Tree Stomatal Regulation and Water Use
in a Changing Climate

From Tropical to Boreal Ecosystems

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Front-page photo: from left to right, Flakaliden research site WTC (© Bengt-Olof Vigren),
Ruhande arboretum, Hawkesbury Forest Experiment WTC and Rwasave nursery (© Thomas
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The author performing gas exchange measurements on trees under fieldwork in Rwanda.

To planet Earth and all its nature

Abstract

Rising levels of atmospheric carbon dioxide concentration ($[CO_2]$) and temperature have the potential to alter stomatal behavior and tree water use, which has implications for forest hydrology and climate. Many models assume decreases in stomatal conductance (g_s) and plant water use under rising $[CO_2]$, which has been invoked as the causes for the positive global trend in river runoff over the past century. Plant water use is, however, also affected by changes in temperature, precipitation, land use and management and climate change-induced alterations in ecosystem structure. Still, there is no consensus about the contribution of different drivers to temporal trends of evapotranspiration (ET) and river runoff. There is great variation in stomatal and photosynthetic responses to $[CO_2]$ and temperature among plant species, and the factors controlling it are still poorly understood, in particular for boreal and tropical tree species.

This thesis investigated the effects of elevated $[CO_2]$ and temperature on the stomatal functioning, tree hydraulics, canopy leaf area and whole-tree water use of mature *Picea abies* and young *Eucalyptus globulus* trees grown in whole-tree chambers in boreal and temperate areas, respectively. In the boreal study, the tree-level experiment was complemented with data on historical trends and patterns in ET of large-scale boreal landscapes, using climate and runoff data from the past 50 years, in order to assess water-use responses to past climate change in Swedish boreal forests. The thesis also explored the temperature responses of photosynthesis as well as the taxonomic and functional controls of the large interspecific variation in stomatal CO_2 responsiveness and photosynthetic capacity in a broad range of tropical woody species.

Results demonstrated that neither mature *P. abies* nor young *E. globulus* saved water under elevated $[CO_2]$, and that warming did not increase their transpiration as decreased g_s cancelled the effect of higher vapour pressure deficit in warmed air. Also, Swedish boreal ET increased over the past 50 years while runoff did not significantly change, with the increase in ET being related to increasing precipitation and forest standing biomass over time. In *E. globulus*, neither elevated $[CO_2]$ nor warming treatment affected g_s, stomatal density or length, or leaf area-specific plant hydraulic conductance. Furthermore, elevated $[CO_2]$ increased both total canopy leaf area and tree water use, while warming did not have any significant influence on either of these variables. In the tropical studies, the optimum temperature for the maximum rate of photosynthetic electron transport (J_{max}) was lower in the native than in the exotic species. The daytime peak leaf temperatures greatly exceeded (by up to 10 °C) the photosynthetic optimum temperatures, in particular in the native montane rainforest species. Lastly, all studied plant taxonomic groups exhibited stomatal closure responses to increased $[CO_2]$, but none of the functional characteristics investigated could explain the variation in stomatal CO_2 responses among tropical woody species. The interspecific variation in photosynthetic capacity was related to within leaf nitrogen allocation rather than to area-based total leaf nutrient content.

The findings of this thesis have important implications for the projections of future water use of forests, showing that changes in tree structural responses (e.g. size, canopy leaf area and hydraulics) are more important than the effects of elevated $[CO_2]$ or warming on leaf transpiration rates. The lack of reductions in g_s under elevated $[CO_2]$ in *P. abies* and *E. globulus* conflicts with the present expectation and model assumption of substantial leaf-level water savings under rising CO_2. In the tropical biome, the evidence of pronounced negative effects of high temperature on the photosynthesis of native montane tree species indicates high susceptibility of these ecosystems to global warming. Furthermore, the results on stomatal and photosynthetic responses in a broad range of tropical species contribute with important data for this comparatively poorly researched biome.

Keywords: Climate change, carbon dioxide, temperature, transpiration, water use, whole-tree chamber, stomata, stomatal conductance, V_{cmax}, J_{max}, tropical, temperate, boreal, trees

List of Papers

This thesis is based in the following papers, referred in the text by roman numerals as follows:

I. **Hasper TB**, Wallin G, Lamba S, Hall M, Jaramillo F, Laudon H, Linder S, Medhurst J, Sigurdsson B, Räntfors M, Uddling J (2015) Water use by Swedish boreal forests in a changing climate. *Functional Ecology,* doi: 10.1111/1365-2435.12546

II. **Hasper TB**, Barton CVM, Crous KY, Quentin AG, Ellsworth DS, Uddling J. Stomatal and water-use responses of *Eucalyptus globulus* to elevated CO_2 and warming. *Manuscript*.

III. Vårhammar A, Wallin G, McLean CM, Dusenge EM, Medlyn BE, **Hasper TB**, Nsabimana D, Uddling J (2015) Photosynthetic temperature responses of tree species in Rwanda: evidence of pronounced negative effects of high temperature in montane rainforest climax species. *New Phytologist*, 206, 1000-1012.

IV. **Hasper TB**, Dusenge EM, Breuer F, Uwizeye FK, Wallin G, Uddling J. Stomatal CO_2 responsiveness and photosynthetic capacity of tropical woody species in relation to phylogeny and functional traits. *Submitted to Oecologia*.

The papers and their respective supplementary material are appended in the end of the thesis and are reproduced with permission from the respective journals.

Abbreviations and Symbols

A_n Net photosynthetic rate (μmol CO_2 m^{-2} s^{-1})
C_A Ambient $[CO_2]$ treatment
C_E Elevated $[CO_2]$ treatment
C_i Intercellular CO_2 concentration (μmol mol^{-1})
CO_2 Carbon dioxide
$[CO_2]$ Carbon dioxide concentration (μmol mol^{-1})
D or VPD   Vapour pressure deficit (kPa)
g_s Stomatal conductance (mol H_2O m^{-2} s^{-1})
g_1 Empirical slope parameter of the combined stomatal–photosynthesis model
              (Medlyn et al. 2011)
g_{smax} Maximum stomatal conductance determined from stomatal density and
              length data (mol H_2O m^{-2} s^{-1})
J_{max} Maximum rate of photosynthetic electron transport (μmol m^{-2} s^{-1})
K_L Leaf area-specific plant hydraulic conductance (mmol m^{-2} s^{-1} MPa^{-1})
LAI   Leaf area index (m^2 canopy leaf area m^{-2} ground area)
LMA   Leaf mass per unit area (g m^{-2})
N   Nitrogen
N_a Nitrogen content per unit leaf area (g m^{-2})
N_m Nitrogen concentration of dry mass (%)
NEP   Net ecosystem production
P   Phosphorus
P_a Phosphorus content per unit leaf area (g m^{-2})
P_m Phosphorus concentration of dry mass (%)
T_A Ambient temperature treatment
T_E Elevated temperature treatment
V_{cmax} Maximum rate of photosynthetic carboxylation (μmol m^{-2} s^{-1})
VPD or D Vapour pressure deficit (kPa)
WUE   Water Use Efficiency (mmol CO_2 mol H_2O^{-1})
ET   Evapotranspiration
ψ_L Leaf water potential (MPa)
ψ_S Soil water potential (MPa)
FACE   Free-air concentration enrichment
WTC   Whole-tree chamber

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1 Introduction

1.1 Climate Change

Atmospheric carbon dioxide concentrations ($[CO_2]$) and temperature have been in continuous change throughout Earth's history. In the last 450 000 years, several cycles of glacial advances and retreats have naturally changed temperatures, $[CO_2]$ and consequently the climate (Fig. 1). Most of these changes were naturally attributed to small variations in Earth's orbit that change the amount of solar energy our planet received. Since the end of the last ice age (ca. 7 000 years ago), temperatures and $[CO_2]$ have been increasing. Adding to that, since the beginning of the Industrial Revolution (1750; Fig. 1), anthropogenic emissions of greenhouse gases and land-use change have been further increasing $[CO_2]$ and temperatures at speeds never recorded before. Emissions of greenhouse gases such as CO_2, but also methane, nitrous oxide and tropospheric ozone, are largely driven by anthropogenic activities, and are the main drivers of the observed warming since 1950 (IPCC 2014a). Since then,

climate change has caused impacts on natural and human systems all around the planet (IPCC 2014b). Continued emission of greenhouse gases will cause further warming and long-lasting changes in all climatic system components, increasing the impacts on Earth's ecosystems.

1.2 Forests and climate

Burning of fossil fuels and anthropogenic land-use change have raised $[CO_2]$ by over 40%, from the pre-industrial 280 µmol mol^{-1} to the current 400 µmol mol^{-1} (Fig. 1; Ciais et al. 2013; Dlugokencky and Tans 2015). Most recent projections estimate that $[CO_2]$ will rise to between 450 and 900 µmol mol^{-1} by the year 2100 (IPCC 2014a), likely reaching concentrations not seen in the past 40 million years (Franks et al. 2013). The rise in atmospheric greenhouse gases concentrations, mainly CO_2 and methane, has already caused an increase of 0.7 °C in global mean temperature since 1900, and recent projections
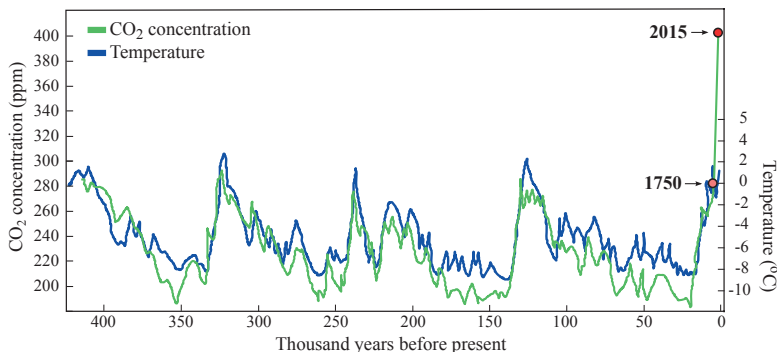

Figure 1. Vostok ice core data for atmospheric $[CO_2]$ and temperature for the last 400 000 years. Temperature changes are relative to the 1960-1990 mean (modified from Petit et al. 1999).

shows that mean global temperatures will further increase by 1 to 4.5 °C by the end of the century (IPCC 2014a). There is also a distinctive pattern of latitudinal and seasonal variation in warming, with the largest temperature increase being expected at higher-latitude regions and winter months. Consequently, Arctic regions might be facing warming of up to 5.5 °C in the summer and 10 °C in the winter (IPCC 2014a).

