambers and M. Ribas-Carbo. Springer, Netherlands, pp. 95-135.

Atkin, O.K. and W.R. Cummins 1994. The effect of nitrogen source on growth, nitrogen economy and respiration of 2 high arctic plant species differing in relative growth rate. Functional Ecology. 8:389-399.

Atkin, O.K., E.J. Edwards and B.R. Loveys 2000. Response of root respiration to changes in temperature and its relevance to global warming. New Phytologist. 147:141-154.

Atkin, O.K., J.R. Evans, M.C. Ball, H. Lambers and T.L. Pons 2000a. Leaf respiration of snow gum in the light and dark. Interactions between temperature and irradiance. Plant Physiology. 122:915-923.

Atkin, O.K., C. Holly and M.C. Ball 2000b. Acclimation of snowgum (*Eucalyptus pauciflora)* leaf respiration to seasonal and diurnal variations in temperature, the importance of changes in the capacity and temperature sensitivity of respiration. Plant Cell & Environment. 23:15-26.

Atkin, O.K., B.R. Loveys, L.J. Atkinson and T.L. Pons 2006a. Phenotypic plasticity and growth temperature: understanding interspecific variability. Journal of Experimental Botany. 57:267-281.

Atkin, O.K., I. Scheurwater and T.L. Pons 2006b. High thermal acclimation potential of both photosynthesis and respiration in two lowland *Plantago* species in contrast to an alpine congeneric. Global Change Biology. 12:500-515.

Atkin, O.K., I. Scheurwater and T.L. Pons 2007. Respiration as a percentage of daily photosynthesis in whole plants is homeostatic at moderate, but not high, growth temperatures. New Phytologist. 174:1-14.

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

Atkin, O.K., Q.S. Zhang and J.T. Wiskich 2002. Effect of temperature on rates of alternative and cytochrome pathway respiration and their relationship with the redox poise of the quinone pool. Plant Physiology. 113:961-965.

Atkinson, L.J., M.A. Hellicar, A.H. Fitter and O.K. Atkin 2007. Impact of temperature on the relationship between respiration and nitrogen concentration in roots: an analysis of scaling relationships, Q_{10} values and thermal acclimation ratios. New Phytologist. 173:110-120.

Azcon-Bieto, J. 1983. Inhibition of photosynthesis by carbohydrates in wheat leaves. Plant Physiology. 73:681-686.

Azcon-Bieto, J., H. Lambers and D.A. Day 1983. Effect of photosynthesis and carbohydrate status on respiratory rates and the involvement of the alternative pathway in leaf respiration. Plant Physiology. 72:598-603.

Azcon-Bieto, J. and C.B. Osmond 1983a. Relationship between photosynthesis and respiration. Plant Physiology. 71:574-581.

Azcon-Bieto, J. and C.B. Osmond 1983b. Relationship between photosynthesis and respiration: The effect of carbohydrate status on the rate of CO_2 production by respiration in darkened and illuminated wheat leaves. Plant Physiology. 71:574-581.

Bassow, S.L. and F.A. Bazzaz 1998. How environmental conditions affect canopy leaf-level photosynthesis in four deciduous tree species. Ecology. 79:2660-2675.

Battaglia, M., C. Beadle and S. Loughhead 1996. Photosynthetic temperature responses of *Eucalyptus globulus* and *Eucalyptus nitens.* Tree Physiology. 16:81-89.

Bazzaz, F.A. 1990. The response of natural ecosystems to the rising global CO_2 levels. Annual Review of Ecology and Systematics. 21:167-196.

Bergh, J., R.E. McMurtrie and S. Linder 1998. Climate factors controlling the productivity of Norway spruce: a model-based analysis. Forest Ecology and Management. 110:127-139.

Berry, J. and O. Bjorkman 1980. Photosynthetic response and adaptation to temperature in higher plants. Annual Review of Plant Physiology 31:491-543.

Bolstad, P.V., K. Mitchell and J.M. Vose 1999. Foliar temperature-respiration response functions for broad-leaved tree species in the southern Appalachians. Tree Physiology. 19:871-878.

Bolstad, P.V., P. Reich and T. Lee 2003. Rapid temperature acclimation of leaf respiration rates in *Quercus alba* and *Quercus rubra*. Tree Physiology. 23:969-976.

Bolstad, P.V., J.M. Vose and S.G. McNulty 2001. Forest productivity, leaf area, and terrain in southern Appalachian deciduous forests. Forest Science. 47:419-427.

Bown, H.E., M.S. Watt, P.W. Clinton, E.G. Mason and B. Richardson 2007. Partitioning concurrent influences of nitrogen and phosphorus supply on photosynthetic model parameters of *Pinus radiata*. Tree Physiology 27:335-344.

Boyce, R.L., J.R. Larson and R.L. Sanford 2006. Phosphorus and nitrogen limitations to photosynthesis in Rocky Mountain bristlecone pine (*Pinus aristata*) in Colorado. Tree Physiology. 26:1477-1486.

Breymeyer, A.I., D.O. Hall, J.M. Melillo and G.I. Argen 1996. Global change: effects of coniferous forests and grasslands. John Wiley & Sons Ltd., New York.

Bruhn, D., J.G. Egerton, B.R. Loveys and M.C. Ball 2007. Evergreen leaf respiration acclimates to long-term nocturnal warming under field conditions. Global Change Biology. 13:1216-1223.

Bunce, J.A. 1992. Light, temperature and nutrients as factors in photosynthetic adjustment to an elevated concentration of carbon dioxide. Plant Physiology. 86:173-179.

Bunce, J.A. 2000. Acclimation of photosynthesis to temperature in eight cool and warm climate herbaceous C_3 species: temperature dependence of parameters of a biochemical photosynthesis model. Photosynthesis Research. 63:59-67.

Byrd, G.T., R.F. Sage and R.H. Brown 1992. A comparison of dark respiration between C_3 and C_4 plants. Plant Physiology. 100:191-198.

Campbell, C., L. Atkinson, J. Zaragoza-Castells, M. Lundmark, O. Atkin and V. Hurry 2007. Acclimation of photosynthesis and respiration is asynchronous in response to changes in temperature regardless of plant functional group. New Phytologist. 176:375-389.

Cannell, M.G.R. and J.H.M. Thornley 2000. Modelling the components of plant respiration: some guiding principles. Annals of Botany. 85:45-54.

Carswell, F.E., P. Meir, E.V. Wandelli, L.C.M. Bonates, B. Kruijt, E.M. Barbosa, A.D. Nobre, J. Grace and P.G. Jarvis 2000. Photosynthetic capacity in a central Amazonian rain forest. Tree Physiology. 20:179-186.

Cheng, L. and L.H. Fuchigami 2000. Rubisco activation state decreases with increasing nitrogen content in apple leaves. Journal of Experimental Botany. 51:1687-1694.

Clark, K.L., W.P. Cropper and H.L. Gholz 2001. Evaluation of modelled carbon fluxes for a slash pine ecosystem: SPM2 simulations compared to eddy flux measurements. Forest Science. 47:52-59.

Covey-Crump, E.M. 2002. Regulation of root respiration in two species of *Plantago* that differ in relative growth rate: the effect of short-and long-term changes in temperature. Plant Cell & Environment. 25:1501-1513.

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

Cramer, W., D.W. Kicklighter and A. Bondeau 1999. Comparing global models of terrestrial net primary productivity (NPP): overview and key results. Global Change Biology. 5:1-15.

Criddle, R.S., M.S. Hopkin, E.D. McArthur and L.D. Hansen 1994. Plant-distribution and the temperature-coefficient of metabolism. Plant Cell & Environment. 17:233-243.

Curtis, P.S. 1996. A meta-analysis of leaf gas exchange and nitrogen in trees under elevated carbon dioxide. Plant Cell & Environment. 19:127-137.

Damesin, C., S. Rambal and R. Joffre 1998. Co-occurrence of trees with different leaf habit: a function approach on Mediterranean oaks. Journal of Ecology. 19:195-204.

Dang, Q.L., H.A. Margolis and G.J. Collatz 1998. Parameterization and testing of a coupled photosynthesis stomatal conductance model for boreal trees. Tree Physiology. 18:141-153.

Day, D.A., K. Krab, H. Lambers, A.L. Moore, J.N. Siedow, A.M. Wagner and J.T. Wiskich 1996. The cyanide-resistant oxidase. To inhibit or not to inhibit, that is the question. Plant Physiology. 110:1-2.

Delieu, T. and D.A. Walker 1981. Polarographic measurement of photosynthetic oxygen evolution by leaf discs. New Phytologist. 89:165-178.

DeLucia, E.H., J.E. Drake, R.B. Thomas and M. Gonzalez-Meler 2007. Forest carbon use efficiency: is respiration a constant fraction of gross primary production? Global Change Biology. 13:1157-1167.

Dewar, R.C., B.E. Medlyn and R.E. McMurtrie 1999. Acclimation of the respiration photosynthesis ratio to temperature: insights from a model. Global Change Biology. 5:615-622.

Diaz-Espejo, A., A.S. Walcroft, J.E. Fernandez, B. Hafidi, M.J. Palomo and I.F. Giron 2006. Modelling photosynthesis in olive leaves under drought conditions. Tree Physiology. 26:1445-1456.

Dreyer, E., X. Le Roux, P. Montpied, F.A. Daudet and F. Mason 2001. Temperature response of leaf photosynthetic capacity in seedlings from seven temperate tree species. Tree Physiology. 21:223-232.

Dungan, R.J. 2003. Seasonal and temperature dependence of photosynthesis and respiration for two co-occurring broad-leaved tree species with contrasting leaf phenology. Tree Physiology. 23:561-568.

Dwyer, S.A., O. Ghannoum, A. Nicotra and S. Von Caemmerer 2007. High temperature acclimation of C4 photosynthesis is linked to changes in photosynthetic biochemistry. Plant Cell & Environment. 30:53-66.

Eagles, C.F. 1967. Variation in the soluble carbohydrate content of climatic races of *Dactylis glomerata* at different temperatures. Annals of Botany. 31:645-651.

Eamus, D., B. Myers, G. Duff and D. Williams 1999. Seasonal changes in photosynthesis of eight savanna tree species. Tree Physiology. 19:665-671.

Escudero, A. and S. Mediavilla 2003. Decline in photosynthetic nitrogen use efficiency with leaf age and nitrogen re-absorption as determinants of leaf life span. Journal of Ecology. 91:880-889.

Evans, J.R. 1989. Photosynthesis and nitrogen relationships in leaves of C_3 plants. Oecologia. 78:9-19.

Farquhar, G.D. and S. von Caemmerer 1982. Modelling of photosynthetic responses to environmental conditions. In Physiological Plant Ecology. II. Encyclopedia of Plants Physiology, New Series, Vol. 12B Berlin, Germany: Springer-Verlag, 549-587

Farquhar, G.D., S. von Caemmerer and J.A. Berry 1980. A Biochemical Model of Photosynthetic CO_2 Assimilation in Leaves of C_3 Species. Planta. 149:78-90.

Farrar, J.F. and M.L. Williams 1991. The effects of increased atmospheric carbon dioxide and temperature on carbon partitioning, source-sink relations and respiration. Plant Cell & Environment. 14:819-830.

Ferrar, P.J., R.O. Slatyer and J.A. Vranjic 1989. Photosynthetic temperature acclimation in *Eucalyptus* species from diverse habitats, and a comparison with *Nerium oleander*. Australian Journal of Plant Physiology. 16:199-217.

Field, C. 1983. Allocating leaf nitrogen for the maximization of carbon gain: leaf age as a control on the allocation program. Oecologia. 56:341-347.

Field, C. and H.A. Mooney 1983. Leaf age and seasonal effects on light, water, and nitrogen use efficiency in a Californian Shrub. Oecologia. 56:348-355.

Field, C.B., P.A. Matson and H.A. Mooney 1992. Responses of terrestrial ecosystems to a changing atmosphere. A resource-based approach. Annual Review of Ecology and Systematics. 23:201-235.

Fiorani, F., A.L. Umbach and J.N. Siedow 2005. The alternative oxidase of plant mitochondria is involved in the acclimation of shoot growth at low temperature. A study of *Arabidopsis* AOX1 a transgenic plant. Plant Physiology. 139:1795-1805.

Foster, D.R. and J.D. Aber 1997. Forest response to disturbance and anthropogenic stress. BioScience. 47:437-445.

Frak, E., X.L. Roux, P. Millard, E. Dreyer, G. Jaouen, B. Saint-Joanis and R. Wendler 2001. Changes in total leaf nitrogen and partitioning of leaf nitrogen drive photosynthetic acclimation to light in fully developed walnut leaves. Plant Cell & Environment. 24:1279-1288.

Fukai, S. and J.H. Silsbury 1977. Responses of sunterranean clover communities to temperature. Effects of temperature on dark respiration rate. Australian Journal of Plant Physiology. 4:159-167.

Gifford, R.M. 1994. The global carbon cycle: a viewpoint on the missing sink. Australian Journal of Plant Physiology. 21:1-5.

Gifford, R.M. 1995. Whole plant respiration and photosynthesis of wheat under increased CO_2 concentration and temperature: long-term vs. short-term distinctions for modelling. Global Change Biology. 1:385-396.

Gifford, R.M. 2003. Plant respiration in productivity models: conceptualisation, representation and issues for global terrestrial carbon-cycle research. Functional Plant Biology. 30:171-186.

Gonzalez-Meler, M., M. Ribas-Carbo, L. Giles and J.N. Siedow 1999. The effect of growth and measurement temperature on the activity of the alternative respiratory pathway. Plant Physiology. 120:765-772.

Grace, J. and R. Zhang 2006. Predicting the effect of climate change on global plant productivity and the carbon cycle. *In* Plant growth and climate change. Blackwell Publishing, USA. Pg: 187-203.

Grassi, G., E. Vicinelli, F. Ponti, L. Cantoni and F. Magnani 2005. Seasonal and inter-annual variability of photosynthetic capacity in relation to leaf nitrogen in a deciduous forest plantation in northern Italy. Tree Physiology. 25:349-360.

Griffin, K.L., D.T. Tissue, M.H. Turnbull, W. Schuster and D. Whitehead 2001. Leaf dark respiration as a function of canopy position in *Nothofagus fusca* trees grown at ambient and elevated CO_2 partial pressures for 5 years. Functional Ecology. 15:497-505.

Griffin, K.L., M. Turnbull and R. Murthy 2002a. Canopy position affects the temperature response of leaf respiration in *Populus deltoides*. New Phytologist. 154:609-619.

Griffin, K.L., M. Turnbull, R. Murthy, G. Lin, J. Adams, B. Farnsworth, T. Mahato, G. Bazin, M. Potasnak and J.A. Berry 2002b. Leaf respiration is differentially affected by leaf vs. stand-level night-time warming. Global Change Biology. 8:479-485.

Griffin, K.L., M.H. Turnbull, D.T. Tissue and D. Whitehead 2000. The onset of photosynthetic acclimation to elevated CO_2 partial pressure in field-grown *Pinus radiata* D. Don. after 4 years. Plant Cell & Environment. 23:1089-1098.

Gunderson, C.A., R.J. Norby and S.D. Wullschleger 2000. Acclimation of photosynthesis and respiration to stimulated climatic warming in northern and southern populations of *Acer saccharum*: laboratory and field evidence. Tree Physiology. 20:87-96.

Gunderson, C.A., J.D. Sholtis, S.D. Wullschleger, D.T. Tissue, P.J. Hanson and R.J. Norby 2002. Environmental and stomatal control of photosynthetic enhancement in the canopy of a sweetgum (*Liquidambar styraciflua* L.) plantation during three years of CO_2 enrichment. Plant Cell & Environment. 25:379-393.

Gunn, S. and J.F. Farrar 1999. Effects of 4 ^{o}C increase in temperature on partitioning of leaf area and dry mass, root respiration and carbohydrates. Functional Ecology. 13:12-20.

Guo, J., W.A. Jermyn and M.H. Turnbull 2002. Carbon partitioning and sucrose metabolism in two field-grown asparagus (*Asparagus officinalis*) cultivars with contrasting yield. Functional Plant Biology. 29:517-526.

Guy, C.L., J.L.A. Huber and S.C. Huber 1992. Sucrose phosphate synthase and sucrose accumulation at low temperature. Plant Physiology. 100:502-508.

Hachiya, T., I. Terashima and K. Noguchi 2007. Increase in respiratory cost at high growth temperature is attributed to high protein turnover cost in Petunia x hybrida petals. Plant Cell & Environment. 30:1269-1283.

Hansen, J., R. Ruedy, J. Glascoe and M. Sato 1999. GISS analysis of surface temperature change. Journal of Geophysical Research. 104:30997-31022.

Hikosaka, K. 1996. Effects of leaf age, nitrogen nutrition and photon flux density on the organisation of the photosynthetic apparatus in leaves of a vine (*Ipomoea tricolor* Cav.) grown horizontally to avoid mutual shading of leaves. Planta. 198:144-150.

Hikosaka, K. 1997. Modelling optimal temperature acclimation of the photosynthetic apparatus in C_3 plants with respect to nitrogen use. Annals of Botany. 80:721-730.

Hikosaka, K., K. Ishikawa, A. Borjigidai, O. Muller and Y. Onoda 2006. Temperature acclimation of photosynthesis: mechanisms involved in the changes in temperature dependence of photosynthetic rate. Journal of Experimental Botany. 57:291-302.

Hikosaka, K., A. Murakami and T. Hirose 1999. Balancing carboxylation and regeneration of ribulose-1, 5-bisphosphate in leaf photosynthesis: temperature acclimation of an evergreen tree, *Quercus myrsinaefolia*. Plant Cell & Environment. 22:841-849.

Hikosaka, K., E. Nabeshina and T. Hiura 2007. Seasonal changes in the temperature response of photosynthesis in canopy leaves of *Quercus crispula* in a cool-temperate forest. Tree Physiology. 27:1035-1041.

Hikosaka, K. and I. Terashima 1995. A model of the acclimation of photosynthesis in the leaves of C_3 plants to sun and shade with respect to nitrogen use. Plant Cell & Environment. 18:605-618.

Hoefnagel, M.H.N., A.H. Millar, J.T. Wiskich and D.A. Day 1995. Cytochrome and alternative respiratory pathways compete for electrons in the presence of pyruvate in soybean mitochondria Archives of Biochemistry and Biophysics. 318:394-400.

Hollinger, D.Y. 1992. Leaf and simulated whole-canopy photosynthesis in two co-occurring tree species. Ecology. 73:1-14.

Hopkins, W.G. 1998. Introduction to Plant Physiology. John Wiley & Sons, INC., New York. pg: 424-442.

Houghton, J.T., Y. Ding and D.J. Griggs 2001. Climate Change 2001: The Scientific basis. Cambridge University Press, Cambridge. pg: 881.

Hurry, V.M., M. Tobiaeson, S. Kromer, P. Gardestrom and G. Oquist 1995. Mitochondria contribute to increased photosynthetic capacity of leaves of winter rye (*Secale cereale* L.) following cold-hardening. Plant Cell & Environment. 18:69-76.

IPCC 2001. Climate Change 2001: The Scientific Basis. Contribution of working group I to the third assessment report of the Intergovernmental Panel of Climate Change (eds. J. T. Houghton et al.). Cambridge University Press, Cambridge, UK and New York, USA.

IPCC 2007. Climate Change 2007: *The Physical Science Basis*. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Eds. S. Solomon, D. Qin, M. Manning, M. Marquis, K. Averyt, M.B. Tignor, H.L. Miller and Z. Chen. Cambridge University Press, United Kingdom and New York.

Ishikawa, K., Y. Onoda and K. Hikosaka 2007. Intraspecific variation in temperature dependence of gas exchange characteristics among *Plantago asiatica* ecotypes from different temperature regimes. New Phytologist. 176:356-364.

Jach, M.E. and R. Ceulemans 2000. Effects of season, needle age and elevated atmospheric CO_2 on photosynthesis in Scots pine (*Pinus sylvestris*). Tree Physiology. 20:145-157.

Jarvis, A.J., V.J. Stauch, K. Schulz and P.C. Young 2004. The seasonal temperature dependency of photosynthesis and respiration in two deciduous forests. Global Change Biology. 10:939-950.

Jurik, T.W. 1986. Seasonal patterns of leaf photosynthetic capacity in successional northern hardwood tree species. American Journal of Botany. 73:131-138.

Kattge, J. and W. Knorr 2007. Temperature acclimation in a biochemical model of photosynthesis: a reanalysis of data from 36 species. Plant Cell & Environment. 30:1176-1190.

Kiener, C.M. and W.J. Bramiage 1981. Temperature effects on the activity of the alternative respiratory pathway in chill-sensitive *Cucumis sativus*. Plant Physiology. 68:1474-1478.

Kimball, J.S., A.R. Keyser and S.W. Running 2000. Regional assessment of boreal forest productivity using an ecological process model and remote sensing parameter maps. Tree Physiology. 20:761-775.

King, A.W., C.A. Gunderson, W.M. Post, D.J. Weston and S.D. Wullschleger 2006. Plant respiration in a warmer world. Science. 312:536-537.

Kirschbaum, M.U.F. and G.D. Farquhar 1984. Temperature dependence of whole-leaf photosynthesis in *Eucalyptus pauciflora* Sieb. ex Spreng. Australian Journal of Plant Physiology. 11:519-538.

Korner, C. 1995. Towards a better experimental basis for upscaling plant responses to elevated CO_2 and climate warming. Plant Cell & Environment. 18:1101-1110.

Korner, C.H. 1989. The nutritional status of plants from high altitudes. A worldwide comparison. Oecologia. 81:379-391.

Kosugi, Y., S. Shibata and S. Kobashi 2003. Parameterisation of the CO_2 and H_2O gas exchange of several temperate deciduous broad-leaved trees at the leaf scale considering seasonal changes. Plant Cell & Environment. 16:285-301.

Krapp, A. and M. Stitt 1995. An evaluation of direct and indirect mechanisms for the 'sink regulation' of photosynthesis in spinach: Changes in gas exchange, carbohydrates, metabolites, enzyme activities and steady-state transcript levels after cold-girdling source leaves. Planta. 195:313-323.

Krause, G.H., E. Grube, O.Y. Koroleva, C. Barth and K. Winter 2004. Do mature shade leaves of tropical tree seedlings acclimate to high sunlight and UV radiation? Functional Plant Biology. 31:743-756.

Kull, O., A. Koppel and A. Noormets 1998. Seasonal changes in leaf nitrogen pool in two *Salix* species. Tree physiology. 18:45-51.

Kurimoto, K., D.A. Day, H. Lambers and K. Noguchi 2004a. Effect of respiratory homeostasis on plant growth in cultivars of wheat and rice. Plant Cell & Environment. 27:853-862.

Kurimoto, K., A.H. Millar, H. Lambers, D.A. Day and K. Noguchi 2004b. Maintenance of growth rate at low temperature in rice and wheat cultivars with a high degree of respiratory homeostasis is associated with a high efficiency of respiratory ATP production. Plant & Cell Physiology. 45:1015-1022.

Lambers, H. 1982. Cyanide-resistant respiration: a non-phosphorylating electron transport pathway acting as an overflow. Plant Physiology. 55:478-485.

Lambers, H. 1985. Respiration in intact plants and tissues: its regulation and dependence on environmental factors, metabolism and invaded organisms. *In* Encyclopedia of Plant Physiology Eds. R. Douce and D.A. Day. Springer-Verlag, Berlin.

Lambers, H. and M. Ribas-Carbo 2005. Plant Respiration: from cell to ecosystem. *In* Respiration in photosynthetic cells: gas exchange components, interactions with photorespiration and the operation of mitochondria in the light. Eds. V. Hurry, A.U. Igamberdiev, O. Keerberg, T. Parnik, O.K. Atkin, J. Zaragoza-Castells and P. Gardestrom. Springer, Netherlands. pg: 43-61.

Larcher, W. 1969. The effect of environmental and physiological variables on the carbon dioxide gas exchange of trees. Photosynthetica. 3:167-198.

Larigauderie, A. and C. Korner 1995. Acclimation of Leaf Dark Respiration to Temperature in Alpine and Lowland Plant Species. Annals of Botany. 76:245-252.

Law, B.E., R.H. Waring and P.M. Anthoni 2000. Measurements of gross and net ecosystem productivity and water vapour exchange of a *Pinus Ponderosa* ecosystem, and an evaluation of two generalised models. Global Change Biology. 6:155-165.

Lawlor, D.W. 1987. Photosynthesis: Metabolism, control and physiology. Longman Scientific and technical, New York, USA.

