Acclimation of boreal Norway spruce to climate change

Empirical and modelling approaches

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To my Guru, Avdoot Baba Shivanand
and to Nitin and Nirvana

You are the creator of your own destiny
-Avdoot Baba Shivanand

Abstract

Climate change impacts forests and forests in turn affect climate change. The long-term responses and climate feedbacks of forests depend on the acclimation of trees to altered conditions. Numerous experiments have been carried out to understand the impact of rising carbon dioxide concentration ([CO_2]) and warming on trees. However, most of them have been on young individuals of temperate locations and limited information is available for mature trees and non-temperate regions. This thesis aims to improve the understanding and predictability of physiological acclimation responses to climate change in Norway spruce (*Picea abies*), a key species of the European part of the boreal biome. Acclimation responses of photosynthesis, respiration and stomatal conductance were determined using continuous measurements of CO_2 and water vapour fluxes of shoots exposed to elevated [CO_2] and/or warming inside whole tree chambers at Flakaliden field site, northern Sweden (Paper I). Furthermore, climate and river runoff data from central and northern Swedish catchments during the last 50 years were used to analyze drivers of temporal trends in evapotranspiration of boreal forests (Paper II). The thesis further explores the relative importance of different types of acclimation (of photosynthesis, respiration, stomatal conductance and phenology, alone or in combinations) for shoot and crown level fluxes of CO_2 and water vapour using the process-based MAESTRA model (Paper III).

Results demonstrated that elevated [CO_2] increased net photosynthesis although it decreased maximum photosynthetic carboxylation capacity. Warming had no significant effect on net photosynthesis or maximum carboxylation capacity. Shoot respiration increased in elevated [CO_2] but was not significantly affected by warming. Shoot respiration was also found to exhibit pronounced seasonal acclimation. Stomatal conductance at light saturation and reference air vapour pressure deficit was not affected by elevated [CO_2] but was significantly decreased by warming. Warming treatment had no effect on transpiration as decreased stomatal conductance compensated for the increase in evaporative demand. Forest evapotranspiration in central Swedish boreal forested landscapes increased over the past 50 years without any significant change in runoff. The positive evapotranspiration trend was related to increasing precipitation and forest standing biomass together with lack of stomatal water savings under rising [CO_2].

Simulations showed that tree physiological acclimation generally acted to dampen the responses of tree net photosynthesis and transpiration. Under a no-acclimation scenario, elevated [CO_2] greatly increased shoot and crown net photosynthesis and decreased transpiration while warming increased transpiration but had little effect on photosynthesis. However, photosynthetic and respiratory acclimation greatly reduce the positive effect of elevated [CO_2] on tree CO_2 assimilation, while stomatal acclimation counteracted the positive effect of warming on tree water consumption.

The findings of this thesis have important implications for the projections of future carbon and water fluxes for the boreal region. The observed boreal long-term acclimation responses of respiration and stomatal regulation differ from those of most temperate regions. Furthermore, the findings guide modellers by quantifying the relative importance of different types of acclimation responses. Physiological acclimation generally dampened instantaneous responses and it is critical to incorporate these responses into vegetation models to improve projections of atmosphere-biosphere interactions of boreal regions in a changing climate.

Keywords: acclimation, dark respiration, evapotranspiration, MAESTRA, phenology, photosynthesis, *Picea abies*, stomatal conductance, V_{cmax}, warming

Sammanfattning

Klimatförändringar påverkar skogar och skogar inverkar i sin tur på klimatet. De långsiktiga responserna och klimatåterkopplingarna från skogar beror på trädens till förändrade förhållanden. Ett antal experiment har genomförts för att förstå effekterna av stigande koldioxidhalt ([CO_2]) och uppvärmning på träd, de flesta av dem på unga individer i områden med temperat klimat. Således finns det begränsad information om vuxna träd och icke-tempererade regioner. Denna avhandling har till syfte att förbättra förståelsen och förutsägbarheten av fysiologiska acklimatiseringsresponser på klimatförändring hos gran (*Picea abies*), en viktig art i det europeiska boreala biomet. Acklimatisering av fotosyntes, respiration och stomatas ledningsförmåga (konduktans) bestämdes med kontinuerliga flödesmätningar av CO_2 och vattenånga hos skott och träd exponerade för förhöjd [CO_2] och/eller uppvärmning i trädkammare vid Flakalidens forskningsstation i norra Sverige (artikel I). Dessutom användes klimat- och flodavrinningsdata från avrinningsområden i centrala och norra Sverige för de senaste 50 åren för att analysera historiska trender och drivkrafter för förändringar i evapotranspiration från boreala skogar (artikel II). Avhandlingen undersöker vidare den relativa betydelsen av olika typer av acklimatisering (av fotosyntes, respiration, stomatakonduktans och fenologi, var för sig eller i kombinationer) för skott- och kronflöden av CO_2 och vattenånga med hjälp av den processbaserade modellen MAESTRA (artikel III).

Resultaten visade att förhöjd [CO_2] ökade nettofotosyntesen även om den minskade maximal fotosyntetisk karboxyleringskapacitet. Uppvärmning hade inga signifikanta effekter på nettofotosyntes eller maximal karboxyleringskapacitet. Respiration från skotten ökade med förhöjd [CO_2] men påverkades inte signifikant av uppvärming. Skottens respiration visade sig också ha en tydlig säsongsbetonad acklimatisering. Stomatakonduktans vid ljusmättnad och referensvärden på luftfuktighet påverkades inte av förhöjd [CO_2] men minskade signifikant av uppvärmning. Uppvärmningsförsöken hade ingen effekt på transpiration eftersom minskad stomatakonduktans kompenserade för den ökade drivkraften för avdunstning i varmare luft. Evapotranspirationen från skogsekosystem ökade under de senaste 50 åren i det boreala skogslandskapet i centrala Sverige utan någon väsentlig förändring av avrinning. Den positiva evapotranspirationstrenden var relaterad till ökad nederbörd och skogsbiomassa i kombination med frånvaron av minskad stomatakonduktans vid förhöjd [CO_2]. Simuleringar visade att trädens fysiologiska anpassning i allmänhet dämpade

responserna hos trädens nettofotosyntes och transpiration. Under ett scenario utan acklimatisering ökade förhöjd [CO_2] kraftigt fotosyntesen hos skott och krona och minskade transpirationen medan uppvärmning ökade transpirationen men hade liten effekt på fotosyntesen. Däremot minskade fotosyntetisk och respiratorisk acklimatisering kraftigt den positiva effekten av förhöjd [CO_2] på CO_2-assimilering hos träd, medan acklimatisering av stomatakonduktansen minskade trädens vattenförbrukning under uppvärmning.

Resultaten i denna avhandling har viktiga konsekvenser för uppskattningar av framtida kol- och vattenflöden för den boreala regionen. Den observerade acklimatiseringen av respiration och stomatakonduktans hos gran skiljer sig väsentligt från responserna hos de flesta tempererade studies. Vidare vägleder resultaten modellerare genom att kvantifiera den relativa betydelsen av olika typer av acklimatiseringsresponser. Fysiologisk acklimatisering dämpade generellt omedelbara responser och det är av stor betydelse att integrera detta i modeller för att förbättra uppskattningar av atmosfär-biosfärinteraktioner i boreala regioner i ett förändrat klimat.

List of Papers

This thesis is based on the following papers, referred in the text by roman numerals. Paper I and II are reproduced with permission from the respective journals.

I. **Lamba S**, Hall M, Räntfors M, Chaudhary N, Linder S, Way D, Uddling J & Wallin G (2018) Physiological acclimation dampens initial effects of elevated temperature and atmospheric CO_2 concentration in mature boreal Norway spruce. *Plant, Cell & Environment*, 41: 300-313.

II. Hasper TB, Wallin G, **Lamba S**, Hall M, Jaramillo F, Laudon H, Linder S, Medhurst J, Sigurdssson B, Räntfors M & Uddling J (2015) Water use by Swedish boreal forests in a changing climate. *Functional Ecology*, 30: 690-699.

III. **Lamba S**, Duursma R A, Hasper TB, Sigurdsson B, Medlyn B, Tarvainen L, Hall M, Linder S, Wallin G, Uddling J (2019) Roles of photosynthetic, respiratory, stomatal and phenological acclimation in controlling carbon and water fluxes of mature Norway spruce in a changing climate. Manuscript.

Other paper not included in this thesis is as follows:

Chaudhary N, Westermann S, **Lamba S**, Shurpali N, Sannel B, Shurgers G, Miller P, Smith B (2019) Peatland carbon dynamics at different temporal scales across the pan-Arctic. In revision.

Abbreviations and symbols

A	Photosynthesis
A_n	Net photosynthesis
C_A	Ambient CO_2 treatment
C_E	Elevated CO_2 treatment
c_i:c_a ratio	Leaf intercellular to surrounding air CO_2 concentration ratio
CO_2	Carbon dioxide
$[CO_2]$	Carbon dioxide concentration
E	Transpiration
ESMs	Earth system models
ET	Evapotranspiration
FACE	Free air carbon dioxide enrichment
g_{ref}	Stomatal conductance at light saturation and a VPD of 1 kPa
g_s	Stomatal conductance
g_0	Stomatal conductance when $A_n = 0$, estimated here as g_s in darkness
g_1	Slope parameter of the combined photosynthesis-stomatal conductance model (Medlyn et al., 2011)
LAI	Leaf area index
N	Nitrogen
N_a	Nitrogen content per unit leaf area
PPFD	Photosynthetic photon flux density
Q_{10}	Parameter representing the relative change in dark respiration as a consequence of increasing the temperature by 10 °C
R	Dark respiration
R_{10}	Dark respiration at 10° C

T_A	Ambient temperature treatment
T_E	Elevated temperature treatment
V_{cmax}	Maximum rate of photosynthetic carboxylation by Rubisco
V_{cmax25}	Maximum rate of photosynthetic carboxylation by Rubisco at 25 °C
VPD	Vapour pressure deficit of air
WTC	Whole tree chamber
WUE	Water use efficiency

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1 Introduction

1 Introduction

1.1 Boreal forests and climate change

Forests give multiple benefits to mankind ranging from ecological to societal, economic and aesthetic services. Climate change influences forests by affecting their growth and productivity. It also affects forests by altering the frequency and intensity of disturbing events such as drought, insect outbreaks and wild fires. Forests in turn, impact climate and climate change by feedback effects, mainly through changes in albedo (i.e. the reflectance of sunlight striking on the earth's surface), the hydrological cycle and atmospheric trace gas composition (Bonan 2008). Climate relevant trace gases affected by forests include primarily carbon dioxide (CO_2) but also methane and nitrous oxide.