Forests feedbacks on climate

Worldwide, forests cover ca. 42 million km^2 in tropical, temperate and boreal regions, representing ca. 30% of the world's land surface (Fig. 2 and 3). They store ca. 45% of the terrestrial carbon (Fig. 2) and contribute with ca. 50% of the terrestrial net primary production (Sabine et al. 2004). However, these figures tend to change depending on the study (e.g. Bonan et al. 2008; Pan et al. 2011; Ciais et al. 2013). Forests provide innumerous services to natural systems and mankind (Hassan et al. 2005). Among them, the most important are biodiversity refuges, food, medicines, forest products, soil protection, hydrological cycle and climate regulation (Bonan 2008). Forests influence global, regional and local climate by exchanging energy, water, CO_2 and other greenhouse gases with the atmosphere.

Figure 2. Land area coverage and total carbon stock (Soil <1m depth, live and dead vegetation, and litter) of the different forest types (Modified from Bonan 2008 and Pan et al. 2011, respectively).

World's forests have not only important feedbacks on climate but also on the rate of ongoing climate change (Bonan 2008). Of the CO_2 currently released from anthropogenic sources, ca. 85% (7.7 PgC y^{-1}) comes from burning of fossil fuels, while ca. 15% (1.4 PgC y^{-1}) comes from deforestation. From the total CO_2 released, ca. 45% (4.1 PgC y^{-1}) stays in the atmosphere, while oceans and forests are able to absorb ca. 26% (2.3 PgC y^{-1}) and 29% (3.0 PgC y^{-1}) respectively (Le Quéré et al. 2009). Forests are thus important net carbon sinks, and remove more carbon from the atmosphere by photosynthesis then they release via respiration and deforestation (Le Quéré et al. 2009; Reich 2011). Forests can also strengthen or mitigate anthropogenic climate change through effects on land albedo and evapotranspiration (ET), depending on the effects of reforestation, afforestation or altered forest composition and structure on these biophysical climate feedbacks in different areas (Bonan 2008).

Tropical forests contain ca. 55% of the carbon in the terrestrial biosphere (Fig. 2) and ca. 50% of global forest net ecosystem production (NEP; Luyssaert et al. 2007). Tropical forest tree species are in general more vulnerable to climate warming and reduction in precipitation than species from other forests biomes (Malhi et al. 2008). Global warming may thus decrease tropical forest productivity and maybe even initiate forest dieback, which may in turn intensify global warming through a positive feedback caused by decreases in evaporative cooling and diminishing forest carbon uptake (Betts et al. 2004). On the other hand, if afforestation and reforestation efforts are successful, forests will mitigate climate change by their high carbon sequestration capacity and evaporative cooling. However, the resilience of future forests to drought, fires, air pollution, and climate change are highly uncertain. Worldwide, loss of tropical forests by logging and clearing to give space to human
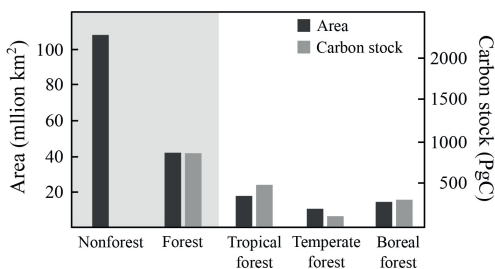
economic activities has been increasing in the last four decades (Malhi et al. 2008). Such land-use pressures are expected to continue into the future and may, together with climate change, turn the Amazonian region, the world's largest tropical forest, into a semi-arid area once a critical threshold of clearing is reached (Bonan 2008).

Temperate forests contain ca. 14% of the terrestrial carbon (Fig. 2) and ca. 35% of the global forest NEP (Luyssaert et al. 2007). Even if current carbon sequestration rates are high, historically, most of the natural temperate forest areas have been carbon sources due to intensive deforestation (Albani et al. 2006). However, in the last decades, socio-economic trends in reforestation and forest-fire control have changed these forests to a carbon sink (IPCC 2013). These forests are under strong pressure from human land use and much of them have already been cleared for agriculture (Bonan 2008). The future of temperate forests is uncertain since they face combined threats from climate change, CO_2 increase, nitrogen eutrophication and land use and management (IPCC 2014b).

In temperate forests, the climate feedbacks are less obvious than in tropical and boreal forests. Temperate forest reforestation and afforestation increase carbon sequestration, but the effects on albedo and evaporative forcings are moderate and likely counteract each other.

Boreal forests contain ca. 32% of the terrestrial carbon (Fig. 2) and ca. 15% of the global forest NEP (Luyssaert et al. 2007). These ecosystems make significant contribution to the Northern Hemisphere terrestrial carbon sink (Reich 2011; IPCC 2013). With global warming, the boreal forest may expand into northern-tundra areas. However it may decline along the southern ecotones, where evergreen trees lose habitat to deciduous-temperate adapted trees. The future boreal forests may also suffer from more frequent fires and insect attacks (Bonan 2008). Expansion or plantation of boreal forests on the tundra would likely strengthen climate warming since the albedo effect is usually stronger than the carbon sequestration effect (boreal forests have low albedo; Bala et al. 2007; Bonan 2008).
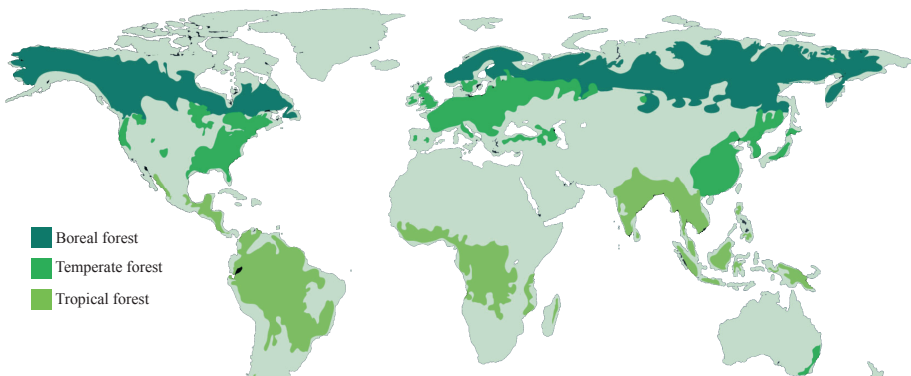

Figure 3. Distribution of Earth's natural tropical, temperate and boreal forests (adapted from illustration by Nicolle Rager Fuller, National Science Foundation).

1.3 Leaf-level responses to rising CO_2 and temperature

The ongoing increase in $[CO_2]$ and temperature have strong impacts on plant physiology, and many studies have investigated the responses of plant carbon and water fluxes to these environmental drives, principally on the leaf level and on relative short-time scales (minutes – months; Fig. 4).

While short-term, leaf-level responses to increased $[CO_2]$ and temperature are fairly well understood; the longer-term (years – decades) and larger-scale responses to these factors are still not so well investigated (Fig. 4). In addition, there is little knowledge on the combined CO_2 and warming responses at any temporal or spatial scale, and to date there have been no studies that looked at the responses to CO_2 and/or temperature at longer temporal scales (decades).

Short-term effects of CO_2

Overall, the direct effects of CO_2 on photosynthesis are well understood. Elevated $[CO_2]$ stimulates the carboxylation rate of Rubisco and suppress its oxygenation function and thus the rate of photorespiration (e.g. Drake et al. 1997). In carboxylation-limited conditions (e.g. high light and/or low internal $[CO_2]$), photosynthesis increases sharply with rising intracellular $[CO_2]$ (C_i) as substrate limitations are relieved. At higher C_i levels, the predominant limitation for CO_2 fixation is the regeneration of Ribulose 1,5 bisphosphate (RuBP) in the Calvin-Benson cycle, which is driven by ATP and NADPH from electron transport in the thylakoid membrane. Even with the 40% increase in $[CO_2]$ in these last 350 years (Fig. 1), plants with C3 photosynthesis (which account for 95% of the global plant species) still typically operate at a C_i that is within the carboxylation-limited phase under full daylight conditions. With this fact, we can expect that further increases in $[CO_2]$ will stimulate net photosynthesis (A_n) in trees, as observed in field experiments with elevated $[CO_2]$ (Ainsworth and Rogers 2007).

Partial stomatal closure, causing a decrease in stomatal conductance (g_s), is another
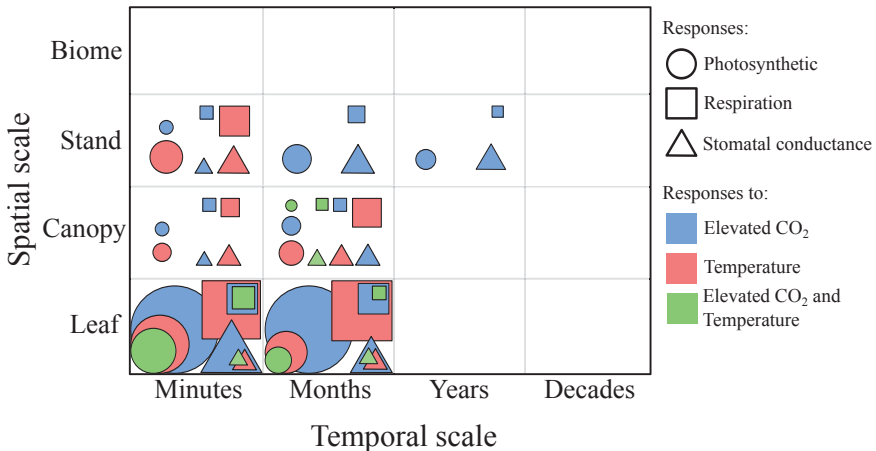

Figure 4. Conceptual diagram illustrating today's confidence in different types of tree physiological responses to elevated $[CO_2]$ and temperature, with higher confidence shown by larger-sized symbols (adapted from Way et al. 2015).

direct plant response to elevated [CO_2] in most plant species investigated (Morison 1985; Ainsworth and Rogers 2007). The direct effects of CO_2 on the pathways determining the stomatal movements are to some extent understood (Kim et al. 2010). However, there are still uncertainties on how stomata senses [CO_2] (Kim et al. 2010; Kollist et al. 2014). There is evidence that stomatal sensitivity to CO_2 vary among plant functionnal types, with angiosperms commonly presenting greater responses than gymnosperms (Medlyn et al. 1999; Brodribb et al. 2009). This greater sensitivity was recently attributed to a more pronounced Ca^{2+}-dependent stomatal signalling pathway in angiosperms (Brodribb and McAdam 2013).