Lawrence, W.T. and W.C. Oechel 1983. Effects of soil temperature on the carbon exchange of taiga seedlings. Root respiration. Canadian Journal of Forest Research. 13:840-849.

Lee, T.D., P.B. Reich and P.V. Bolstad 2005. Acclimation of leaf respiration to temperature is rapid and related to specific leaf area, soluble sugars and leaf nitrogen across temperate deciduous tree species. Functional Ecology. 19:640-647.

Lennon, A.M., U.H. Neueschwander, M. Ribas-Carbo, L. Giles, J.A. Ryals and J.N. Siedow 1997. The effects of salicylic acid and TMV infection upon the alternative oxidase of tobacco. Plant Physiology. 115:783-791.

Leuning, R. 1995. A critical appraisal of a combined stomatal-photosynthesis model for C_3 plants. Plant Cell & Environment. 18:339-357.

Leuning, R. 2002. Temperature dependence of two parameters in a photosynthesis model. Plant Cell & Environment. 25:1205-1210.

Lewis, J.D., M. Lucash, D.M. Olszyk and D.T. Tingey 2004. Relationships between needle nitrogen concentration and photosynthetic responses of Douglas-fir seedlings to elevated CO_2 and temperature. New Phytologist. 162:355-364.

Long, S.P. and F.I. Woodward 1988. Plants and temperature. *In* Effects of low temperature on respiratory metabolism by plants Eds. T. Rees, M.M. Burrell, T.G. Entwistle, J.B. Hammond, D. Kirk and N.J. Kruger. The Company of Biologists Ltd., Cambridge, pp. 377-393.

Loveys, B.R., L.J. Atkinson, D.J. Sherlock, R.L. Roberts, A.H. Fitter and O.K. Atkin 2003. Thermal acclimation of leaf and root respiration: an investigation comparing inherently fast- and slow-growing plant species. Global Change Biology. 9:895-910.

Loveys, B.R., I. Scheurwater, T.L. Pons, A.H. Fitter and O.K. Atkin 2002. Growth temperature influences the underlying components of relative growth rate: an investigation using inherently fast- and slow-growing plant species. Plant Cell & Environment. 25:975-988.

Lundmark, T., J.E. Hallgren and J. Heden 1988. Recovery from winter depression of photosynthesis in spruce and pine. Trees: 110-114.

Luo, Y., S. Wan and D. Hui 2001. Acclimatization of soil respiration to warming in a tall grass prairie. Nature. 413:624-625.

Luomala, E.M., K. Laitinen, S. Kellomaki and E. Vapaavuori 2003. Variable photosynthetic acclimation in consecutive cohorts of Scots Pine needles during 3 years of growth at elevated CO_2 and elevated temperature. Plant Cell & Environment. 26:645-660.

Lyons, J.M. 1973. Chilling injury in plants. Annual Review of Plant Physiology. 24:445-466.

Lyons, J.M. and J.K. Raison 1970. Oxidative activity of mitochondria isolated from plant tissues sensitive and resistant to chilling injury. Plant Physiology. 45:386-389.

Machler, F., A. Oberson, A. Grub and J. Nosberger 1988. Regulation of photosynthesis in nitrogen-deficient wheat seedlings. Plant Physiology. 87

Makela, A., P. Hari, F. Berninger, H. Hanninen and E. Nikinmaa 2004. Acclimation of photosynthetic capacity in Scots pine to the annual cycle of temperature. Tree Physiology. 24:369-376.

Makela, A., J. Landsberg and A.R. Ek 2000. Process-based models for forest ecosystem management: current state of the art and challenges for practical implementation. Tree Physiology. 20:289-298.

Makino, A., H. Nakano and T. Mae 1994. Effects of Growth Temperature on the Responses of Ribulose-1, 5-Bisphosphate Carboxylase, Electron Transport Components, and Sucrose Synthesis Enzymes to Leaf Nitrogen in Rice, and their Relationships to Photosynthesis. Plant Physiology. 105:1231-1238.

Martindale, W. and R.C. Leegood 1997. Acclimation of photosynthesis to low temperatures in *Spinacia oleraceae* L. II. Effects of nitrogen supply. Journal of Experimental Botany. 48:1873-1880.

Maxwell, D.P., Y. Wang and L. Mcintosh 1999. The alternative oxidase lowers mitochondrial reactive oxygen production in plant cells. PNAS. 96:8271-8276.

McCutchan, C. and R. Monson 2001. Effects of tissue-type and development on dark respiration in two herbaceous perennials. Annals of Botany. 87:355-364.

McDonnell, E. and J.F. Farrar 1993. Respiratory characteristics of isolated barley mitochondria and intact barley roots. Journal of Experimental Botany. 44:1485-1490.

McNulty, A.K. and W.R. Cummins 1987. The relationship between respiration and temperature in leaves of the arctic plant *Saxifraga cernua.* Plant Cell & Environment. 10:319-325.

Medlyn, B.E., E. Dreyer, D. Ellsworth, M. Forstreuter, P.C. Harley, M.U.F. Kirschbaum, X. Le Roux, P. Montpied, J. Strassemeyer, A. Walcroft, K. Wang and D. Loustau 2002a. Temperature response of parameters of a biochemically based model of photosynthesis. II. A review of experimental data. Plant Cell & Environment. 25:1167-1179.

Medlyn, B.E., D. Loustau and S. Delzon 2002b. Temperature response of parameters of a biochemically based model of photosynthesis. I. Seasonal changes in mature maritime pine (*Pinus pinaster* Ait.). Plant Cell & Environment. 25:1155-1165.

Millar, A.H., O.K. Atkin, R.I. Menz, B. Henry, G. Farquhar and D.A. Day 1998. Analysis of respiratory chain regulation in roots of soybean seedlings. Plant Physiology. 117:1083-1093.

Misson, L., K.P. Tu, R.A. Boniello and A.H. Goldstein 2006. Seasonality of photosynthetic parameters in a multi-specific and vertically complex forest ecosystem in Sierra Nevada of California. Tree Physiology. 26:729-741.

Miyazawa, Y., A.A. Suzuki, K. Sone and I. Terashima 2004. Relationships between light, leaf nitrogen and nitrogen remobilisation in the crowns of mature evergreen *Quercus glauca* trees. Tree Physiology. 24:1157-1164.

Monson, R.K., J.P. Sparks, T.N. Rosenstiel, L.E. Scott-Denton, T.E. Huxman, P.C. Harley, A.A. Turnipseed, S.P. Burns, B. Backlund and J. Hu 2005. Climatic influences on

ecosystem CO_2 exchange during the transition from wintertime carbon source to springtime carbon sink in a high-elevation, subalpine forest. Oecologia. 146:130-147.

Monson, R.K., A.A. Turnipseed, J.P. Sparks, P.C. Harley, L.E. Scott-Denton, K. Sparks and T.E. Huxman 2002. Carbon sequestration in a high-elevation, subalpine forest. Global Change Biology. 8:459-478.

Mooney, H.A. and M. West 1964. Photosynthetic acclimation to temperature in dessert shrub, *Larrea divaricata*. Carbon dioxide exchange characteristics of intact leaves. Plant Physiology. 61:406-410.

Morison, J.I.L. and M.D. Morecroft 2006. Plant growth and climate change. *In* Significance of temperature in plant life. Ed. C. Korner. Blackwell Publishing, USA. Pg: 48-66.

Nagel, O.W., S. Waldron and H.G. Jones 2001. An off-line implementation of the stable isotope technique for measurements of alternative respiratory pathway activities. Plant Physiology. 127:1279-1286.

Niinemets, U., A. Cescatti, M. Rodeghiero and T. Tosens 2005. Leaf internal diffusion conductance limits photosynthesis more strongly in older leaves of Mediterranean evergreen broad-leaved species. Plant Cell & Environment. 28:745-752.

Nogochi, K., C.S. Go, I. Terashima, S. Ueda and T. Yoshinari 2001. Activities of the cyanide-resistant respiratory pathway in leaves of sun and shade species. Australian Journal of Plant Physiology. 28:27-35.

Noguchi, K., C.S. Go, I. Terashima, S. Ueda and T. Yoshinari 2001a. Activities of the cyanide-resistant respiratory pathway in leaves of sun and shade species. Australian Journal of Plant Physiology. 28:27-35.

Noguchi, K., N. Nakajima and I. Terashima 2001b. Acclimation of leaf respiratory properties in *Alocasia odora* following reciprocal transfer of plants between high- and low-light environments. Plant Cell & Environment. 24:831-839.

Noguchi, K. and I. Terashima 1997. Different regulation of leaf respiration between *Spinacia oleracea*, a sun species, and *Alocasia odora*, a shade species. Physiologia Plantarum. 101:1-7.

Noguchi, K. and I. Terashima 2006. Responses of spinach leaf mitochondria to low N availability. Plant Cell & Environment. 29:710-719.

Onoda, Y., K. Hikosaka and T. Hirose 2005. The balance between RuBP carboxylation and RuBP regeneration: a mechanism underlying the interspecific variation in acclimation of photosynthesis to seasonal change in temperature. Functional Plant Biology. 32:903-910.

Ow, L.F., K.L. Griffin, D. Whitehead, A.S. Walcroft and M.H. Turnbull 2008a. Thermal acclimation of leaf respiration but not photosynthesis in *Populus deltoides* x *nigra*. New Phytologist. 178:123-134.

Ow LF, Whitehead D, Walcroft AS, Turnbull MH 2008b. Thermal acclimation of photosynthesis and respiration in *Pinus radiata*. Functional Plant Biology. 35(6): 448-461.

Pavlik, B.M. 1983. Nutrient and productivity relations of the dune grasses *Ammophila arenaria* and *Elymus mollis*. I. Photosynthesis and nitrogen use efficiency in the laboratory and field. Oecologia. 57:227-232.

Pons, T.L. and R.A.M. Welschen 2002. Overestimation of respiration rates in commercially available clamp-on leaf chambers. Complications with measurement of net photosynthesis. Plant Cell & Environment. 25:1367-1372.

Poorter, H., C. Vijver, R.G. Boot and H. Lambers 1995. Growth and carbon economy of a fast-growing and slow-growing grass species as dependent on nitrate supply. Plant Soil. 171:217-227.

Potter, C., J. Bubier and P. Crill 2001. Ecosystem modelling of methane and carbon dioxide fluxes for boreal forest sites. Canadian Journal of Forest Research. 31:208-223.

Purvis, A.C. and R.L. Shewfelt 1993. Does the alternative pathway ameliorate chilling injury in sensitive plant tissues? Physiologia Plantarum. 88:712-718.

Rachmilevitch, S., B. Huang and H. Lambers 2006. Assimilation and allocation of carbon and nitrogen of thermal and nonthermal *Agrotis* species in response to high soil temperature. New Phytologist. 170:479-490.

Raghavendra, A.S., K. Padmasree and K. Saradadevi 1994. Interdependence of photosynthesis and respiration in plant cells - interactions between chloroplasts and mitochondria. Plant Science. 97:1-14.

Reich, P.B., D.S. Ellsworth and M.B. Walters 1998a. Leaf structure (specific leaf area) modulates photosynthesis-nitrogen relations: evidence from within and across species and functional groups. Functional Ecology. 12:948-958.

Reich, P.B., J. Oleksyn and M.G. Tjoelker 1996. Needle respiration and nitrogen concentration in Scots Pine populations from a broad latitudinal range: a common garden test with field-grown trees. Functional Ecology. 10:768-776.

Reich, P.B., M.G. Tjoelker, J.L. Machado and J. Oleksyn 2006. Universal scaling of respiratory metabolism, size and nitrogen in plants. Nature. 439:457-461.

Reich, P.B., M.B. Walters and D.S. Ellsworth 1991. Leaf age and season influence the relationships between leaf nitrogen, leaf mass per area and in maple and oak trees. Plant Cell & Environment. 14:251-259.

Reich, P.B., M.B. Walters, D.S. Ellsworth, J.M. Vose, J.C. Volin, C. Gresham and W.D. Bowman 1998b. Relationships of leaf dark respiration to leaf nitrogen, specific leaf area and leaf life-span: a test across biomes and functional groups. Oecologia. 114:471-482.

Rey, A. and P.G. Jarvis 1998. Long-term photosynthetic acclimation to increased atmospheric CO_2 concentration in young birch (*Betula pendula*) trees. Tree Physiology. 18:441-450.

Ribas-Carbo, M., J.A. Berry, D. Yakir, L. Giles, S.A. Robinson, A.M. Lennon and J.N. Siedow 1995. The regulation of the electron partitioning between that cytochrome and alternative oxidase in plant mitochondria. Plant Physiology. 109:829-837.

Ribas-Carbo, M., A. Ricardo, A. Miquel, M. Gonzalez, J.J. Irigoyen and M. Sanchez-Diaz 2000. The electron partitioning between the cytochrome and alternative respiratory pathways during chilling recovery in two cultivars of maize differing in chilling sensitivity. Plant Physiology. 122:199-204.

Robakowski, P., P. Montpied and E. Dreyer 2002. Temperature response of photosynthesis of silver fir (*Abies alba* Mill.) seedlings. Annals of Forest Science 59:163-170.

Rogers, A., B.U. Fischer, J. Bryant, M. Frehner, H. Blum, C.A. Raines and S.P. Long 1998. Acclimation of photosynthesis to elevated CO_2 under low- nitrogen nutrition is affected by the capacity for assimilate utilisation. Perennial ryegrass under free-air CO_2 enrichment. Plant Physiology. 118:683-689.

Rook, D.A. 1969. The influence of growing temperature on photosynthesis and respiration of *Pinus radiata* seedlings. New Zealand Journal of Botany. 7:43-55.

Rustad, L. 2001. Matter of time on the prairie. Nature. 413:578-579.

Ryan, M.G. 1991. Effects of climate change on plant respiration. Ecological Applications. 1:157-167.

Ryan, M.G. 1995. Foliar maintenance respiration of sub-alpine and boreal trees and shrubs in relation to nitrogen content. Plant Cell & Environment. 18:765-772.

Ryan, M.G., R.M. Hibberd, S. Pongracic, R.J. Raison and R.M. McMurtrie 1996. Foliage, fine root, woody tissues and stand respiration in *Pinus radiata* in relation to nitrogen status. Tree Physiology. 16:333-343.

Ryan, M.G., S. Linder, J.M. Vose and R.M. Hubbard 1994. Dark respiration of pines. Ecological Bulletins. 43:50-63.

Sage, R.F. and D.S. Kubien 2007. The temperature response of C_3 and C_4 photosynthesis. Plant Cell & Environment. 30:1086-1106.

Salmon, J.T. 1999. The trees in New Zealand: Exotic trees (The broadleaves). Reed Books, Auckland, New Zealand. pg: 29-43.

Salmon, J.T. 2000. The trees in New Zealand: Exotic trees (The conifers). Reed Books, Auckland, New Zealand. pg: 114-123.

Salvucci, M.E., K.W. Osteryoung, S.J. Crafts-Brandner and E. Vierling 2001. Exceptional sensitivity of rubisco activase to thermal denaturation *in vitro* and *in vivo.* Plant Physiology. 127:1053-1064.

Schimel, D.S., J.I. House, K.A. Hibbard, P. Bousquet, P. Ciais, P. Peylin, B.H. Braswell, M.J. Apps, D. Baker, A. Bondeau, J. Canadell, G. Churkina, W. Cramer, A.S. Denning, C.B. Field, P. Friedlingstein, C. Goodale, M. Heimann, R.A. Houghton, J.M. Melillo, B. Moore III, D. Murdiyarso, I. Noble, S.W. Pacala, I.C. Prentice, M.R. Raupach, P.J. Rayner, R.J. Scholes, W.L. Steffen and C. Wirth 2001. Recent patterns and mechanisms of carbon exchange by terrestrial ecosystems. Nature. 414:169-172.

Schoettle, A.W. and W.K. Smith 1999. Interrelationships among light, photosynthesis and nitrogen in the crown of mature *Pinus contorta* ssp. *latifolia*. Tree Physiology. 19:13-22.

Schulze, E.D. and M.M. Caldwell 1994. Ecophysiology of photosynthesis. Springer-Verlag, Berlin. pg 560-576.

Sharkey, T.D., M.R. Badger, S. von Caemmerer and T.J. Andrews 2001. Increased sensitivity of photosynthesis in tobacco plants with reduced Rubisco activase. Photosynthesis Research. 67:147-156.

Sharkey, T.D., C.J. Bernacchi, G.D. Farquhar and E.L. Singsaas 2007. Fitting photosynthetic carbon dioxide response curves for C_3 leaves. Plant Cell & Environment. 30:1035-1040.

Sholtis, J.D., C.A. Gunderson, R.J. Norby and D.T. Tissue 2004. Persistent stimulation of photosynthesis by elevated CO_2 in a sweetgum (*Liquidambar styraciflua*) forest stand. New Phytologist. 162:343-354.

Siedow, J.N. and A.L. Umbach 1995. Plant mitochondrial electron transfer and molecular biology. Plant Cell 7:821-831.

Sitch, S., B. Smith and I.C. Prentice 2003. Evaluation of ecosystem dynamics, plants geography and terrestrial carbon cycling in the LPJ dynamic global vegetation model. Global Change Biology. 9:161-185.

Slator, A. 1906. Studies in fermentation. I. The chemical dynamics of alcoholic fermentation by yeast. Journal of the Chemical Society. 89:136-142.

Slatyer, R.O. 1977. Altitudinal variation in photosynthetic characteristics of snow gum, *Eucalyptus pauciflora* Sieb. ex spreng. Temperature response of material grown in contrasting thermal environments. Australian Journal of Plant Physiology. 4:301-312.

Smith, E.M. and E.B. Hadley 1974. Photosynthetic and respiratory acclimation to temperature in *Ledum groenlandicum* populations. Arctic and Alpine Research. 6:13-27.

Sorensen, F.C. and W.K. Ferrell 1972. Photosynthesis and growth of Douglas-fir seedlings when grown in different environments. Canadian Journal of Botany. 51:1689-1698.

Steingrover, E. 1981. The relationship between cyanide-resistant root respiration and the storage of sugars in the taproot of *Daucus carota* L. Journal of Experimental Botany. 32:911-919.

Stewart, C.R., B.A. Martin, L. Reding and S. Cerwick 1990. Respiration and alternative oxidase in corn seedling tissues during germination at different temperatures. Plant Physiology. 92:755-760.

Stitt, M. and Hurry V. 2002. A plant for all seasons: alterations in photosynthetic carbon metabolism during cold acclimation in Arabidopsis. Current Opinions in Plant Biology. 5:199-206.

Strain, B.R., K.O. Higginbotham and J.C. Mulroy 1976. Temperature preconditioning and photosynthetic capacity of *Pinus taeda* L. Photosynthetica. 10:47-53.

Strand, A., V. Hurry, P. Gustafsson and P. Gardestrom 1997. Development of *Arabidopsis thaliana* leaves at low temperatures releases the suppression of photosynthesis and photosynthetic gene expression despite the accumulation of soluble carbohydrates. The Plant Journal. 12:605-614.

Strand, A., V. Hurry, S. Henkes, N. Huner, P. Gustafsson, P. Gardestrom and M. Stitt 1999. Acclimation of *Arabidopsis* leaves developing at low temperatures. Increasing cytoplasmic volume accompanies increased activities of enzymes in the Calvin cycle and in the sucrose-biosynthesis pathway. Plant Physiology. 119:1387-1397.

Sullivan, N.H., P.V. Bolstad and J.M. Vose 1996. Estimates of net photosynthetic parameters for twelve tree species in mature forests of the Southern Appalachians. Tree Physiology. 16:397-406.

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

Talts, P., T. Parnik, P. Gardestrom and O. Keerburg 2004. Respiratory acclimation in *Arabidopsis thaliana* leaves at low temperature. Plant Physiology. 161:573-579.

Teskey, R.O. and R.E. Will 1999. Acclimation of loblolly pine (*Pinus taeda)* seedlings to high temperatures. Tree Physiology. 19:519-525.

Thornley, J.H.M. and M.G.R. Cannell 2000. Modelling the components of plant respiration: Representation and Realism. Annals of Botany. 85:55-67.

Thornley, J.H.M. and I.R. Johnson 2000. Plant and Crop Modelling: A mathematical approach to plant and crop physiology. The Blackburn Press, Caldwell, New Jersey, USA. pg: 3-44.

Tissue, D.T. 2002. Leaf respiration at different canopy positions in sweetgum (*Liquidambar styraciflua*) grown in ambient and elevated concentrations of carbon dioxide in the field. Tree Physiology. 22:1157-1166.

Tissue, D.T., M.M. Barbour, J.E. Hunt, M.H. Turnbull, K.L. Griffin, A.S. Walcroft and D. Whitehead 2006. Spatial and temporal scaling of intercellular CO_2 concentration in a temperate rain forest dominated by *Dacrydium cupressinum* in New Zealand. Plant Cell & Environment. 29:497-510.

Tissue, D.T., K.L. Griffin, M.H. Turnbull and D. Whitehead 2001. Canopy position and needle age affect photosynthetic response in field-grown *Pinus radiata* after five years of exposure to elevated carbon dioxide partial pressure. Tree Physiology. 21:915-923.

Tissue, D.T. and S.J. Wright 1995. Effect of seasonal water availability on phenology and the annual shoot carbohydrate cycle of tropical forest shrubs. Functional Ecology. 9:518-527.

Tjoelker, M.G., J. Oleksyn and P.B. Reich 1999a. Acclimation of respiration to temperature and CO_2 in seedlings of boreal tree species in relation to plant size and relative growth rate. Global Change Biology. 5:679-692.

Tjoelker, M.G., J. Oleksyn and P.B. Reich 2001. Modelling respiration of vegetation: evidence for a general temperature-dependent Q_{10}. Global Change Biology. 7:223-230.

Tjoelker, M.G., P.B. Reich and J. Oleksyn 1999b. Changes in leaf nitrogen and carbohydrates underlie temperature and CO_2 acclimation of dark respiration in five boreal tree species. Plant Cell & Environment. 22:767-778.

Tjoelker MG, Oleksyn J, Reich PB, Zytkowiak R 2008. Coupling of respiration, nitrogen, and sugars underlies convergent temperature acclimation in *Pinus banksiana* across wide-ranging sites and populations. Global Change Biology. 14: 782-797.

Turnbull, M., D.T. Tissue, K.L. Griffin, S.J. Richardson, D.A. Peltzer and D. Whitehead 2005. Respiration characteristics in temperate rainforest tree species differ along a long-term soil-development chronosequence. Oecologia. 143:271-279.

Turnbull, M.H., D. Doley and J.Y. Yates 1993. The dynamics of photosynthetic acclimation to changes in light quantity and quality in three Australian rainforest tree species. Oecologia. 94:218-228.

Turnbull, M.H., R. Murthy and K.L. Griffin 2002a. The relative impacts of daytime and night-time warming on photosynthetic capacity in *Populus deltoides*. Plant Cell & Environment. 25:1729-1737.

Turnbull, M.H., D.T. Tissue, K.L. Griffin, G.N.D. Rogers and D. Whitehead 1998. Photosynthetic acclimation to long-term exposure to elevated CO_2 concentration in *Pinus radiata* D. Don. is related to age of needles. Plant Cell & Environment. 21:1019-1028.

Turnbull, M.H., D.T. Tissue, R. Murthy, X. Wang, A.D. Sparrow and K.L. Griffin 2004. Nocturnal warming increases photosynthesis at elevated CO_2 partial pressure in *Populus deltoides*. New Phytologist. 161:819-826.

Turnbull, M.H., D. Whitehead, D.T. Tissue, W.S.F. Schuster, K.J. Brown, V.C. Engel and K.L. Griffin 2002b. Photosynthetic characteristics in canopies of *Quercus prinus* and *Acer rubrum* differ in response to soil water availability. Oecologia. 130:515-524.

Turnbull, M.H., D. Whitehead, D.T. Tissues, W.S.F. Schuster, K.J. Brown and K.L. Griffin 2003. Scaling foliar respiration in two contrasting forest canopies. Functional Ecology. 17:101-114.

Usami, T., J. Lee and T. Oikawa 2001. Interactive effects of increased temperature and CO_2 on the growth of *Quercus myrsinaefolia* saplings. Plant Cell & Environment. 24:1007-1019.

Vanlerberghe, G.C. and L. McIntosh 1992. Lower growth temperature increases alternative pathway capacity and alternative oxidase protein in tobacco. Plant Physiology. 100:115-119.

Volder, A., E.J. Edwards, J.R. Evans, B.C. Robertson, M. Schortemeyer and R.M. Gifford 2004. Does greater night-time, rather than constant, warming alter growth of managed pasture under ambient and elevated atmospheric CO_2? New Phytologist. 10:1-15.

