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Acclimation of photosynthesis to different growth temperatures in *Betula pendula*

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Abstract

Over the past century the global climate has been undergoing rapid changes and atmospheric CO_2 concentrations are projected to rise further throughout the 21st century. This accumulation of greenhouse gases in the atmosphere is associated with an elevation of global mean temperatures, at a currently unprecedented rate. How this abrupt warming will affect the inherent biosphere remains poorly understood. However, especially vegetation providing temporary carbon sequestration has to be considered in the context of climate change. Being closely interwoven with climate, plants will not only be passively affected by altered environmental factors, but they will adjust to new conditions and *vice versa* influence the persistent climate. Thus, in order to make reliable predictions of future climates, increased knowledge about metabolic plant processes, such as photosynthesis, is crucial. Key factors that need to be investigated are temperature responses of photosynthetic capacities in trees, which are believed to take up 33% of anthropogenic carbon emissions, as well as their potential for acclimation to new environmental conditions.

This study aimed to examine impacts of long-term temperature treatment on plant growth in terms of thermal acclimation. The deciduous tree *Betula pendula* was grown in climate chambers of 15 °C and 25 °C, respectively, and photosynthesis measurements were performed at different temperatures (15 to 40 °C) in each treatment. In order to round up those gas exchange measurements, underlying leaf properties such as nitrogen content and leaf mass per area, leaf thickness, as well as lipid composition and chlorophyll *a* fluorescence were investigated and related to the respective photosynthetic performance. Eventually, modeled parameters describing maximum rates of photosynthetic carboxylation capacity (V_{cmax}) and electron transport (J_{max}) and their temperature responses could be analyzed from different angles. Furthermore, morphology and biomass characteristics were derived from the birches to estimate the impact of growth temperature on carbon allocation.

It could be concluded that low-temperature grown *Betula pendula* exhibits increased photosynthetic capacities per area, compared to birches grown in high temperatures. This difference in V_{cmax} and J_{max} between the birches could be traced back to their disparity in nitrogen mass per area (NMA), which was higher in trees grown in 15 °C compared to those grown in 25 °C. NMA was positively correlated to leaf mass per area (LMA) and leaf thickness. A shift in optimum temperature was not observed for the maximum rate of carboxylation (V_{cmax}), which showed no optimum temperature within the measurement range. In contrast, the maximum rate of electron transport (J_{max}) revealed lower optimum temperature in case the birches were grown in colder conditions. An associated dampened increase of J_{max} at high leaf temperatures could be explained by altered lipid composition of the thylakoid membrane, implying relatively more unsaturated fatty acids and increased membrane fluidity in cool-grown birches. Besides that, chlorophyll *a* fluorescence signals might allude to a decreased functioning of PSII in the birches grown in 15 °C, which is related to J_{max} as well. Regarding biomass and carbon allocation, the low-temperature grown birches

exhibited significantly lower dry mass, whereas birches grown in elevated temperatures generally displayed enhanced growth, which was accompanied by higher dry mass.

Taken together, when grown in warmer conditions, birches appeared to invest in leaf area, rather than thickness, revealing lower photosynthetic performance per area. Nevertheless, due to thermal membrane acclimation and unlike trees adjusted to a cold environment, their photosynthetic capacity could increase unhindered with elevating temperature and their growth was generally enhanced, resulting in greater carbon sequestration. Hence, with respect to a warmer future climate, these findings might result in attenuated increase of atmospheric CO_2 concentrations.

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Abbreviations

A_c: Rate of carboxylation limited photosynthesis per leaf area (μmol m^{-2} s^{-1})

A_j: Rate of RuBP regeneration limited photosynthesis per leaf area (μmol m^{-2} s^{-1})

A_n: Net rate of photosynthesis per leaf area (μmol m^{-2} s^{-1})

A_{pred}: Model-predicted net rate of photosynthesis per leaf area (μmol m^{-2} s^{-1})

birches^{15}: *Betula pendula* grown in climate chambers of 15 °C

birches^{25}: *Betula pendula* grown in climate chambers of 25 °C

C_i: Intercellular CO_2 concentration (ppm)

F_v: Variable fluorescence

F_m: Maximum fluorescence

GHG: greenhouse gas

g_s: Stomatal conductance per leaf area (mol m^{-2} s^{-1})

g_m: Mesophyll conductance per leaf area (mol m^{-2} s^{-1})