Short-term effects of warming

Short-term photosynthetic temperature responses are usually characterized by a curve that peaks at intermediate temperatures, which depend on the growth environment (Fig. 5; Sage and Kubien 2007; Yamori et al. 2014). Temperature affects photosynthesis primarily by affecting enzyme functioning and membrane integrity and fluidity. All components of photosynthesis are influenced by temperature, including the maximum carboxylation rate by Rubisco (V_{cmax}), the Michaelis-Menten constants for carboxylation and oxygenation, the photosynthetic electron transport, and the CO_2 supply to the chloroplast via changes in g_s and mesophyll conductance (Bernacchi et al. 2001; Crous et al. 2013; Bernacchi et al. 2002; Lin et al. 2012). Leaves functioning below their thermal optimum will benefit from warming, while leaves frequently operating at or above its photosynthetic thermal optimum will suffer reductions in photosynthesis and carbon gain. So, depending on the initial temperature and its magnitude, warming can stimulate, suppress or have little effect on photosynthetic rates (Yamori et al. 2014).
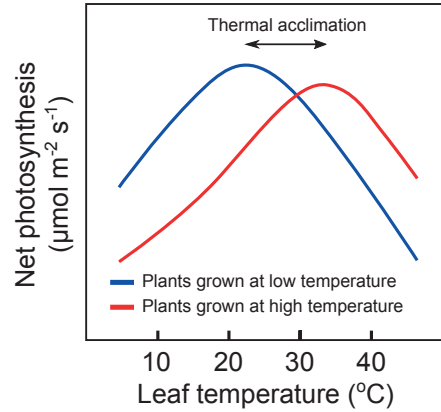

Figure 5. Thermal acclimation of the response of net photosynthesis to temperature (adapted from Yamori et al. 2014).

Generally, temperature is considered to have little direct effect on g_s, with studies only indicating a temperature effect via altered water viscosity (Fredeen and Sage 1999; Peak and Mott 2011; Rockwell et al. 2014). However, changes in temperatures are usually positively correlated with changes in vapour pressure deficit (VPD). Also, changes in VPD usually have direct effects on transpiration (Kupper et al. 2011). So, increased VPD generally causes a reduction in g_s, which may partially or fully compensate for the positive effect of VPD on transpiration. In so called 'isohydric' species (including many tree species) and at high levels of VPD, the effects of increasing VPD and decreasing g_s often offset each other to maintain transpiration at a relatively constant value (Oren et al. 1999). If this fails to happen, the leaf water supply may not be able to keep pace with the increase in transpiration, which in turn may cause xylem cavitation. At lower levels of VPD, however, VPD has a positive effect on transpiration.

Increased temperature and VPD will usually cause a decrease in water-use efficiency (WUE). However, predictions on how WUE

will be affected by changes in leaf temperature are more challenging than how it will be affected by increased [CO_2]. Changes in WUE will mainly depend on if photosynthesis will be stimulated, not affected or suppressed by warming. In addition, the WUE response will also depend on if g_s will be regulated to maintain a constant transpiration rate, or if transpiration will substantially increase in warmer conditions (Way et al. 2015).

Long-term effects of CO$_2$

In the long term, biochemical and structural acclimation of leaves to the new environmental conditions commonly reduce photosynthetic responses to elevated [CO_2] (Fig. 6a; Sage 1994; Medlyn et al. 1999; Ainsworth and Long 2005; Ainsworth and Rogers 2007). This down-regulation of the photosynthetic capacity may be caused by an increase in leaf carbohydrate production causing a shift in the balance between carbon sources and sinks in the tree (Moore et al. 1999). In addition, the degree of photosynthetic capacity down-regulation is linked to leaf nitrogen (N) content, which is typically

reduced in elevated [CO_2] (Ainsworth and Rodgers 2007). Field CO_2 experiments often found significant down-regulation of photosynthetic capacity because of reductions in the leaf N content (Sage 1994; Curtis and Wang 1998; Ellsworth et al. 2004). However, it should be noted that the down-regulation of photosynthetic capacity is usually not large enough to offset the positive effect of increased substrate availability on A_n (Fig. 6a).

Overall, leaves of plants grown in elevated [CO_2] had ca. 20% lower g_s than leaves grown in ambient conditions (Fig. 6b; Medlyn et al. 2001; Ainsworth and Rogers 2007). In general, the g_s response to elevated [CO_2] is typically stronger in broadleaf trees than in conifers (Medlyn et al. 2001; Ward et al. 2013). Furthermore, the long-term g_s response to increased [CO_2] seems to be similar to the short-term g_s response (Fig. 7), indicating that plant species that exhibit a pronounced direct stomatal closure response to a short-term increase in [CO_2] also develop a long-term g_s reduction. Usually, leaves that developed under increased [CO_2] exhibit a similar intracellular to ambient [CO_2] ratio
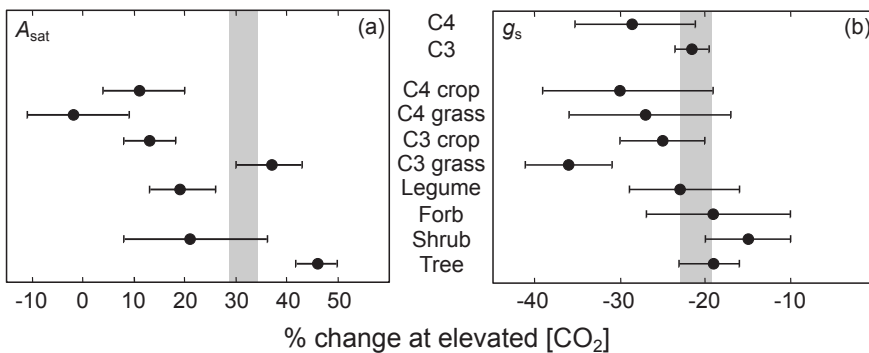

Figure 6. Responses of light-saturated CO_2 uptake (A_{sat}; a) and of g_s (b) to elevated [CO_2] in FACE experiments. Grey bars represent the overall mean and 95% confidence interval (CI) of all data. Dark symbols represent the mean response (95% CI) of C3 and C4 species and different plant functional groups (adapted from Ainsworth and Rogers 2007).
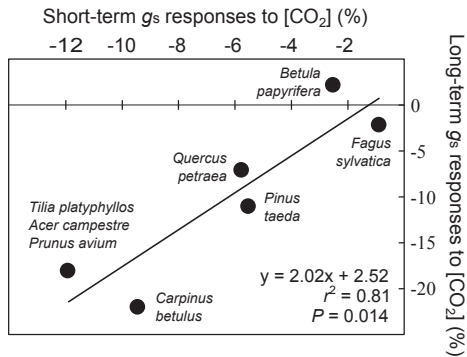
Short-term g_s responses to [CO_2] (%)

Long-term g_s responses to [CO_2] (%)

Betula papyrifera

Fagus sylvatica

Quercus petraea

Pinus taeda

Tilia platyphyllos
Acer campestre
Prunus avium

$y = 2.02x + 2.52$
$r^2 = 0.81$
$P = 0.014$

Carpinus betulus

Figure 7. Relationship between the short-term response of g_s to elevated [CO_2] and the long-term effect of growth under elevated [CO_2] on g_s in temperate forest free-air CO_2 enrichment (FACE) experiments. Regression statistics are shown in the figure. Based on data from Cech et al. (2003), Keel et al. (2007), Maier et al. (2008), Domec et al. 2009, Onandia et al. (2011) and Tor-ngern et al. (2015).

(C_i/C_a) as leaves developed in ambient [CO_2], an assumption that is also incorporated into models (Leuning 1995; Medlyn et al. 2011).

Long-term effects of warming

As for responses to increased [CO_2], short-term leaf responses to warming can also be modified to the new environmental conditions due to longer-term thermal acclimation (Sage and Kubien 2007; Yamori et al. 2014). Compared with the direct effect of short-term changes in leaf temperature, thermal acclimation can further stimulate or inhibit photosynthesis depending on the initial leaf temperature and the direction and magnitude of temperature change (Way and Yamori 2014). In general, however, thermal acclimation acts to maintain a homeostatic rate of carbon fixation at the typically prevailing growth temperature (e.g. Battaglia et al. 1996; Ghannoum et al. 2010). Long-term exposure to warmer environments frequently leads to an adjustment of the optimum leaf temperature for photosynthesis to a higher value (Fig. 5; Way et al. 2015; Kattge and Knorr 2007), while the values of V_{cmax} and

maximum rate of photosynthetic electron transport (J_{max}) at a standard leaf temperature (often 25 °C) have been found to be quite conservative (Way and Oren 2010). Overall, photosynthetic temperature acclimation to warming has been shown to vary among plant functional types, with evergreen tree species commonly being less capable to acclimate photosynthesis and to maintain homeostatic levels of carbon gain in response to warming compared to deciduous trees (Way and Yamori 2014; Yamori et al. 2014). In addition, temperature acclimation of photosynthesis is more likely to occur in younger leaves, due to new leaves greater capacity to adjust anatomical and structural characteristics to warmer growth conditions (Campbell et al. 2007). However, this is not always the case and several studies found no significant photosynthetic acclimation at all to warming (e.g. Warren 2008; Silim et al. 2010).

Studies of long-term acclimation of g_s to warming are rare (Way et al. 2015), and existing studies indicate limited effects of warming on g_s measured at a constant set of environmental conditions (e.g. Phillips et al. 2011; Lewis et al. 2013). However, exceptions exist and g_s (at standard measurement conditions) is sometimes reduced in trees grown in warmer temperatures (Mäenpää et al. 2011). Warming studies showed variable results on stomatal patterning, from decreased (e.g. Luomala et al. 2005) to unaltered (e.g. Hovenden 2001; Lewis et al. 2002) or increased stomatal density, stomatal index and stomatal aperture area in trees grown at increased temperature (e.g. Pandey et al. 2007; Sadras et al. 2012; Zheng et al. 2013).