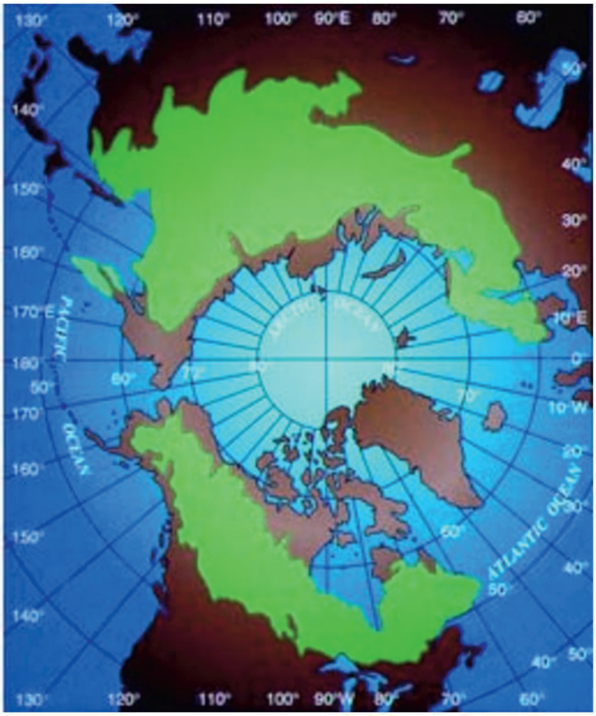


Figure 1. *Global distribution of boreal forests. Green colour represents boreal forests (Source: Hare and Ritchie, 1972, used with permission).*

Boreal forests are estimated to contain 32% of the carbon stock of the world's forests amounting to 272 ± 23 Pg C (Pan et al., 2011). They are located south of the Arctic cycle and extend through most of Canada, Russia and Scandinavia (Figure 1). Northward shifting of species distribution (Koven, 2013), lengthening of growing season (Liu et al., 2018; Park et al., 2016), recent insect outbreaks (Pureswaran et al., 2018) and increase in wild fires (Veraverbeke et al., 2017) are some of the examples by which climate change is already impacting boreal forests. Boreal forests consist of few species, mostly conifers such as spruces, firs and pines along with some deciduous trees mainly along the rivers and lakes and as a transitory stage after fires (Oliver and Larson, 1996).

1.2 Norway spruce

Norway spruce (*Picea abies* (L.) Karst.) is found from central to northern and eastern Europe all the way from the Alps to the Arctic circle and the Ural mountains. It is a key species of the Scandinavian boreal forest biome and is highly important, both from ecological and economic viewpoints in this region. It can grow up to 50-60 m in height with a trunk diameter of up to 150 cm and living for 200-300 years (Caudullo et al., 2016). However, in production forest (forest managed primarily for economic reasons e.g. production of solid wood for timber, pulpwood for paper etc.) it is normally cut at an age of 60-100 years.

1.3 Short-term leaf level responses to elevated [CO_2] and warming

A short-term response can be defined as "the initial plant response to an environmental stimulus that manifests before any type of physiological, structural or biochemical adjustment or limitation" (Smith and Dukes 2013). Three fundamental plant responses at the leaf level are those of photosynthesis (A), respiration (R; here used for dark respiration) and stomatal conductance (g_s). These are all sensitive to temperature (Figure 2).

Photosynthesis shows an optimum-shaped response to temperature with declining rates at both low and high temperatures (Berry and Björkman 1980). Dark respiration shows an exponential response to increased temperature until it collapses and reaches zero at the leaf lethal limit. The response of g_s to increased temperature is typically associated with the effect of higher vapour pressure deficit of the air (VPD; Figure 2), which is the driving force for transpiration. Since temperature and VPD co-vary in nature it is often difficult to separate the effect of temperature from that of VPD.

Photosynthesis is stimulated by elevated [CO_2] within seconds (Curtis 1996; Drake et al., 1997; Norby et al., 1999) whereas earlier claims of a direct negative response of R turned out to be an artifact (Ainsworth and Rogers 2007). In most species, the g_s decreases when a leaf is exposed to a short-term increase in [CO_2] (Morison, 1987).

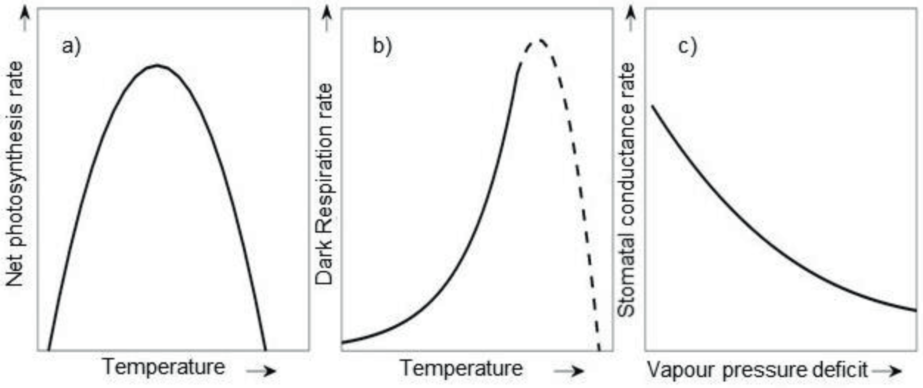


Figure 2: *Short-term response rate of net photosynthesis (a) and dark respiration (b) to temperature and of stomatal conductance (c) to vapour pressure deficit. Shapes of the responses vary among species and environmental conditions and are drawn here only schematically.*

1.4 Acclimation: altered short-term response function

Plant acclimation can be defined as a physiological, structural or biochemical adjustment of an individual in response to a change in its environment (Figure 3; Smith and Dukes 2013). These adjustments make the plant more fit to the new environmental conditions and many of them are manifested as modifications in the short-term responses of physiological processes. Acclimation should not be confused with adaptation as the latter is a population response that occurs over generations whereas the former is the response of an individual organism within its lifetime.

Several meta-analyses have been carried out to investigate the long-term acclimated responses of A, R and g_s (Table 1). In long term stand scale free-air CO_2 enrichment (FACE) experiments, elevated [CO_2] increased net photosynthesis (A_n) although the actual biochemical photosynthetic capacity (V_{cmax}) was downregulated (Ainsworth and Rogers, 2007). Warming experiments on trees, performed mostly with different types of chambers and thus having less field realism than FACE experiments, showed no overall impact on photosynthetic capacity (Way and Oren 2010). Leaf dark respiration and the shape of its temperature response (Q_{10}) showed no general response to elevated [CO_2] (Slot and Kitajima 2015; Way et al., 2015) whereas *R* at a standard temperature was found to decrease with warming (Atkin et al., 2015; Slot and Kitajima 2015). The response of Q_{10} (of *R*) to warming exhibited no clear pattern (Slot and Kitajima 2015). The g_s at light saturation and similar VPD conditions (g_{ref}) was typically decreased in elevated [CO_2] (Ainsworth and Rogers 2007) while no general response pattern has been found for warming (Way et al., 2015). Warming caused an earlier start of the growing season (Jeong et al., 2011). Note that meta-analyses, and therefore the responses described above, are based on experiments with primarily temperate trees. A limited number of experiments have been carried out for non-temperate trees, leading to considerable uncertainty regarding tree responses in boreal and tropical regions (Fisher et al., 2018; Jones et al., 2014).

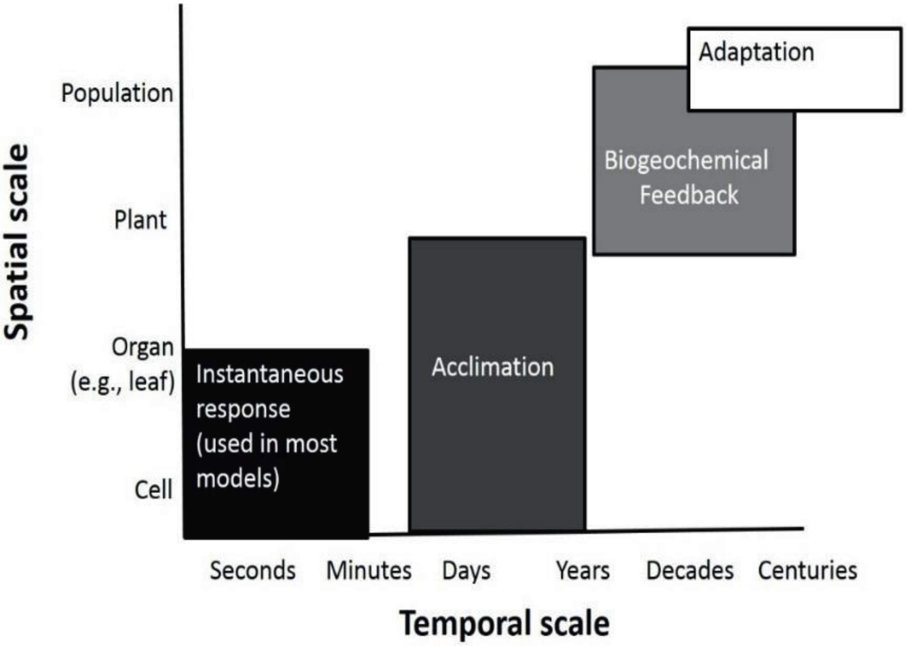
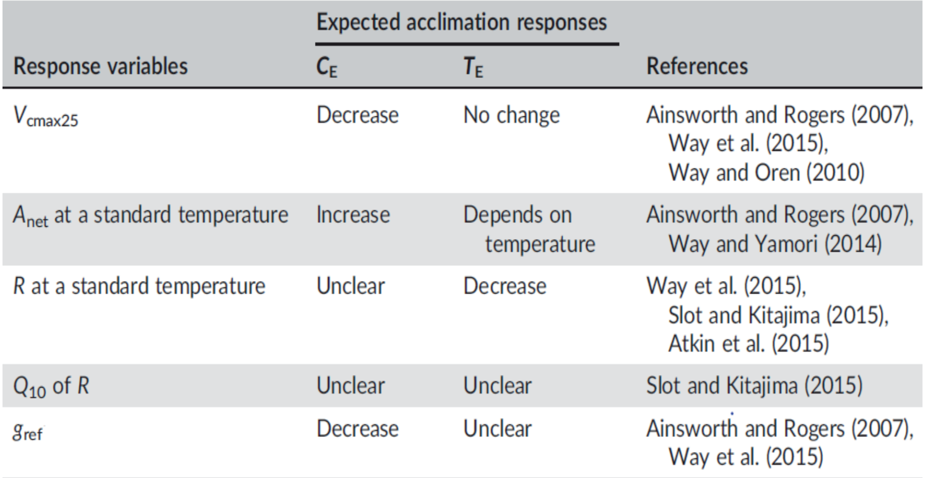