H_a: Rate of exp. increase of the peaked Arrhenius function below the optimum (kJ mol^{-1})

H_d: Rate of decrease of the peaked Arrhenius function above the optimum (kJ mol^{-1})

IRGA: Infra-red-gas-analyzer

J: Rate of photosynthetic electron transport per leaf area (μmol m^{-2} s^{-1})

J_{max}: Maximum rate of photosynthetic electron transport per leaf area (μmol m^{-2} s^{-1})

K_c: Michaelis-Menten coefficient of the activity of Rubisco with respect to CO_2

K_o: Michaelis-Menten coefficient of the activity of Rubisco with respect to O_2

k_{obs}: Observed value of V_{cmax} or J_{max} as a function of temperature (μmol m^{-2} s^{-1})

k_{opt}: Value of V_{cmax} or J_{max} at T_{opt} (μmol m^{-2} s^{-1})

k_{pred}: Predicted value of V_{cmax} or J_{max} as a function of temperature (μmol m^{-2} s^{-1})

LMA: Leaf mass per area (g m^{-2})

NMA: Nitrogen mass per leaf area (g m^{-2})

N%: Percentage of nitrogen per leaf dry mass

NPP: Net primary production

O_i**:** Intercellular concentration of O_2 (ppm)

Q**:** Photosynthetic active photon flux density (μmol m^{-2} s^{-1})

R**:** Universal gas constant (8.314$mol^{-1}K^{-1}$)

r^2**:** Squared error term

R_d**:** Dark respiration (μmol m^{-2} s^{-1})

RH: Relative humidity (%)

Rubisco: Ribulose-1,5-bisphosphate carboxylase/oxygenase

RuBP: Ribulose-1,5-bisphosphate

T_{growth}**:** Plant growing temperature (°C)

T_{meas}**:** Temperature of the leaf within the IRGA measurement chamber (°C)

T_{leaf}**:** Leaf temperature (°C)

T_k**:** Actual temperature (K)

T_{opt}**:** Optimal temperature of photosynthesis (V_{cmax} or J_{max}) (°C or K)

V_{cmax}**:** Maximum rate of carboxylation per leaf area (μmol m^{-2} s^{-1})

VPD: Vapour pressure deficit

α**:** Quantum yield of electron transport (mol electrons mol^{-1} photons)

Γ^***:** CO_2 compensation point in absence of mitochondrial respiration (μmol mol^{-1})

Θ**:** Curvature of the light response curve

Introduction

Global climate change

Climate change increasingly gained importance as a global issue of concern. By now, it is without doubt that climatic alteration is primarily attributed to increasing anthropogenic greenhouse gas (GHG) emissions, implying the combustion of fossil fuels and changes in land use. Since pre-industrial times, annual emissions of carbon dioxide (CO_2), which is regarded as the most important anthropogenic GHG, have increased around 80% regarding the period between 1970 and 2004. According to the most recent, forth assessment report (AR4) published by the *Intergovernmental Panel on Climate Change* (IPCC) , the atmospheric CO_2 concentration will continue to increase from the current concentration of 390 ppm to approximately 700 ppm by the end of 2100, depending on the assumptions of different scenarios and models the predictions are based upon. Moreover, in terms of an associated greenhouse effect, global mean temperatures are predicted to rise by approximately 3 °C approaching the end of the 21st century. Irrespective of regional, seasonal and diurnal heterogeneity, global warming occurs worldwide, heating up air, land and oceans. It is accompanied by altered wind and precipitation regimes, as well as melting ices and rising sea levels (IPCC 2007). As stated above, the entire reaction chain of these environmental changes is mainly traced back to the human-induced CO_2 emissions, which are apparently hard to foresee. Although scenarios of climate models account for numerous factors, such as population size, economic growth, technological development or political and social aims, accurate predictions of future atmospheric CO_2 concentration and average temperature, respectively, are vague. Predictions especially appear to be complicated, since the persistent global warming itself might influence the biogeochemical fluxes of carbon, meaning that warming affects the biosphere, which adjusts to the new condition and *vice versa* influences the climate in a certain way (Newman *et al*. 2011).