1.4 Canopy-level responses to rising CO_2 and temperature

Effects of CO_2

The physiological responses at the leaf level lead to cumulative effects on plant structure

that may strengthen or dampen the initial effects of elevated $[CO_2]$ and warming on photosynthesis and transpiration. Results from long-term studies have shown that leaf area index (LAI) of trees is commonly increased by elevated $[CO_2]$ (McCarthy et al. 2007; Uddling et al. 2009; McCarthy et al. 2010; Norby et al. 2010). The strength of the stimuli is related to the initial LAI of the trees, and it is stronger in ecosystems where LAI is low (Palmroth et al. 2006). In addition, the degree to which LAI will increase under elevated $[CO_2]$ will be constrained by water and nutrient availability in the environment (Woodward 1990; McCarthy et al. 2007). Since mature leaves need to be self-sufficient for carbohydrates, the increase in LAI under elevated $[CO_2]$ is possible partially because of the decrease in the light compensation point (the light intensity at which the rate of photosynthesis equals the rate of respiration) of photosynthesis in elevated $[CO_2]$ (Way et al. 2015). As $[CO_2]$ increases, photorespiration is suppressed and leaves from lower canopy layers will get a more favorable carbon balance, causing increased LAI under elevated $[CO_2]$ (Hirose et al. 1997).

As elevated CO_2-induced leaf-level water-saving translate into reduced tree water use, this will lead to enhanced soil moisture and runoff on large geographical scales (e.g. Gedney et al. 2006; Betts et al. 2007; Cao et al. 2010). Studies claiming that stomatal responses to $[CO_2]$ explain temporal increases in global runoff during the last century (Gedney et al. 2006; Betts et al. 2007; Cao et al. 2010) conflict with studies suggesting that this increase was primarily caused by rising temperature and precipitation (Labat et al. 2004; Huntington 2008; Wisser et al. 2010; Alkama et al. 2011) or to a combination of these climatic effects and land-use changes (Piao et al. 2007; Raymond et al. 2008; Jaramillo and Destouni 2014). However, reductions in leaf-level g_s can be counterbalanced by higher LAI and changes in tree hydraulic functioning in higher $[CO_2]$ (Schäfer et al. 2002; Wullschleger et al. 2002; Domec et al. 2009; Leuzinger and Bader 2012). The forest water-use responses to increased $[CO_2]$ will also depend on possible changes in tree species composition (Warren et al. 2011).

The impact of $[CO_2]$ increase on tree WUE depends on the responses of A_n and transpiration at the canopy level. Eddy flux measurements in forests have shown a significant increase in WUE as $[CO_2]$ increased over the past 18 years (Keenan et al. 2013), agreeing with tree ring derived WUE estimates from different biomes based on stable carbon isotope methodology that showed an average 20% increase in WUE since 1960 (Peñuelas et al. 2011).

Effects of warming

The impacts of warming on canopy-level processes are much less studied than the impacts of increased $[CO_2]$, making the task to scale up from leaf to canopy level much more challenging. In general, warming has positive effects on tree species growth in cool climate and negative effects on tree species from warmer areas (Way and Oren 2010; Ghannoum and Way 2011). While in cool climates, tree water use may be stimulated by increased VPD and growing season prolongation, responses of plant growth and transpiration in warm climates will likely depend on the proximity of initial temperatures to upper thermal thresholds (Doughty and Goulden 2009; Crous et al. 2013) and constraints from soil water availability (Chung et al. 2013). In many experiments with seedlings, warming stimulates plant developmental rates and past studies typically report on positive effects of increased temperature on plant growth and canopy leaf area (e.g. Tjoelker et al. 1999; Way and Sage 2008; Way et al. 2013). However, warming-induced differences in canopy leaf area are likely considerably smaller in stands with

closed canopies because of within-canopy self-shading (Way et al. 2015).

Combined CO_2 and warming effects

Plants growing in a future warmer environment will also be growing in a CO_2-enriched world (Fig. 1). Compared with the number of studies manipulating just one of these factors, there are much fewer studies looking at the responses of trees to both [CO_2] increase and warming (Fig. 4). It was only in the last 20 years that these studies became more common, producing valuable data on the combined impact of [CO_2] and temperature on trees (e.g. Zha et al. 2005; Hall et al. 2009; Ghannoum et al. 2010; Phillips et al. 2011; Zeppel et al. 2012; Crous et al. 2013; Sigurdsson et al. 2013; Duan et al. 2014). At the leaf level, the combined effect of increased [CO_2] and temperature can interact in ways that either strengthens or cancel their independent effects (Long 1991; Sage and Kubien 2007; Lewis et al. 2013; Sigurdsson et al. 2013; Duan et al. 2014), with no clear patterns on when, where or why to expect a certain type of interaction. The performance of trees grown under both elevated [CO_2] and temperature will depend both on the balance between the individual responses to [CO_2] and temperature and on these poorly investigated interactions (Way et al. 2015).

1.5 Knowledge gaps

The great majority of studies have so far mostly looked at the effects of increased [CO_2] and temperature on plants at low temporal (minutes to months) and spatial (leaf) scales (Fig. 4). Therefore, there is an urgent need of data from longer experiments (years to decades) looking at larger spatial scales (canopy to stand), especially regarding warming responses. Since plants growing in a higher [CO_2] atmosphere will much likely also experience a warmer environment, it is also crucial that future experiments look at the combined impacts of these two global change factors. Factorial experiments on [CO_2] and temperature are few, mainly due to the elevated operational costs involved in these experiments. However, such studies are critical for better understanding and prediction of how plants and terrestrial ecosystems will respond to climate change. Furthermore, it is important that such experiments investigate how these climate change factors affect not only plant physiological processes but also integrated responses that are important for ecosystem functioning and ecosystem climate feedbacks (i.e. plant carbon and water balance, biomass magnitude and allocation, canopy structure and LAI). In addition, there is a critical need to better understand how [CO_2] increase and warming will affect tree species in understudied tropical and boreal ecosystems.

2 Aims and Hypotheses

The overall aim of this thesis was to increase the understanding of stomatal and water-use responses of ecologically and economically important boreal, temperate and tropical tree species to increased $[CO_2]$ and temperature. In addition, the specific papers of the thesis had the following aims and hypotheses:

Paper I

This study aimed to explore the climate change responses of boreal forest water use by using both experimental data and long-term monitoring.

The hypotheses were: (i) stomatal conductance will decreased under elevated $[CO_2]$; (ii) boreal trees and forests will save water under elevated $[CO_2]$; and (iii) boreal trees and forests will use more water under elevated temperature.

Paper II

This study aimed to improve the understandding of the effects of elevated $[CO_2]$ and warming on stomatal regulation and water use of young *Eucalyptus globulus* trees grown in a warm humid temperate area in southeast Australia.

The hypotheses were: (i) short- and long-term responses of g_s to elevated $[CO_2]$ are linked, such that the direct stomatal response translate into a similar long-term effect of growth in elevated $[CO_2]$ on g_s; (ii) long-term responses of g_s to experimental treatments are coordinated with the responses of stomatal patterning (density and size) and leaf area-specific hydraulic conductance; (iii) tree water use is decreased by elevated $[CO_2]$ and increased by warming, reflecting direct leaf-level responses; or (as an alternative to iii) (iv) changes in canopy leaf area compensate for possible changes in leaf-level water use for little net change in tree water use under elevated $[CO_2]$ and/or warming.

Paper III

This study aimed to improve the limited understanding of temperature responses of photosynthesis in tropical tree species by providing the first temperature response assessments of photosynthetic capacity (i.e. J_{max} and V_{cmax}) in tropical tree species. In addition, the study also explored the role of leaf energy balance in assessing high temperature sensitivity.

The hypotheses were: (i) J_{max} is more sensitive to high temperature than V_{cmax}, as has been found in temperate and boreal tree species; (ii) cold-adapted native montane rainforest species have lower photosynthetic optimum leaf temperature than warm-adapted exotic plantation species; and (iii) the optimum leaf temperatures of photosynthesis are commonly exceeded in the cool-adapted native montane tropical tree species but not in the warm-adapted exotic plantation species when grown in an intermediate temperature common garden.

Paper IV

This study aimed to improve the poor understanding of the taxonomic and functional controls of the large interspecific variation in stomatal CO_2 responsiveness and photosynthetic capacity (i.e. J_{max} and V_{cmax}) among tropical woody species by examining leaf physiological, chemical and structural traits in an evolutionary broad cross-section of mature woody seed plants in a tropical arboretum in Rwanda.

The hypotheses were: (i) different major taxonomical groups will have different short-term stomatal response to increased $[CO_2]$,

and differences will be particularly prono-unced between gymnosperms and angios-perm groups; (ii) the variation in stomatal behavior (i.e. short-term CO_2 response and the empirical slope parameter of the combi-ned stomatal–photosynthesis model, g_1) among groups can be linked to certain plant functional characteristics; and (iii) within-leaf nutrient allocation is more important than total area-based leaf nutrient content in controlling the interspecific variation in pho-tosynthetic capacity among tropical woody species.

3 Materials and Methods

The methodologies of all experiments are described in detail in each of the original studies on which this thesis is based. Here, however, a brief description of the sites at which the experiments were made is provided, as well as a description of the methodology used and measurements conducted in each study (Table 1).

3.1 Sites

Flakaliden Research site, Sweden (*Paper I*)

The Flakaliden Research site is located in northern Sweden (64°06′48″N 19°28′32″E) and it was established in a Norway spruce (*Picea abies*) stand (Fig. 8a) in 1963. Since the late 1980s, it has been used for several long-term manipulation experiments addressing questions regarding nutrient limitations and the possible impacts of climate change on the structure and function of boreal forests (Ryan 2013). Data used in **Paper I** are from the experiment that investigated the effects of elevated [CO_2] and/or temperature on ca. 40-year-old Norway spruce trees using 12 whole-tree chambers (WTC) (Medhurst et al. 2006). Detailed description of the experimental site, the design and performance of the WTC, and the biomass components of trees used in the experiment can be found in Medhurst et al. (2006) and Sigurdsson et al. (2006).