***Figure 3**. Plant responses at different scales of time and space (Source: Modified from Smith and Dukes, 2013, used with permission).*

Table 1: *Expected acclimation responses for the photosynthetic, respiratory and stomatal response variables based on earlier meta-analyses (taken from Paper I)*



	Expected acclimation responses		
Response variables	C_E	T_E	References
V_{cmax25}	Decrease	No change	Ainsworth and Rogers (2007), Way et al. (2015), Way and Oren (2010)
A_{net} at a standard temperature	Increase	Depends on temperature	Ainsworth and Rogers (2007), Way and Yamori (2014)
R at a standard temperature	Unclear	Decrease	Way et al. (2015), Slot and Kitajima (2015), Atkin et al. (2015)
Q_{10} of R	Unclear	Unclear	Slot and Kitajima (2015)
g_{ref}	Decrease	Unclear	Ainsworth and Rogers (2007), Way et al. (2015)

Symbols: C_E=Elevated CO_2 treatment; T_E=Elevated temperature treatment; V_{cmax25}=maximum rate of Rubisco carboxylation at 25ºC; A_{net}=net photosynthesis; R=dark respiration; Q_{10}=parameter describing the temperature response of dark respiration; g_{ref}=stomatal conductance at light saturation and same vapour pressure deficit

Few studies have assessed the timescale of acclimation. It differs depending on the species and the variable studied. For example, photosynthetic acclimation to elevated temperature may occur over a period of a few days to up to two weeks (Bjorkman & Badger, 1979; Gunderson et al., 2010; Rogers et al., 2017; Slatyer & Ferrar, 1977). It is also not clear whether full potential acclimation requires new tissues formation (Atkin and Tjoelker 2003) or if it can take place in already existing tissue (Bruhn et al., 2007). More thorough investigation is needed to assess the timescale of acclimation for different species and physiological variables.

1.5 Tree and stand level responses

While short-term leaf level responses to increased [CO_2] and warming are relatively well known, responses at the tree and stand level require more attention (Way et al., 2015). It has been suggested that responses observed at a smaller scale are dampened at higher spatio-temporal scales (Leuzinger et al., 2011). However, larger-scale and longer-term manipulation experiments and response measurements are technically and financially difficult. The limited number of such experiments cause uncertainty regarding larger scale responses of forests in a changing climate. Furthermore, most studies measure leaf gas exchange on healthy and sun-exposed leaves at light saturation and during the peak of the growing season. This may lead to the wrong impression of the responses of entire trees over the full growing season. Continuous gas exchange measurements at leaf/shoot and tree/stand levels have the potential to reduce the uncertainties associated with scaling up leaf level responses in time and space.

Elevated [CO_2] generally increased tree growth and canopy leaf area index (LAI) in FACE experiments (Ainsworth and Long, 2005). Tree N uptake increased considerably less than biomass accumulation, causing decreased plant tissue N concentration at both leaf and whole plant levels (Ainsworth and Long 2005; Feng et al, 2015; Finzi et al., 2007; Luo et al., 2006). This indicates N constraints on the elevated [CO_2] induced tree growth stimulation and has been attributed to the negative effects of elevated [CO_2] on plant N acquisition (Feng et al., 2015).

Elevated [CO_2] might also lead to changes in tree water use. FACE experiments with young and fast-growing deciduous tree species showed increased stand-level water use under elevated [CO_2] (Aspen-FACE and POP/EuroFACE; Uddling et al., 2008, Tricker et al., 2009) whereas experiments in older forests reported no significant changes (Web-FACE and Duke FACE experiments; Cech et al., 2003, Schäfer et al., 2002) or decreased stand water use in elevated [CO_2] (ORNL experiment; Wullschleger et al., 2002),

Uncertainty regarding how tree or stand responses compare with leaf level responses are even larger for warming than for elevated [CO_2] (Way et al., 2015). This is because free air warming experiments have only been conducted on seedlings using infrared heating, which is less ecologically realistic since plant surface heating differs from air warming due to self-shading and effects of variation in wind speed (Reich et al., 2015). It has been suggested that warming should increase tree growth in boreal regions as their trees are currently mostly operating below their thermal optimum (Way and Oren, 2010). A meta-analysis reported increased growth (more in deciduous species than in evergreen trees) in warming experiments mostly conducted on temperate species (Way and Oren, 2010). It also found relatively higher allocation of biomass to leaves and less to roots as well as increased height at a given stem diameter under warming. Of course, effects of warming will be constrained by associated possible changes in the availability of water and nutrients.

Warming (with associated increase in VPD) is expected to increase transpiration and water use. Total transpiration is affected not only by influences of temperature and VPD on transpiration rates but also by increasing growing season length in a warmer climate (Chung et al., 2013; Kirschbaum et al., 2018).

1.6 Climate feedbacks of boreal forests

Forests exert feedbacks on climate and climate change through changes in albedo, evapotranspiration (ET) and biogeochemical cycles controlling atmospheric trace gas composition. Boreal forests are known for having lower albedo than a treeless landscape, particularly during the snowy season. This causes positive climate forcing, i.e. warming. Tree carbon sequestration and evaporative cooling is lesser in boreal forests than in tropical and temperate forests (Figure 4).

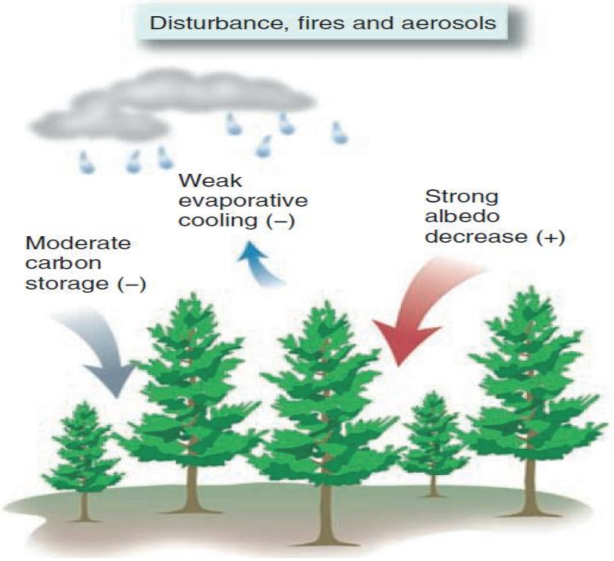


***Figure 4**. Climate feedbacks of boreal forests. Moderate, weak and strong is in comparison with tropical and temperate forests. Negative (-) and positive symbols (+) refer to the temperature feedbacks of boreal forests compared to a reference treeless landscape, implying cooling and warming, respectively (Source: Bonan 2008, used with permission).*

Nevertheless, both these feedbacks contribute to negative climate forcing for the boreal regions, i.e. cooling. Strong albedo decrease outweighs effects of carbon sequestration and ET in boreal forests, which implies net warming of intact forests compared to landscapes without trees. However, impacts of fire, disturbance and aerosols might interfere and must also be taken into account (Bonan, 2008).

1.7 Vegetation models

Terrestrial vegetation models simulate vegetation responses to changing environmental conditions. These models provide input to Earth system models (ESMs; Collins et al., 2011), which are used by the Intergovernmental Panel on Climate Change for predictions of future climate change. Present vegetation responses in the ESMs are largely based on knowledge about plant responses at small spatial (e.g., leaves) and temporal scales (e.g., minutes), while longer-term acclimated responses are not often represented (Smith and Dukes, 2013). A review of 17 vegetation models including many belonging to the current generation ESMs, found that none of the 17 models included acclimated responses for both photosynthesis and respiration to increased [CO_2] and warming (Smith and Dukes, 2013). Current work is going on to include various acclimation responses in the vegetation models but it needs more observational data for model parameterization and evaluation (Rogers et al., 2017).

1.8 Knowledge gaps

Climate change predictions of current terrestrial vegetation models heavily rely on data from temperate trees. Their accuracy for non-temperate regions therefore remains unclear due to data shortage (Fisher et al., 2018; Jones et al., 2014). It is likely that the vegetation responses to climate change predicted by models are not accurate for boreal regions if the acclimated long-term physiological responses to elevated [CO_2] and/or warming differ between boreal and temperate trees. A recent study highlighted the need for species- or plant functional type-specific physiological parameters accounting for long-term acclimation responses to improve vegetation processes in ESMs (Rogers et al., 2017).

Additional knowledge gaps are caused by the low number of experiments with mature field-grown trees and the rarity of studies documenting the effects of both elevated [CO_2] and warming. The effects of combined treatment of elevated [CO_2] and warming on leaf physiology may interact in ways that will either increase or cancel their independent effects (Lewis et al., 2013; Long 1991; Sage and Kubien 2007; Sigurdsson et al., 2013). This causes substantial uncertainty in predicting the impacts of climate change on trees and forests (Way et al., 2015).

With respect to modelling, considerable uncertainty is caused by the inclusion of one or two types of acclimation responses only in most studies to date (Smith and Dukes, 2013). This is perhaps not surprising since the relative contributions of different types of physiological acclimation on tree carbon and water fluxes is poorly known.