Climate change and biosphere interactions

Photosynthesis and respiration are the two main physiological processes that link the biosphere and the atmosphere in terms of an everlasting cycle of carbon. During photosynthesis plants use the primary energy source of the sun, take up atmospheric CO_2 and form complex carbon structures, namely living tissues. Therefore, vegetation with its photosynthesis plays an important role in the spacious cycle of carbon, providing temporary sequestration (Gurevitch *et al*. 2006). Besides that, plants influence our prevailing climate by exchanging energy, water and other chemical species with the atmosphere. Regarding carbon sinks, particularly forests have to be considered, as they constitute 45% of the terrestrial carbon storage and contribute about 50% to terrestrial net primary production (NPP). They are expected to take up approximately 33% of anthropogenic carbon emissions (Bonan 2008).

Necessity of investigating plant acclimation to improve climate predictions

Taken together, it appears to be evident that responses of trees embedded in a changing climate have to be accounted for in our models, if we are to make accurate predictions of atmospheric carbon dioxide concentrations and future rates of climate change. Basically, plants can respond to environmental changes in three different ways. They can adjust through phenotypic plasticity, evolve and adapt through natural selection, or migrate to more suitable habitats (Nicotra *et al.* 2010). However, many possibly important responses of plants remain poorly understood, and thus have not been incorporated in climate models comprehensively (Arneth *et al*. 2010). Therefore, we have to gain knowledge about plant's adjustment to a changing climate, which would allow assumptions of potential feedbacks, and lately improve our climate predictions. In order to examine their capacity of adjusting, namely acclimation, plants are experimentally exposed to a certain environmental factor for a longer time period, and the resulting carbon fluxes involving photosynthesis are measured. These investigations are based on model parameters simulating photosynthesis. In order to realistically assess the net carbon assimilation rate, the model parameters have to be adjusted according to the acclimation of photosynthesis. Smith and Dukes (2010) reviewed that the estimated carbon flux between vegetation and the atmosphere can tremendously differ, depending on whether the model included acclimation of plants or not.

Modeling photosynthesis

Leaves embody the evolutionary young organs of plants that are specialized to perform photosynthesis. Acting as a flat solar panel, they house the cellular and biochemical machinery required for photosynthesis, capturing sunlight and determining the carbon uptake of the entire plant. Eventually, they define the gross carbon acquisition provided by an ecosystem (Beerling 2007). C_3 plants, which, in contrast to C_4 plants, assimilate CO_2 directly via the Calvin cycle, constitute approximately 95% of all plants on earth (Vu 2005). Hence, in order to investigate carbon assimilation in the context of global climate change, a biochemical leaf model of C_3 photosynthesis proposed by Farquhar, von Caemmerer and Berry (1980) is commonly used.

In order to apply this model, the net carbon uptake provided by a leaf has to be measured at different CO_2 concentrations. Accounting for variations caused by the opening of stomata, measured assimilation rates are usually referred to intercellular CO_2 concentrations. Finally, obtained rates for the net photosynthetic assimilation rate (A_n) can be plotted against varying intercellular CO_2 concentrations (C_i), resulting in a so called "AC_i curve". The Farquhar *et al*. model assumes that throughout the measurement, the net photosynthetic carbon uptake is limited by one of two major processes, namely by the activity of the carbon fixing enzyme ribulose-1,5-bisphosphate corboxylase/oxygenase (Rubisco), or by the regenratation of ribulose-1,5-bisphosphate (RuBP), which is the CO_2 accepting compound. RuBP regeneration is dependent on the energy-rich molecules ATP and NADPH, derived from the electron transport chain. At low amounts of accessible CO_2, the main limiting factor for A_n is the rate of carboxylation, whereas at higher CO_2 concentrations it is the electron transport chain that

cannot keep pace with regenerating and preparing RuBP for further carbon uptake. The model parameter V_{cmax}, representing the maximum carboxylation capacity, can be derived from the initial slope of the AC_i curve, whereas the parameter J_{max}, indicating the RuBP regeneration capacity and the respective maximum rate of electron transport, refers to the trend of the AC_i curve at higher CO_2 concentrations (Sharkey *et al*. 2007). The limitation of A_n by a third process, which implies the use of triose phosphate, is rare and thus will not be accounted for in this study.