Central Swedish catchments (*Paper I*)

The four catchments, Dalälven, Ljusnan, Ljungan and Indalsälven are situated within the southern and middle boreal sub-zones (Fig. 9). Forests with Norway spruce and/or Scots pine (*Pinus sylvestris*) dominate all four catchments, covering 73% of the total area. Around 98% of the forested area is managed, with clear-cutting as the dominating harvest method and an approximate rotation length of 90 years. Grassland, lakes and wetlands cover 6, 7 and 8% of this area, respectively. The central region of Sweden, in which these four catchment areas are situated, is sparsely inhabited with the main urban areas found along the coast of Gulf of Bothnia. A detailed description of the catchments can be found in **Paper I**.

Svartberget/Krycklan Research Catchment, Sweden (*Paper I*)

The Svartberget/Krycklan Research Catchment site is located in northern Sweden (64°14′39″N, 19°45′58″E) in an area of 47 ha. It is dominated by mixed forest stands (82% of the total area), mostly consisting of Norway spruce and Scots pine (Buffam et al. 2007). This site is an old forest stand that has not been subject to forest management in the last century and provides a representative forest landscape hydrology of the area (Fig. 8b). A detailed description of the site can be found in Laudon et al. (2013).

Hawkesbury Forest Experiment, Australia (*Paper II*)

The Hawkesbury Forest Experiment site is situated in Richmond, Australia, (33°36′40″S, 150°44′26″E) in an area of 5 ha established in a paddock, which had been converted from native pasture grasses in the late 1990s (Fig. 8c). Data used in **Paper II** was from an experiment that investigated the effects of elevated [CO_2] and/or temperature on young *E. globulus* trees using the same 12 WTC that were used in the Flakaliden CO_2 and warming experiment. A detailed description of the site and the WTC characteristics and functionality can be found in Barton et al. (2010) and more information on the experimental trees and performance of the WTC were provided by Crous et al. (2013) and Quentin et al. (2015).

Figure 8. Photos of the sites where the studies in this thesis where conducted or data was obtained from. Flakaliden Research site (a); Svartberget/Krycklan Research Catchment (b); Hawkesbury Forest Experiment (c); Rwasave Nursery (d); and Ruhande Arboretum (e). Photos by Bengt-Olof Vigren (a); Hjalmar Laudon (b); and Thomas B. Hasper (c, d, e).

*Rwasave Nursery, Rwanda (**Paper III**)*

The Rwasave nursery is located on the edge of the Ruhande Arboretum in Butare, Rwanda (2°36′28″S, 29°45′28″E) and produces seedlings of native and exotic tree species for the Ruhande arboretum and farmers (Fig. 8d).

*Ruhande Arboretum (**Paper IV**)*

The Ruhande Arboretum is located adjacent to the University of Rwanda, Huye district, southwestern Rwanda (2°36′54″S, 29°44′53″E) and is managed by the Rwanda Agriculture Board. The arboretum was established in 1934 and since then has gathered 227 tree species (50 native to Rwanda) planted, in most part, as replicated monospecific 50 x 50 m plots within its 200 ha plantation area (Fig. 8e). More information about the arboretum can be found in Nsabimana et al. (2009).
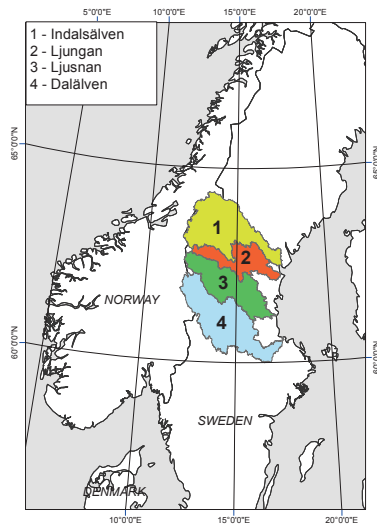

Figure 9. Map of the central Swedish catchments used in **Paper I**.

Table 1. Overview of experiments in Papers I, II, III and IV.

ASPECTS	PAPER I	PAPER II	PAPER III	PAPER IV
Location	Flakaliden, Sweden Svartberget/Krycklan, Sweden Central Swedish catchments	Richmond, Australia	Huye, Rwanda	Huye, Rwanda
Climate zone	Boreal	Warm humid temperate	Tropical	Tropical
Tree species	*Picea abies*	*Eucalyptus globulus*	*Carapa grandiflora* *Entandrophragma excelsum* *Hagenia abyssinica* *Cedrela serrata* *Eucalyptus maidenii* *Eucalyptus microcorys*	*Araucaria angustifolia* *Cupressus lusitanica* *Pinus patula* *Podocarpus latifolius* *Podocarpus falcatus* *Phoenix reclinata* *Dendrocalamus giganteus* *Bambusa vulgaris* *Heliconia rostrata* *Musa sapientum* *Macaranga kilimandscharica* *Prunus careta* *Eucalyptus maculata* *Carapa grandiflora* *Cedrela serrata* *Brachychiton acerifolius* *Jacaranda mimosifolia* *Cordia lucidum* *Ligustrum lucidum* *Cyphomandra betaceae* *Tithonia diversifolia*
Tree age	Mature	Juvenile	Seedlings	Mature
Growth environment	Whole-tree chamber, planted in the soil	Whole-tree chamber, planted in the soil	Nursery, planted in pots	Arboretum, planted in the soil
Treatment or groups compared	Temperature, CO_2	Temperature, CO_2	Climate of species origin	Plant taxonomic group
Response measurements	Leaf- and canopy-level g_s, transpiration and leaf area; Large-scale and long-term data of temperature, precipitation, runoff, growing season length, forest biomass	Responses of g_s to $[CO_2]$; Leaf size, LMA, stomatal density and length, leaf N and P concentration, leaf water potential; Tree leaf area and water use	A-C_i curves, g_s; plant height, leaf size, LMA, leaf N and P concentration	Responses of g_s and A_n (A-C_i curves) to $[CO_2]$; Leaf size, LMA, stomatal density and length, leaf N and P concentration, leaf water potential, wood density

3.2 Measurements

Gas exchange measurements

Gas exchange measurements were performed in all studies of this thesis in order to measure different leaf-, shoot- and canopy-level physiological responses to elevated $[CO_2]$ and temperature (Fig. 8a and 10). Leaf gas exchange instruments measured the leaf fluxes of CO_2 and water vapour to determine plant physiological characteristics such as A_n, g_s, transpiration and C_i. The LI 6400 (Li-Cor Inc., Lincon, NE, USA) instrument used in **Papers II**, **III** and **IV** has control over $[CO_2]$, radiation and temperature, allowing for controlled response measurements to these variables. In **Paper I**, gas exchange was measured at both shoot and canopy levels, using shoot cuvettes and the WTC system.

Structural and chemical measurements

Measurements of structural and chemical leaf trait were conducted to assess the treatment effects on these variables, as well as to explore their influences on plant physiological responses. Table 1 shows which data that were collected in each experiment. Leaf traits included size, length, thickness, mass per unit area (LMA), N and phosphorus (P) content (mass- and area-based) and chlorophyll content. In addition, measurements were conducted to determine stomatal density and guard cell length, and the maximal g_s anatomically possible based on these data (g_{smax}). Measurements of leaf water potential (Ψ_L; pre-dawn and midday) were taken using Scholander type pressure bombs to estimate leaf area-specific plant hydraulic conductance (K_L). Structural measurements at the branch and tree levels included wood density and total canopy leaf area, respectively.

Forest hydroclimatic measurements

Measurements of precipitation, air temperature and river runoff of forest-dominated catchment areas in central Sweden (Dalälven, Ljusnan, Ljungan and Indalsälven) used in **Paper I** were obtained from the Swedish Meteorological and Hydrological Institute *Vattenweb* database. Catchment scale ET was calculated by water balance, subtracting river runoff from precipitation, assuming no changes in annual water storage. Measurements of growing season length for central-northern Sweden were calculated from long-term meteorological data from the Swedish Meteorological and Hydrological Institute meteorological database.

Figure 10. Leaf gas exchange measurement on *Carapa grandiflora* for Paper IV. Photo by Thomas B. Hasper.

4 Findings and Discussion

Paper I

Elevated [CO_2] treatment had no short- or long-term effect on g_s of mature Norway spruce trees in the Flakaliden WTC experiment (Fig. 11a, d), and water-balance constrained large-scale ET estimates significantly increased (18%) in forested areas of central Sweden during the past half-century (Fig. 12). These results agreed with previous studies showing that conifers often lack or have weak stomatal responses to elevated [CO_2] (Medlyn et al. 2001; Brodribb et al. 2009). Considering that Norway spruce and Scots pine (which also exhibit weak g_s responses to elevated [CO_2]; Sigurdsson et al. 2002; Wang and Kellomäki 1997) are the two most abundant tree species in the Scandinavian part of the boreal biome, it is unlikely that these forests will save water under rising [CO_2]. This contradicts projections of CO_2-induced plant water-savings made by climate and dynamic global vegetation models that have incorporated combined stomatal–photosynthesis equations (Betts et al. 2007; Luo et al. 2008). The results of this study also agreed with results from FACE experiments that have indicated that the effect of elevated [CO_2] on forest water use is small under ecologically realistic conditions (Leuzinger and Körner 2010). In fact, stand-level transpiration was more commonly increased (Uddling et al. 2008; Tricker et al. 2009) or not significantly affected (Cech et al. 2003; Leuzinger and Bader 2012; Tor-ngern et al. 2015) than de-crease (Wullschleger and Norby 2001) in forest FACE experiments.