2 Aim and Objectives:

The aim of this thesis is to improve the understanding and predictability of the long-term responses of boreal trees to elevated [CO_2], warming and their combination, as well as to separate and quantify the importance of different types of acclimation responses for tree carbon and water fluxes in a changing climate.

The specific objectives of the thesis are to:

i) Determine the long-term physiological acclimation responses of mature boreal Norway spruce trees to elevated [CO_2] and warming, alone and in combination (Paper I).

ii) Determine the water use responses of mature boreal Norway spruce trees and large-scale forested landscapes to experimental [CO_2] enhancement and warming as well as long-term temporal climatic trends (Paper II).

iii) Simulate different acclimation responses using MAESTRA model and assess their relative importance for carbon and water fluxes of shoot as well as entire crown of mature boreal Norway spruce trees grown under elevated [CO_2] and warming, alone and in combination (Paper III).

3 Material and methods

3.1 Study sites and experimental design

Study sites used in this thesis are Flakaliden, northern Sweden (Paper I, II and III), central Swedish catchments (Paper II) and Svartberget/Krycklan research catchment, northern Sweden (Paper II) (Figure 5). Flakaliden research site was used to carry out the experiment to determine the long-term responses of mature boreal Norway spruce trees to elevated [CO_2], warming and their combined treatment. Central Swedish catchments and Svartberget/Krycklan research catchment were used to determine the historical trends and patterns of ET in these large scale boreal forested landscapes. Detailed descriptions are provided in each of the papers on which this thesis is based. A brief description of the experimental sites along with the methodology are provided below (text + Table 2).

Flakaliden Research Site, Sweden (Paper I, II and III)

Flakaliden is a long-term field experimental site (Figure 5) in north of Sweden (64°07′N,19°27′E, 310 m above sea level). It was established in 1986 in a Norway spruce (*P. abies* [L.] Karst) stand, planted in 1963 with four-year old seedlings of local provenance (Linder, 1995; Bergh et al., 1999). It lies in the middle boreal sub zone (Sjörs, 1999). The site has a tough boreal climate. It has previously been used for several long-term manipulation experiments. Data used in Paper I, II and III comes from the factorial experiment that examined the effects of elevated [CO_2] and/or warming (and associated increase in VPD) on mature Norway spruce trees grown in whole tree chambers (WTCs) during 2001-2004. The experiment consisted of three tree replicates (n=3) in a factorial combination of ambient and elevated temperature and CO_2.

Central Swedish catchments (Paper II)

Patterns and trends in forest runoff and ET were investigated using data from large catchments in central Sweden (i.e., Dalälven, Ljusnan, Ljungan and Indalsälven; Figure 5), lying within the southern and middle boreal sub-zones. The catchments are dominated by Norway spruce and/or Scots pine forests, accounting for 73% of the total area. Almost all forests in the region are managed, using clear cutting as dominating harvesting method. Approximate rotation length is 90 years. Grassland, lakes and wetlands form about 6, 7 and 8 % of the total area, respectively. The catchment areas are sparsely inhabited. For further details, see Paper II.

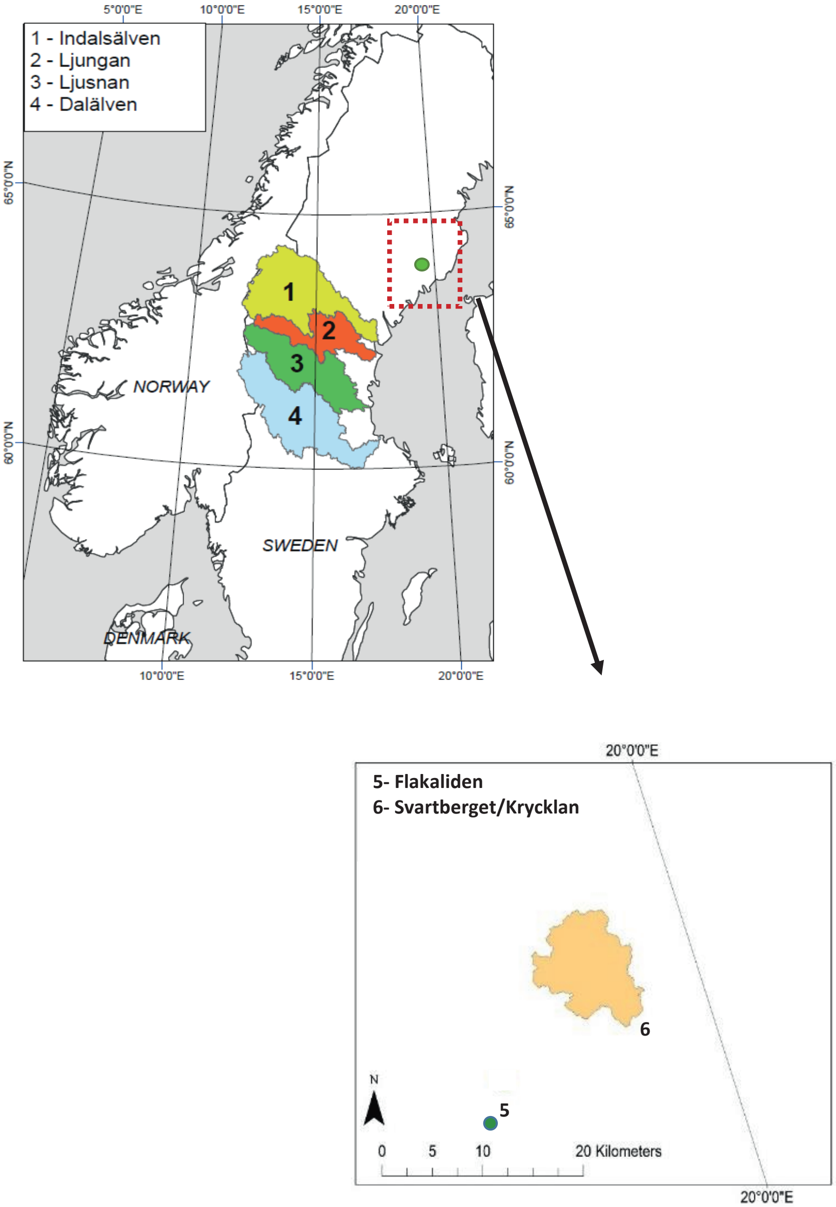


Figure 5: *Maps showing the research sites used in this thesis. Numbers 1-4 represent central Swedish catchments, 5 represents Flakaliden experimental site, northern Sweden and 6 represents Svartberget/Krycklan research catchment, northern Sweden.*

***Table 2**: Outline of experiments in Paper I, II and III*

Aspects	**Paper I**	**Paper II**	**Paper III**
Location	Flakaliden , Sweden	Flakaliden , Sweden Svartberget/Krycklan catchment, Sweden Central Swedish catchments	Flakaliden , Sweden
Climate zone	Boreal	Boreal	Boreal
Tree species	*Picea abies*	*Picea abies* (Flakalidenexperiment) *Picea abies* and *Pinus sylvestris* (Catchments)	*Picea abies*
Tree age	Mature	Mature	Mature
Growth Environment	Whole tree chamber, planted in soil	Whole tree chamber, planted in soil (Flakalidenexperiment) Trees freely rooted in soil(Catchments)	Whole tree chamber, planted in soil
Treatment or groups compared	Warming and/or elevated [CO_2]	Warming and/or elevated [CO_2]	Warming and/or elevated [CO_2]
Response variables	A_n (at 20 ˚C), V_{cmax25}, R (at 15 ˚C), Q_{10}, g_{ref}, c_i:c_a ratio, g_1, g_0, seasonal responses of V_{cmax25} and R_{10} (all at shoot level)	g_s and E (shoot and canopy level), tree leaf area, large scale and long-term data of temperature, precipitation, runoff, ET, growing season length, forest biomass	A_n, E, WUE (at both shoot and crown level)

Symbols: A_n= net photosynthesis; V_{cmax25} = maximum carboxylation capacity at 25 °C; R = dark respiration; Q_{10} = parameter describing the shape of the temperature response of dark respiration; g_{ref} = stomatal conductance at light saturation and VPD of 1 kPa; c_i:c_a ratio = leaf intercellular to surrounding air CO_2 concentration ratio; g_1 = slope parameter describing the combined stomatal-photosynthesis model (Medlyn et al., 2011); g_0= stomatal conductance when A_n=0, estimated here as g_s in darkness; R_{10} = dark respiration at 10 °C; g_s=stomatal conductance; E = transpiration; ET= Evapotranspiration; WUE = water use efficiency

Svartberget/Krycklan research catchment, Sweden (Paper II)

The Svartberget/Krycklan research catchment (Figure 5) is located in northern Sweden (64°14′39″N, 19°45′58″E) covering an area of 47 ha. It is mostly covered by mixed forest stands dominated by Norway spruce and Scots pine, comprising 82% of the total area (Buffam et al., 2007). Forest management interventions have not taken place in this site during the last century.

3.2 Measurements

Gas exchange measurements

CO_2 and H_2O flux measurements in the Flakaliden elevated [CO_2] by warming experiment were carried out at both shoot (Paper I, II, III) and whole tree levels (Paper II, III) using shoot cuvettes (SC) and whole tree chambers (WTC), respectively (Figure 6). Measurements of shoot level fluxes were performed on one one-year-old shoot per tree during the entire experiment. Whole tree fluxes were performed on the aboveground part of the tree, with 12 WTCs functioning as big flux chambers. Data used were from the non-frost affected periods of 2002-2004 (Paper I) or 2003-2004 (Paper II, III).