Thermal acclimation in the photosynthesis model

The net photosynthetic assimilation rate of plants is dependent on temperature. In the context of the Farquhar *et al.* model, A_n is determined by Rubisco's carboxylation capacity (V_{cmax}) and the electron transport capacity (J_{max}), as mentioned above. Both the maximum rate of carboxylation and the maximum rate of electron transport have been shown to increase with rising temperatures up to an optimum temperature (T_{opt}), after which they rapidly decline again (Kattge and Knorr 2007). Hence, derived at different temperatures and plotted against the respective measurement temperature, A_n displays a so-called "temperature response curve". In lower temperatures, A_n is rather limited by the activity of the carbon fixing enzyme Rubisco, whereas at high temperatures it is the electron transport that limits A_n (von Ceammerer 2000). In between, namely at the optimum temperature of A_n, the plant's rate of photosynthesis is maximized (Berry and Björkmann 1980). This optimum temperature might shift towards higher temperatures, when the growth temperature is elevated, allowing the plant to perform photosynthesis and thus grow most efficiently in its current climate. The collection of biochemical, structural or physiological responses underlying the optimum temperature shift is termed thermal acclimation (Smith and Duces 2013).

Von Caemmerer and Farquhar (1981) demonstrated, that a long-term temperature-induced change might be associated with altered *in vitro* activity of Rubisco and altered *in vitro* rate of electron transport. Therefore, they suggested that these alterations could be modeled simply by the parameters V_{cmax} and J_{max}. According to Berry and Björkmann (1980), the major factors affecting the temperature responses of the model parameters V_{cmax} and J_{max} is growth temperature, as well as genotype or species. Besides that, several other factors have been shown to influence the parameters, such as plant nutrition, light conditions or leaf age. The observation that photosynthetic capacity in terms of V_{cmax} and J_{max} at various temperatures is dependent on the temperature the plant was grown in implies thermal acclimation. Farquhar and von Caemmerer (1982) argued that a shift in the photosynthetic optimum temperature is based on changes in the balance between the processes of carboxylation and RuBP regeneration. Under this assumption, the capacity of the partial process revealing the lower optimum temperature would increase relative to the capacity of the other partial process, resulting in a shift towards higher optimum temperatures (the intersection of the two respective temperature response curves of V_{cmax} and J_{max}). However, by now, several studies showed that the shift in optimum temperature is not only attributed to an altered balance between V_{cmax} and J_{max}. Growth temperature rather affects the temperature dependence of the partial processes *per se* (Hikosaka *et al*. 1999; Bunce 2000).

In a study published in 2002, Medlyn *et al*. parameterized the commonly used photosynthesis model from 19 gas exchange studies on crop species, deciduous trees and evergreen trees (2002b). Their main aim was to investigate alterations in the temperature response of the model parameters V_{cmax} and J_{max} due to different long-term temperature-exposures. Taking growth environment and plant type into consideration, the review proposes particular rates for V_{cmax} and J_{max} in order to improve the parameter choice when modeling photosynthesis. Regarding the relationship between parameter values and growth temperature, particularly *Betula pendula* exhibited distinctly different temperature responses, when the same genus was grown in diverse temperatures. This alludes to a high acclimation capacity of the tree, as well as the fact that *Betula pendula* is a widespread European tree tolerating various temperature regimes. Therefore, to investigate thermal acclimation in terms of the photosynthetic parameters V_{cmax} and J_{max}, *Betula pendula* was chosen for the conducted experiments.

Analysis of nitrogen content, lipid composition and chlorophyll a fluorescence

In order to round up gas exchange measurements providing rates for V_{cmax} and J_{max} potentially underlying leaf characteristics can be examined by applying various biochemical analysis. Here, nitrogen content and lipid analysis, as well as chlorophyll *a* fluorometry are frequently used.

It is widely believed that the photosynthetic capacity of C_3 plants is closely linked to the amount of foliar nitrogen, which, being a crucial component of proteins and enzymes, is a representative for the presence of enzymes. RuBP carboxylase, the crucial enzyme of the Calvin cycle, as well as proteins of the thylakoid membrane, such as pigment-protein/ reaction center complexes and components of the electron transport chain (especially the complexes cytochrome b/f and ferredoxin NADP reductase) constitute the majority of nitrogen present in the leaf. Thus, it is evident that the maximum rates of carboxylation and electron transport are highly related to the amount of foliar nitrogen (Evans 1989), and that V_{cmax} and J_{max} should be examined with respect to the corresponding nitrogen content.