Large-scale water-balance constrained ET was not dependent on mean temperature from May to September or growing season length (Fig. 13a, b) and warming did not increase transpiration (Fig. 11b) in the Flaka-
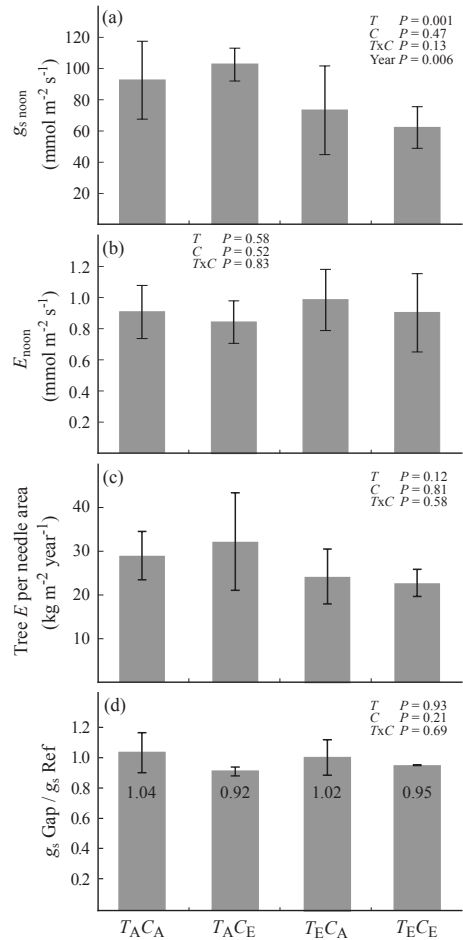

Figure 11. Shoot stomatal conductance at noon ($g_{s\,noon}$; a), shoot transpiration at noon (E_{noon}; b), tree transpiration per unit total projected needle area (tree E per needle area; c) and relative stomatal conductance during a CO_2 fumigation gap compared to preceding and following reference days (g_s Gap / g_s Ref; d). From **Paper I**.

liden WTC experiment, since g_s reductions compensated for the increased VPD (Fig. 11a). Annual ET did, however, increase with increasing annual precipitation (Fig. 13c).

These results indicate that forest ET in the southern and middle part of boreal Sweden is more strongly limited by water availability than by atmospheric evaporative demand.
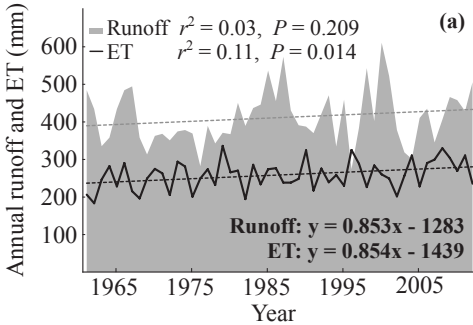

Figure 12. Temporal trends of annual runoff and ET for large forested catchments of central Sweden. Modified from **Paper I**.

The finding that shoot transpiration was unaffected by temperature conflicts with projections made by ecosystem models employing combined stomatal–photosynthesis models, which predict that transpiration increases with increased temperature and VPD in boreal Sweden (e.g. Luo et al. 2008). In addition, the lack of positive effects of warming on shoot and tree water use in the Flakaliden experiment contrasts with results from a Scots pine warming experiment in Finland. In that experiment warming had a positive effect on shoot g_s and transpiration at high levels of temperature and VPD, as a result of reduced sensitivity of g_s to decreasing leaf water potential (Kellomaki and Wang 1996; Wang and Kellomaki 1997).

The reason why ET did not increase with growing season temperature in the Flakaliden experiment may be that Norway spruce exhibits tight stomatal control over transpiration, being a so called "isohydric" species (Leuzinger and Bader 2012). In such species, transpiration may not increase much as temperature (and VPD) increases from moderate to high values. Peak summer transpiration
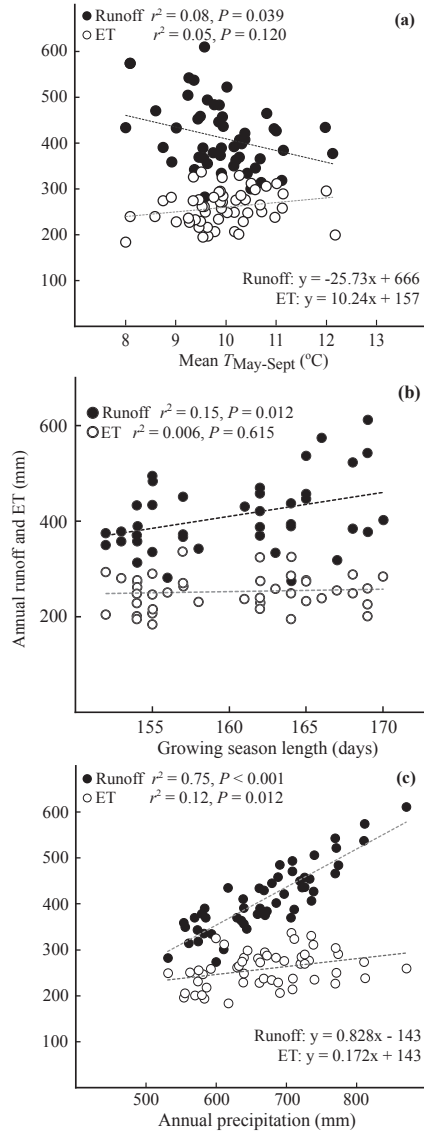

Figure 13. Linear regressions of annual runoff and evapotranspiration (ET) plotted against mean temperature from May to September ($T_{May–Sept}$; a), growing season length (b) and annual precipitation (c) for large forested catchments of central Sweden. Modified from **Paper I**.

may thus not differ much between a normal and an unusually warm summer. Early and late growing season transpiration has minor

contributions to annual total ET as judged by Flakaliden transpiration data, possibly explaining the lack of significant relationship between growing season length and annual ET (Fig. 13b).

Paper II

The direct g_s response to a short-term change in [CO_2] (from 400 to 640 µmol mol^{-1}) was strong (-32%) and similar in all treatments (Fig. 14), but the g_s measured under growth conditions was not significantly affected by elevated [CO_2] or warming treatments. The observed short-term stomatal closure response to increased [CO_2] thus did not translate into a reduction in g_s in trees grown under elevated [CO_2], indicating that guard cell CO_2 responsiveness is not an important determinant of the long-term effect of elevated [CO_2] on g_s in young *E. globulus* trees. These findings contrasts with observations from previous FACE experiments (Cech et al. 2003; Keel et al. 2007; Domec et al. 2009; Maier et al. 2008; Onandia et al. 2011; Tor-ngern et al. 2015), where short- and long-term stomatal responses were positively and significantly related (r^2 = 0.81; P = 0.014; Fig. 1 in **Paper IV**). As discussed below, the likely reason why short- and long-term g_s responses to elevated [CO_2] were uncoupled in this study was that structural responses dominated over the direct stomatal closure response in determining the long-term effect of elevated [CO_2] on g_s in young trees of our fast-growing species.

While the short- and long-term responses of g_s were uncoupled, the lack of effects of elevated [CO_2] and warming on g_s measured at growth conditions was matched by constancy in leaf area-specific hydraulic conductance and stomatal patterning across treatments (Fig. 4 in **Paper II**). This is in line with the general notion that changes in g_s and leaf hydraulic conductance are typically well coordinated (e.g. Brodribb and Jordan 2008). Similarly, stomatal density and length and

the g_{smax} values based on these variables were unaffected by both CO_2 and warming treatment. These findings agree with the lack of treatment effects on g_s measured at growth conditions. However, the results are in conflict with the negative correlation between [CO_2] and stomatal density in the fossil record, demonstrating an overall reduction of 2 to 4% in stomatal density per 100 µmol mol^{-1} increase in [CO_2] across species (Franks et al. 2012). However, responses among [CO_2] experiments are inconsistent (Gerhart and Ward 2010), and FACE experiments with trees found no overall effect of elevated [CO_2] on stomatal density (Ainsworth and Rogers 2007).
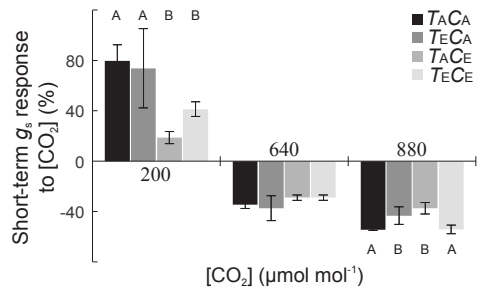

Figure 14. Responses of stomatal conductance (g_s) to short-term changes in [CO_2] relative to the g_s value at 400 µmol mol^{-1} [CO_2] (%). Different letters indicate statistically significant ($P \le 0.05$) differences in responses among treatments. Treatment combinations of ambient (T_A) or elevated (T_E) air temperature and ambient (C_A) or elevated (C_E) [CO_2] are indicated as $T_A C_A$, $T_E C_A$, $T_A C_E$ and $T_E C_E$. Error bars represent standard errors of the means ($T_A C_A$, n = 3; other treatments, n = 2). From **Paper II**.

At the canopy level, elevated [CO_2] significantly increased canopy leaf area by 96% and tree water use by 51% (Fig. 15a, b). Consequently, tree water use per unit canopy leaf area showed a 23% decrease in elevated [CO_2] (Fig. 15c), which was likely a result of a higher fraction of shaded leaves in larger tree crowns. Therefore, the expectation that plants will use less water under higher [CO_2] does not hold for young *E. globulus* trees. In

fact, stand-level tree water savings under elevated $[CO_2]$ seems to be a rare phenomenon under ecologically realistic conditions (Wullschleger and Norby 2001). Overall, stand-level tree water use in forest FACE experiments was more commonly increased (Uddling et al. 2008; Tricker et al. 2009) or not significantly affected (Cech et al. 2003; Leuzinger and Bader 2012; Tor-ngern et al. 2015) than decreased (Wullschleger and Norby 2001) by elevated $[CO_2]$.

Warming treatments had no significant effects on any of the measured response variables in this study (Fig. 15 and figures in **Paper II**). These findings conflict with the expectation that transpiration would increase at higher VPD, and may be related to the observed reduction in biomass allocation to coarse roots in the warming treatment of the experiment (Quentin et al. 2015).