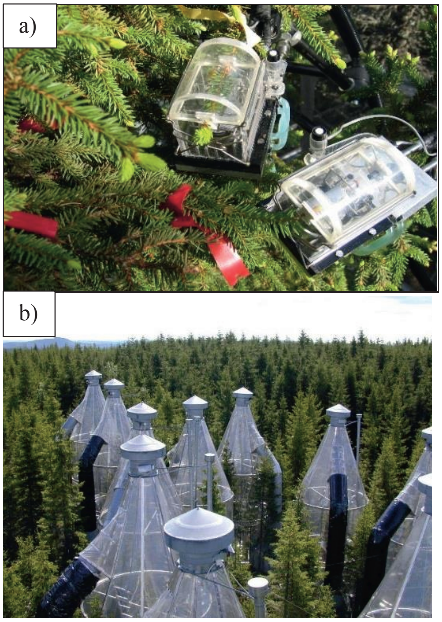


Figure 6*: Shoot cuvettes (SCs; a) and Whole tree chambers (WTCs; b) from Flakaliden’s* CO_2 *by warming experiment. Photos: a) Göran Wallin b) Bengt-Olof Vigren*

Meteorological and hydrological measurements

Measurements of precipitation, air temperature and river runoff in the four large catchment areas of central Sweden (Dalälven, Ljusnan, Ljungan and Indalsälven; Paper II) were available from the Vattenweb database of the Swedish Meteorological and Hydrological Institute. Annual ET (Paper II) was calculated based on the annual water balance of the basin by subtracting river runoff from precipitation, thus assuming that annual water storage changes were negligible.

Structural and chemical measurements

Tree dimensions for all the 12 WTC trees (i.e. crown radius, crown height and leaf area) were used in Paper III. Needle area and N concentration data from the harvest were available for four different needle age classes (current year =C, C+1 year, C+2 years, C+3 years) at five vertical crown layers of equal length. Only minor variation in area based needle N content (N_a) was found amongst different needle age classes within a crown layer. Therefore, all the needle age classes were pooled together in each layer (Paper III). Response data reported in Paper I (V_{cmax25}, A_n at 20 °C, R at 15 °C, Q_{10}, g_{ref}, c_i:c_a ratio) are for the second layer of the crown from above. For further details, see Paper I and III.

3.3 Simulations

Simulations were carried out both at the shoot and at the entire crown level (Paper III) using a detailed, three-dimensional model, MAESTRA (Duursma and Medlyn 2012; Medlyn 2004). It is a process based model with sub models of radiation, g_s and A_n to simulate carbon and water vapour fluxes for leaf or tree or stand (Figure 7).

Results of Paper I formed the major part of the model input parameters for the modelling. Simulation experiments were carried out to determine the effects of elevated [CO_2] and/or warming on CO_2 and water vapour fluxes in different physiological acclimation scenarios (photosynthetic, respiratory, stomatal and phenological). Both individual and combined acclimation scenarios were simulated. Further details on acclimation scenarios and simulations are provided in Paper III.

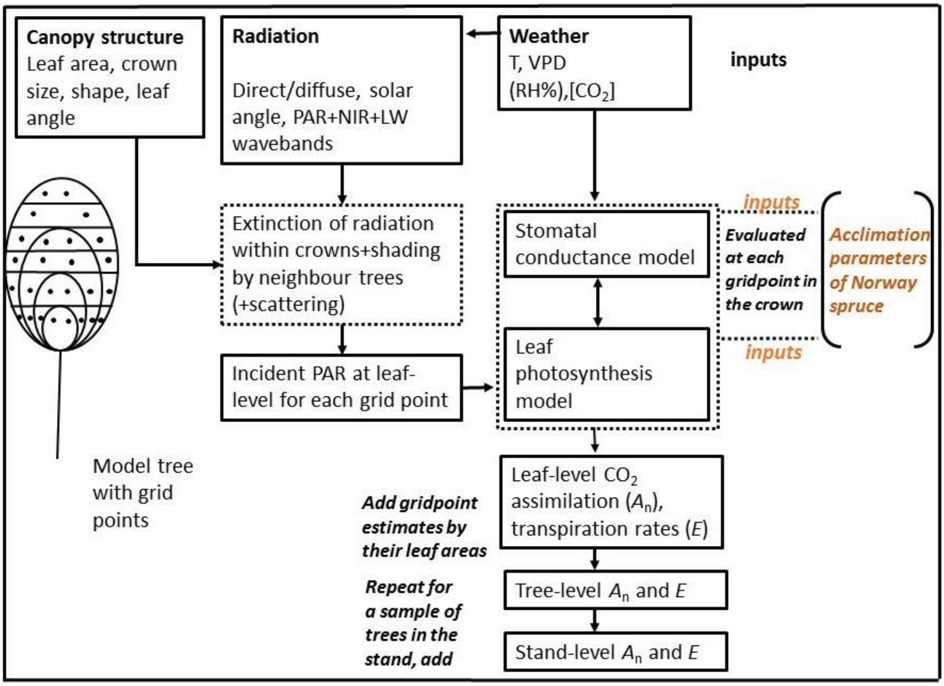


***Figure 7**: Flow chart diagram of MAESTRA model. Input data in terms of canopy structure, radiation and weather are used in sub-models of radiation interception and combined stomatal conductance-photosynthesis regulation (Modified from Duursma and Medlyn, 2012, used with permission). MAESTRA is capable of producing output in the form of A_n and E at leaf-level, tree level and stand level. Long-term acclimation parameters of Norway spruce have been used in the combined stomatal conductance-photosynthesis model used in MAESTRA for simulations in this thesis.*

4 Findings and Discussion

Paper I

Photosynthesis

As expected, light-saturated A_n was significantly increased in elevated [CO_2] (Figure 8b). This occurred in spite of a significant 23% reduction in V_{cmax25} by elevated [CO_2] (Figure 8a). This downregulation of V_{cmax} is in line with previous meta-analyses (Ainsworth & Long, 2005 and Medlyn et al., 1999). However, the magnitude of response is larger (10-13%) compared to these earlier studies. Our results on V_{cmax} and area based needle N content (N_a) indicated that both lower fractional investment in Rubisco (lower V_{cmax} at similar N_a) and decreased N_a (in T_EC_E) contributed to the decrease in V_{cmax} under elevated [CO_2].

There was no significant impact of warming on V_{cmax25} (Figure 8a), in line with a previous meta-analysis (Way & Oren, 2010). Moreover, there was no seasonal acclimation of V_{cmax25} (Figure 9) between the early and late part of the summer, which further supports that this parameter was unaffected by changes in temperature. However, spring shoot carbon assimilation was increased by warming through prolongation of the growing season by almost 10 days (Hall et al., 2009; Hall et al., 2013; Slaney et al., 2007).

Dark respiration

Elevated [CO_2] increased R by 64% (Figure 8c) and decreased Q_{10} by 20% (Figure 8d), reflecting a constant enhancement of R by ~0.2 µmol m^{-2} s^{-1} over the entire temperature range measured (5-22˚C) (Figure 10). Increased respiration rates in elevated [CO_2] may be a result of higher energy demand from increased mobilization of starch and phloem loading. Previous findings from this experiment have shown increased soil CO_2 efflux in elevated [CO_2] (Comstedt et al., 2006). It is thus probable that in elevated [CO_2], much of the surplus carbohydrates formed during the growing season were translocated belowground with associated increased energy costs in the shoots. A previous branch-bag study at the same site also found increased R in elevated [CO_2], but the effect was smaller than in the present study (Roberntz and Stockfors, 1998). A recent meta-analysis found no overall pattern regarding the impact of elevated [CO_2] on

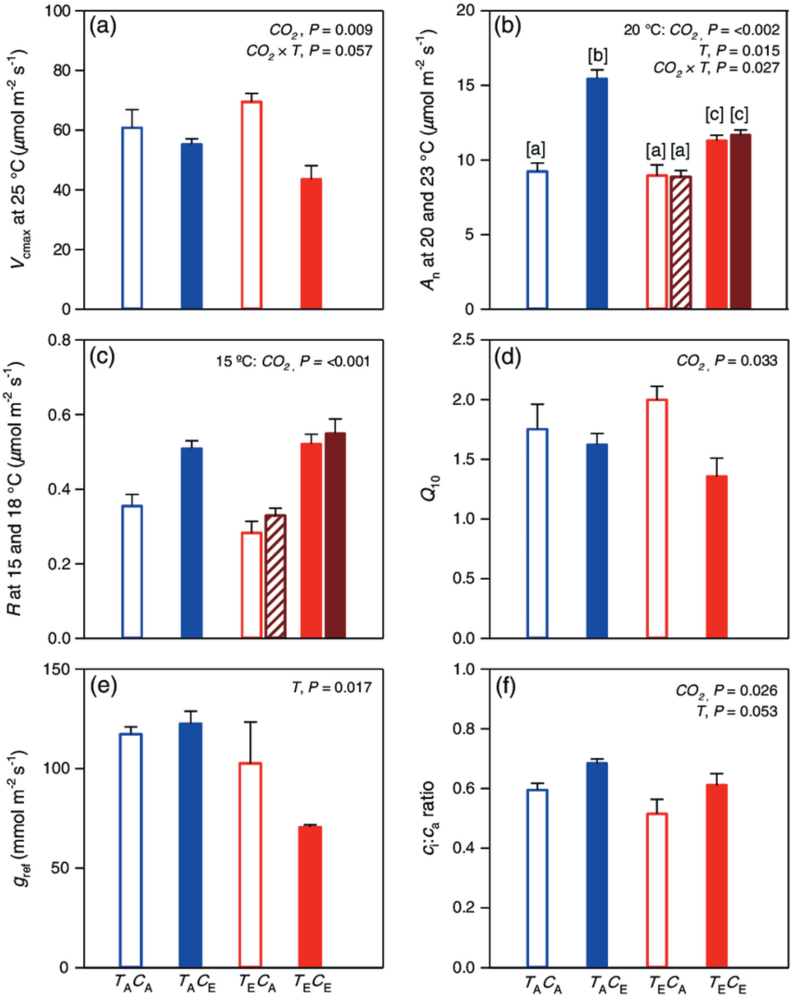


Figure 8. *Maximum rate of Rubisco carboxylation at 25 ºC (V_{cmax25}; a), net photosynthesis at 20 ºC and 23 ºC (in T_E) (A_n; b), dark respiration at 15 ºC and 18 ºC (in T_E) (R; c), parameter describing the shape of the temperature response of dark respiration at 15 ºC (Q_{10}; d), stomatal conductance at light saturation and a vapour pressure deficit of 1 kPa (g_{ref}; e) the leaf intercellular to surrounding air CO_2 concentration ratio (c_i:c_a ratio; e) for shoots of mature Norway spruce trees exposed to ambient (T_A) or elevated (T_E) temperature and ambient (C_A) or elevated (C_E) CO_2. The extra bars in darker red colour in (b) and (c) indicate results for the higher temperature given on the y axes. Error bars represent standard errors of the mean (n=3). Significant ($P \leq 0.05$) or marginally significant ($0.05 < P \leq 0.10$) P values are shown in the figure. For the significant temperature by CO_2 interaction in b, letters in squared brackets indicate significant differences among treatment combinations according to Tukey's post hoc test. From Paper I.*

respiration (Way et al., 2015). Suggestions that effects of elevated [CO_2] on respiration are associated with effects on N_a (Way et al., 2015) are not supported by the present study since R but not N_a was increased in elevated [CO_2].