Not only has the nitrogen content in the leaf been shown to determine the photosynthetic performance, but also characteristics of the thylakoid membrane. In its very essence, oxygenic photosynthesis is a membrane-bound process. The protein-pigment complexes that mediate the primary light absorbtion are bound to the thylokoid membrane and the membrane houses the components for electron transportation. Moreover, the proton gradient driving ATP synthase is built across the thylakid membrane. It is thus comprehensible that the chloroplast of higher plants is a very membrane rich organelle, and that the thylakoid membrane properties, such as lipid composition and membrane viscosity appear to affect the temperature response of the whole electron transport chain (Mikami and Murata 2003). In case plants are grown at various temperatures, acclimation of the membrane with altered characteristics may occur, eventually shifting the temperature optimum (Berry and Björkmann 1980).

Furthermore, when investigating the electron transport capacity, chlorophyll *a* fluorescence signals can accompany gas exchange measurements. They provide estimations of the electron

transport rate in the chloroplast *in vivo* (Edwards and Baker 1993). Fluorescence characteristics are widely used in order to assess the efficiency of PSII activity (Krause and Weis 1991). There are different fluorescence parameters that can be determined by exposing the leaf to brief, saturated light pulses and measuring its re-emission of energy in form of fluorescence (Maxwell and Johnson 2000). In the present study the ratio of the variable fluorescence (F_v) over the maximum fluorescence (F_m) is used to give an indication about the functioning of PSII.

Aims of the study

The current study is conducted in the context of climate change regarding the necessity of accounting for biosphere responses to eventually improve predictions of future ecosystems and their surrounding atmosphere. Ultimately the aim goes back to the scale of leaf photosynthesis and carbon allocation in a C_3 deciduous tree with potentially high acclimation capacity, namely *Betula pendula*. This research intends to gain knowledge about thermal acclimation to various growth temperatures, in terms of the Farquhar et al. (1980) model parameters V_{cmax} and J_{max}. That is, the purpose of this study is to investigate whether *Betula pendula* reveals alterations in V_{cmax} and J_{max} due to different growth temperatures. Questions that have to be answered are:

- Do birches grown in low temperatures achieve different rates for V_{cmax} and J_{max} compared to birches grown in high temperatures?
- Does the amount of nitrogen per area differ between the warmed and unwarmed trees and is there a potential correlation between nitrogen content and photosynthetic capacity?
- In case of an obtained difference in nitrogen content, can it be explained by diverse leaf mass per area and leaf thickness, and how are those parameters related to photosynthesis?
- Does thermal acclimation in terms of a shift in optimum temperature occur for V_{cmax} and J_{max}?
- Is there a difference between the birches regarding lipid composition and chlorophyll *a* fluorescence that might explain obtained values for the temperature responses of V_{cmax} and J_{max}?
- How are V_{cmax} and J_{max} related to each other and is there a difference in their balance induced by various growth temperatures?
- Do the birches grown in high and low temperatures reveal different morphologies and biomass characteristics with respect to potential carbon allocation in upcoming warmer climates?

Taken together, the present study intends to combine gas exchange measurements with underlying leaf properties and aims to give an indication of carbon sequestration, to eventually provide a bigger picture of the impacts long-term temperature treatment can have on plant growth.

Material and Methods

Plant material and growth conditions

The species chosen for this study was *Betula pendula* (silver birch). It is a deciduous tree of the family Betulaceae, which naturally grows in cooler, more northerly habitats of Europa. However, its distribution ranges into southwest Asia in the mountains of northern Turkey and the Caucasus. Being native in higher latitudes, the tree is adapted to regimes with greater seasonal and diurnal temperature variations and thus can be posited to have relatively large capacities for acclimation (Cunningham and Read 2002).