Vu, J.C.V., L.H. Allen Jr, K.J. Boote and G. Bowes 1997. Effects of elevated CO_2 and temperature on photosynthesis and rubisco in rice and soybean. Plant Cell & Environment. 20:68-76.

Wagner, A.M. and K. Krab 1995. The alternative respiration pathway in plants: role and regulation. Plant Physiology 95:318-325.

Walcroft, A.S., D. Whitehead, W.B. Silvester and F.M. Kelliher 1997. The response of photosynthetic model parameters to temperature and nitrogen concentration in *Pinus radiata* D. Don. Plant Cell & Environment. 20:1338-1348.

Walther, G.R., C.A. Burga and P.J. Edwards 2001. "Fingerprints" of climate change: Adapted behaviour and shifting species ranges. Kluwer Academic, Boston, USA.

Wang, K.Y., T. Zha and S. Kellomaki 2002. Measuring and stimulating crown respiration of Scots Pine with increased temperature and carbon dioxide enrichment. Annals of Botany. 90:325-335.

Warren, C.R. 2008. Does growth temperature affect the temperature responses of photosynthesis and internal conductance to CO_2 ? A test with *Eucalyptus regnans*. Tree Physiology. 28:11-19.

Warren, C.R. and M.A. Adams 2004. Evergreen trees do not maximise instantaneous photosynthesis. Trends in Plant Science. 9:270-274.

Weston, D.J. and W.L. Bauerle 2007. Inhibition and acclimation of C_3 photosynthesis to moderate heat: a perspective from thermally contrasting genotypes of *Acer rubrum* (red maple). Tree Physiology. 27:1083-1092.

Whitehead, D., K.L. Griffin, M.H. Turnbull, D.T. Tissue, V.C. Engel, K.J. Brown, W.S.F. Schuster and A.S. Walcroft 2004. Response of total night-time respiration to differences in total daily photosynthesis for leaves in a *Quercus rubra* L. canopy: implications for modelling canopy CO_2 exchange. Global Change Biology. 10:925-938.

Whitehead, D. and A.S. Walcroft 2005. Forest and shrubland canopy carbon uptake in relation to foliage nitrogen concentration and leaf area index: a modelling analysis. Annals of Forest Science. 62:525-535.

Wilson, K.B., D.D. Baldocchi and P.J. Hanson 2000. Spatial and seasonal variability of photosynthetic parameters and their relationship to leaf nitrogen in a deciduous forest. Tree Physiology. 20:565-578.

Wilson, K.B., D.D. Baldocchi and P.J. Hanson 2001. Leaf age affects the seasonal pattern of photosynthetic capacity and net ecosystem exchange of carbon in a deciduous forest. Plant Cell & Environment. 24:571-583.

Wright, I.J., P.B. Reich, O.K. Atkin, C.H. Lusk, M.G. Tjoelker and M. Westoby 2006. Irradiance, temperature and rainfall influence leaf dark respiration in woody plants: evidence from comparisons across 20 sites. New Phytologist. 169:309-319.

Wullschleger, S.D. 1993. Biochemical limitations to carbon assimilation in C_3 plants-a retrospective analysis of the A/C_i curves from 109 species. Journal of Experimental Botany. 44:907-920.

Wythers, K.R., P.B. Reich, M.G. Tjoelker and P.V. Bolstad 2005. Foliar respiration acclimation to temperature and temperature variable Q_{10} alter ecosystem carbon balance. Global Change Biology. 11:435-449.

Xiong, F.S., E.C. Mueller and T.A. Day 2000. Photosynthetic and respiratory acclimation and growth response of Antarctic vascular plants to contrasting temperature regimes. American Journal of Botany. 87:700-710.

Xu, C.Y. and K.L. Griffin 2006. Seasonal variation in the temperature response of leaf respiration in *Quercus rubra*: foliage respiration and leaf properties. Functional Ecology. 20:778-789.

Xu, C.Y., W.S.F. Schuster and K.L. Griffin 2006. Seasonal variation of temperature response of respiration in invasive Japanese barberry (*Berberis thunbergii*) and two co-occurring native understorey shrubs in a northeastern US deciduous forest. Oecologia. 153: 809-819.

Xu, L.K. and D.D. Baldocchi 2003. Seasonal trends in photosynthetic parameters and stomatal conductance of blue oak (*Quercus douglasii*) under prolonged summer drought and high temperature. Tree Physiology. 23:865-877.

Xu, M., T.A. DeBiase, A. Goldstein, Z. Liu and Y. Qi 2001. Ecosystem respiration in young ponderosa pine plantation in the Sierra Nevada Mountains, California. Tree Physiology. 21:309-318.

Yamori, W., K. Noguchi and I. Terashima 2005. Temperature acclimation of photosynthesis in spinach leaves: analyses of photosynthetic components and temperature dependencies of photosynthetic partial reactions. Plant Cell & Environment. 28:536-547.

Yamori, W., K. Suzuki, K. Noguchi, M. Nakai and I. Terashima 2006. Effects of Rubisco kinetics and Rubisco activation state on the temperature dependence of the photosynthetic rate in spinach leaves from contrasting growth temperatures. Plant Cell & Environment. 29:1659-1670.

Yasumura, Y., K. Hikosaka and T. Hirose 2006. Seasonal changes in photosynthesis, nitrogen content and nitrogen partitioning in *Lindera umbellata* leaves grown under high and low irradiance. Tree Physiology. 26:1315-1323.

Yelle, S., R.C. Beeson, M.J. Trudel and A. Gosselin 1989. Acclimation of two tomato species to high temperatures. Plant Physiology. 90:1465-1472.

Zaragoza-Castells, J., D. Sanchez-Gomez, F. Valladares, V. Hurry and O.K. Atkin 2007. Does growth irradiance affect temperature dependence and thermal acclimation of leaf respiration? Insights from a Mediterranean tree with long-lived leaves. Plant Cell & Environment. 30:820-833.

Zarter, C.R., W.W. Adams, V. Ebbert, D.J. Cuthbertson, I. Adamska and B. Demmig-Adams 2006a. Winter down-regulation of intrinsic photosynthetic capacity coupled with up-regulation of Elip-like proteins and persistent energy dissipation in a subalpine forest. New Phytologist. 172:272-282.

Zarter, C.R., B. Demmig-Adams, V. Ebbert, I. Adamska and W.W. Adams 2006b. Photosynthetic capacity and light harvesting efficiency during winter-to-spring transition in subalpine conifers. New Phytologist. 172:283-292.

Ziska, L.H. and J.A. Bunce 1997. The role of temperature in determining the stimulation of CO_2 assimilation at elevated carbon dioxide concentration in soybean seedlings. Physiologia Plantarum. 100: 126-132.

Ziska, L.H., J.A. Bunce and E.W. Goins 2004. Characterization of an urban-rural CO_2/temperature gradient and associated changes in initial plant productivity during secondary succession. Oecologia. 139: 454-458.

APPENDIX

Thermal acclimation of leaf respiration but not photosynthesis in *Populus deltoides* × *nigra*

Lai Fern Ow[1], Kevin L. Griffin[2], David Whitehead[3], Adrian S. Walcroft[4] and Matthew H. Turnbull[1]

[1]School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch, New Zealand; [2]Department of Earth and Environmental Sciences, Lamont-Doherty Earth Observatory, Columbia University, Palisades, NY 10964-8000, USA; [3]Landcare Research, PO Box 40, Lincoln 7640, New Zealand; [4]Landcare Research, Private Bag 11052, Manawatu Mail Centre, Palmerston North 4442, New Zealand

Author for correspondence:
Lai Fern Ow
Tel: +64 3 364 2987
Fax: +64 3 364 2590
Email: genow@starhub.net.sg

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Summary

• Dark respiration and photosynthesis were measured in leaves of poplar *Populus deltoides* × *nigra* ('Veronese') saplings to investigate the extent of respiratory and photosynthetic acclimation in pre-existing and newly emerged leaves to abrupt changes in air temperature.

• The saplings were grown at three temperature regimes and at high and low nitrogen availabilities. Rates of photosynthesis and dark respiration (R_d) were measured at the initial temperature and the saplings were then transferred to a different temperature regime, where the plants remained for a second and third round of measurements on pre-existing and newly emerged leaves.

• Acclimation of photosynthesis was limited following transfer to warmer or cooler growing conditions. There was strong evidence of cold and warm acclimation of R_d to growth temperature, but this was limited in pre-existing leaves. Full acclimation of R_d was restricted to newly emerged leaves grown at the new growth temperature.

• These findings indicate that the extent of thermal acclimation differs significantly between photosynthesis and respiration. Importantly, pre-existing leaves in poplar were capable of some respiratory acclimation, but full acclimation was observed only in newly emerged leaves. The R_d/A_{max} ratio declined at higher growth temperatures, and nitrogen status of leaves had little impact on the degree of acclimation.

Key words: alternative oxidase, carbon balance, cytochrome oxidase, nitrogen (N) availability, *Populus deltoides* × *nigra* ('Veronese'), temperature, thermal homeostasis.

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Introduction

Photosynthesis and respiration are the two major biological components regulating the exchange of carbon between the atmosphere and the terrestrial biosphere. A small difference between these two fluxes is the defining aspect of the carbon balance of an ecosystem (Schimel *et al.*, 2001). A change in temperature can result in an immediate change in the rates of photosynthesis and respiration, with the magnitudes being determined by the short-term sensitivity of each process to temperature. The temperature sensitivity of foliar photosynthesis differs from that of respiration (Morison & Morecroft, 2006), and hence the balance between the two processes may be altered following a short-term change in temperature (Dewar *et al.*, 1999; Loveys *et al.*, 2002; Atkin *et al.*, 2006b). This balance, in turn, has a major impact on net CO_2 emissions and carbon storage in terrestrial ecosystems.

In contrast to instantaneous responses, the effect of long-term changes in growth temperatures on rates of photosynthesis and respiration depends on the degree to which these processes acclimate. Acclimation is defined as adjustments in physiological processes to allow plant performance or fitness to remain the same at a new growth temperature (Berry

& Bjorkman, 1980; Hopkins, 1998). The degrees to which photosynthesis and respiration acclimate are clearly important determinants of long-term plant responses to environmental change, but they are poorly understood. Our limited understanding indicates that the degree of acclimation differs between species (i.e. higher in fast and slower in slow growing species); some species acclimate strongly while others are incapable of even partial acclimation (Berry & Bjorkman, 1980; Larigauderie & Korner, 1995; Loveys *et al.*, 2003).

Rates of respiration typically increase exponentially with short-term increases in temperature (minutes to hours). The precise shape of this exponential relationship is dependent on both the antecedent temperature conditions and the degree of acclimation, which can be quite rapid, occurring within hours to days (Atkin *et al.*, 2000b). In turn, the extent of acclimation depends on the environmental conditions to which the plant is genetically adapted (Larigauderie & Korner, 1995; Tjoelker *et al.*, 2001). Hence, predicting rates of respiration as a function of temperature requires knowledge of both previous temperature conditions and the rate (and extent) of acclimation.

While the instantaneous temperature response of respiration may be exponential over a range of temperatures, the long-term (days to weeks) response is rarely so. Instead, the long-term response usually results in an upward shift of the respiratory temperature response curve with plants grown at cooler temperatures and a downward shift of the response curve as plants are grown in warmer temperatures (Lee *et al.*, 2005). Atkin & Tjoelker (2003) proposed mechanisms for two acclimation scenarios, which they term type I and type II acclimation, to explain both the short- and long-term changes in respiration in response to changes in temperature. For type I acclimation, changes in growth temperature may result in relatively rapid changes in Q_{10} (proportional change in respiration for a 10°C change in temperature) with no change in respiration rates at low temperatures. This form of acclimation occurs predominantly among mature tissues (in this case, pre-existing leaves). By contrast, type II acclimation leads to changes in respiration at both low and high temperatures (i.e. an overall shift of the temperature response curve) and is considered to occur over longer periods (Zaragoza-Castells *et al.*, 2007) or in leaves that have developed at the new temperature. In this present study, we used temperature-treated (for weeks to months) pre-existing and newly developed leaves to test for the occurrence of type I and type II acclimation, respectively.

Photosynthesis is also strongly affected by temperature and, in the short term, rates of photosynthesis typically increase in response to temperature, reach a maximum value at an optimum temperature and then decline at higher temperatures (Sage & Kubien, 2007). At temperatures above the optimum value, rates of photosynthesis often decrease sharply (rates of respiration are typically found to increase beyond this point). Photosynthetic acclimation to a long-term change in temperature may result in a change in the shape of the response curve or a shift of the entire curve, thus changing the absolute rate and/or the temperature optimum (Berry & Bjorkman, 1980; Turnbull *et al.*, 2002a; Sage & Kubien, 2007). For example, rates of photosynthesis in plants exposed to low temperatures may be reduced initially but, with subsequent acclimation, photosynthetic rates may increase as a result of a higher degree of unsaturation of membrane lipids and a greater concentration of proteins regulating photosynthetic capacity (Berry & Bjorkman, 1980; Stitt & Hurry, 2002; Yamori *et al.*, 2005; Sage & Kubien, 2007). By contrast, long-term exposure of plants to temperatures above those normally experienced in their growing conditions may result in reduced rates of photosynthesis resulting from the inactivation of Rubisco (Salvucci *et al.*, 2001) and changes in photosynthetic membrane composition (Sharkey *et al.*, 2001).

Tissue N is an important determinant of the rate of key physiological processes in plants (Lewis *et al.*, 2004; Takashima *et al.*, 2004) and is also temperature-sensitive, varying with changes in growth temperature (Rachmilevitch *et al.*, 2006). There is strong evidence for a correlation between the rate of photosynthesis, respiration and foliar N concentration (photosynthesis: (Field & Mooney, 1983; Reich *et al.*, 1991; Turnbull *et al.*, 2002b; Morison & Morecroft, 2006); respiration: (Tjoelker *et al.*, 1999b; Griffin *et al.*, 2002; Loveys *et al.*, 2003). However to date, very few studies (Field *et al.*, 1992; Krapp & Stitt, 1995; Martindale & Leegood, 1997; Atkinson *et al.*, 2007) have focused on the role of leaf N in photosynthetic and respiratory acclimation, particularly in tree species. Many previous studies (see earlier references) have confirmed earlier findings of a strong relationship between N and rates of photosynthesis and respiration, and have not extended their investigation to the role of N in thermal acclimation.

Here we report the results of an experiment which demonstrates the effects of step temperature changes on the rate of both photosynthesis and respiration of *Populus deltoides* × *nigra* saplings. During the course of this 3-month experiment, day and night growth temperatures were adjusted by 5–10°C, well within the growing range experienced by this species in the field. The objectives of our study were to examine the extent of photosynthetic and respiratory acclimation in pre-existing leaves and leaves developed under new temperature conditions; and to establish the impact of leaf nitrogen status (manipulated using low and high nitrogen availabilities) on the potential for thermal acclimation. We hypothesized that both photosynthetic and respiratory acclimation to temperature change would be apparent in this fast-growing deciduous species, but that it would be limited in pre-existing leaves and leaves with low N status. A key objective was to determine the extent of error involved when short-term temperature responses of respiration are used to predict long-term responses.

Materials and Methods

Growth conditions and experimental design

This experiment was conducted using growth chambers at the University of Canterbury, Christchurch, New Zealand, from October to December 2005. The chambers (Contherm 630, climate simulator) were set at a constant photosynthetically active irradiance of 700 μmol m^{-2} s^{-1} and 70% relative humidity. The photoperiod was maintained at 10 : 14 h dark : light using 400 W metal halide lamps. The temperature treatments were night : day 10 : 15°C, 15 : 20°C and 20 : 25°C and the three treatments were allocated randomly across the chambers.

Populus deltoides × *nigra* ('Veronese') stem cuttings were obtained from Oxford (50 km northwest of Christchurch). Eight plants growing in 9 l pots were widely spaced in each chamber, allowing good air flow and light penetration during the experiment. The growing medium consisted of a mixture of bark and chip (coarse organic material) with a ratio of 8 : 2 supplemented with a base quantity of slow-release fertilizer (8.8 N : 5.5 P : 10.6 K plus trace elements) and the plants were watered to saturation and allowed to drain daily. Half the plants (high nitrogen, HN) were supplied with 7 g of compacted 1–2 mm granules of a nitrogen-rich (38% N) fertilizer (Enduro, Rosenlew RKW, Finland) that was well mixed with the potting mix. The remainder was designated as low nitrogen (LN).

Before the experiment, plants were established in a well lit glasshouse for 1.5 months at ambient daytime temperatures of 15–20°C, after which they were transferred to growth cabinets. The initial temperature treatment regimes described earlier were imposed for 1 wk, after which the first physiological measurements were made. This allowed us to construct 'acclimated' temperature response curves (at the growth temperatures) before the shift of plants to new temperature regimes. Following these initial measurements, plants from the 10 : 15°C (night : day) temperature treatment were transferred to the 15 : 20°C treatment, plants from the initial 15 : 20°C treatment were moved to the 20 : 25°C treatment, and plants from the 20 : 25°C treatment were transferred to the 10 : 15°C treatment. The three temperature transfers will hereafter be denoted by the respective day or night temperatures (15 to 20°C, 20 to 25°C and 25 to 15°C for photosynthesis or 10 to 15°C, 15 to 20°C and 20 to 10°C for respiration). Following a week in the new conditions, a second set of physiological measurements were taken on leaves that were present before the temperature changes. After another 5 wk in the new growing conditions, a final set of measurements was taken on all plants using new leaves that had emerged and developed in the new temperature conditions. Space constraints did not allow us to have a constant temperature control ('control' plants) but the three-way transfer regime allowed us to be confident that temperature change was the driving factor in plant responses. Leaves used for measurements were 3–4 wk old and leaf areas were within the range of 40–90 cm^2 (obtained using a Li-3100C area meter; Li-Cor Inc., Lincoln, NE, USA). Measurements were always conducted on leaves that were close to the apex and receiving full illumination. A large-leaved poplar variety was used, hence the leaf area may be higher than that normally reported in studies working on poplar.

Measurements of respiration, photosynthesis and leaf characteristics

Leaf-level gas exchange measurements were made with two cross-calibrated, portable open-path gas-exchange systems with CO_2 control (Li-6400, Li-Cor Inc.) with the standard 2 by 3 cm chamber equipped with blue-red light-emitting diodes mounted on the top of the cuvette. Environmental controls within the cuvette were maintained to match the growing conditions, unless otherwise specified. Curves of the response of photosynthesis, A, to intercellular CO_2 concentration, C_i, were generated by changing the external CO_2 concentration, C_a, in 14 steps from 150 to 0 Pa at a constant photosynthetically active irradiance, Q, of 1500 μmol m^{-2} s^{-1}. Measurements were taken at each C_a set point when photosynthesis reached a constant value (when the coefficient of variation for the CO_2 concentration differential between the sample and reference analysers was below 1% and visibly stable). This condition was typically achieved within 1–2 min after a stable set point had been reached. Curves of the response of photosynthesis to irradiance were generated by changing incident Q in 10 steps from 2000 μmol m^{-2} s^{-1} to darkness. During the light response curves, the external CO_2 partial pressure was held constant at 37 Pa. Measurements of photosynthesis in pre-existing and new leaves were made at the daytime growth temperatures of 15, 20 and 25°C. Temperatures were maintained using thermoelectric coolers (of the Li 6400), and air saturation deficit in the cuvette was maintained between 1.0 and 1.5 kPa. Measurements from the A/C_i curves were used to determine values for the maximum rate of carboxylation (V_{cmax}) and the apparent maximum rate of electron transport at saturating irradiance (J_{max}). The A/C_i response data were analysed using the biochemical model of photosynthesis as described by Farquhar *et al.* (1980) and fitted using the Marquardt-Levenberg algorithm (Sigma Plot, Software, version 8.0; SPSS Inc. Chicago, IL, USA). Measurements from the A/Q curves were fitted using a rectangular hyperbola (Thornley & Johnson, 2000) and used to determine values of maximum photosynthesis (A_{max}) at saturating irradiance and at ambient CO_2 concentration. Unlike the analysis of respiration, calculations of photosynthetic parameters from A/C_i and A/Q curves did not include a correction factor for the gasket effect (Pons & Welschen, 2002). Although we assumed that CO_2 exchange processes only occurred in the part of the leaf enclosed in the leaf chamber, it was unlikely to result in the underestimation of

photosynthetic rates, because the gasket effect was found to be absent when measurements were made at saturating light (present study at 1500 μmol m^{-2} s^{-1}) and the error was negligible in comparison to the high photosynthetic rate (photosynthetic parameters in this study were in the following ranges: A_{max}, 8.1–24.4; J_{max}, 37.1–167.4; V_{cmax}, 11.2–80.2) (Pons & Welschen, 2002).

Measurements of the rate of dark respiration, R_d, were taken at the end of the dark period on excised leaves collected before onset of illuminated chamber conditions. Measurements were made on fully expanded leaves (with intact petioles) in a separate growth chamber. Upon excision, the ends of the petiole were wrapped in a moist paper towel, sealed in a small plastic bag and placed in the dark – measurements were completed within 2–3 h. Previous measurements have shown that leaf respiration remains stable under these conditions for several hours (Turnbull *et al.*, 2005) and this was confirmed here. The leaf area used to calculate respiration rate included a correction factor to account for darkened leaf material under the cuvette gasket following Pons & Welschen (2002). As the poplar leaves almost always extended beyond the gasket, we assumed that half the leaf area under the gasket (approx. 3 cm^2) contributed respired CO_2 into the chamber, which was added to our total leaf area calculations to avoid an overestimation of dark respiration rate. To avoid disturbance, the portable gas-exchange system was kept in the dark growth chamber during the measurements and controlled externally from a laptop computer. The instantaneous response of dark respiration was determined by taking measurements of R_d at temperatures of 10, 15, 20 and 25°C in pre-existing and new leaves before and after transfer in all three temperature treatments. The growth chamber and leaf chamber temperature conditions were controlled similarly and the leaves were allowed to equilibrate to the new temperature conditions for 30–45 min before the onset of the respiration measurements. Values of R_d at each temperature were calculated from the mean of five measurements made over 3 min. Curves of the response of R_d to temperature, T_l (K), were analysed as described in Atkin *et al.* (2005b), where:

$$R_d = R_{10} Q_{10}^{[(T_l - T_0)/10]} \quad \text{Eqn 1}$$

where R_{10} is the respiration rate at the base temperature, T_0 (here 10°C or 283 K), and Q_{10} is a parameter describing the change in respiration with a 10°C increase in temperature. The curves were fitted using the Marquardt-Levenberg algorithm (Sigma Plot, version 8.0).

The relative enzymatic activities for dark respiration via the cytochrome (COX) and alternative (AOX) oxidase pathways were measured using oxygen electrodes with dual digital controllers (Model 20, Rank Brothers Ltd). Rates of oxygen uptake were measured for leaf samples placed in buffer solution (Delieu & Walker, 1981; McCutchan & Monson, 2001) for 5–10 min using data acquisition software (TracerDAQ, version 1.7; Measurement Computing Corporation, Norton, MA, USA). Leaf discs measuring 26 mm^2 were incubated in running buffer containing 30 mM salicylhydroxamic acid (SHAM, an inhibitor of AOX activity) for 1.5 h in darkness for the measurement of COX activity. In addition, AOX activity was measured following 1.5 h incubation of leaf discs in 3 mM potassium cyanide (KCN, an inhibitor of COX activity). Appropriate controls (without inhibitors) were also incubated in a similar way before measurements were taken. Residual respiratory activity was determined following incubation of separate discs in both inhibitors (average 11.6% (±0.004) of total respiration; this was subtracted from each value before calculating activity as a percentage of total uninhibited respiration). Leaf discs incubated in 50 μM carbonylcyanide m-chlorophenylhydrazone (CCCP) were used to assess the fully uncoupled rate of oxygen consumption. The activity of each pathway was calculated as a percentage of the fully uncoupled rate.

Total nitrogen concentration of leaves on an area and mass basis (N_a and N_m) were determined on leaves dried at 70°C, weighed, finely ground in a ball mill and analysed using a CNS analyser (Carlo Erba Na 1500, Milan, Italy). Specific leaf area, S (m^2 kg^{-1}), was calculated from measurements of leaf area and dry mass.

Statistical analysis

Two-way analyses of variance (ANOVA) were used to test for the effects of temperature and nitrogen supply on S, A, R_d and N (SAS Institute, software version 8.2, Cary, NC, USA). Differences were considered significant if $P \leq 0.05$. Treatment means were separated by least-significant-difference tests at $P \leq 0.05$. Differences between means in Fig. 4 were evaluated with a one-way ANOVA.