Experimental warming did not affect R or Q_{10} (Figure 8c and 8d). However, substantial seasonal acclimation of R was detected by comparing rates between early summer and late summer periods (Figure 9). Dark respiration measured at 10 °C (R_{10}) was found to be 46% higher in the early as compared to the late period. There was also a seasonal change in Q_{10} and was on average 104% higher in the early compared to the late period. Previous studies on seasonal acclimation (Tjoelker et al., 2008) support the lower R_{10} during the warmer part of the growing season. Thermal acclimation might be the reason for part of this seasonal change in R_{10} but it is probably also linked to seasonal changes in needle starch content. The starch content gets stored successively in spring until bud breaks in early

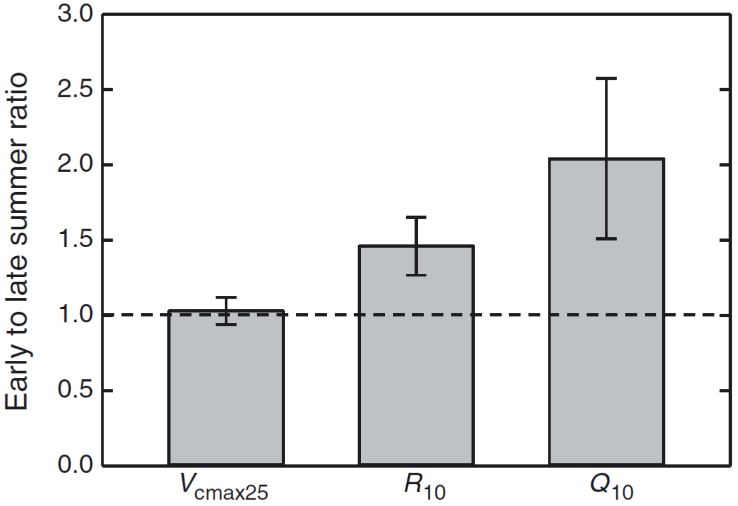


Figure 9. *Early to late seasonal ratios of maximum Rubisco carboxylation rate at 25 °C (V_{cmax25}), dark respiration at 10 °C (R_{10}) and parameter describing the shape of the temperature response of dark respiration at 10 °C (Q_{10}) for shoots of mature Norway spruce trees. Data from 2003-2004 are pooled across all experimental treatments since the treatments did not significantly affect these seasonal ratios. Error bars represent 95% confidence interval (n=12). From Paper I.*

June, after which it declines during new shoot growth (Hall et al., 2009; Linder 1995; Slaney et al., 2007). The higher R_{10} during early period might thus largely be a consequence of higher energy demand related to starch mobilization and phloem loading during the translocation of carbohydrates from older shoots to the developing new shoots (Bouma et al., 1995).

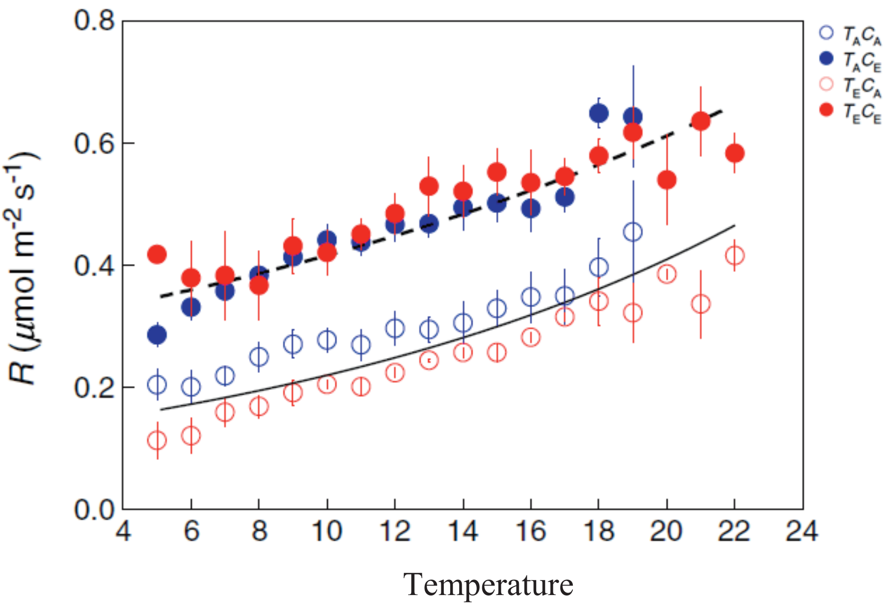


Figure 10*: Temperature responses of shoot dark respiration (R) of mature Norway spruce trees exposed to ambient (T_A) or elevated (T_E) temperature and ambient (C_A) or elevated (C_E) CO_2. The solid and dashed lines show temperature response functions of trees in C_A and C_E, respectively. Data were pooled across temperature treatments since warming did not significantly affect R. From Paper I.*

Stomatal Conductance

Stomatal conductance at light saturation and a VPD of 1 kPa (g_{ref} in Figure 8e) was unaffected by elevated [CO_2]. This agrees with observations that stomatal responses to elevated [CO_2] are weak or absent in gymnosperms (Brodribb et al., 2009; Hasper et al., 2017; Medlyn et al., 2001), as also reported for northern broadleaf tree species (Sigurdsson et al., 2002; Uddling et al., 2009). The leaf intercellular to surrounding air CO_2 concentration ratio (c_i:c_a ratio) increased by 17% in elevated [CO_2] (Figure 8f). This is in line with c_i:c_a ratio estimates based on stable carbon isotope data in a previous CO_2 by fertilization experiment in

Flakaliden (Marshall & Linder 2013). The c_i:c_a ratio also increased in a WTC experiment with Scots pine in Finland (Kellomäki & Wang 1996). Similar results were also found in branch bag experiments with Scots pine and Norway spruce (Sigurdsson et al., 2002).

Unchanged stomatal conductance and increased c_i:c_a ratio in elevated [CO_2] implies increased g_1 (see Figure 5c in Paper), the key parameter in combined stomatal-photosynthesis models (Katul et al., 2010; Leuning et al., 1995; Medlyn et al., 2011). This finding is in conflict with the assumption of constant c_i:c_a ratio and g_1 under elevated [CO_2] in these models. It therefore challenges projections of considerable plant water savings projected by vegetation models that use the combined stomatal-photosynthesis model (Betts et al., 2007; Gedney et al., 2006 and Luo et al., 2008).

Warming and the associated increase in VPD reduced g_{ref}, c_i:c_a ratio and g_1 (Figure 8e-f and Figure 5c in Paper I). This reduced g_{ref} response is opposite to that found in a recent field study on temperate deciduous tree seedlings (Marchin et al., 2016) where g_{ref} increased with long-term increase in temperature and VPD.

Paper II

Experimental effects on tree water use

Mature Norway spruce trees in the Flakaliden WTC experiment did not show any change in shoot g_s at noon in elevated [CO_2] treatment (Figure 11a). This is in agreement with Paper I results and challenges predictions of plant water savings by current vegetation models (Betts et al., 2007; Gedney et al., 2006 and Luo et al., 2008) in higher [CO_2].

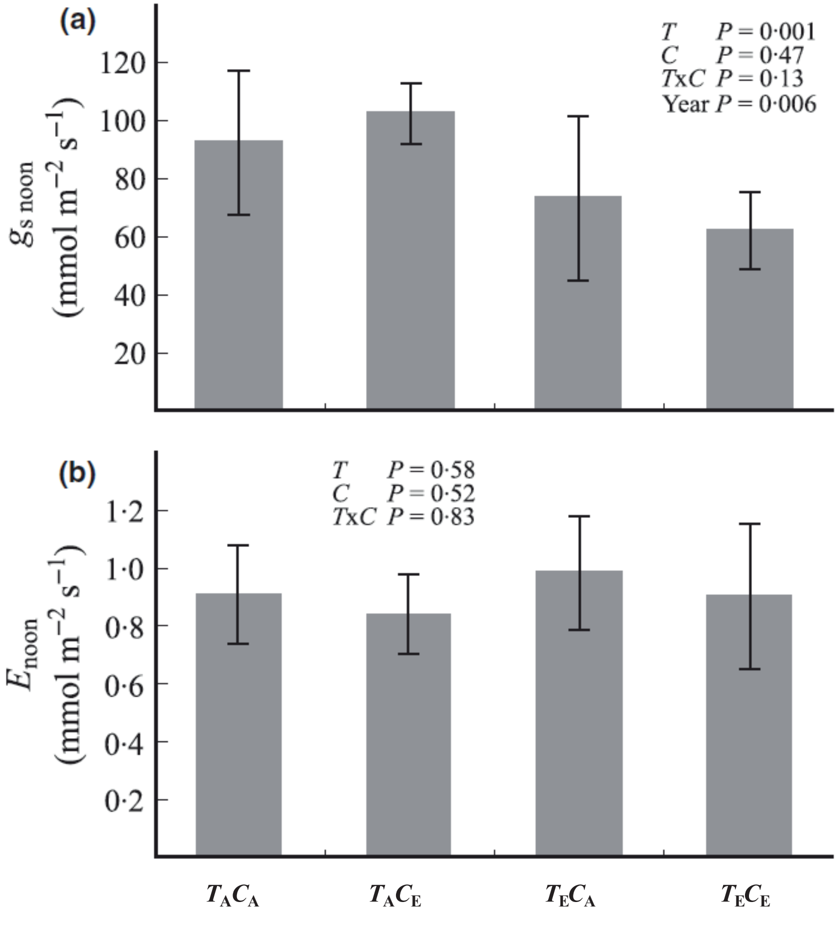


Figure 11: *Shoot stomatal conductance at noon ($g_{s\ noon}$; a) and shoot transpiration at noon (E_{noon}; b) of mature Norway spruce trees in the Flakaliden CO_2 by warming WTC experiment. Bars represent mean values for 2003-2004 combined. Error bars represent one SD. n=3. Symbols: T_A=Ambient temperature; C_A=Ambient CO_2; C_E=Elevated CO_2; T_E=Elevated temperature. From Paper II.*

Mature Norway spruce trees in the Flakaliden WTC experiment did not show any effect of warming on shoot *E* at noon (Figure 11b) as the decrease in g_s (Figure 11a) compensated for the increased VPD. This disagrees with a temperate field study on deciduous tree seedlings which found increased *E* in two out of three seedlings under long-term warming and higher VPD (Marchin et al., 2016).