Paper III

Cool-adapted native montane species had lower (5–7 °C) optimum leaf temperature for the maximum rate of electron transport (J_{max}) (but not for V_{cmax}) than the warm-adapted exotic plantation species (Fig. 16). This implies that there are genetically controlled differences in thylakoid membrane temperature responses between the studied native and exotic species that are related to their adaptations at origin climatic conditions. In native species, but not in exotics, the optimal temperature was lower for J_{max} than for V_{cmax} (Fig. 16). The result for native species conforms to the concept of a greater dependence of electron transport than of Rubisco carboxylation on thylakoid membrane stability and, thus, higher sensitivity to high temperatures (e.g. Sage and Kubien 2007). The optimum leaf temperature values for J_{max} in the exotic species were in the upper range of those previously reported (Kattge and Knorr 2007), indicating that the photosynthesis of these species are well adapted to future warming at the experimental site.
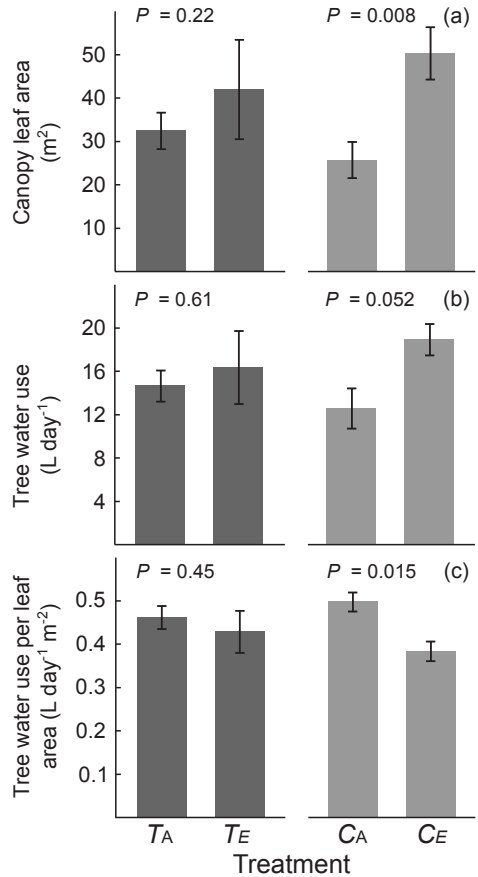
Figure 15. Canopy leaf area (a), daily tree water use (b) and daily tree water use per unit leaf area (c) under ambient (T_A) or elevated temperature (T_E) and ambient (C_A) or elevated $[CO_2]$ (C_E). From **Paper II**.

This study also showed that the photosynthetic optimum leaf temperature of native montane species were frequently and greatly surpassed by their leaf temperatures, but less so the exotic plantation species, adapted to a warmer and more variable climate. This was demonstrated by the observed and modeled leaf temperatures being ca. 10 °C higher than the optimum leaf temperature for A_n at C_i of 272 μmol mol^{-1} (A_{n272}) at the hottest hour of the day in the two climax montane species (*Carapa grandiflora* and *Entandrophragma*

excelsum), compared with smaller but signifycant exceedances in the other species (Fig. 17). This difference in exceedances was related to differences in leaf energy balance, rather than differences in A_n optimum temperatures (Fig. 17 and Table 3 in **Paper III**), with the two native climax species exhibiting low g_s (causing low transpiratory cooling) and large leaf size (causing inefficient heat dissipation). The large interspecific variation in leaf temperature in the common garden highlights the importance of considering leaf traits influencing the leaf energy balance (i.e. leaf size and g_s) when evaluating plant sensitivity to warming. When grown together, different species may have similar physiological temperature responses but, as a result of differences in their leaf energy budgets, still experience different degrees of heat stress.

The finding that tropical climax species were particularly sensitive to high temperature is in line with findings from investigations of warming effects on the growth of tropical South American seedlings (e.g. Cheesman and Winter 2013). While not conclusive or directly applicable to mature forest, this suggests that warmer air temperatures have the potential to threaten seedling growth and recruitment and potentially migration and survival rates of tropical montane rainforest climax species (Medjibe et al. 2014; Zhu et al. 2014). Global warming will probably affect the competitive balance between tropical tree species with different magnitudes of optimum temperature exceedances. Here, as in other studies (Cheesman and Winter 2013; Zhu et al. 2014), there was an indication that such effects would be to the disadvantage of native montane species, which may be outcompeted by species exhibiting smaller warming-induced reductions in photosynthesis (e.g. pioneers or warm-adapted species; Fig 17).
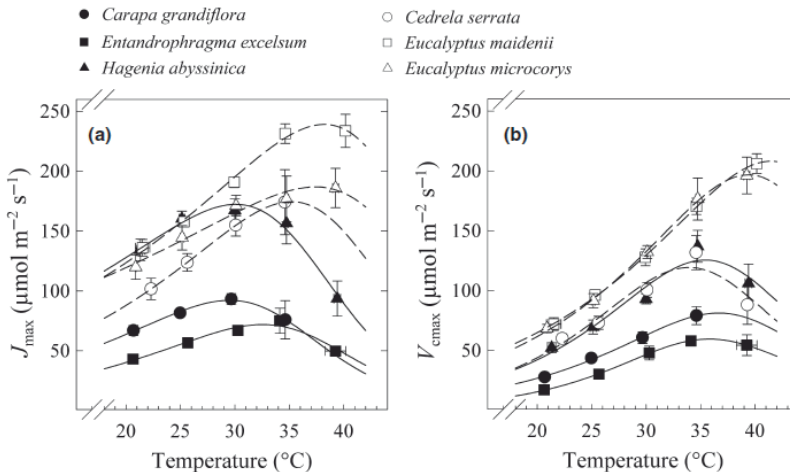

Figure 16. Responses of maximum electron transport rate (J_{max}; a) and maximum rate of Rubisco carboxylation (V_{cmax}; b) to temperature. Species include native tropical montane species (closed symbols and solid lines) and exotic species (open symbols and dashed lines). Error bars indicate ± SE. Lines represent the fitted peaked Arrhenius equations. From **Paper III**.
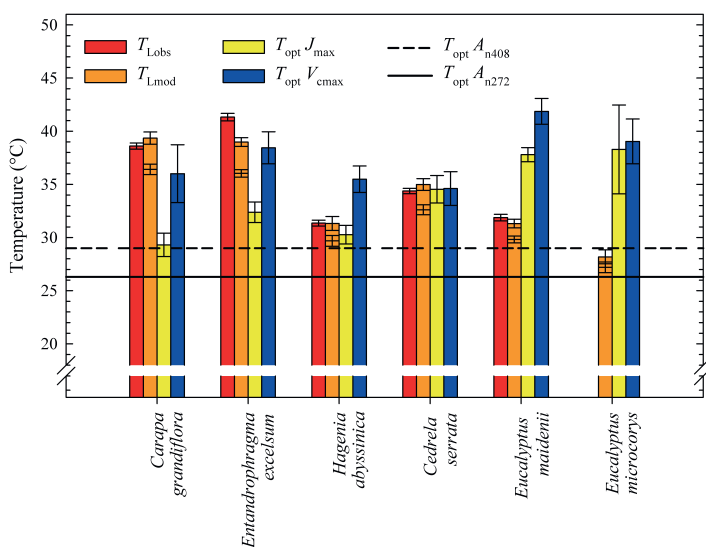
Figure 17. Leaf temperatures in relation to photosynthetic optimum temperatures. Leaf temperatures are observations from four clear days (T_{Lobs}; red; 28.3°C air temperature at 1 m above ground) or modeled estimates based on mean annual weather data (T_{Lmod}; orange; upper values for 15:00 h weather; lower values for mean 06:30–18:00 h weather) for the native tropical montane species *Carapa grandiflora*, *Entandrophragma excelsum* and *Hagenia abyssinica* and the exotic plantation species *Cedrela serrata*, *Eucalyptus maidenii* and *Eucalyptus microcorys*. Also shown are optimum temperature (T_{opt}) of maximum electron transport rate (J_{max}; yellow) and maximum Rubisco carboxylation rate (V_{cmax}; blue). Error bars indicate ± SE. Horizontal lines indicate the optimum temperature for net photosynthesis at a constant internal [CO_2] of 272 (T_{opt} for A_{n272}; solid line; 26.3°C) and 408 μmol mol^{-1} (T_{opt} for A_{n408}; dashed line; 29°C). From **Paper III**.

Paper IV

Species of all taxonomic groups significantly decreased g_s in response to a short-term increase in [CO_2], with gymnosperms exhibiting weaker responses (-12% at 600 μmol mol^{-1} compared to 400 μmol mol^{-1} [CO_2]) than monocots (-29%) and asterids and rosids being intermediate and not significantly different from any other group (Fig. 18a). These results are generally in line with earlier observations that stomatal CO_2 responsiveness is lower in gymnosperm compared to angiosperm species (Saxe et al. 1998; Medlyn et al. 2001; Brodribb et al. 2009). However, results did not support the claim that stomata of gymnosperms lack primary responses to increased [CO_2] (e.g. Brodribb et al. 2011). Rather, they support recent studies that also found significant stomatal closure responses to increased [CO_2] in gymnosperms (Haworth et al. 2013), and molecular studies showing that the genetic tool kits necessary to respond to environmental cues such as [CO_2] were present already in early land plants and are not exclusive to angiosperms (Chater et al. 2013).

The interspecific variation in short-term stomatal CO_2 responsiveness across species was not significantly related to any of the measured structural or functional plant traits. However, gymnosperm g_s responses got stronger with increasing stomatal density

(Fig. 18b), agreeing with observations that stomatal sensitivity to other environmental cues is generally greater in species with higher stomatal density (Franks et al. 2009). In addition, there was a significant relationship between A_n at constant C_i of 280 µmol mol^{-1} (A_{n280}) and stomatal CO_2 responsiveness in monocots (Fig. 18c), indicating that responsiveness may be linked to the metabolic activity of the leaf in this taxonomic group where stomatal CO_2 responses were strong (Fig. 18a). It was also found that g_1 was significantly related to K_L (Fig. 18d). This likely reflects a dependence of the marginal carbon cost of water use (which is inversely related to the square of g_1) on the hydraulic capacity of trees, such that species with high hydraulic efficiency (i.e. high K_L) have a low car-bon cost per water use (i.e. a high g_1).