Results

Foliar characteristics

There were significant differences in nitrogen concentration, on both a mass and area basis, N_m and N_a, respectively, between high (HN) and low (LN) nitrogen plants (Fig. 1a,b). N_m was significantly greater in HN plants than in LN plants. New leaves in the HN and LN treatments showed a significant increase in N_a in response to cold transfer (25 to 15°C; Fig. 1b). There was no significant change in N_a in pre-existing and newly emerged leaves in either the HN or LN treatments in response to warm transfer (15 to 20°C and 20 to 25°C; Fig. 1b). A significant decrease in S was observed in response to cold transfer in newly emerged leaves in the HN treatment only (25 to 15°C; Fig. 1c). Following warm transfer (15 to 20°C and 20 to 25°C; Fig. 1c), a significant increase was found in newly emerged leaves only, but also in LN pre-existing leaves shifted from 15 to 20°C.

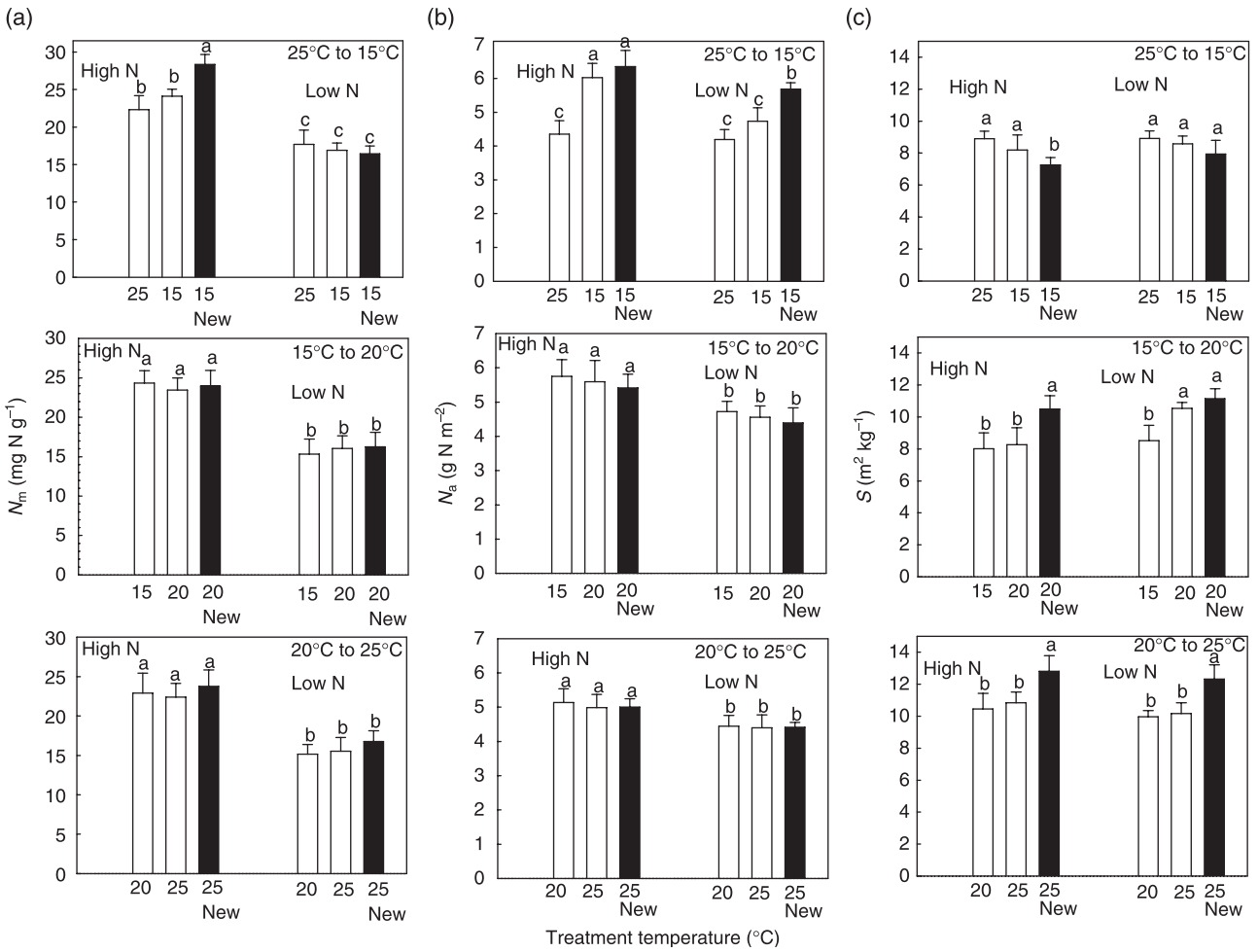

Fig. 1 Variation in foliar nitrogen concentration on mass (N_m) (a), area (N_a) basis (b) and specific leaf area, (S) (c), in *Populus deltoides* × *nigra* ('Veronese') plants under three temperature transfer regimes (25–15°C, 15–20°C and 20–25°C). Each panel displays the response of pre-existing leaves at the initial growth temperature and 1 wk after plants were transferred to the new temperature (open bars). The closed bar represents the response of new leaves that expanded in the new temperature. Data are presented for plants grown under high and low nitrogen availability. All values are means ± SE; $n = 8$. Different letters indicate means are significantly different at the $P < 0.05$ level.

Photosynthesis

The maximum response of photosynthesis (A_{max}) at saturating irradiance and ambient CO_2 concentration decreased significantly in pre-existing and newly emerged leaves in response to cold transfer (25 to 15°C; Fig. 2a). A 60 and 50% reduction in A_{max} was observed in newly emerged leaves from HN and LN plants, respectively. There was no significant change in A_{max} values of warm-transferred (15 to 20°C and 20 to 25°C; Fig. 2a) plants in the HN treatment, but a significant increase was exhibited in pre-existing and newly emerged leaves of LN plants. Quantum yield was insensitive to temperature transfer (data not shown). V_{cmax} decreased significantly in both HN and LN treatments in response to cold transfer (25 to 15°C; Fig. 2b), with pre-existing leaves displaying a similar response to newly emerged leaves. V_{cmax} increased initially in response to warm transfer (15 to 20°C and 20 to 25°C; Fig. 2b) in pre-existing leaves, but was followed by subsequent partial readjustment in newly emerged leaves. J_{max} in cold-transferred plants generally displayed a similar response to that of V_{cmax}; values did not change significantly in response to the 20 to 25°C transfer (Fig. 2c). The J_{max}/V_{cmax} ratio increased significantly following cold transfer (25 to 15°C; Fig. 2d) and generally decreased in response to warm transfer (15 to 20°C and 20 to 25°C; Fig. 2d). The long-term response of A_{max} and V_{cmax} to temperature (determined from values at the three growth temperatures before and after transfer) did not differ greatly between the initial (pre-existing leaves before transfer) and temperature responses after transfer (Fig. 3a,b). Overall the R_d/A_{max} ratio of pre-existing and new leaves declined with increasing temperature both before and after transfer, but this was more accentuated in new leaves (Fig. 3c).

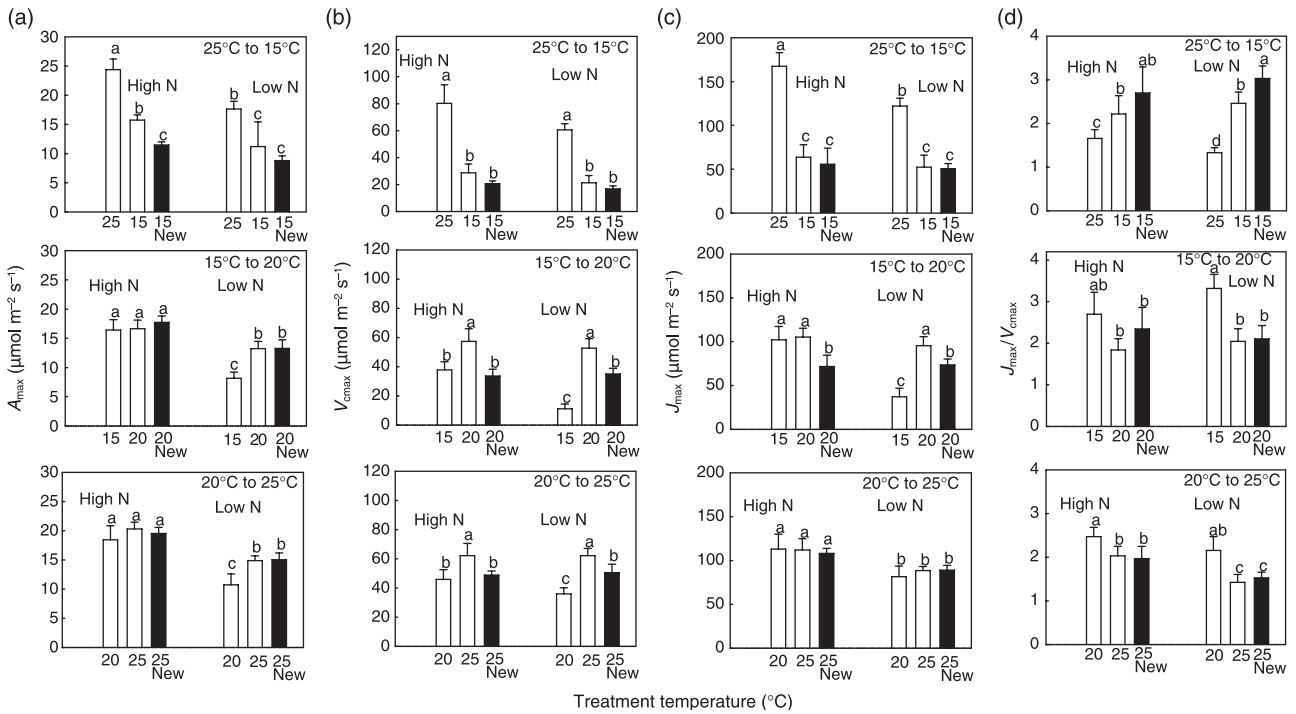

Fig. 2 Photosynthetic parameters calculated from *A*/*Q* (curves of the response of photosynthesis to irradiance) responses A_{max} (a) and from *A*/C_i (curves of the response of photosynthesis, *A*, to intercellular CO_2 concentration, C_i) responses V_{cmax} (b), J_{max} (c) and J_{max}/V_{cmax} (d) in *Populus deltoides* × *nigra* ('Veronese') plants under three temperature transfer regimes (25 to 15°C, 15 to 20°C and 20 to 25°C). Each panel displays the response of pre-existing leaves at the initial growth temperature and 1 wk after plants were transferred to the new temperature (open bars). The closed bar represents the response of new leaves that expanded in the new temperature. Data are presented for plants grown under high and low nitrogen availability. All values are means ± SE; $n = 8$. Different letters indicate means are significantly different at the $P < 0.05$ level.

Respiration

The instantaneous temperature response of R_d displayed a characteristic exponential increase with increasing temperature (Fig. 4a–c). Newly emerged leaves after cold transfer (20 to 10°C; Fig. 5a) displayed a significant increase in R_{10} values in both HN and LN plants, while no significant change was seen in pre-existing leaves. Newly emerged leaves in warm-transferred plants (10 to 15°C and 15 to 20°C; Fig. 5a) exhibited a significant decrease in R_{10} in both HN and LN treatments. Temperature transfer had no significant effect on Q_{10} (within the range of 10–20°C) in either cold- and warm-transferred plants (Fig. 5b). The long-term (acclimated) respiratory response to temperature, as determined by actual values of R_d at the respective growth temperatures (solid lines, Fig. 4), was less pronounced than the instantaneous responses. The degree of acclimation in newly emerged leaves (Fig. 4c) was greater than that for pre-existing leaves (Fig. 4a,b), such that R_d in newly emerged leaves did not differ across the 10°C range ('thermal homeostasis'). Accordingly, we predicted rates of leaf respiration at the new temperatures from the response obtained at the initial temperature (before the plants were transferred) and compared these predicted values to actual values determined for pre-existing and newly emerged leaves at the new growth temperatures (Table 1). The initial response overestimated the acclimated R_d of new leaves following an increase in temperature from 15 to 20°C by as much as 69%, and underestimated the response to a decrease in temperature from 20 to 10°C by up to 44% (Table 1).

The enzymatic activity of the COX pathway generally did not change significantly in response to warm transfer (10 to 15°C or 15 to 20°C) but was reduced significantly in newly emerged leaves of cold-transferred plants (20 to 10°C; Table 2). AOX activity in cold-transferred plants (20 to 10°C) increased significantly in pre-existing leaves and remained high in newly emerged leaves (Table 2). In warm-transferred plants (10 to 15°C and 15 to 20°C), AOX activity increased in pre-existing and new leaves, but no change was found in HN 15 to 20°C plants (Table 2).

Discussion

Our results clearly show strong acclimation of respiration to growth temperature in *Populus deltoides*, regardless of the nitrogen status of the leaves. However, the degree of acclimation in pre-existing leaves was limited compared with newly emerged leaves, which displayed the capacity to acclimate fully (thermal homeostasis). The comparison between predicted and actual rates of respiration indicates a distinct

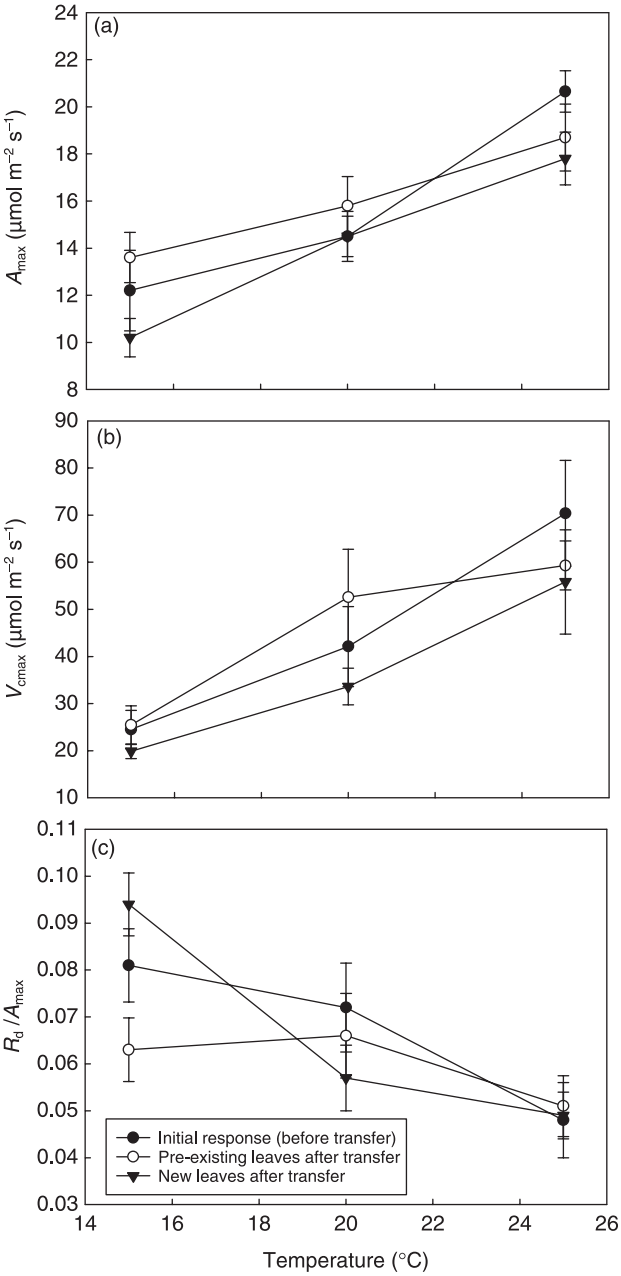

Fig. 3 Temperature response of A_{max} (a), V_{cmax} (b) and R_d/A_{max} (c) at three daytime growth temperatures (15, 20 and 25°C) for *Populus deltoides* × *nigra* ('Veronese') plants before and following transfer between three temperature regimes (means are averaged (n = 8) across nitrogen (N) treatments).

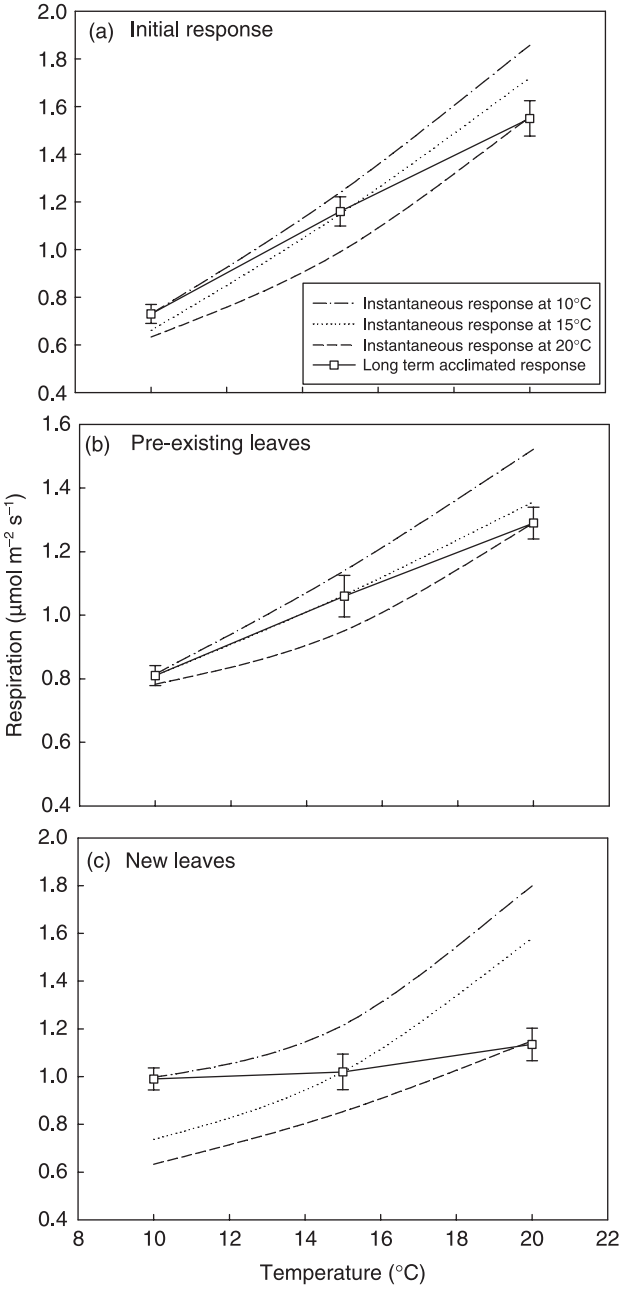

Fig. 4 Instantaneous (determined over a range of temperatures within a 3 h period) and 'acclimated' (actual values of R_d at the respective night-time growth temperatures) responses of respiration to temperature for: (a) pre-existing leaves at the initial night-time temperature (before transfer of plants to new temperature regimes); (b) pre-existing leaves at the new night-time temperature; and (c) newly expanded leaves at the new night-time temperature. Values at the night-time growth temperatures are means ± SE. One-way ANOVA tests indicated significant differences in R_d at each growth temperature (P = 0.001 for 10°C, P = 0.05 for 15°C and P = 0.001 for 20°C) (data presented are averaged (n = 8) across nitrogen (N) treatments).

limitation in the use of instantaneous temperature response curves to predict long-term respiratory responses in pre-existing and new leaves. In contrast to respiration, we found little evidence for acclimation of photosynthetic parameters to growth temperature (in the range 15–25°C). Taken together, these findings have important implications for the carbon balance of deciduous species in the face of temperature variation.

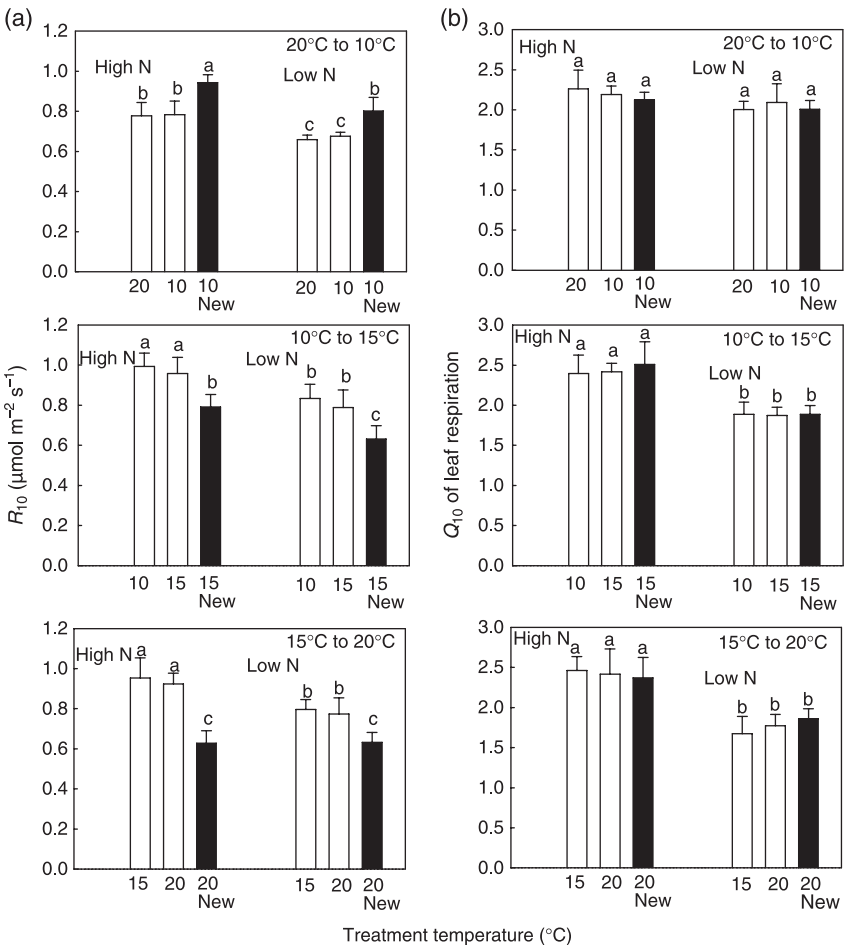

Fig. 5 Temperature response parameters of dark respiration (R_{10} – modelled respiration at a base temperature of 10°C (a) and Q_{10} (b) under three temperature transfer regimes (20 to 10°C, 10 to 15°C and 15 to 20°C). For detailed explanation on how values were derived, refer to the Materials and Methods section. Each panel displays the response of pre-existing leaves at the initial growth temperature and 1 wk after plants were transferred to the new temperature (open bars). The closed bar represents the response of new leaves that expanded in the new temperature. Data are presented for plants grown under high and low nitrogen availabilities. All values are means ± SE; $n = 8$. Different letters indicate means are significantly different at the $P < 0.05$ level.

Table 1 Rate of leaf respiration in *Populus deltoides* × *nigra* plants transferred between various growth temperatures

	Respiration (μmol m^{-2} s^{-1})		
Temperature transfer treatments	Predicted rates	Actual rates	% difference
Pre-existing leaves			
10 to 15°C	1.24	1.06	17.0
15 to 20°C	1.72	1.29	33.3
20 to 10°C	0.63	0.82	–23.2
Newly emerged leaves			
10 to 15°C	1.24	0.99	25.3
15 to 20°C	1.72	1.02	68.6
20 to 10°C	0.63	1.13	–44.2

Rates are quoted as values predicted based upon the temperature response curves of leaves before transfer (Fig. 4a) and actual rates determined in pre-existing leaves after transfer (Fig. 4b) and new leaves formed after transfer to the appropriate growth temperatures (Fig. 4c). Using the predicted and actual rates derived from Fig. 4, a value of % difference was obtained. Values with negative signs indicate an underestimation of respiration (data averaged across N treatments).