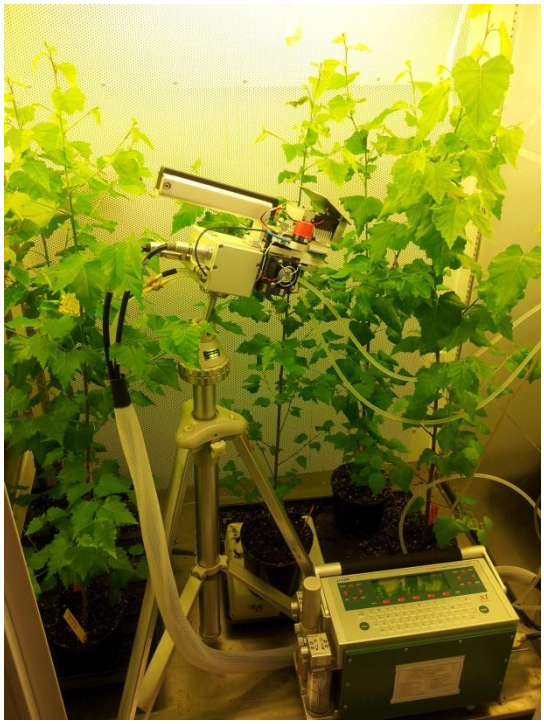
Two year old silver birches obtained from a nursery were grown in 3 L plastic pots in growth chambers (Department of Biological and Environmental Sciences, Carl Skottsbergs gata 22B, 40530 Gothenburg, Sweden). Two different climate chambers were used, each representing one growth temperature (T_{growth}). The climate chambers exhibiting 15 °C are referred to as low temperature treatment, while the temperature in the other climate chambers was held constant at 25 °C and thus stands for high growth temperature conditions. The sample size for each long-term temperature-treatment was four. Except for the growth temperature, all other environmental factors were similar between the climate chambers. Day and night lengths were 16 and 8 h, respectively. During the light period the photosynthetic active photon flux density (Q) was 200 µmol m^{-2} s^{-1}. The relative humidity (RH) was high and fluctuated between 70 and 80%. Besides the fact that other environmental factors than temperature did not vary between the two greenhouses, the plants did not suffer drought stress or nutrient depletion, such that the study could focus on the impacts of temperature on plant growth.

The four birches grown in 15 °C (birches^{15}) and the four birches grown in 25 °C (birches^{25}) developed leaves at different time points, yet the bud break occurred approximately after two weeks. Growth being enhanced in 25 °C implied that the birches^{25} revealed more plant material in a shorter amount of time. Taking this into consideration, the birches were watered and fertilized according to their developmental state and their respective demand. More precisely, the trees were irrigated and fertilized with a balanced, custom made solution containing all necessary micro- and macro-nutrients three times a week. The nutrient solution was based on nitrogen content (100%) and contained 65% K, 13% P, 7% Ca, 8.5% Mg, 9% S, 1% Fe and several other micro-nutrients. This mixture corresponded to 100 mg N per week for the cool-grown birches and 200 mg N per week for the fast developing warm-grown birches.

Two of the birches in the 15 °C climate chambers were transiently exposed to aphids. However, since it was a small number of insects, they could be removed manually.

Gas exchange measurements

In order to examine and compare photosynthetic capacities, one leaf per tree was measured in each growth chamber. The leaves were taken from the upper part of the canopy, which was constituted by the stem that developed within the climate chambers. Further criteria when choosing a leaf to measure were that the leaf should be overall healthy and reveal certain maturation, implying that it is sufficiently adjusted to the respective growth temperature. These criteria were preconditioned for all foliar investigations that followed the LI-6400XT measurements (analysis of lipid composition, chlorophyll *a* fluorescence and morphology). The gas exchange measurements were started after a growing period in the climate chambers of seven weeks and were performed within three weeks, with one measurement being repeated two weeks afterwards.



Figur 1. *Betula pendula* with a leaf inside the IRGA chamber during a measurement. Photo: Martina Lutz

For investigations of gas fluxes provided by the leaf the photosynthesis portable system LI-6400XT was used (LI-COR Inc., Lincoln, NE, USA). Its technique is based on the comparison of two air streams with set environmental conditions. One air stream serves as a reference, while the other represents the sample air and passes through a chamber, which encases the gas exchanging leaf. Hence, by applying an infra-red-gas-analyzer (IRGA) and comparing the absolute CO_2 concentrations of the reference and the sample air, the net photosynthetic carbon assimilation rate of the enclosed leaf can be examined. Since the transpiration rate is measured equivalently in terms of a difference in water content, further parameters such as stomata conductance (g_s) and intercellular CO_2 concentration (C_i) can be calculated, as well. Figure 1 shows the setting of a measurement in the 25 °C climate chambers, with a leaf being enclosed in the LI-6400XT IRGA chamber.