Trends in forest water use

Estimates of ET of forested catchments in central Sweden showed a significant increase of 18% during the past half century (Figure 12). No significant change was found in runoff which had considerably higher inter-annual variability. Standing forest biomass (Figure S7 in Paper II) increased by 31% over the past 50 years. The increase in biomass of central Swedish forests is likely to be a major driver of the increase in ET.

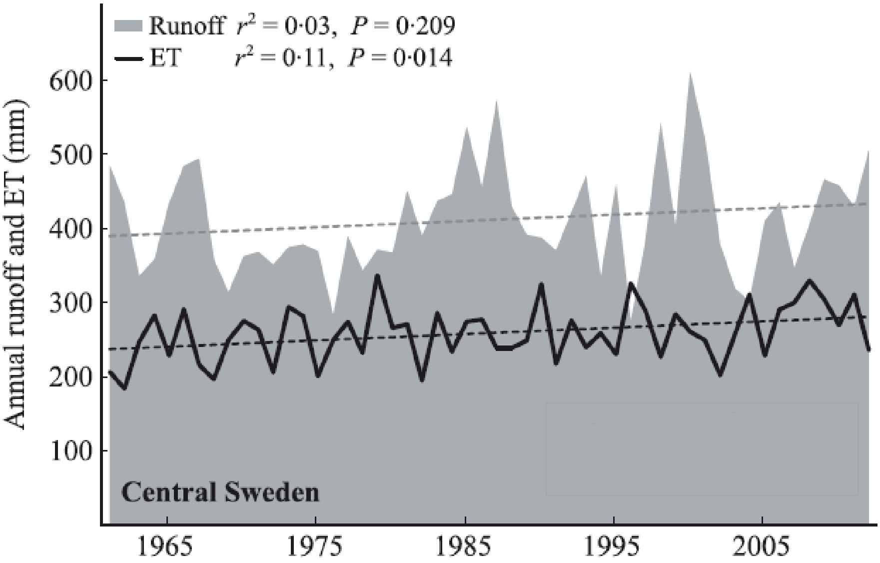


Figure 12: *Temporal trends in annual runoff and evapotranspiration (ET) for large forested catchments of central Sweden. From Paper II.*

Annual ET was not significantly related to the average growing season temperature during May September ($T_{\text{May-Sept}}$; Figure 13a) the length of growing season (Figu 13b). However, it was positive related to annual precipitation (Figu 13c).

Thus, the increase in ET was related to higher precipitation and a steady increase in forest standing biomass over time, together with lack of stomatal closure responses to elevated [CO_2]. The results from this work have important implications for predictions of water use responses of European boreal forests. The findings suggest that changes in precipitation and standing biomass of Swedish boreal forests are more important for *E* than the impacts of elevated [CO_2] or warming (as shown above).

Our result of increasing ET over time contrasts with those of a meta-analysis study, reporting decreasing forest ET trends using the Eddy covariance technique (Keenan et al., 2013). However, those trends were not significant for the boreal sites in the study and that none of the sites used more than 10 years of data.

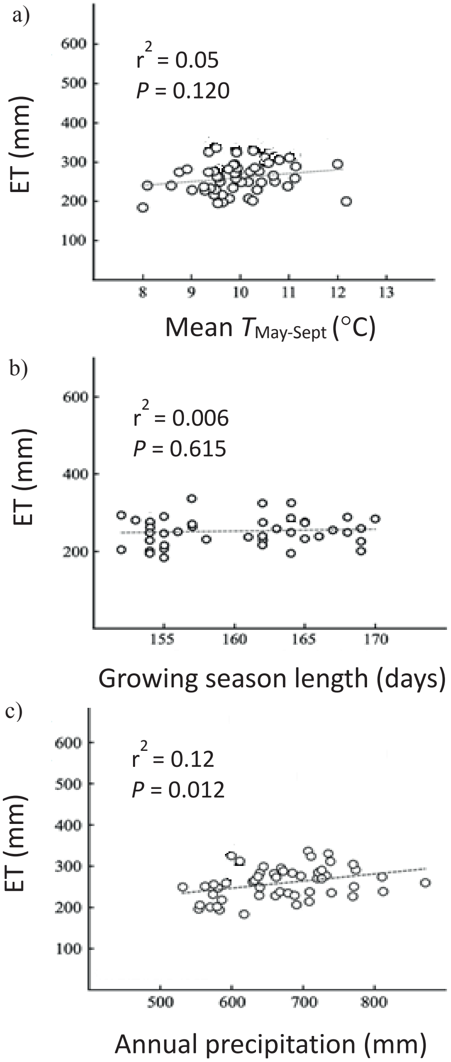


***Figure 13**: Linear regressions of annual evapotranspiration (ET) against mean temperature from May to September (Mean$T_{May\text{-}Sept}$ in ºC; a), growing season length in days; (b), annual precipitation in mm; (c) for large forested catchments of central Sweden. Redrawn from Paper II*

Paper III

Net photosynthesis

Impact of elevated [CO_2]

Elevated [CO_2] increased both shoot and crown A_n (Figure 14a and 14b) in all the acclimation scenarios. However, the response is considerably smaller in the photosynthetic acclimation scenario as compared to the no acclimation scenario. The difference is due to the 23% V_{cmax} downregulation under elevated [CO_2]. This is a common but unusually large response to elevated [CO_2] as compared to previous tree meta-analyses (-13% in Ainsworth & Long 2005 and -10% in Medlyn et al., 1999).

Respiratory acclimation to elevated [CO_2] caused an additional reduction of A_n. This reduction was substantial at crown level but not at shoot level (Figure 14). Studies to date have revealed no clear pattern regarding the impact of [CO_2] on leaf dark respiration (Way et al., 2015). This highlights the need for more research to better parametrize the effect of elevated [CO_2] on R in vegetation models. The results of Paper III show that failure to include the acclimation responses of photosynthesis and dark respiration can cause considerable overestimation of A_n in models projecting forest CO_2 fluxes of the European part of the boreal biome.

Impact of warming

Stomatal acclimation as well as phenological acclimation had the largest effects on shoot and crown A_n under warming (Figure 14a and 14b). The negative effect of stomatal acclimation (i.e. reduced g_1) was similar in magnitude as the positive effect of phenological acclimation (earlier spring recovery of A_n; Wallin et al., 2013). A meta-analysis on controlled tree warming experiments showed boreal trees to be presently operating below their thermal optimum with respect to growth (Way and Oren, 2010). However, this study for field-grown mature Norway spruce trees show that the positive metabolic effects of warming may be cancelled by stomatal acclimation. This highlights the importance of including both warming-induced acclimation of stomatal regulation and dynamic phenology in vegetation models to better estimate CO_2 fluxes in rapidly warming boreal regions.

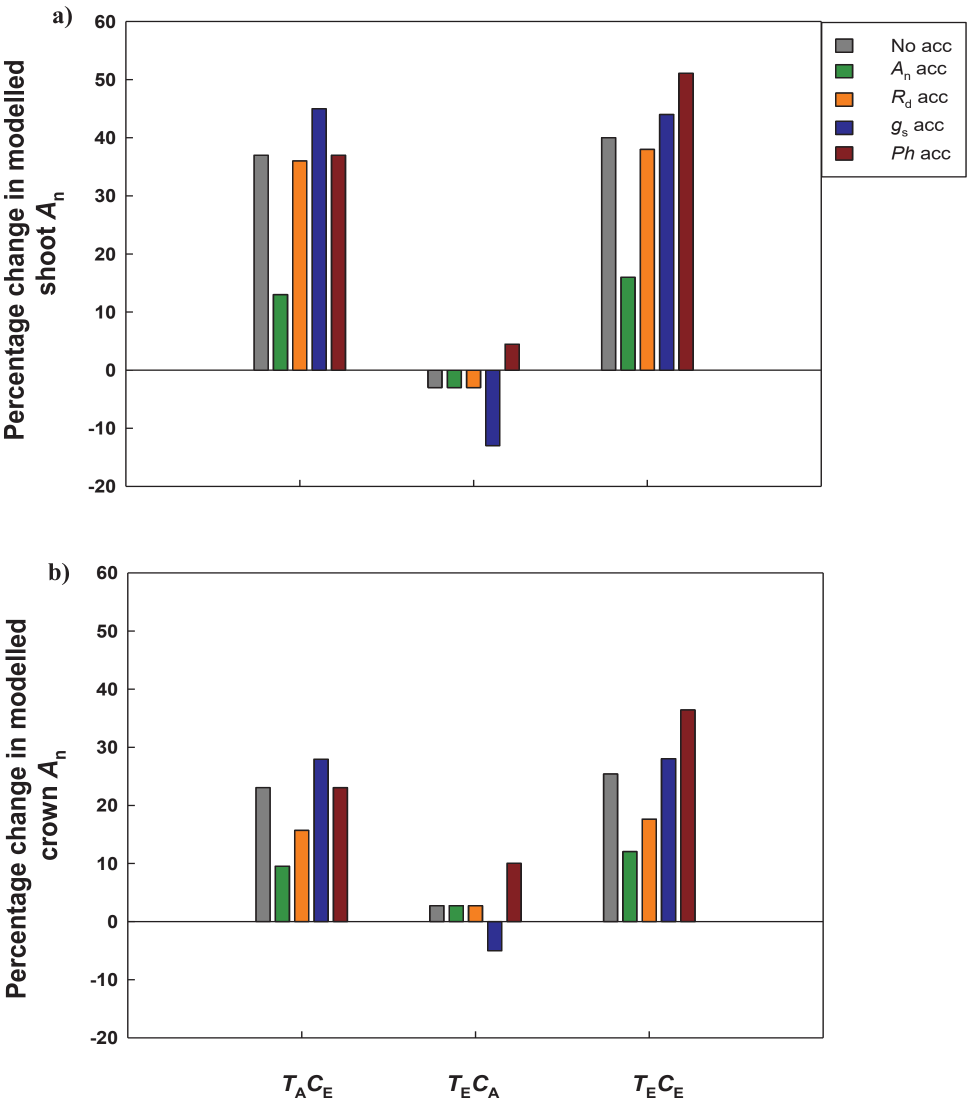


Figure 14: *Modelled effects of different single factor acclimation scenarios in relation to the control treatment T_AC_A on (a) shoot and (b) crown net photosynthesis (A_n) of mature Norway spruce exposed to ambient (T_A) or elevated (T_E) temperature and ambient (C_A) or elevated (C_E) CO_2. Effects are averaged across 2 years, 2003-2004. Acclimation scenarios: no-acclimation (No acc), photosynthesis acclimation (A_n acc), respiratory acclimation (R_d acc), stomatal acclimation (g_s acc) and phenological acclimation (Ph acc). From Paper III.*