Photosynthetic response to changes in growth temperature

Previous work suggests high variability in the degree of photosynthetic and respiratory acclimation among species, with some species exhibiting full acclimation while others appear incapable of even partial acclimation (Atkin *et al.*, 2005a, 2006b). Our results (Fig. 2a) show that 10°C cold transfer resulted in a reduced photosynthetic capacity, despite increasing tissue nitrogen content (Fig. 1b), while 5°C increases

Table 2 Activity of the cytochrome (COX) and alternative oxidase (AOX) pathways as a percentage of total respiratory activity in leaf discs of *Populus deltoides* × *nigra* plants exposed to both increasing and decreasing temperatures

	Cytochrome pathway activity (% of total respiratory activity)			Alternative oxidase pathway activity (% of total respiratory activity)		
Treatment	Pre-existing leaves	Pre-existing leaves after transfer	Newly emerged leaves	Pre-existing leaves	Pre-existing leaves after transfer	Newly emerged leaves
20 to 10°C						
LN	101.5 (4.0) b	76.0 (10.5) ab	48.8 (8.5) a	16.3 (5.9) a	38.9 (9.0) b	56.1 (11.7) c
HN	100.5 (19.0) b	98.1 (5.8) b	67.5 (19.0) a	16.0 (5.5) a	52.8 (5.0) b	47.7 (6.3) b
10 to 15°C						
LN	100.5 (4.0) a	98.3 (5.0) a	91.5 (13.7) a	3.7 (5.5) a	59.1 (8.1) c	40.1 (16.4) b
HN	64.4 (6.7) a	110.2 (7.3) b	111.4 (17.5) b	9.8 (2.9) a	66.1 (14.6) c	30.1 (9.4) b
15 to 20°C						
LN	84.2 (1.8) a	87.6 (9.1) a	69.1 (5.5) a	19.5 (7.8) a	48.6 (15.5) b	49.1 (2.3) b
HN	101.1 (6.4) a	72.3 (11.6) a	71.9 (10.8) a	29.4 (8.0) a	34.6 (13.1) a	28.4 (4.9) a

Measurements were made in pre-existing leaves at the initial temperature and 7 d after transfer to the new growth temperature; and in new leaves at the new growth temperature. Plants were grown under low (LN) and high (HN) nitrogen supply.
Values shown are means (± standard error of the mean, SEM) where $n = 4$.
Different letters within rows indicate statistically different values at $P < 0.05$ using the least-significant-difference test of treatment means.

in temperature led to small increases in photosynthetic capacity in LN plants only. These results are consistent with previous work by Rook (1969), who found a 25% decrease in photosynthetic rates of *Pinus radiata* seedlings after transfer to cooler temperatures. But in a recent study, Yamori *et al.* (2006) found that cold-grown spinach leaves typically exhibit greater photosynthetic capacities than their warm-grown counterparts and suggested that acclimation of Rubisco kinetics to the lower growth temperature should result in increased photosynthetic rates. Clearly, the response of photosynthesis to cooler temperatures is not universal and may depend on species. In the present study, the long-term temperature response of photosynthesis over the range 15–25°C remained remarkably constant despite changes in growth temperature (Fig. 3a,b). This suggests that, in stark contrast to respiration (Fig. 4), photosynthetic processes in poplar are incapable of significant acclimation to temperature changes. Although the measurements carried out in this study do not allow us to identify an optimum temperature for this species, we cannot rule out possible changes in T_{opt}. Nonetheless, the absence of photosynthetic acclimation is an important finding and is consistent with the notion that photosynthesis can generally operate effectively between 0 to 30°C (Sage & Kubien, 2007).

Rates of photosynthesis are limited either by ribulose-1,5-bisphosphate (RuBP) carboxylation or by RuBP regeneration (Berry & Bjorkman, 1980). As RuBP carboxylation-limited photosynthesis does not have the same temperature dependence as RuBP regeneration-limited photosynthesis, temperature change may result in an imbalance between the two processes and a change in the J_{max}/V_{cmax} ratio (Farquhar & von Caemmerer, 1982; Hikosaka *et al.*, 1999; Onoda *et al.*, 2005). Here, the ratio of the capacities of RuBP regeneration to carboxylation (J_{max}/V_{cmax}) increased in cold-transferred plants, while it decreased in warm-transferred plants (Fig. 2d). This is consistent with previous work by Dreyer *et al.* (2001) and Onoda *et al.* (2005), although in the latter study this response was not universal among species. The increase in the J_{max}/V_{cmax} ratio at low growth temperatures is considered to relieve the limitation of RuBP regeneration on photosynthetic rate, while a decrease in the ratio with increasing temperature is a result of a greater activation energy for V_{cmax} than for J_{max} (Onoda *et al.*, 2005). The decrease in the J_{max}/V_{cmax} ratio in response to increased temperatures is highly conserved in a wide range of species, but the inter-specific differences in the response of J_{max}/V_{cmax} to growth temperature are not known.

Respiratory responses to changes in growth temperature

Our results show that respiration in poplar is strongly sensitive to short-term changes in temperature, but acclimation reduces the magnitude of the response over longer periods

(days, weeks or months). While this has been demonstrated previously, the extent to which it occurs in different tissues and species is still poorly understood (Larigauderie & Korner, 1995; Atkin *et al.*, 2000; Atkin & Tjoelker, 2003; Wright *et al.*, 2006). Cold-transferred poplar displayed a significant increase in R_d while warm-transferred plants (15 to 20°C and 20 to 25°C; Fig. 5a) exhibited a significant decrease. As a result, the acclimated response of R_d at the actual growth temperature in new leaves was much less pronounced than the instantaneous response with nearly identical rates of respiration at contrasting temperatures, indicating full respiratory homeostasis over a 10°C temperature range (Fig. 4c).

Our findings also show that acclimation potential for respiration in both warm- and cold-transferred plants is limited in pre-existing leaves and that full acclimation is only possible in new leaves that emerged after the change in temperature. This supports work by others (Hurry *et al.*, 1995; Loveys *et al.*, 2002; Atkin *et al.*, 2005a; Atkin *et al.*, 2006a) showing that limited acclimation of respiration can occur in pre-existing leaves but full acclimation is restricted to leaves emerging after the change in temperature. However, this finding is by no means universal and is likely to be especially variable in species with long-lived leaves (Bruhn *et al.*, 2007; Zaragoza-Castells *et al.*, 2007), which, if given more time (e.g. 60 d), may express a greater degree of acclimation in pre-existing leaves. In the present study, respiratory acclimation was achieved, in part, through changes in S (i.e. the amount of metabolizing tissue per unit area). This highlights the importance of leaf structural changes in acclimation to changing growth temperature, and provides a strong explanation for the limited response in pre-existing leaves. While limited changes in S are possible following a change in environment, this parameter is largely determined at the time of leaf development and expansion. Importantly for our understanding of the likely potential for respiratory acclimation under field conditions, we find that, while tissue N content clearly regulates rates of respiration, it has little or no impact on the extent of thermal acclimation (at least within the range of N concentrations experienced here).

These responses may be placed in the context of the acclimation mechanisms suggested by Atkin *et al.* (2005a). Pre-existing leaves displayed a response that was partly consistent with the type I scenario proposed by Atkin *et al.* (2005a), although Q_{10} was rather insensitive. Previous studies have shown that the Q_{10} often decreases with increasing growth temperature. Our results are, however, consistent with those of Tjoelker *et al.* (1999a), showing very little change in Q_{10} after cold or warm transfer (Fig. 5b). By contrast, new leaves (Fig. 4c) clearly displayed characteristics of type II acclimation, which is often associated with temperature-mediated changes in respiratory capacity achieved through the development of new tissues (Atkin & Tjoelker, 2003). Type II acclimation may also be associated with enzymatic changes (e.g. changes in the relative amounts of enzymes or proteins invested into the respiratory chain), resulting in the relative change of AOX and COX activity (Atkin *et al.*, 2005a). This was especially evident in cold-transferred plants, where COX activity declined while AOX activity increased (increase in AOX activity was consistent in both cold- and warm-transferred plants). This switching between the AOX and COX pathways has been shown in spinach, beans and maize grown at different intensities of irradiance and different chilling conditions (Ribas-Carbo *et al.*, 2000; Noguchi *et al.*, 2001). AOX is highly sensitive to changes in temperature, and engagement of the nonphosphorylating AOX pathway may be advantageous to plant acclimation to cold temperature where energy that would otherwise be conserved as ATP is converted to heat (Ribas-Carbo *et al.*, 2000). For example, Atkin *et al.* (2005b) showed that changes in growth temperature can alter the partitioning of electrons between different respiratory pathways, and the engagement of AOX may vary with age, growth temperature, irradiance or phosphate supply (Gonzalez-Melers *et al.*, 1999; Ribas-Carbo *et al.*, 2000). It is also worth noting that this study provides estimates of maximum rates of *in vivo* AOX and COX activity only, and the results should not be taken as true *in vivo* capacity of the two pathways or *in vivo* switching between these pathways. The more reliable methodology presently available to study electron partitioning between AOX and COX without the use of inhibitors is the use of the oxygen-isotope fractionation which measures the differences in the isotopic fractionation of ^{18}O between the two terminal oxidases (Ribas-Carbo *et al.*, 2005). Clearly our findings suggest directions for future work in this area.

Balance between photosynthesis and respiration

Inclusion of partial and full acclimation of respiration into models may significantly affect calculations of long-term plant and ecosystem carbon balance. The importance of acclimation to calculations of respiration is demonstrated in Table 1, where predicted rates of respiration in pre-existing and newly emerged leaves at new temperatures were very different from the actual rates observed, showing that the instantaneous response is a poor predictor of long-term acclimated responses of respiration to changes in temperature. In the absence of respiratory acclimation, models of the impacts of climate warming would predict greater increases in respiration than photosynthesis (Dewar *et al.*, 1999; Atkin *et al.*, 2000b; Gifford, 2003). Our results show that R_d/A_{max} at the leaf level decreased in response to increasing temperature (Fig. 3c). Although our results deviate from those of Loveys *et al.* (2003), who proposed that the R_d/A_{max} ratio is insensitive to environmental conditions, our findings are, however, consistent with those of Xu *et al.* (2006), showing significant seasonal variation in the ratio for three shrub species. Such responses in the R_d/A_{max} ratio suggest that increases in temperature over a moderate range may favour

the uptake of carbon in trees rather than its loss to the atmosphere.

In conclusion, the present study has highlighted that poplar, a deciduous species with a high amount of developmental plasticity, has considerable potential for respiratory acclimation to changes in growth temperature. By contrast, there was little evidence of temperature acclimation in photosynthetic characteristics. Nitrogen status of leaves had little, if any, impact on the degree of acclimation. This is important for our understanding of respiratory responses of trees under conditions of varying soil nutrition. The presence of respiratory acclimation in the face of increasing atmospheric temperatures would serve to reduce the potential for positive feedback of respiration in the carbon cycle. Hence, future work on different plant types (e.g. evergreen species; species with different potentials for generating new tissues) is needed to gain a full understanding of the extent of thermal acclimation in a range of plant species.

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References

Atkin OK, Bruhn D, Hurry VM, Tjoelker MG. 2005a. Evans review no. 2: the hot and the cold: unravelling the variable response of plant respiration to temperature. *Functional Plant Biology* **32**: 87–105.

Atkin OK, Bruhn D, Tjoelker MG. 2005b. Response of plant respiration to changes in temperature: mechanisms and consequences of variations in Q_{10} values and acclimation. In: Lambers H, Ribas-Carbo M, eds. *Plant respiration*. Dordrecht, the Netherlands: Springer, 95–135.

Atkin OK, Edwards EJ, Loveys BR. 2000. Response of root respiration to changes in temperature and its relevance to global warming. *New Phytologist* **147**: 141–154.

Atkin OK, Holly C, Ball MC. 2000b. Acclimation of snowgum (*Eucalyptus pauciflora)* leaf respiration to seasonal and diurnal variations in temperature, the importance of changes in the capacity and temperature sensitivity of respiration. *Plant, Cell & Environment* **23**: 15–26.

Atkin OK, Loveys BR, Atkinson LJ, Pons TL. 2006a. Phenotypic plasticity and growth temperature: understanding interspecific variability. *Journal of Experimental Botany* **57**: 267–281.

Atkin OK, Scheurwater I, Pons TL. 2006b. High thermal acclimation potential of both photosynthesis and respiration in two lowland *Plantago* species in contrast to an alpine congeneric. *Global Change Biology* **12**: 500–515.

Atkin OK, Tjoelker MG. 2003. Thermal acclimation and the dynamic response of plant respiration to temperature. *Trends in Plant Science* **8**: 343–351.

Atkinson LJ, Hellicar MA, Fitter AH, Atkin OK. 2007. Impact of temperature on the relationship between respiration and nitrogen concentration in roots: an analysis of scaling relationships, Q_{10} values and thermal acclimation ratios. *New Phytologist* **173**: 110–120.

Berry J, Bjorkman O. 1980. Photosynthetic response and adaptation to temperature in higher plants. *Annual Review of Plant Physiology* **31**: 491–543.

Bruhn D, Egerton JG, Loveys BR, Ball MC. 2007. Evergreen leaf respiration acclimates to long-term nocturnal warming under field conditions. *Global Change Biology* **13**: 1216–1223.

Delieu T, Walker DA. 1981. Polarographic measurement of photosynthetic oxygen evolution by leaf discs. *New Phytologist* **89**: 165–178.

Dewar RC, Medlyn BE, McMurtrie RE. 1999. Acclimation of the respiration photosynthesis ratio to temperature: insights from a model. *Global Change Biology* **5**: 615–622.

Dreyer E, Le Roux X, Montpied P, Daudet FA, Mason F. 2001. Temperature response of leaf photosynthetic capacity in seedlings from seven temperate tree species. *Tree Physiology* **21**: 223–232.

Farquhar GD, von Caemmerer S. 1982. Modelling of photosynthetic responses to environmental conditions. In: Lange OL, Nobel PS, Osmond CB, Ziegler H, eds. *Physiological Plant Ecology. I. Encyclopedia of Plant Physiology, New Series, Vol. 12B*. Berlin, Germany: Springer-Verlag, 549–587.

Farquhar GD, von Caemmerer S, Berry JA. 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. *Planta* **149**: 78–90.

Field C, Mooney HA. 1983. Leaf age and seasonal effects on light, water, and nitrogen use efficiency in a Californian shrub. *Oecologia* **56**: 348–355.

Field CB, Matson PA, Mooney HA. 1992. Responses of terrestrial ecosystems to a changing atmosphere. A resource-based approach. *Annual Review of Ecology and Systematics* **23**: 201–235.

Gifford RM. 2003. Plant respiration in productivity models: conceptualisation, representation and issues for global terrestrial carbon-cycle research. *Functional Plant Biology* **30**: 171–186.

Gonzalez-Melers M, Ribas-Carbo M, Giles L, Siedow JN. 1999. The effect of growth and measurement temperature on the activity of the alternative respiratory pathway. *Plant Physiology* **120**: 765–772.

Griffin KL, Turnbull M, Murthy R. 2002. Canopy position affects the temperature response of leaf respiration in *Populus deltoides*. *New Phytologist* **154**: 609–619.

Hikosaka K, Murakami A, Hirose T. 1999. Balancing carboxylation and regeneration of ribulose-1,5-bisphosphate in leaf photosynthesis: temperature acclimation of an evergreen tree, *Quercus myrsinaefolia*. *Plant, Cell & Environment* **22**: 841–849.

Hopkins WG. 1998. *Introduction to plant physiology*. New York, NY, USA: John Wiley.

Hurry VM, Tobiaeson M, Kromer S, Gardestrom P, Oquist G. 1995. Mitochondria contribute to increased photosynthetic capacity of leaves of winter rye (*Secale cereale*) following cold-hardening. *Plant, Cell & Environment* **18**: 69–76.

Krapp A, Stitt M. 1995. An evaluation of direct and indirect mechanisms for the 'sink regulation' of photosynthesis in spinach: changes in gas exchange, carbohydrates, metabolites, enzyme activities and steady-state transcript levels after cold-girdling source leaves. *Planta* **195**: 313–323.

Larigauderie A, Korner C. 1995. Acclimation of leaf dark respiration to temperature in alpine and lowland plant species. *Annals of Botany* **76**: 245–252.

Lee TD, Reich PB, Bolstad PV. 2005. Acclimation of leaf respiration to temperature is rapid and related to specific leaf area, soluble sugars and leaf nitrogen across temperate deciduous tree species. *Functional Ecology* **19**: 640–647.

Lewis JD, Lucash M, Olszyk DM, Tingey DT. 2004. Relationships between needle nitrogen concentration and photosynthetic responses of douglas-fir seedlings to elevated CO_2 and temperature. *New Phytologist* **162**: 355–364.

Loveys BR, Atkinson LJ, Sherlock DJ, Roberts RL, Fitter AH, Atkin OK. 2003. Thermal acclimation of leaf and root respiration: an investigation

comparing inherently fast-and slow-growing plant species. *Global Change Biology* **9**: 895–910.

Loveys BR, Scheurwater I, Pons TL, Fitter AH, Atkin OK. 2002. Growth temperature influences the underlying components of relative growth rate: an investigation using inherently fast- and slow-growing plant species. *Plant, Cell & Environment* **25**: 975–988.

Martindale W, Leegood RC. 1997. Acclimation of photosynthesis to low temperatures in S*pinacia oleraceae*. Effects of nitrogen supply. *Journal of Experimental Botany* **48**: 1873–1880.

McCutchan C, Monson R. 2001. Effects of tissue-type and development on dark respiration in two herbaceous perennials. *Annals of Botany* **87**: 355–364.

Morison JIL, Morecroft MD, eds. 2006. *Plant growth and climate change. Significance of temperature in plant life*. Boston, MA, USA: Blackwell Publishing.

Noguchi K, Go CS, Terashima I, Ueda S, Yoshinari T. 2001. Activities of the cyanide-resistant respiratory pathway in leaves of sun and shade species. *Australian Journal of Plant Physiology* **28**: 27–35.

Onoda Y, Hikosaka K, Hirose T. 2005. The balance between RuBP carboxylation and RuBP regeneration: a mechanism underlying the interspecific variation in acclimation of photosynthesis to seasonal change in temperature. *Functional Plant Biology* **32**: 903–910.

Pons TL, Welschen RAM. 2002. Overestimation of respiration rates in commercially available clamp-on leaf chambers. Complications with measurement of net photosynthesis. *Plant, Cell & Environment* **25**: 1367–1372.

Rachmilevitch S, Huang B, Lambers H. 2006. Assimilation and allocation of carbon and nitrogen of thermal and nonthermal *Agrotis* species in response to high soil temperature. *New Phytologist* **170**: 479–490.

Reich PB, Walters MB, Ellsworth DS. 1991. Leaf age and season influence the relationships between leaf nitrogen, leaf mass per area and in maple and oak trees. *Plant, Cell & Environment* **14**: 251–259.

Ribas-Carbo M, Ricardo A, Miquel A, Gonzalez M, Irigoyen JJ, Sanchez-Diaz M. 2000. The electron partitioning between the cytochrome and alternative respiratory pathways during chilling recovery in two cultivars of maize differing in chilling sensitivity. *Plant Physiology* **122**: 199–204.

Ribas-Carbo M, Robinson SA, Giles L. 2005. Application of the oxygen isotope technique to assess respiratory pathway partitioning. In: Lambers H, Ribas-Carbo M, eds. *Plant Respiration: from cell to ecosystem*. Advances in Photosynthesis & Respiration, vol. 18. Berlin, Germany: Springer, 31–42.

Rook DA. 1969. The influence of growing temperature on photosynthesis and respiration of *Pinus radiata* seedlings. *New Zealand Journal of Botany* **7**: 43–55.

Sage RF, Kubien DS. 2007. The temperature response of C_3 and C_4 photosynthesis. *Plant, Cell & Environment* **30**: 1086–1106.

Salvucci ME, Osteryoung KW, Crafts-Brandner SJ, Vierling E. 2001. Exceptional sensitivity of rubisco activase to thermal denaturation *in vitro* and *in vivo*. *Plant Physiology* **127**: 1053–1064.

Schimel DS, House JI, Hibbard KA, Bousquet P, Ciais P, Peylin P, Braswell BH, Apps MJ, Baker D, Bondeau A *et al.* 2001. Recent patterns and mechanisms of carbon exchange by terrestrial ecosystems. *Nature* **414**: 169–172.

Sharkey TD, Badger MR, von Caemmerer S, Andrews TJ. 2001. Increased sensitivity of photosynthesis in tobacco plants with reduced rubico activase. *Photosynthesis Research* **67**: 147–156.

Stitt M, Hurry V. 2002. A plant for all seasons: alterations in photosynthetic carbon metabolism during cold acclimation in Arabidopsis. *Current Opinions in Plant Biology* **5**: 199–206.

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

Thornley JHM, Johnson IR. 2000. *Plant and crop modelling: a mathematical approach to plant and crop physiology*. Caldwell, NJ, USA: The Blackburn Press.

Tjoelker MG, Oleksyn J, Reich PB. 1999a. Acclimation of respiration to temperature and CO_2 in seedlings of boreal tree species in relation to plant size and relative growth rate. *Global Change Biology* **5**: 679–692.

Tjoelker MG, Oleksyn J, Reich PB. 2001. Modelling respiration of vegetation: evidence for a general temperature-dependent Q_{10}. *Global Change Biology* **7**: 223–230.

Tjoelker MG, Reich PB, Oleksyn J. 1999b. Changes in leaf nitrogen and carbohydrates underlie temperature and CO_2 acclimation of dark respiration in five boreal tree species. *Plant, Cell & Environment* **22**: 767–778.

Turnbull MH, Murthy R, Griffin KL. 2002a. The relative impacts of daytime and night-time warming on photosynthetic capacity in *Populus deltoides*. *Plant, Cell & Environment* **25**: 1729–1737.

Turnbull M, Tissue DT, Griffin KL, Richardson SJ, Peltzer DA, Whitehead D. 2005. Respiration characteristics in temperate rainforest tree species differ along a long-term soil-development chronosequence. *Oecologia* **143**: 271–279.

Turnbull MH, Whitehead D, Tissue DT, Schuster WSF, Brown KJ, Engel VC, Griffin KL. 2002b. Photosynthetic characteristics in canopies of *Quercus prinus* and *Acer rubrum* differ in response to soil water avaliability. *Oecologia* **130**: 515–524.

Wright IJ, Reich PB, Atkin OK, Lusk CH, Tjoelker MG, Westoby M. 2006. Irradiance, temperature and rainfall influence leaf dark respiration in woody plants: evidence from comparisons across 20 sites. *New Phytologist* **169**: 309–319.

Xu CY, Schuster WSF, Griffin KL. 2006. Seasonal variation of temperature response of respiration in invasive japanese barberry (*Berberis thunbergii*) and two co-occuring native understorey shrubs in a northeastern U.S. deciduous forest. *Oecologia* **153**: 809–819.

Yamori W, Noguchi K, Terashima I. 2005. Temperature acclimation of photosynthesis in spinach leaves: analyses of photosynthetic components and temperature dependencies of photosynthetic partial reactions. *Plant, Cell & Environment* **28**: 536–547.

Yamori W, Suzuki K, Noguchi K, Nakai M, Terashima I. 2006. Effects of rubisco kinetics and rubisco activation state on the temperature dependence of the photosynthetic rate in spinach leaves from contrasting growth temperatures. *Plant, Cell & Environment* **29**: 1659–1670.

Zaragoza-Castells J, Sanchez-Gomez D, Valladares F, Hurry V, Atkin OK. 2007. Does growth irradiance affect temperature dependence and thermal acclimation of leaf respiration? Insights from a mediterranean tree with long-lived leaves. *Plant, Cell & Environment* **30**: 820–833.

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Thermal acclimation of respiration but not photosynthesis in *Pinus radiata*

Lai Fern Ow[A,D], *David Whitehead*[B], *Adrian S. Walcroft*[C] *and Matthew H. Turnbull*[A]

[A]School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch, New Zealand.
[B]Landcare Research, PO Box 40, Lincoln 7640, New Zealand.
[C]Landcare Research, Private Bag 11052, Manawatu Mail Centre, Palmerston North 4442, New Zealand.
[D]Corresponding author. Email: genow@starhub.net.sg

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Abstract. *Pinus radiata* L. were grown in climate-controlled cabinets under three night/day temperature treatments, and transferred between treatments to mimic changes in growth temperature. The objective was to determine the extent to which dark respiration and photosynthesis in pre-existing and new needles acclimate to changes in growth temperatures. We also assessed whether needle nitrogen influenced the potential for photosynthetic and respiratory acclimation, and further assessed if short-term (instantaneous, measured over a few hours) respiratory responses are accurate predictors of long-term (acclimated, achieved in days–weeks) responses of respiration to changing temperature. Results show that respiration displayed considerable potential for acclimation. Cold and warm transfers resulted in some acclimation of respiration in pre-existing needles, but full acclimation was displayed only in new needles formed at the new growth temperature. Short-term respiratory responses were poor predictors of the long-term response of respiration due to acclimation. There was no evidence that photosynthesis in pre-existing or new needles acclimated to changes in growth temperature. N status of leaves had little impact on the extent of acclimation. Collectively, our results indicate that there is little likelihood that respiration would be significantly stimulated in this species as night temperatures increase over the range of 10–20°C, but that inclusion of temperature acclimation of respiration would in fact lead to a shift in the balance between photosynthesis and respiration in favour of carbon uptake.