The purpose of the gas exchange measurements was to examine the photosynthetic rate with increasing intercellular CO_2 concentrations, resulting in an AC_i curve, from which photosynthetic capacities could be derived later on. Hereby, the ultimate aim was to run this procedure at different leaf temperatures, such that the photosynthetic capacities collected at various temperatures displayed a "temperature response curve", which eventually could be compared between the two growth chambers. That is, the leaf experienced an increase of CO_2 concentration at 15, 25, 32.5 and 40 °C, respectively. At every temperature step, the series of CO_2 concentrations in the air entering the leaf chamber was determined to 400, 0, 60, 125, 250, 400, 800, 1500 and 2000 µmol mol^{-1}. Those measurement concentrations were framed by a measurement at 400 µmol mol^{-1} to detect potential variation of photosynthetic rates

throughout the measurement. The respective air temperature within the IRGA chamber during the measurements and, thus, the leaf temperature (T_{meas}), were controlled by a surrounding metal block, namely the 6400-88 Expanded Temperature Control Kit (LI-COR Inc., Lincoln, NE, USA). In order to reach the desired leaf temperatures, the metal block had to be supported and connected to an external water bath with greater potential temperature range.

Variations in the vapor pressure deficit (VPD) and the respective relative humidity (RH) within the leaf chamber due to different temperatures were minimized by adjusting the water content of incoming air, while possible condensation and stomata closure had to be considered. However, the aspired RH of 60% within the leaf chamber could not be avoided to decrease to a minimum of 20% at 40 °C. Nevertheless, the collected data can be considered as reliable, since the stomata conductance did not fall below a value of 0.07 µmol m^{-1} s^{-1}. The photosynthetic active photon flux density (Q) provided by a LED light source was held constant at 1000 µmol m^{-2} s^{-1} during the whole measurements.

Before collecting data, the desired environmental conditions within the IRGA chamber were determined, such that the leaf could acclimate for at least 20 min to the new measurement temperature. Regarding the warm-grown birches, the measurements conducted at higher temperatures appeared to be affected by a preceding measurement at 15 °C. Hence, the four temperature steps were split up, with the measurements at 25 °C and 15 °C being conducted on one day and those at 32.5 °C and 40 °C conducted at the following day. Thereby the plants recovered from the former temperature exposure, as seen when comparing their photosynthetic rate at their respective growth temperature between the days. In order to maintain the same protocol, the cool-grown birches were measured in an equivalent way, starting with the measurement at their growth temperature of 15 °C, which was followed by 25 °C, and conducting the measurements at 32.5 °C and 40 °C the next day.

Modelling photosynthesis based on the parameters V_{cmax} and J_{max}

The output data obtained from the LI-6400XT measurements, involving net photosynthetic assimilation rate and intercellular CO_2 concentrations at different leaf temperatures, were analyzed using a biochemical model of C_3 leaf photosynthesis. This AC_i curve fitting model was proposed by Farquhar *et al*. (1980) and modified by Sharkey et al. (2007). It assumes that photosynthesis is limited by two major processes.

At low CO_2 concentrations, it is the carboxylation reaction performed by the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) that occurs as a photosynthesis limiting factor. The corresponding assimilation rate in case photosynthesis was only limited by carboxylation (A_c), can be estimated by using the equation

$$A_c = \frac{V_{c\max}(C_i - \Gamma^*)}{\left[C_i + K_c\left(1 + \frac{O_i}{K_o}\right)\right]} \qquad (1)$$

where V_{cmax} is the maximum rate of carboxylation, Γ^* is the CO_2 compensation point in absence of mitochondrial respiration, K_c and K_o are Michaelis-Menten constants of the activity of Rubisco with respect to CO_2 and O_2, and O_i is the intercellular concentration of O_2. Since this study intended to focus on temperature responses of photosynthesis, the parameters Γ^*, K_c and K_o were not considered to be constant, but estimated, accounting for the particular measurement temperature. Therefore, an Arrhenius equation was incorporated into the model according to Bernacchi (2001).