Transpiration

Impact of elevated [CO_2]

Stomatal acclimation to elevated [CO_2], as manifested through increased g_1 had the largest effect on shoot and crown E as it offset the large water savings projected in the non-acclimated scenario (Figure 15 a and 15b). Increased g_1 is in conflict with assumptions of unchanged g_1 (and reduced g_s) by the combined photosynthesis-stomatal conductance models (e.g. Medlyn et al., 2011) used by contemporary vegetation models. Thus, the plant water savings under rising [CO_2] predicted by such models (Betts et al., 2007; Gedney et al., 2006; Luo et al., 2008) will likely not be realized for this key species of the European boreal biome. This result is in accordance with Paper II results showing steady increases in ET in boreal forested catchments in central Sweden over the past 50 years.

Impact of warming

Stomatal acclimation to warming, as manifested through decreased g_1 had the largest effect on shoot and crown E, cancelling the positive effect of increased VPD and growing season prolongation (Figure 15a and 15b). This disagrees with projections of increased transpiration in warming by contemporary models assuming constant g_1 under warming (Luo et al., 2008; Medlyn et al., 2011). Our finding calls for more research on stomatal acclimation responses under warming as they will have major consequences for land hydrology in a changing climate.

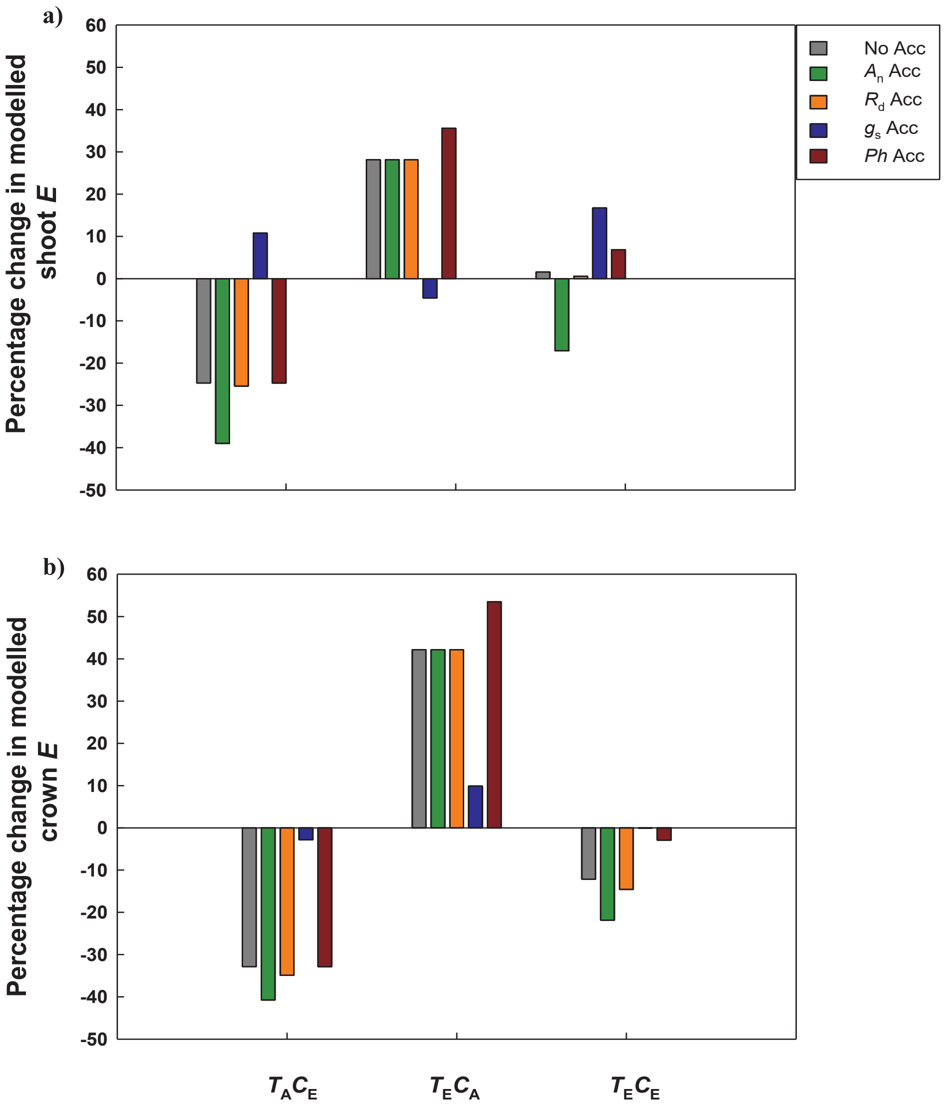


Figure 15: *Modelled effects of different single factor acclimation scenarios in relation to the control treatment T_AC_A on (a) shoot and (b) crown transpiration (E) of mature Norway spruce exposed to ambient (T_A) or elevated (T_E) temperature and ambient (C_A) or elevated (C_E) CO_2. Effects are averaged across 2 years, 2003-2004. Acclimation scenarios: no-acclimation (No acc), photosynthesis acclimation (A_n acc), respiratory acclimation (R_d acc), stomatal acclimation (g_s acc) and phenological acclimation (Ph acc). From Paper III.*

5 Conclusions

Paper I

Values of V_{cmax} at a given temperature decreased in elevated [CO_2] but were not significantly affected by warming. Dark respiration at a given temperature was greatly increased under elevated [CO_2] but not significantly affected by warming. Stomatal conductance on the other hand was not changed by elevated [CO_2] but was substantially decreased by warming and higher VPD. Shoot respiration showed seasonal acclimation while V_{cmax} did not.

The observed boreal long-term acclimation responses of respiration and stomatal regulation differed from those of most observations in temperate regions. These results highlight the importance of incorporating boreal-specific acclimation responses in vegetation models for predicting future carbon and water fluxes of this region.

Most of the acclimation responses observed acted to dampen the instantaneous responses to elevated temperature or [CO_2]. Thus, accounting for long-term acclimation responses in current state vegetation models is important to avoid overestimation of the impacts of elevated [CO_2] and warming on carbon and water fluxes of the European part of the boreal biome.

Paper II

Evapotranspiration over boreal central Sweden has steadily increased since 1960. The increase was linked to the positive effects of higher precipitation, lack of stomatal water savings in the presence of elevated [CO_2] and the increasing standing forest biomass during this period. This historical increase in ET cannot be entirely attributed to forest management strategies as it also happened in the old unmanaged forest in the Svartberget catchment. These results contradict current projections of reduced stomatal conductance and tree water savings under rising atmospheric [CO_2] for the European part of the boreal forest biome.

Paper III

Physiological acclimation of trees to elevated [CO_2] and warming generally dampened non-acclimated responses. Photosynthetic and respiratory acclimation largely reduced the positive effect of elevated [CO_2] on tree carbon assimilation while stomatal and phenological acclimation had large influences on annual water consumption under warming. The study identified the type of acclimation response that mattered the most in elevated [CO_2] and warming. These finding have important implications for vegetation models for projections of future carbon and water fluxes for the boreal region.

6 Outlook

The acclimation responses observed were mostly different from temperate regions. Incorporating these boreal acclimation responses in future modelling studies is therefore crucial in order to improve estimations of CO_2 and water fluxes for the European part of the boreal biome. Also, the observed field responses of mature trees were quite different from those reported from a glass house experiment with seedlings of the same species (Kroner and Way 2016). This emphasizes the need for more field experiments with mature trees.

It should be noted that the responses reported in this thesis are for one age class belonging to particular crown strata (second from top) of individual trees growing in WTCs. In future experiments, measurements could be made on multiple age classes at several crown positions. A comparative experiment with Scots pine, another abundant species of the boreal biome could also be carried out with a similar set up to provide its long term responses. This could highlight interspecies differences, if any. Flakaliden has served as the major field experimental site for Scandinavian boreal trees and forest stands. Additional experimental sites are needed to explore possible site differences. Since experimental data are scarce for the boreal region, studies with different methodologies should be planned for this region. This could include studies along natural temperature gradient as well as, ideally, FACE experiment. The results show that effects of combined treatments of elevated [CO_2] and warming were mostly additive but there is an indication that these two factors might interact with respect to photosynthesis. Combined experiments of elevated [CO_2] and warming are further needed for the boreal regions to better understand the responses.

Modelling experiments could be planned out in large scale vegetation models, modifying the combined stomatal photosynthesis conductance model based on water use responses of Norway spruce to find out the impact on water fluxes in a changing atmosphere and climate. Inter-comparison modelling studies could be carried out with the present state of models with and without acclimation responses to explore possible difference in the influences on carbon estimations and water use projections.

The work also has key implications to inform vegetation models about the relative importance of different kinds of acclimation responses for boreal vegetation. Future work could scale up responses beyond the tree crown, to explore the implications of different physiological acclimation responses at the stand or regional scales. Inter-model comparison studies could be carried out with the acclimated responses of Norway spruce to identify uncertainties in scaling up using different models.

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