Additional keywords: N availability, Monterrey (radiata) pine, temperature, thermal homeostasis.

Introduction

With the concern for global warming has come a heightened interest in the effects of elevated temperatures on physiological processes in trees and the productivity of forests (Teskey and Will 1999). The carbon balance of forest ecosystems is defined by the difference between photosynthetic carbon gain and respiratory carbon loss. On an annual basis, respiration can consume between 30 and 70% of photosynthetic carbon fixation (Ryan *et al*. 1994, 1996; Turnbull *et al*. 2005). In addition, given that plant respiration results in the release of 60 Gt of carbon annually (King *et al*. 2006), 80% which is from forest trees (i.e. pines) (Ryan *et al*. 1994), it is essential that respiratory responses of forest trees to environmental variables is better understood. With global warming, higher respiratory costs may cause lower productivity in forest and may enhance the release of stored carbon into the atmosphere, potentially adding to the greenhouse effect.

Temperature has a large influence on the processes of photosynthesis and respiration. In the short term (minutes–hours), photosynthesis increases exponentially with increasing temperature, eventually displaying a declining rate of increase such that an optimum temperature can be identified, after which rates start to decline. By contrast, short-term responses of dark respiration tend to remain exponential until close to lethal temperatures. Despite being highly sensitive to short-term changes in temperature, photosynthetic and respiratory responses to longer term (days–weeks) temperature change is commonly reduced in magnitude.

The degree to which respiration and photosynthesis change with temperature is highly variable, and is influenced by a range of external and internal factors (Larigauderie and Korner 1995; Atkin *et al*. 2000*a*, 2000*b*; Tjoelker *et al*. 2001). Acclimation to temperature occurs when the rate of a physiological process adjusts in response to a change in temperature over the course of hours to weeks. Rates of photosynthesis have long been known to acclimate to prevailing temperature (Berry and Bjorkman 1980), but the degree of photosynthetic acclimation is highly variable between species as well as within genera (Atkin *et al*. 2006). Acclimation can change the shape of the photosynthetic temperature response curve or result in a shift in the curve such that the absolute rate and the optimum temperature changes. Similarly, rates of respiration have also been found to acclimate rapidly (hours–days) following a change in prevailing temperature (Rook 1969;

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Atkin and Tjoelker 2003). As with photosynthesis, respiratory acclimation potential varies between species (Atkin *et al*. 2000*a*, 2005*a*, 2005*b*; Loveys *et al*. 2003). The short-term temperature response of respiration is often exponential, but in the longer term it is seldom so (Gifford 2003). Respiratory acclimation to higher and lower temperatures often results in the downward and upward shift of the temperature response curve of respiration, respectively.

Based on the background above, it is clear that the ratio of respiration to photosynthesis is also likely to be sensitive to temperature. Over a short period, this ratio is generally considered to increase with increasing temperature. By contrast, it has been suggested that the longer-term response of the respiration to photosynthesis ratio tends to be homeostatic but with some exceptions across a range of temperatures and species (Gifford 1995, 2003). Although the specific responses of photosynthesis and respiration are thought to be independent, both respiration and photosynthesis are physiologically linked (Turnbull *et al*. 2004; Whitehead *et al*. 2004) and are also known to respond to a range of environmental variables. If the balance between photosynthesis and respiration were to be altered as a result of increasing temperature, there is a risk that respiration would be stimulated, releasing stored carbon into the atmosphere and acting as a positive feedback to the greenhouse effect. Therefore, an in-depth understanding of how respiratory and photosynthetic processes will respond to temperature in the short and long term is important if models of forests or ecosystem carbon exchange are to accurately predict plant responses to climate change.

Rates of dark respiration and photosynthesis often correlate with tissue N content across diverse taxa and environments [respiration (Ryan 1995; Reich *et al*. 1998, 2006; Wright *et al*. 2006), and photosynthesis (Field 1983; Reich *et al*. 1991; Turnbull *et al*. 2002; Morison and Morecroft 2006)]. However, few studies (Field *et al*. 1992; Krapp and Stitt 1995; Martindale and Leegood 1997; Atkinson *et al*. 2007) have focussed on the role of leaf N in photosynthetic and respiratory acclimation, particularly in tree species. Many previous studies (references above) have confirmed earlier findings of a strong relationship between N and rates of photosynthesis and respiration, but have not extended their investigation into the role of N in thermal acclimation.

In this study we assess the extent of photosynthetic and respiratory acclimation to changing temperature in pre-existing and new needles of *Pinus radiata* L. We have previously investigated the responses of photosynthesis and respiration in poplar in response to step changes in temperature under controlled conditions (Ow *et al*. 2008). This investigation showed significant thermal acclimation in respiration but not photosynthesis. In the present study, day and night-time growth temperatures were adjusted up or down by 5–10°C. The objectives of our study were to: (1) examine the extent of photosynthetic and respiratory acclimation in pre-existing and new needles – our hypothesis being that a high degree of both photosynthetic and respiratory acclimation would not be expressed in pre-existing needles (at the new temperature) as pines (and other evergreen species) are slow-growing, with a low potential for phenotypic plasticity; and (2) establish whether leaf nutritional status (manipulated using low and high levels of N availability) influences the potential for acclimation – we hypothesised that low leaf N would limit acclimation potential. A key objective was to determine the extent to which short-term predictions of the temperature responses of respiration can be used to accurately predict long-term responses to changing temperature.

Materials and methods

Plant material and environmental conditions

This experiment was conducted at the University of Canterbury in Christchurch, New Zealand (Latitude 43°25′E, longitude 172°58′N, at sea level) from August to November 2006. Small saplings of *Pinus radiata* L. were grown from cuttings (New Zealand Forest Research Institute, Rotorua, New Zealand) in 9-L pots which contained a mixture of bark and chip (coarse organic material) with a ratio of 8 : 2, respectively. The potting mix contained a base level of slow release fertilizer (N : P : K in the proportions 8.8 : 5.5 : 10.6 + trace elements). The plants were initially maintained in a glasshouse [photon flux density (PFD) at ~1000–1100 $\mu mol\ m^{-2}\ s^{-1}$] before the experiment at temperatures in the middle of the experimental range (i.e. 15–20°C) and watered to capacity every 4–5 days. Half the saplings were supplemented with 7 g of compacted 1–2 mm grain nitrogen-rich (38% N) fertiliser (Enduro, Rosenlew RKW, Finland). The experiment was conducted in three growth chambers (Contherm 630, climate simulator, Petone, Wellington, New Zealand) set at a PFD of 700 $\mu mol\ m^{-2}\ s^{-1}$ and 70% relative humidity. The lighting regime was maintained at a 10/14 h dark/light period, using 400 W metal halide lamps. This experiment consisted of a total of three treatments, randomly allocated amongst the chambers, which each contained eight well spaced plants to allow for thorough air flow and light penetration. The temperature treatments were (night/day) 10/15°C, 15/20°C and 20/25°C.

The initial temperature treatment regimes described above were imposed for 1 week after which the first gas-exchange measurements were made. This allowed us to construct 'acclimated' temperature response curves (at the growth temperatures) before the transfer of plants to new temperature regimes. Following the initial gas-exchange measurement, the plants were transferred between the three temperature treatments whereby plants from the 10/15°C (night/day) temperature regime were transferred to the 15/20°C temperature regime, plants from the initial 15/20°C temperature regime were moved to the 20/25°C temperature regime, and plants from the 20/25°C temperature regime were transferred to the 10/15°C growth environment. The three temperature transfers will hereafter be denoted in the text by the respective day or night temperatures (15–20 and 20–25 and 25–15 for photosynthesis or 10–15 and 15–20 and 20–10 for respiration). Following a week in the new environment a second set of gas exchange measurements were made. Although the timing of transfers was clearly artificial, it is consistent with previous rates of acclimation (e.g. Atkin *et al*. 2000*b*). We have also confirmed that the timing is appropriate with measurements of seasonal variation in photosynthesis and respiration in this species (data not shown) in the field. After an additional 8 weeks in these growth conditions, a final round of gas-exchange measurements was made on new needles which

developed under the new temperature regime. Space constraints did not allow us to have constant temperature ('control') plants, but the three-way transfer regime allowed us to be confident that temperature change was the driving factor in plant responses.

Gas-exchange measurements

Leaf-level gas-exchange measurements were made on current year needles (2–6 months old) with two cross-calibrated, portable open-path gas-exchange systems with CO_2 control (Li-6400, Li-Cor Inc., Lincoln, NE, USA) using the standard 2×3-cm chamber equipped with blue-red light-emitting diodes mounted on the top of the cuvette. Six needles from two fascicles on secondary branches were used for each measurement. Environmental controls within the cuvette were maintained to match the chamber conditions, unless otherwise specified. Measurements of the responses of photosynthesis (A) to intercellular CO_2 partial pressure (C_i) response curves were generated by altering the external CO_2 concentration (C_a) in 14 steps from 150 to 0 Pa at a constant PFD of 1500 μmol m^{-2} s^{-1}. Measurements were made at each C_a set point when photosynthetic gas exchange had equilibrated (taken to be when the coefficient of variation (CV) for the CO_2 concentration differential between the sample and reference analysers was below 1% and visibly stable). This condition was typically achieved within 1–2 min after a stable set point had been reached. The response of photosynthesis (A) to irradiance (Q) were generated by altering the incident PFD in 10 steps from 2000 to 0 μmol m^{-2} s^{-1} using a light source consisting of blue-red light-emitting diodes mounted on the top of the cuvette. During the light response curves, the external CO_2 partial pressure was held constant at 37 Pa. The desired needle temperature for the measurement of photosynthesis (the respective daytime growth temperatures of 15, 20 and 25°C) was maintained using thermoelectric coolers (of the Li 6400) and water vapour pressure deficit was maintained between 1.0 and 1.5 kPa. A/C_i response curves were used to determine values for the maximum rate of carboxylation (V_{cmax}) and the apparent maximum rate of electron transport at saturating irradiance (J_{max}). The A/C_i response data were analysed using the biochemical model of photosynthesis as described by Farquhar *et al*. (1980) with appropriate parameter corrections for temperature (Bernacchi *et al*. 2003). AQ – light response curves was used to determine values of maximum assimilation (A_{max}) at saturating light and at ambient CO_2. The AQ response is described by a rectangular hyperbola as in Thornley and Johnson (2000). Needle surface area was determined from measurements of fascicle diameter and needle length according to the equation in Turnbull *et al*. (1998). Surface areas were within the range of 20–45 cm^2 and measurements were always conducted on needles that were close to the apex, receiving full illumination. Calculations of photosynthetic parameters from A/C_i and A/Q curves did not include a gasket correction factor (Pons and Welschen 2002). We assumed that CO_2 exchange processes only occurred in the part of the sample enclosed in the chamber hence unlikely to result in the underestimation of photosynthetic rates. Moreover, the gasket effect was found to be absent when measurements were made at saturating light (present study at 1500 μmol m^{-2} s^{-1}) because the error was negligible in comparison to the high photosynthetic rate (photosynthetic parameters in this present study ranged between, A_{max} : 8.1 and 18.9, J_{max} : 62.3 and 147.5 and V_{cmax} : 31.2 and 77.2) (Pons and Welschen 2002).

Measurements of leaf dark respiration (R_d) were made at the end of the dark period on excised needles removed before onset of daytime (illuminated) chamber conditions. Measurements were made on current-year needles (2–6 months old) in a separate growth chamber to control measurement temperature. Upon excision, the ends of the fascicles were wrapped in moist paper towel and sealed in a plastic bag and placed in the darkened growth chamber. Previous measurements have shown that leaf respiration remains stable under these conditions for many hours (Turnbull *et al*. 2005) and this was confirmed here. Measurements were completed within 2–3 h of removal of needles. The surface area used to calculate respiration rate included a correction factor to account for darkened leaf material under the cuvette gasket (Lambers and Ribas-Carbo 2005). We assumed that ~2 cm^2 of sample under the gasket contributed respired CO_2 into the chamber which was added to our total surface area calculations to avoid an overestimation of dark respiration rate. The Li-6400 portable gas-exchange system was itself kept in the darkened growth chamber during the measurements and controlled externally from a lap-top computer. The instantaneous response of dark respiration was determined by making measurements of R_d at temperatures of 10, 15, 20 and 25°C in pre-existing and new needles before and after transfer in all three temperature treatments. The growth chamber and leaf chamber temperature conditions were similarly controlled and the needles were allowed to equilibrate to the new temperature conditions for 30–45 min before the onset of the respiration measurements. The rate of dark respiration was calculated from the mean of five measurements made over 3 min. Respiratory temperature response curves were analysed as previously described (Atkin *et al*. 2005*b*), where dark respiration rate at a given temperature is given by:

$$R = R_{10} Q_{10}^{(T_1 - T_0/10)} \quad (1)$$

where R_{10} is the respiration rate at the base temperature, T_0 (here 10°C or 283 K), T_1 is foliage temperature (K) and Q_{10} is a parameter describing the proportional change in respiration with a 10°C increase in temperature. Non-linear curve fitting was performed using the Marquardt–Levenberg algorithm (Sigma Plot, Software version 8.0 SPSS Inc. Chicago, IL, USA). Nitrogen was determined on needles dried at 70°C, ground to powder and measured with a CNS analyser (Carlo Erba Na 1500, Milan, Italy). Specific leaf area (S) was calculated following the measurement of individual surface area and dry mass.

Oxygen electrode measurements

The relative enzymatic activities for dark respiration via the cytochrome (COX) and alternative (AOX) oxidase pathways were measured using oxygen electrodes with dual digital controllers (Model 20, Rank Brothers Ltd, Bottisham, Cambridge, UK). Rates of oxygen consumption were monitored in needle samples in buffer (Delieu and Walker

1981; McCutchan and Monson 2001) for 5–10 min using data acquisition software (TracerDAQ, version 1.7; Measurement Computing Corporation, Norton, MA, USA). Needle segments measuring 1 cm were incubated in running buffer containing 30 mM salicylhydroxamic acid (SHAM, an inhibitor of AOX activity) for 1.5 h in darkness for the measurement of COX activity. In addition, AOX activity was measured following 1.5-h incubation of needles in 3 mM potassium cyanide (KCN, an inhibitor of COX activity). Appropriate controls (without inhibitors) were also incubated in a similar way before measurements were made. Residual respiratory activity was determined following incubation of separate needles in both inhibitors [average 11.6% (±0.008) of total respiration was subtracted from each value before calculating activity as a percentage of total uninhibited respiration]. Needles incubated in 50 μM carbonylcyanide *m*-chlorophenylhydrazone (CCCP) were used to assess the fully uncoupled rate of oxygen consumption. The activity of each pathway was calculated as a percentage of the fully uncoupled rate.

Statistical analysis

A two-way ANOVA was used to test for the main effects and interactions of temperature and nutritional content [high-N (HN), low-N (LN)] on photosynthetic parameters, needle N, respiratory parameters, *S*, AOX and COX activity (SAS Institute, software version 9, Cary, NC, USA). Differences were considered significant when $P < 0.05$. Treatment means were compared by least significant difference to determine whether means of the dependent variable were significantly different at $P = 0.05$. Differences between means in Fig. 3 were evaluated with a one-way ANOVA.

Results

Needle characteristics

The values of *S* recorded over the range of temperatures varied from 15 to 23.5 $m^2 kg^{-1}$. Analysis of variance revealed significant differences in *S* of pre-existing and new needles at the new temperature in response to both cold and warm transfer (Table 1). Cold-transferred pre-existing and newly developed needles (Table 1; 25–15) had significantly lower *S*. Newly developed needles, following transfer to the cooler environment, had lower *S* values than their pre-existing counterparts at the same temperature. By contrast, warm-transferred pre-existing and new needles (Table 1; 15–20 and 20–25) had significantly higher *S* values than the values observed at the initial temperature. The values of N recorded over the range of temperatures varied from 2.24 to 3.95 g m^{-2}. An N-treatment effect was evident between HN and LN plants on both a mass and area basis (N_a: Table 1). Cold-transferred pre-existing and new needles (Table 1; 25–15) showed a significant increase in leaf N, and warm-transferred pre-existing and new needles (Table 1; 15–20 and 20–25) displayed a significant decrease in leaf N, with the exception of warm-transferred HN plants (Table 1; 20–25) where no significant change was observed at the new temperature.

Photosynthesis

A_{max} recorded over the range of temperatures varied from 8 to 19 μmol $m^{-2} s^{-1}$. Cold-transfer of plants resulted in no significant change in A_{max} for HN plants but a significant decrease was found in pre-existing and new needles of LN plants after transfer (Fig. 1*a*; 25–15). Warm-transferred plants displayed no significant change in A_{max} values in either pre-existing or new needles (Fig. 1*a*; 15–20 and 20–25). Values of V_{cmax} over the range of temperatures varied from 31 to 78 μmol $m^{-2} s^{-1}$. Cold transfer resulted in a large decrease in V_{cmax} in both HN and LN plants (Fig. 1*b*; 25–15). By contrast, following warm transfer, a large (~2-fold) increase was observed in pre-existing and new needles (Fig. 1*b*; 15–20 and 20–25). J_{max} ranged from 60 to 148 μmol $m^{-2} s^{-1}$. J_{max} declined significantly following cold transfer (Fig. 1*c*; 25–15), although pre-existing needles were limited in their ability to achieve values obtained by new needles. J_{max} displayed a significant increase in response to warm transfer (Fig. 1*c*; 15–20 and 20–25). As was found for

Table 1. Specific leaf area (S $m^2 kg^{-1}$) and nitrogen content per unit leaf area (N_a g m^{-2}) in pre-existing and new needles for *Pinus radiata* plants exposed to both increasing and decreasing temperatures

Measurements were made in pre-existing needles at the initial temperature, 7-days after transfer to the new temperature and in new needles at the new temperature. Plants were grown under low N (LN) and high N (HN) availability. Values shown are means (± s.e. of the mean, s.e.m.) where $n = 8$. Different letters within a given temperature transfer indicate statistically different values at $P < 0.05$ using least significant difference test of treatment means

Temperature transfer	S ($m^2 kg^{-1}$)				N_a ($g m^{-2}$)			
	Pre-existing leaves at initial temperature	Pre-existing leaves at new temperature	New leaves at new temperature	ANOVA statistics (P)	Pre-existing leaves at initial temperature	Pre-existing leaves at new temperature	New leaves at new temperature	ANOVA statistics (P)
25 to 15°C								
High N	22.9 (0.5)a	19.3 (0.3)c	15.9 (0.4)d	<0.05	2.53 (0.1)b	3.27 (0.1)a	3.20 (0.1)a	<0.05
Low N	21.7 (0.4)b	19.2 (0.2)c	15.1 (0.2)d		2.24 (0.1)c	2.60 (0.1)b	2.68 (0.1)b	
15 to 20°C								
High N	19.5 (0.2)d	22.1 (0.3)b	23.0 (0.3)a	<0.05	3.95 (0.1)a	2.88 (0.1)b	2.93 (0.1)b	<0.05
Low N	19.3 (0.3)d	20.6 (0.2)c	21.9 (0.2)b		2.85 (0.1)b	2.40 (0.1)c	2.33 (0.1)c	
20 to 25°C								
High N	22.2 (0.2)c	23.3 (0.4)ab	23.5 (0.3)a	<0.05	2.75 (0.04)a	2.62 (0.1)a	2.70 (0.04)a	<0.05
Low N	20.7 (0.2)d	22.6 (0.4)bc	22.3 (0.3)c		2.59 (0.1)a	2.28 (0.1)b	2.25 (0.04)b	

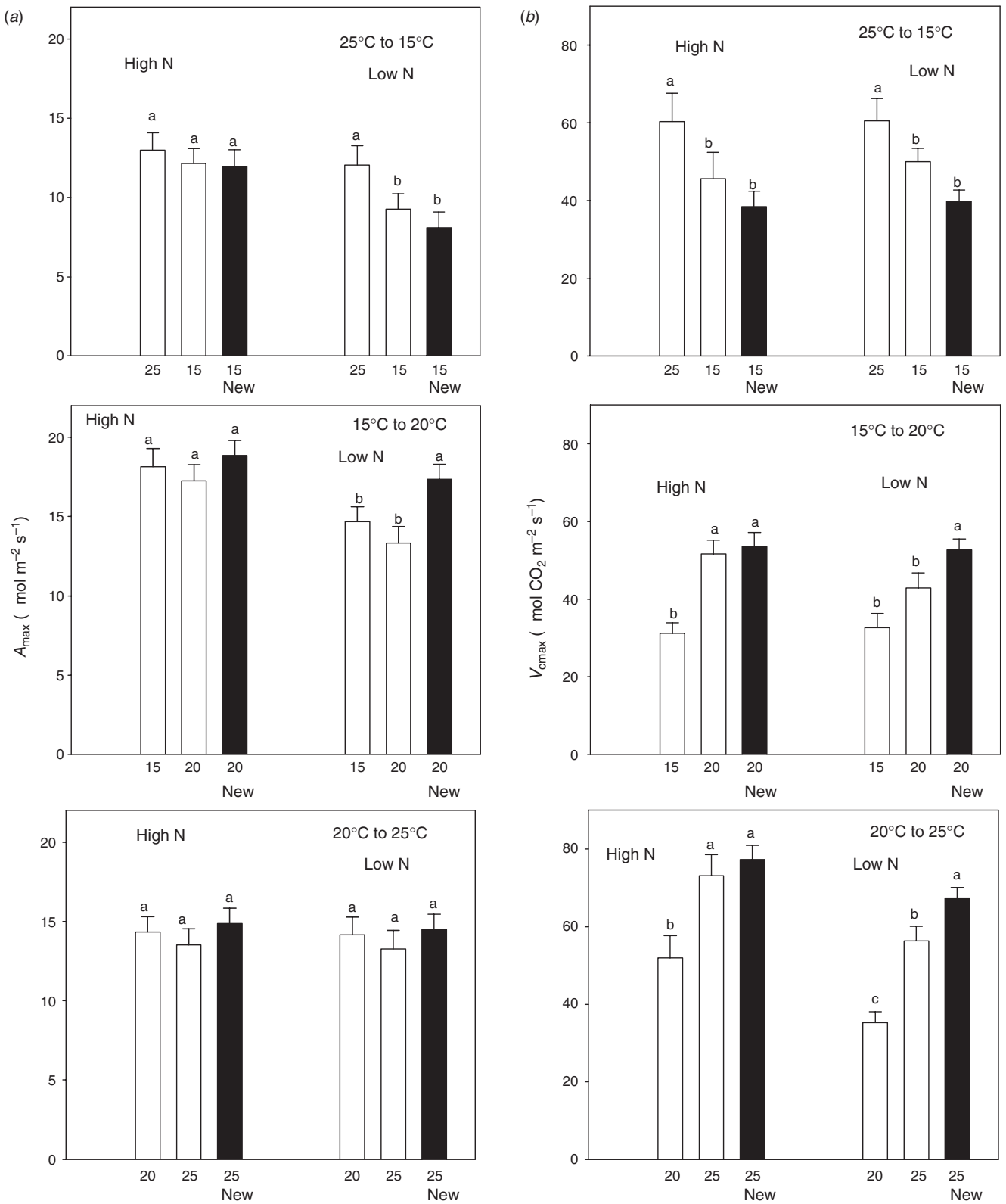

Fig. 1. Photosynthetic parameters calculated from the A/C_i and A/Q response in *Pinus radiata* plants (A_{max}, V_{cmax}, J_{max} and J_{max}/V_{cmax}) under three temperature transfer regimes (25–15°C, 15–20°C and 20–25°C). Each frame displays the photosynthetic response of pre-existing needles at the initial growth temperature and 1 week after the plants were transferred to the new temperature (open bars). The closed bar represents the responses of newly expanded needles in the new temperature. Data are presented for plants grown under high nitrogen (HN) and low nitrogen (LN) availability. All values are means ± s.e.; $n = 8$. Different letters indicate means are significantly different at $P < 0.05$.