The large interspecific variation in photosynthetic capacity was not explained by total leaf area-based nutrient content, in agreement with observations from previous tropical studies (Coste et al. 2005; Houter and Pons 2014; Dusenge et al. 2015). Instead, the differences in photosynthetic capacity among species were found to be strongly linked to the fractional investment of leaf N into compounds maximizing these capacities (Fig. 19). These results agreed with previous results from studies with tropical montane tree species (Coste et al. 2005; Dusenge et al. 2015) and a global meta-analysis (Ali et al. 2015). Moreover, there was a near-significant negative relationship ($P = 0.085$) between leaf N investments into Rubisco and bioenergetics (N_R+N_B) versus light harvesting compounds (N_{LH}; Fig. 6d in **Paper IV**), corroborating the suggestion by Dusenge et al. (2015) that there is a N allocation tradeoff such that species with large fractional leaf N investments into light harvesting have low investments into compounds maximizing photosynthetic capacity, and vice versa. These results imply that current vegetation models, which often assume that V_{cmax} and
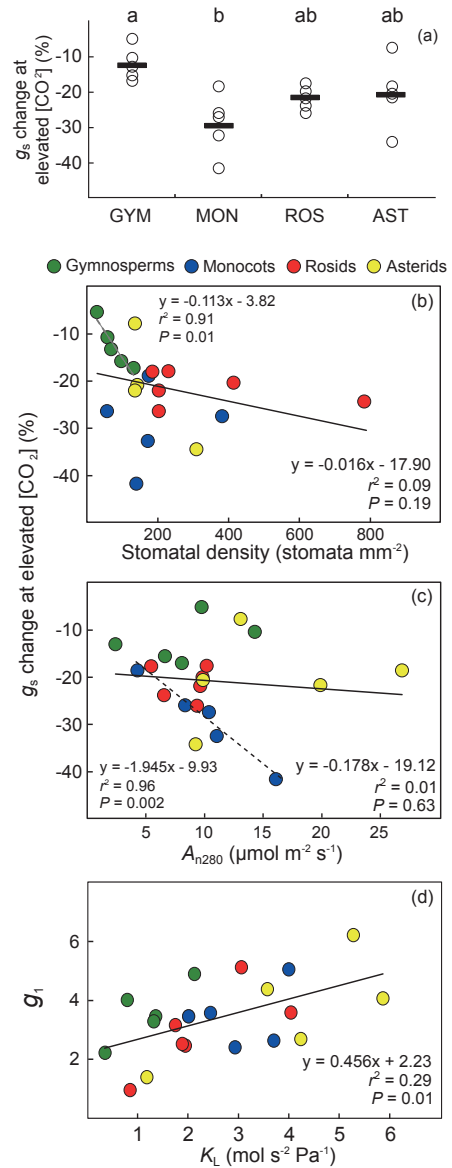

Figure 18. Short-term g_s response to increased [CO_2] (600 vs. 400 µmol mol^{-1}) in relation to taxonomic group identity (a), stomatal density (b), and net photosynthetic rate at 280 µmol mol^{-1} intercellular [CO_2] (A_{n280}; c), as well as and the combined stomatal–photosynthesis parameter g_1 in relation to leaf area-specific plant hydraulic conductance (K_L; d). Modified from **Paper IV**.

J_{max} are plant functional type-specific functions of area-based leaf N content (e.g. Rogers 2014), would be much improved if they also account for differences in within-leaf N allocation.
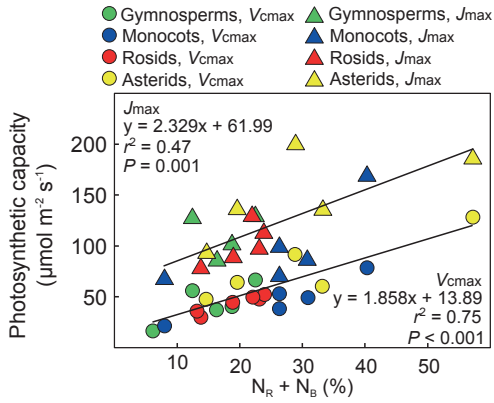

Figure 19. Photosynthetic capacity (i.e. V_{cmax} and J_{max}) in relation to the fraction of leaf N allocated to Rubisco and photosynthetic bioenergetics (N_R+N_B). From **Paper IV**.

5 Conclusions

Paper I

Evapotranspiration in boreal central Sweden has increased by 18% over the past 50 years, and this increase can be linked to the positive effects of increasing precipitation, to the lack of elevated CO_2-induced stomatal water savings, and to the 31% increase in standing forest biomass during this period. The historical increase in evapotranspiration cannot be fully attributed to intensified forest management, since it occurred also in the old unmanaged forest in the Svartberget catchment.

Due to future expected climatic and economic developments, it is likely that the past trend of increasing evapotranspiration in central Sweden will continue into the future. Therefore, the results of this study have important implications for the projections of future boreal biosphere–atmosphere interacttions, indicating that current projections of reduced g_s and tree water use under rising $[CO_2]$ will not hold for the European part of the boreal forest biome.

Paper II

The pronounced direct guard cell stomatal closure response to a short-term increase in $[CO_2]$ did not translate into a similar long-term effect of elevated $[CO_2]$ on g_s. Instead, the lack of CO_2 and warming treatment effects on g_s was in agreement with constancy in both stomatal patterning (density and size) and leaf area-specific plant hydraulic conductance. At the canopy level, increased canopy leaf area together with lack of leaf-level water savings resulted in enhanced tree water use under elevated $[CO_2]$, while warming treatment did not significantly influence any of these response variables.

Taken together, these results demonstrated that structural responses dominated over direct stomatal guard cell responses in determining the impact of elevated $[CO_2]$ on g_s and water use of young *E. globulus* trees, implying that expectations of elevated CO_2-induced tree water savings and reduced drought vulnerability will not be realized for young stands with this species.

Paper III

The photosynthetic apparatus of seedlings of cool-adapted montane rainforest climax tree species was very sensitive to high temperature; with leaf temperatures of sunlit foliage greatly exceeding the photosynthetic optimum temperatures at the hottest hour of the day, to a large extent as a result of low transpiratory cooling (i.e. low g_s) and inefficient heat dissipation (i.e. large leaf size).

These results suggest that montane rainforest climax species may be particularly sensitive to future global warming and highlights the urgent need for more research on thermal responses of photosynthesis (considering biochemical and stomatal limitations as well as leaf energy balance) in tropical trees in order to better assess their sensitivity to warming.

Paper IV

All analysed species significantly decreased g_s in response to a short-term increase in $[CO_2]$. Responses were weaker in gymnosperms (-12%) than in monocots (-29%), and rosids and asterids (-21.4% and 20.7%, respectively) did not significantly differed from any other group.

Within-leaf N allocation was more important than the total leaf N content in controlling the interspecific variation in photosynthetic capacity among tropical woody species, and there was an indication of a trade-

off between leaf N investments into compounds maximizing photosynthetic capacity versus light harvesting. These findings imply that current vegetation models, which often assume that V_{cmax} and J_{max} are plant functional type-specific functions of area-based leaf N content, would be much improved if they could also account for differences in within-leaf N allocation.

6 Outlook

In this section there are examples of how the findings of the studies in this thesis could be used and further developed.

Swedish boreal forests have been experiencing significant hydrological changes during the last century. Results from **Paper I** showed that tree g_s and water-use were unaffected by elevated CO_2 and warming treatments and that large-scale ET has been increasing over the last 50 years, contradicting existing ecosystem models that assume substantial plant water-savings under rising CO_2. These results have important implications for guiding model development in order to increase the reliability in projecting future boreal hydrology.

Some research questions could be further explored based on these results. It would be interesting to further investigate and quantify the influences of direct climatic effects, changes in human land use and management, and plant ecological responses to climate change (e.g. plant water saving responses under rising $[CO_2]$) in driving changes in land ET and runoff in Sweden and elsewhere. Furthermore, observation-based ET results at catchment scale could be compared with simulations of ET and runoff using different types of models (ecosystem models, dynamic vegetation models and climate models) and satellite-based methodologies. Such comparison could serve to evaluate and develop models, which in turn would increase confidence in projections of future hydroclimatic change under different climate and land-use change scenarios.

Results from **Paper II** were from young *E. globulus* trees and showed that this fast-growing species responded to increased $[CO_2]$ by greatly increasing canopy leaf area and tree water use. Eucalyptus species are generally fast growing, especially in young stages of development. Therefore, the responses observed in this study might be transient, and cannot be generalized for adult individuals and/or other Eucalyptus species. However, the findings make important contribution to the understanding of how young *E. globulus* (a widely used and ecologic and economic important species) trees will perform in a future climate.

Research to explore the impacts of elevated $[CO_2]$ on mature Eucalyptus forests (mix of Red gum (*Eucalyptus tereticornis*), Cabbage gum (*Eucalyptus amplifolia*) and other species) has recently started at the EucFACE experiment, in Richmond, Australia. FACE studies are ecologically realistic experiments examining the effects of $[CO_2]$ on plant growth and ecosystem functioning at the stand scale. EucFACE will provide important knowledge on how a mature native Eucalyptus forest will respond to the future increase in $[CO_2]$.

Results from **Paper III** showed that the photosynthetic apparatus of seedlings of cool-adapted montane rainforest climax tree species are very sensitive to high temperature, indicating that these species will be particularly sensitive to future global warming. This pioneering result stress the need for more research on thermal responses of photosynthesis in tropical trees in order to better assesses their sensitivity to warming.

An important question concerning the potential impacts of global warming on tropical forests is whether or not tropical tree species are close to an upper thermal limit above which they will experience heat stress and growth declines. Future studies could explore how increased temperatures affects tropical montane forest tree species by studying

the same species planted in replicated common gardens along elevation gradients in tropical forests. Such studies would be able to assess differences in acclimation capacity among species (e.g. high-, mid- and low-elevation species, native vs. exotic, climax vs. pioneer species). If conducted at stand scale, such study could also examine if warm-adapted lower-elevation species outcompete cool-adapted montane rainforest species when grown at lower elevations (representing climate change scenarios).

Results from **Paper IV** showed direct stomatal closure responses to a short-term increase in $[CO_2]$ in all analysed tropical woody plant species. These findings contradicted previous studies that did not find any response of g_s to increased $[CO_2]$ in gymnosperms.

Also, **Paper IV** results showed that within-leaf N allocation was more important than the total leaf N content in controlling the interspecific variation in photosynthetic capacity among the analysed species. This finding implies that current vegetation models would be much improved if they could also account for differences in within-leaf N allocation.

7 Acknowledgments

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