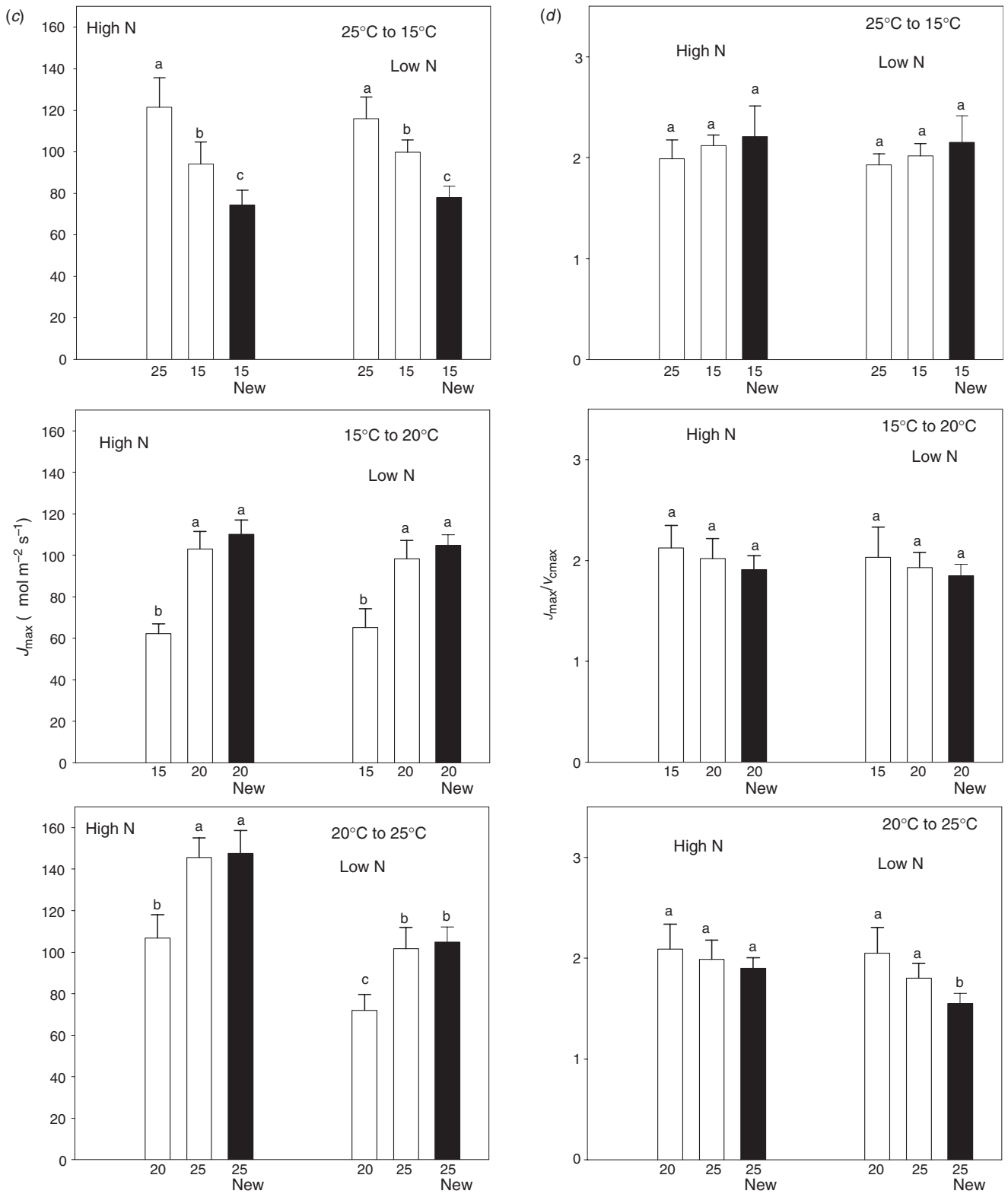

Fig. 1. (*continued*)

V_{cmax}, pre-existing needles at the new temperature achieved values very close to those observed in new needles. Generally, changes in the response of the J_{max}/V_{cmax} ratio to temperature were very limited (in both HN and LN plants) (Fig. 1*d*; 25–15, 15–20 and 20–25). The long-term responses of V_{cmax} and J_{max} as a function of temperature (determined by taking J_{max} and V_{cmax} at the three growth temperatures before and after transfer) did not change following transfer (Fig. 2*a*, *b*).

Respiration

The instantaneous response of respiration in the dark (R_d) to temperature was sensitive to temperature change (Fig. 3*a*–*c*). Following transfer in pre-existing needles, little to no difference in the values of respiration at low temperatures was observed between treatments (Fig. 3*b*). Differences between treatments were found only at moderate to higher temperatures, with the highest values of respiration observed in the 10°C treatment. In new needles R_d was greater in cold-grown plants at both low and high measurement temperatures (Fig. 3*c*). The long-term response of respiration (determined by actual R_d at the respective growth temperatures – solid line Fig. 3*a*–*c*) was less pronounced than that of the instantaneous response in pre-existing needles after transfer (Fig. 3*b*). However, in newly-formed needles, the long-term response of R_d was virtually insensitive to temperature (thermal homeostasis; Fig. 3*c*).

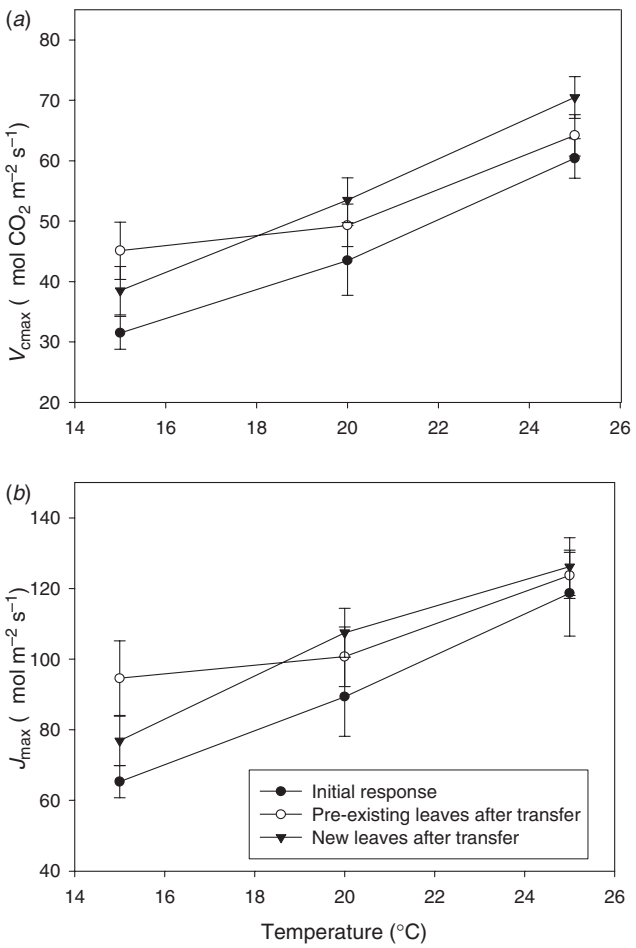

Fig. 2. Photosynthetic temperature response of (*a*) V_{cmax} (*b*) J_{max} at three daytime growth temperatures (15, 20 and 25°C) for *Pinus radiata* plants (means are averaged across N treatments) ($n = 8$).

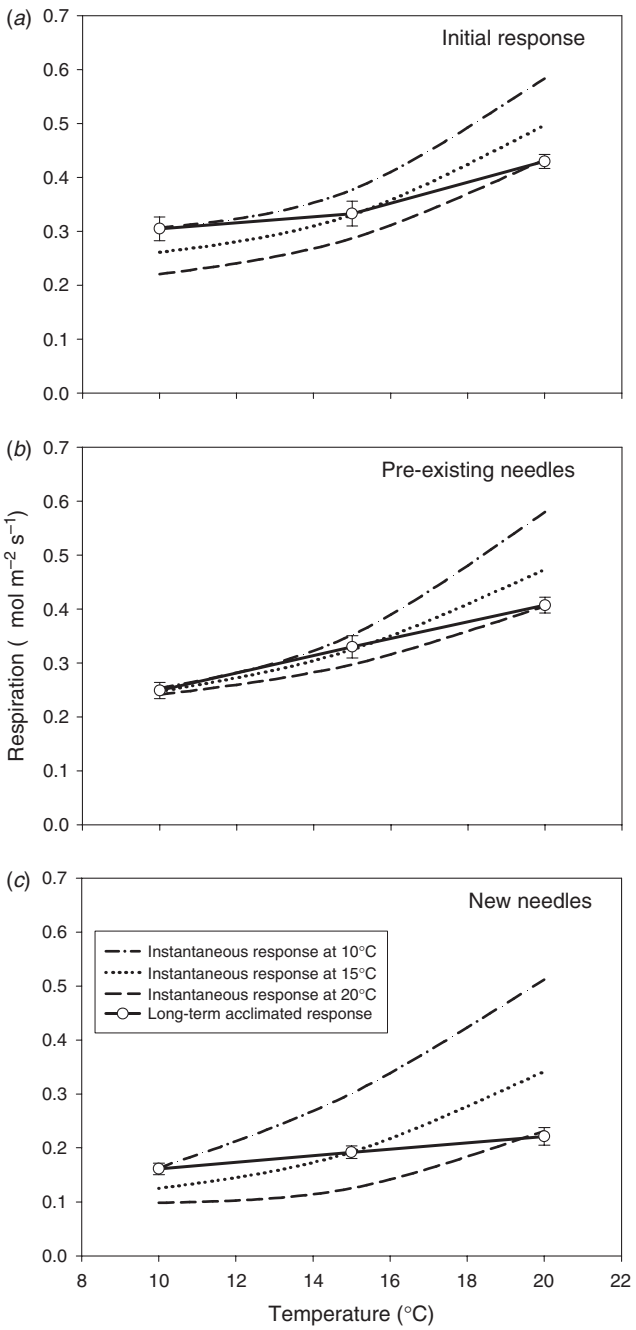

Fig. 3. The instantaneous respiratory temperature response of (*a*) pre-existing needles of *Pinus radiata* at the initial night-time temperature (before transfer of plants to new temperature regimes), (*b*) pre-existing needles at the new night-time temperature, and (*c*) new needles at new night-time temperature. The acclimated response (dark solid line) is determined by taking actual values of R_d at the respective night-time growth temperatures. Values at the night-time growth temperatures are means ± s.e. One-way ANOVA tests indicated significant differences in R_d at each growth temperature ($P = 0.0005$ for 10°C, $P = 0.00051$ for 15°C and $P = 0.0005$ for 20°C) (data presented are averaged across N treatments).

Changes in the temperature response characteristics of R_d following growth temperature transfer (calculated from the instantaneous curves) show that leaf respiration increased significantly in response to cold transfer (R_{10} values lower at 20°C than at 10°C in new needles (Fig. 4*a*; 20–10). Although the shift of plants to a cool temperature increased rates of respiration, the shift of plants to warmer temperatures resulted in a decline in R_{10} in new needles (Fig. 4*a*; 10–15 and 15–20). The acclimation potential of pre-existing and new needles was clearly different. Significant changes in R_{10} in response to either cold and warm transfer (Fig. 4*a*; 20–10, 10–15 and 15–20) were achieved only in new needles formed at the new temperature, but pre-existing needles at the new temperature were found to show little to no change in R_{10} when compared with the initial response. The values of Q_{10} over the 10–20°C range varied from 2.0 to 3.2. Q_{10} was sensitive to temperature transfer, although the magnitude of change varied between treatments. Following cold transfer, no significant response was observed in Q_{10} (Fig. 4*b*; 20–10). By contrast, with warm transfer, Q_{10} values generally declined with increasing temperature (Fig. 4*b*; 10–15 and 15–20), although this was most pronounced in plants that had the lowest initial growth temperature (10–15) and significantly so only in new needles.

The respiration/photosynthesis ratio (R_d/A_{max}) generally declined with warm transfer and increased following cold transfer. Following cold transfer, there was very little response in pre-existing needles, but new needles displayed a significant increase in the ratio relative to the initial response (Fig. 4*c*; 20–10). Similarly, warm transfers led to no change in the ratio in pre-existing needles at the new temperature but a significant decrease in the ratio was seen in new needles (Fig. 4*c*; 10–15 and 15–20). Changes in the long term (determined by taking R_d/A_{max} at the three growth temperatures before and after transfer) response of R_d/A_{max} to growth temperature were absent before transfer and in pre-existing leaves after transfer (Fig. 5). Changes to R_d/A_{max} were limited to new needles, in which a greater than 2-fold increase was observed at 10°C relative to the ratio observed at 15 and 20°C (Fig. 5).

Enzymatic activity of the cytochrome and alternative oxidase pathways

The enzymatic activity of the COX and AOX pathways (as a percentage of total respiratory activity in leaves) is shown in Table 2. COX pathway activity remained remarkably consistent in response to warm transfer (Table 2; 10–15 and 15–20) but declined significantly in cold-transferred plants (Table 2; 20–10). This was especially evident in new needles. AOX activity in cold-transferred plants increased significantly in pre-existing needles and was sustained in new needles (Table 2; 20–10). In warm-transferred plants, there was a less pronounced and transient increase in AOX activity in pre-existing needles of LN plants – this response was sustained in new needles of HN plants only (Table 2; 10–15 and 15–20).

Discussion

Our study has provided an analysis of the components of needle characteristics and gas exchange that undergo change when *P. radiata* acclimates to a range of contrasting growth temperatures. The findings of this study clearly show that (1) photosynthetic parameters tend to retain a relatively fixed response to temperature and do not acclimate following changes in growth temperature; (2) acclimation of R_d does occur, but the degree of acclimation exhibited by pre-existing and new needles differs – in new needles, near perfect thermal homeostasis of respiration was observed over a 10°C temperature range – this was absent from pre-existing needles at the new temperature; and (3) thermal acclimation displayed in new needles results in a change in the R_d/A ratio which favours respiration at low temperatures and photosynthesis at high temperatures. These results are important to our understanding of tree function in response to changing environmental conditions and to modelling efforts to predict vegetation responses to global climate change.

Photosynthetic response to temperature transfer

Studies investigating the temperature response of photosynthesis report great variability in the degree of photosynthetic acclimation among species, with some species exhibiting full acclimation whilst others are incapable of even partial acclimation (Berry and Bjorkman 1980; Atkin *et al*. 2005*a*). Our photosynthetic data suggest that cold transfer resulted in a reduced photosynthetic capacity in *P. radiata* (despite increasing tissue nitrogen content) although 5°C increases in temperature led to significant increases in photosynthetic capacity. Our observations of decreased photosynthetic rates in response to a cold transfer are consistent with previous work by Rook (1969) who found a 25% decrease in photosynthetic rates of *P. radiata* seedlings following transfer to cooler temperatures. Overall, the long-term temperature response of photosynthesis over the range of 15–25°C (Fig. 2*a*, *b*) shows that photosynthesis retains its instantaneous sensitivity to temperature, as evidenced by an increase in values for V_{cmax} and J_{max} with increasing growth temperature. The shift to a new temperature did not elicit significant change in the shape or position of the long-term photosynthetic temperature response curve. These observations indicate that only a very limited degree of photosynthetic acclimation occurs in *P. radiata* in the face of changes in temperature. This confirms our previous finding for poplar grown under controlled conditions (Ow *et al*. 2008).

We found that the ratio of the capacities of ribulose-1,5-bisphosphate (RuBP) regeneration to RuBP carboxylation (J_{max}/V_{cmax}) was insensitive to temperature change (Fig. 1*d*). This is consistent with previous work by Onoda *et al*. (2005). Similarly, Medlyn *et al*. (2002) also observed that the J_{max}/V_{cmax} ratio did not change with changes in growth temperature in *Pinus taeda* and *Pinus pinaster*. In a previous study, Hikosaka (1997) posed the hypothesis that temperature acclimation is a result of a re-allocation of nitrogen between the electron transport and carboxylation processes so as to ensure that both processes are co-limiting at ambient conditions. Under this hypothesis, it is predicted that the J_{max}/V_{cmax} ratio at a given temperature should be lower for plants exposed to low temperatures. In our study however, the transfer to cooler conditions (25–15°C) led to very little change in this ratio. Other studies investigating acclimation to growth temperature

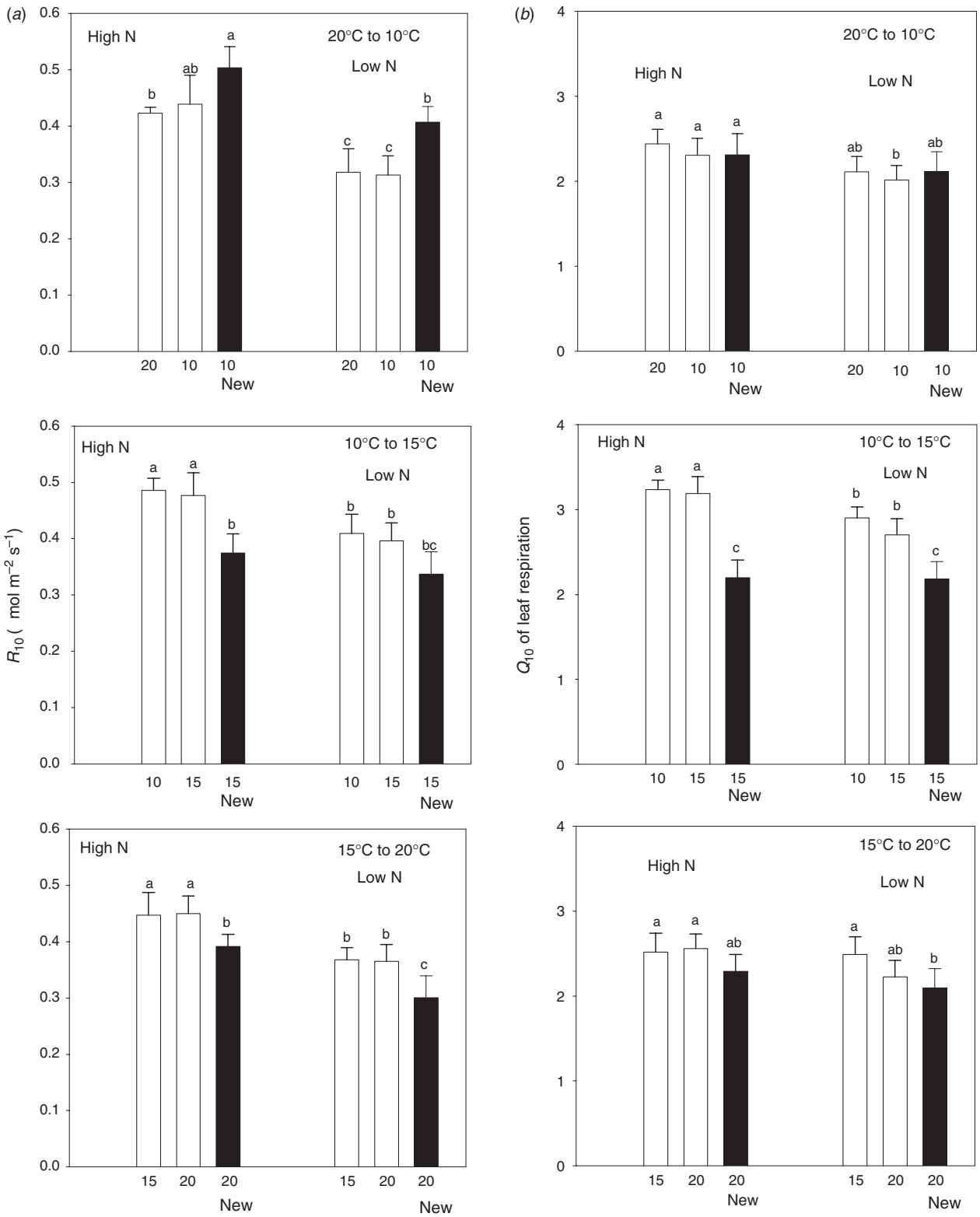

Fig. 4. Temperature response parameters of dark respiration (R_{10}, Q_{10} and R_d/A_{max}) under three temperature transfer regimes (20–10°C, 10–15°C and 15–20°C). Each frame displays the respiratory response of pre-existing needles of *Pinus radiata* at the initial growth temperature and 1 week after the plants were transferred to the new temperature (open bars). The closed bar represents the response of newly expanded needles in the new temperature. Data are presented for plants grown under high nitrogen (HN) and low nitrogen (LN) availability. All values are means ± s.e.; $n = 8$. Different letters indicate means are significantly different at $P < 0.05$.

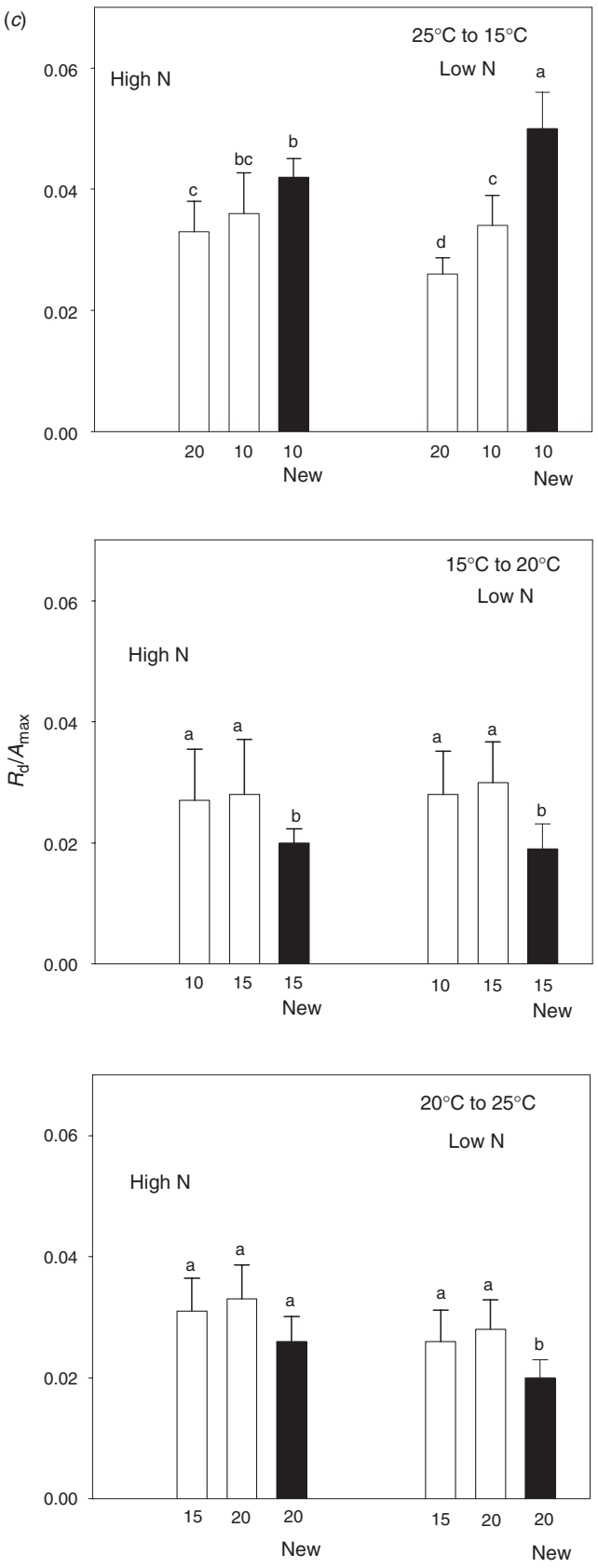

Fig. 4. (*continued*)

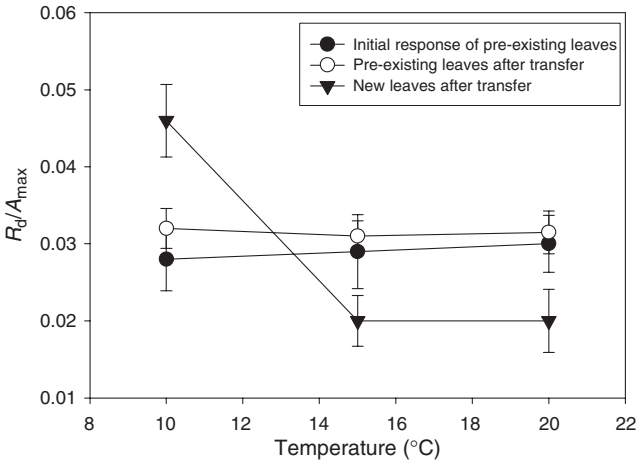

Fig. 5. Ratios of needle R in darkness (R_{dark}) to light saturated photosynthesis at ambient CO_2 (A_{max}) at three night-time growth temperatures (10, 15 and 20°C) for *Pinus radiata* plants (means are averaged across N treatments) ($n = 8$).

have similarly failed to find any evidence of such a change (Bunce 2000; Medlyn *et al*. 2002). Across a range of temperatures (10–35°C), rates of photosynthesis are limited either by RuBP carboxylation or by RuBP regeneration (Berry and Bjorkman 1980). An increase in the J_{max}/V_{cmax} ratio at low growth temperatures is considered to relieve the limitation of RuBP regeneration on photosynthetic rate, whilst a decrease in the ratio with increasing temperature is a result of a greater activation energy for V_{cmax} than for J_{max} (Onoda *et al*. 2005). J_{max} has been found to possess a lower optimum temperature than V_{cmax} (Dreyer *et al*. 2001), and this may explain the imbalance between the two processes when growth temperature changes, resulting in changes in the J_{max}/V_{cmax} ratio (Farquhar and von Caemmerer 1982; Onoda *et al*. 2005). However, a change in the J_{max}/V_{cmax} ratio does not always indicate an acclimation response as it may be simply due to the differences in the short term responses of J_{max} and V_{cmax} to temperature. Regardless of the factors involved in temperature acclimation of photosynthesis, Onoda *et al*. (2005) posed the hypothesis that some species (i.e. pines) may have photosynthesis that is limited by RuBP carboxylation over a wide range of temperatures, but does not have plasticity in the J_{max}/V_{cmax} ratio. This may have the advantage of incurring less cost in reconfiguration of the photosynthetic apparatus with temperature acclimation.

Respiratory responses to changes in temperature

In the present study, our findings show that respiration in *P. radiata* is strongly temperature sensitive in the short term (increasing approximately exponentially with temperature), but generally shows compensatory adjustments (acclimation) over a longer period (weeks and months). These findings are consistent with previous findings for a range of largely non-tree species (Larigauderie and Korner 1995; Atkin *et al*. 2000; Atkin and Tjoelker 2003; Wright *et al*. 2006). Cold transferred plants (Fig. 4*a*; 20–10) displayed a significant increase in rates of R_d in new needles only, and warm transferred plants (Fig. 4*a*; 15–20

Table 2. Enzymatic capacity of the cytochrome (COX) and alternative oxidase (AOX) pathways as a percentage of total respiratory capacity in needles of *Pinus radiata* plants exposed to both increasing and decreasing temperatures

Measurements were made in pre-existing needles at the initial temperature and 7-days after transfer to the new temperature and in new needles at the new temperature. Plants were grown under low and high N availability (see Table 1 for leaf N contents). Values shown are means (± s.e. of the mean, s.e.m.) where $n = 4$. Different letters within rows indicate statistically different values at $P < 0.05$ using least significant difference test of treatment means

Temperature transfer	Cytochrome pathway capacity (% of total respiratory capacity)			Alternative oxidase pathway capacity (% of total respiratory capacity)		
	Existing leaves	Existing leaves after transfer	New leaves	Existing leaves	Existing leaves after transfer	New leaves
20 to 10°C						
Low N	102 (12)^{a}	75 (13)^{ab}	57 (8)^{b}	22 (6)^{b}	45 (10)^{a}	44 (11)^{a}
High N	98 (16)^{a}	60 (12)^{b}	50 (11)^{b}	27 (7)^{b}	48 (10)^{a}	53 (13)^{a}
10 to 15°C						
Low N	91 (12)^{a}	91 (18)^{a}	102 (19)^{a}	16 (3)^{b}	40 (8)^{a}	16 (4)^{b}
High N	85 (18)^{a}	81 (16)^{a}	79 (19)^{a}	19 (5)^{b}	41 (8)^{a}	38 (6)^{a}
15 to 20°C						
Low N	89 (20)^{a}	81 (15)^{a}	86 (17)^{a}	17 (5)^{b}	29 (7)^{a}	15 (4)^{b}
High N	95 (19)^{a}	93 (11)^{a}	91 (14)^{a}	19 (5)^{a}	31 (6)^{a}	27 (5)^{a}

and 20–25) exhibited a significant decrease in rates in new needles. Rates of respiration at growth temperatures were greater than predicted by the instantaneous response in cold acclimated needles, and less than predicted by the instantaneous response in warm acclimated needles. As a result, the long-term (acclimated) response of R_d in pre-existing and new needles was much less pronounced than the instantaneous response (Fig. 3*a*–*c*). This was especially evident in new needles, with near identical rates of respiration at contrasting temperatures, indicating full respiratory homeostasis over a 10°C temperature range (Fig. 3*c*). Similar to earlier conclusions (Ow *et al*. 2008), data presented in Fig. 3 shows that the instantaneous response is a poor predictor of long-term acclimated responses of respiration to changes in temperature. Accordingly we concur with Atkin and Tjoelker (2003) and Atkin *et al*. (2005*a*) that short-term temperature response curves may be poor indicators of long-term respiratory CO_2 release and therefore should only be used with caution.

Our findings clearly suggest that acclimation potential may be limited in pre-existing needles in *P. radiata*, and that full acclimation may only be achieved in new needles grown under the new temperature. Moreover, this limitation is exhibited in both warm- and cold-transferred plants. The majority of studies investigating acclimation of leaf respiration have investigated plants with short-lived leaves (Loveys *et al*. 2003; Armstrong *et al*. 2006*a*, 2006*b*). These studies have concluded that acclimation is developmentally dependent (i.e. new leaves are required to develop at the new growth temperature for full acclimation of dark respiration). There are, however, remarkably few supporting studies involving tree species, and even fewer involving conifers. Interestingly, our findings differ from those by Bolstad *et al*. (2003), who found a high degree of thermal acclimation in dark respiration in fully expanded, pre-existing leaves of deciduous species such as *Quercus alba* and *Quercus rubra*. Other studies have also reported evidence of substantial acclimation in leaves of other long-lived species (Bolstad *et al*. 1999, 2001; Atkin *et al*. 2000*b*). Such studies have proposed that full acclimation can occur in pre-existing leaves, but only if the tissues are sufficiently long-lived to allow for sufficient time for cellular and biochemical changes to take place within pre-existing leaves. This is often absent from short-lived species as the changes cannot take place due to senescence.

Although there is strong evidence from previous studies showing that tissue N content clearly regulates rates of respiration, it had little or no impact on the extent of thermal acclimation in this present study (at least within the range of N concentrations experienced here). This was further confirmed in a recent study we conducted on poplar (Ow *et al.* 2008). Here, respiratory acclimation was achieved, in part, through changes in S (i.e. the amount of metabolising tissue per unit area). This highlights the importance of leaf structural changes to thermal acclimation. However, despite significant changes in S following transfer to a new temperature, the response of R_d in pre-existing leaves was limited. This leads us to suggest that physiological changes are largely determined at the time of leaf development and expansion, and involve alteration of structure (anatomy/morphology), enzymatic capacity and chemical composition (Atkin *et al*. 2006*a*). This is important for our understanding of respiratory responses of trees under conditions of varying soil nutrition.

Although respiration responds to temperature on both short-and long-term time scales, the Q_{10} of respiration describes the short-term sensitivity of respiration to temperature (i.e. seconds–hours). In this present study, despite changes in R_d with cold transfer, the Q_{10} of leaf respiration did not change significantly. However, warm transfers led to a significant decrease in Q_{10} values (Fig. 4*b*; 10–15 and 15–20). This is consistent with previous studies which have shown that the Q_{10} of respiration often decreases with increasing temperature (Tjoelker *et al*. 2001). The relative responses of R_{10} and Q_{10} in pre-existing and new needles in this study allow us to assess the extent to which acclimation involves Type I and Type II mechanisms (Atkin and Tjoelker 2003). Type I acclimation

(changes in respiration at moderate to higher temperatures) corresponds well with our findings for pre-existing needles. By contrast, new needles clearly displayed characteristics of Type II acclimation (change in respiration at high and low temperatures), which is often associated with temperature–mediated changes in respiratory capacity achieved through the development of new tissues with altered anatomy and morphology (Atkin and Tjoelker 2003). Analysis of enzymatic activity of the COX and AOX pathways (Table 2) supports the notion that Type II acclimation may result in changes in the relative amounts of enzymes or proteins invested into the respiratory chain (Atkin *et al*. 2005*a*). This was especially evident in cold transferred plants, where COX activity declined whilst AOX activity increased significantly. This switching between the AOX and COX pathways is well documented in a range of species such as *Alocasia odora*, *Spinacia oleracea*, *Zea mays* and *Phaseolus vulgaris* (Ribas-Carbo *et al*. 2000; Nagel *et al*. 2001; Nogochi *et al*. 2001) and have been confirmed in our recent study involving poplar (Ow *et al*. 2008). Furthermore, the AOX pathway is highly sensitive to changes in temperature and engagement of this pathway may have advantages for plant acclimation to colder temperature, whereby energy that should otherwise be conserved as ATP is converted to heat (Ribas-Carbo *et al*. 2000). The nature of our measurements (using inhibitors of COX and AOX) only allow us to draw conclusions regarding enzymatic activity and not actual *in vivo* capacity. However, the findings here should still prove a useful directive to future work aimed at understanding enzymatic changes that accompany respiratory acclimation in changing environments.

Balance between photosynthesis and respiration

Given the tight coupling that exists between photosynthetic and respiratory metabolism (Atkin *et al*. 2006) but the different temperature sensitivities of the two processes, a change in the respiration/photosynthesis ratio can occur following a change in growth temperature. Although the R_d/A ratio varied with cold and warm transfers (Fig. 4*c*), in the long term we observed a strong negative relationship with temperature in new needles (i.e. the ratio was greater at low temperature and decreased at high temperatures). The ratio increased at low temperature due to increasing dark respiration (a result of acclimation) and simultaneously decreasing photosynthesis, although the lower ratio resulted from lower R_d (a result of acclimation) but greater photosynthesis at higher temperatures. These results suggest that as temperatures increase over the moderate range of temperatures in this study, CO_2 uptake in *P. radiata* will be favoured over CO_2 release. The overall impact of this would be a reduction in the potential for positive feedback of respiration in the carbon cycle as temperature increases, in contrast to previous suggestions that the opposite might be the case (Cox *et al*. 2000; Houghton *et al*. 2001; Luo *et al*. 2001). At present, most climate C cycle models assume that rates of photosynthesis and respiration will increase with increasing temperature in a predictable way that will remain constant over time [i.e. they will not acclimate to changes in temperature (Rustad 2001)]. The result of this may be an overestimate of the effects of global warming on respiratory CO_2 release over long periods, particularly in models that assume a positive feedback of global warming on rates of respiration.

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References

Armstrong AF, Logan DC, Atkin OK (2006*a*) On the developmental dependence of leaf respiration: responses to short- and long-term changes in growth temperature. *American Journal of Botany* **93**, 1633–1639. doi: 10.3732/ajb.93.11.1633

Armstrong AF, Logan DC, Tobin AK, O'Toole P, Atkin OK (2006*b*) Heterogeneity of plant mitochondrial responses underpinning respiratory acclimation to the cold in *Arabidopsis thaliana* leaves. *Plant, Cell & Environment* **29**, 940–949. doi: 10.1111/j.1365-3040.2005.01475.x

Atkin OK, Tjoelker MG (2003) Thermal acclimation and the dynamic response of plant respiration to temperature. *Trends in Plant Science* **8**, 343–351. doi: 10.1016/S1360-1385(03)00136-5

Atkin OK, Edwards EJ, Loveys BR (2000) Response of root respiration to changes in temperature and its relevance to global warming. *New Phytologist* **147**, 141–154. doi: 10.1046/j.1469-8137.2000.00683.x

Atkin OK, Evans JR, Ball MC, Lambers H, Pons TL (2000*a*) Leaf respiration of snow gum in the light and dark. Interactions between temperature and irradiance. *Plant Physiology* **122**, 915–923. doi: 10.1104/pp.122.3.915

Atkin OK, Holly C, Ball MC (2000*b*) Acclimation of snowgum (*Eucalyptus pauciflora*) leaf respiration to seasonal and diurnal variations in temperature, the importance of changes in the capacity and temperature sensitivity of respiration. *Plant, Cell & Environment* **23**, 15–26. doi: 10.1046/j.1365-3040.2000.00511.x

Atkin OK, Bruhn D, Hurry VM, Tjoelker MG (2005*a*) *Evans Review No. 2*: the hot and the cold: unravelling the variable response of plant respiration to temperature. *Functional Plant Biology* **32**, 87–105. doi: 10.1071/FP03176

Atkin OK, Bruhn D, Tjoelker MG (2005*b*) Response of plant respiration to changes in temperature: mechanisms and consequences of variations in Q_{10} values and acclimation. In 'Plant respiration'. (Eds H Lambers, M Ribas-Carbo) pp. 95–135. (Springer: Netherlands)

Atkin OK, Loveys BR, Atkinson LJ, Pons TL (2006) Phenotypic plasticity and growth temperature: understanding interspecific variability. *Journal of Experimental Botany* **57**, 267–281. doi: 10.1093/jxb/erj029

Atkin OK, Scheurwater I, Pons TL (2006*a*) High thermal acclimation potential of both photosynthesis and respiration in two lowland *Plantago* species in contrast to an alpine congeneric. *Global Change Biology* **12**, 500–515. doi: 10.1111/j.1365-2486.2006.01114.x

Atkinson LJ, Hellicar MA, Fitter AH, Atkin OK (2007) Impact of temperature on the relationship between respiration and nitrogen concentration in roots: an analysis of scaling relationships, Q_{10} values and thermal acclimation ratios. *New Phytologist* **173**, 110–120. doi: 10.1111/j.1469-8137.2006.01891.x

Bernacchi CJ, Pimentel C, Long SP (2003) *In vivo* temperature response functions of parameters required to model RuBP-limited photosynthesis. *Plant, Cell & Environment* **26**, 1419–1430. doi: 10.1046/j.0016-8025.2003.01050.x

Berry J, Bjorkman O (1980) Photosynthetic response and adaptation to temperature in higher plants. *Annual Review of Plant Physiology* **31**, 491–543. doi: 10.1146/annurev.pp.31.060180.002423

Bolstad PV, Mitchell K, Vose JM (1999) Foliar temperature-respiration response functions for broad-leaved tree species in the southern Appalachians. *Tree Physiology* **19**, 871–878.

Bolstad PV, Vose JM, McNulty SG (2001) Forest productivity, leaf area, and terrain in southern Appalachian deciduous forests. *Forest Science* **47**, 419–427.

Bolstad PV, Reich P, Lee T (2003) Rapid temperature acclimation of leaf respiration rates in *Quercus alba* and *Quercus rubra*. *Tree Physiology* **23**, 969–976.

Bunce JA (2000) Acclimation of photosynthesis to temperature in eight cool and warm climate herbaceous C_3 species: temperature dependence of parameters of a biochemical photosynthesis model. *Photosynthesis Research* **63**, 59–67. doi: 10.1023/A:1006325724086

Cox PM, Betts RA, Jones CD, Spall SA, Totterdell IJ (2000) Acceleration of global warming due to carbon-cycle feedbacks in coupled climate model. *Nature* **408**, 184–187. doi: 10.1038/35041539

Delieu T, Walker DA (1981) Polarographic measurement of photosynthetic oxygen evolution by leaf discs. *The New Phytologist* **89**, 165–178. doi: 10.1111/j.1469-8137.1981.tb07480.x

Dreyer E, Le Roux X, Montpied P, Daudet FA, Mason F (2001) Temperature response of leaf photosynthetic capacity in seedlings from seven temperate tree species. *Tree Physiology* **21**, 223–232.

Farquhar GD, von Caemmerer S (1982) 'Modelling of photosynthetic responses to environmental conditions. In 'Physiological plant ecology. II. Encyclopedia of plant physiology. New series'. pp. 549–587. (Springer-Verlag: Berlin)

Farquhar GD, von Caemmerer S, Berry JA (1980) A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. *Planta* **149**, 78–90.

Field C (1983) Allocating leaf nitrogen for the maximization of carbon gain: leaf age as a control on the allocation program. *Oecologia* **56**, 341–347. doi: 10.1007/BF00379710

Field CB, Matson PA, Mooney HA (1992) Responses of terrestrial ecosystems to a changing atmosphere. A resource-based approach. *Annual Review of Ecology and Systematics* **23**, 201–235. doi: 10.1146/annurev.es.23.110192.001221

Gifford RM (1995) Whole plant respiration and photosynthesis of wheat under increased CO_2 concentration and temperature: long-term vs. short-term distinctions for modelling. *Global Change Biology* **1**, 385–396. doi: 10.1111/j.1365-2486.1995.tb00037.x

Gifford RM (2003) Plant respiration in productivity models: conceptualisation, representation and issues for global terrestrial carbon-cycle research. *Functional Plant Biology* **30**, 171–186. doi: 10.1071/FP02083

Hikosaka K (1997) Modelling optimal temperature acclimation of the photosynthetic apparatus in C_3 plants with respect to nitrogen use. *Annals of Botany* **80**, 721–730. doi: 10.1006/anbo.1997.0512

Houghton JT, Ding Y, Griggs DJ (2001) 'Climate change 2001: the scientific basis.' (Cambridge University Press: Cambridge)

King AW, Gunderson CA, Post WM, Weston DJ, Wullschleger SD (2006) Plant respiration in a warmer world. *Science* **312**, 536–537. doi: 10.1126/science.1114166

Krapp A, Stitt M (1995) An evaluation of direct and indirect mechanisms for the 'sink regulation' of photosynthesis in spinach: changes in gas exchange, carbohydrates, metabolites, enzyme activities and steady-state transcript levels after cold-girdling source leaves. *Planta* **195**, 313–323. doi: 10.1007/BF00202587

Lambers H, Ribas-Carbo M (2005) (Eds) 'Plant respiration: from cell to ecosystem. Respiration in photosynthetic cells: gas exchange components, interactions with photorespiration and the operation of mitochondria in the light.' (Springer: Netherlands)

Larigauderie A, Korner C (1995) Acclimation of leaf dark respiration to temperature in alpine and lowland plant species. *Annals of Botany* **76**, 245–252. doi: 10.1006/anbo.1995.1093

Loveys BR, Atkinson LJ, Sherlock DJ, Roberts RL, Fitter AH, Atkin OK (2003) Thermal acclimation of leaf and root respiration: an investigation comparing inherently fast- and slow-growing plant species. *Global Change Biology* **9**, 895–910. doi: 10.1046/j.1365-2486.2003.00611.x

Luo Y, Wan S, Hui D (2001) Acclimatization of soil respiration to warming in a tall grass prairie. *Nature* **413**, 624–625.

Martindale W, Leegood RC (1997) Acclimation of photosynthesis to low temperatures in *Spinacia oleraceae* L. Effects of nitrogen supply. *Journal of Experimental Botany* **48**, 1873–1880.

McCutchan C, Monson R (2001) Effects of tissue-type and development on dark respiration in two herbaceous perennials. *Annals of Botany* **87**, 355–364. doi: 10.1006/anbo.2000.1341

Medlyn BE, Dreyer E, Ellsworth D, Forstreuter M, Harley P *et al.* (2002) Temperature response of parameters of a biochemically based model of photosynthesis. II. A review of experimental data. *Plant, Cell & Environment* **25**, 1167–1179. doi: 10.1046/j.1365-3040.2002.00891.x

Morison JIL, Morecroft MD (2006) (Eds) 'Plant growth and climate change. Significance of temperature in plant life.' (Blackwell Publishing: New York)

Nagel OW, Waldron S, Jones HG (2001) An off-line implementation of the stable isotope technique for measurements of alternative respiratory pathway activities. *Plant Physiology* **127**, 1279–1286. doi: 10.1104/pp.127.3.1279

Nogochi K, Go CS, Terashima I, Ueda S, Yoshinari T (2001) Activities of the cyanide-resistant respiratory pathway in leaves of sun and shade species. *Australian Journal of Plant Physiology* **28**, 27–35.

Onoda Y, Hikosaka K, Hirose T (2005) The balance between RuBP carboxylation and RuBP regeneration: a mechanism underlying the interspecific variation in acclimation of photosynthesis to seasonal change in temperature. *Functional Plant Biology* **32**, 903–910. doi: 10.1071/FP05024

Ow LF, Griffin KL, Whitehead D, Walcroft AS, Turnbull MH (2008) Thermal acclimation of leaf respiration but not photosynthesis in *Populus deltoides* × *nigra*. *New Phytologist* **178**, 123–134. doi: 10.1111/j.1469-8137.2007.02357.x

Pons TL, Welschen RAM (2002) Overestimation of respiration rates in commercially available clamp-on leaf chambers. Complications with measurement of net photosynthesis. *Plant, Cell & Environment* **25**, 1367–1372. doi: 10.1046/j.1365-3040.2002.00911.x

Reich PB, Walters MB, Ellsworth DS (1991) Leaf age and season influence the relationships between leaf nitrogen, leaf mass per area and in maple and oak trees. *Plant, Cell & Environment* **14**, 251–259. doi: 10.1111/j.1365-3040.1991.tb01499.x

Reich PB, Walters MB, Ellsworth DS, Vose JM, Volin JC, Gresham C, Bowman WD (1998) Relationships of leaf dark respiration to leaf nitrogen, specific leaf area and leaf life-span: a test across biomes and functional groups. *Oecologia* **114**, 471–482. doi: 10.1007/s004420050471

Reich PB, Tjoelker MG, Machado JL, Oleksyn J (2006) Universal scaling of respiratory metabolism, size and nitrogen in plants. *Nature* **439**, 457–461. doi: 10.1038/nature04282

Ribas-Carbo M, Ricardo A, Miquel A, Gonzalez M, Irigoyen JJ, Sanchez-Diaz M (2000) The electron partitioning between the cytochrome and alternative respiratory pathways during chilling recovery in two cultivars of maize differing in chilling sensitivity. *Plant Physiology* **122**, 199–204. doi: 10.1104/pp.122.1.199

Rook DA (1969) The influence of growing temperature on photosynthesis and respiration of *Pinus radiata* seedlings. *New Zealand Journal of Botany* **7**, 43–55.

Rustad L (2001) Matter of time on the prairie. *Nature* **413**, 578–579. doi: 10.1038/35098162

Ryan MG (1995) Foliar maintenance respiration of sub-alpine and boreal trees and shrubs in relation to nitrogen content. *Plant, Cell & Environment* **18**, 765–772. doi: 10.1111/j.1365-3040.1995.tb00579.x

Ryan MG, Linder S, Vose JM, Hubbard RM (1994) Dark respiration of pines. *Ecological Bulletins* **43**, 50–63.

Ryan MG, Hibberd RM, Pongracic S, Raison RJ, McMurtrie RM (1996) Foliage, fine root, woody tissues and stand respiration in *Pinus radiata* in relation to nitrogen status. *Tree Physiology* **16**, 333–343.

Teskey RO, Will RE (1999) Acclimation of loblolly pine (*Pinus taeda*) seedlings to high temperatures. *Tree Physiology* **19**, 519–525.

Thornley JHM, Johnson IR (2000) 'Plant and crop modelling: a mathematical approach to plant and crop physiology.' (The Blackburn Press: Caldwell, NJ)

Tjoelker MG, Oleksyn J, Reich PB (2001) Modelling respiration of vegetation: evidence for a general temperature-dependent Q_{10}. *Global Change Biology* **7**, 223–230. doi: 10.1046/j.1365-2486.2001.00397.x

Turnbull M, Tissue DT, Griffin KL, Richardson SJ, Peltzer DA, Whitehead D (2005) Respiration characteristics in temperate rainforest tree species differ along a long-term soil-development chronosequence. *Oecologia* **143**, 271–279. doi: 10.1007/s00442-004-1803-0

Turnbull MH, Tissue DT, Griffin KL, Rogers GND, Whitehead D (1998) Photosynthetic acclimation to long-term exposure to elevated CO_2 concentration in Pinus radiate D. Don. is related to age of needles. *Plant Cell Environment* **21**, 1019–1028.

Turnbull MH, Whitehead D, Tissue DT, Schuster WSF, Brown KJ, Engel VC, Griffin KL (2002) Photosynthetic characteristics in canopies of *Quercus prinus* and *Acer rubrum* differ in response to soil water availability. *Oecologia* **130**, 515–524. doi: 10.1007/s00442-001-0842-z

Turnbull MH, Tissue DT, Murthy R, Wang X, Sparrow AD, Griffin KL (2004) Nocturnal warming increases photosynthesis at elevated CO_2 partial pressure in *Populus deltoides*. *New Phytologist* **161**, 819–826. doi: 10.1111/j.1469-8137.2004.00994.x

Whitehead D, Griffin KL, Turnbull MH, Tissue DT, Engel VC, Brown KJ, Schuster WSF, Walcroft AS (2004) Response of total night-time respiration to differences in total daily photosynthesis for leaves in a *Quercus rubra* canopy: implications for modelling canopy CO_2 exchange. *Global Change Biology* **10**, 925–938. doi: 10.1111/j.1529-8817.2003.00739.x

Wright IJ, Reich PB, Atkin OK, Lusk CH, Tjoelker MG, Westoby M (2006) Irradiance, temperature and rainfall influence leaf dark respiration in woody plants: evidence from comparisons across 20 sites. *New Phytologist* **169**, 309–319. doi: 10.1111/j.1469-8137.2005.01590.x

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