At high CO_2 concentrations, the regeneration of the carbon dioxide binding compound ribulose-1,5-bisphosphate (RuBP) becomes the dominating factor that limits photosynthesis, as RuBP has to be prepared for further carbon uptake. Its regeneration is dependent on the electron transport chain implying the formation of ATP and NADPH. The equivalent assimilation rate (A_j), in case photosynthesis was only limited by the rate of electron transport is given by

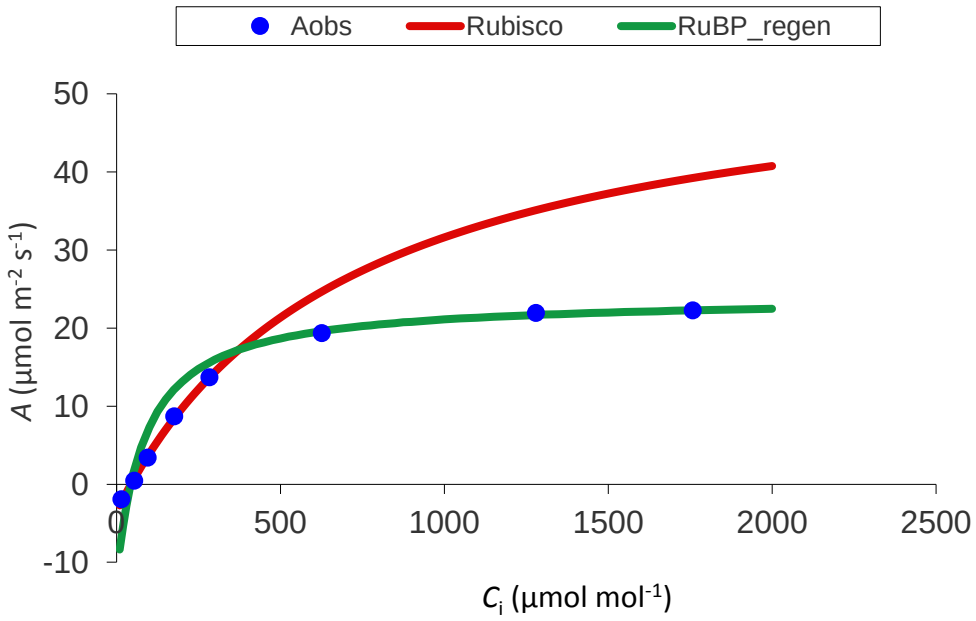
$$A_j = \left(\frac{J}{4}\right) \times \frac{(C_i - \Gamma^*)}{(C_i - 2\Gamma^*)} \qquad (2)$$

where J is the rate of electron transport.

According to the model, the net photosynthetic rate observed throughout a measurement with increasing CO_2 concentrations is determined by the minimum of those two biochemical processes. The final predicted photosynthetic rate (A_{pred}) can thus be described as

$$A_{pred} = \min(A_c, A_j) - R_d \qquad (3)$$

with R_d being the dark respiration performed by mitochondria. Eventually, the model plots the rates of A_n derived from the LI-COR measurement against the respective values of C_i. Primarily setting V_{cmax} to 50, J to 100 and R_d to 1 µmol m^{-1} s^{-1}, the model solves the equations of A_c and A_j and adds the predicted AC_i curves (A_c and A_j against C_i) to the observed data. Finally the mathematically modeled curves are fitted to the results of the intrinsic gas exchange measurements (Figure 2).



Figur 2. Photosynthetic rates (Aobs) derived from a gas exchange measurements at various intercellular CO_2 concentrations (C_i) conducted at a leaf temperature of 25 °C in the cold climate chambers. Two modeled AC_i curves are fitted to the observed data points and represent assimilation rates, in case photosynthesis was only limited by carboxylation (Rubisco) at low C_i concentrations and electron transport (RuBP_regen) at higher C_i concentrations, respectively.

In order to fit the predictions to the observed data, the deviation of the modeled A_c and A_j from the measurement results (A_n) is minimized by adjusting the parameters V_{cmax} and J in the respective equations. This is done by minimizing the sum of squares (Σr^2) defined as

$$\sum r^2 = \sum (obs - pred)^2 \qquad (4)$$

where *obs* represents A_n and *pred* stands for A_{pred}. Thereby, values for the maximum carboxylation capacity, as well as rates for the electron transport can be derived from the gas exchange measurement. J is related to the maximum electron transport capacity (J_{max}) as

$$J_{max} = \frac{\Theta J^2 - \alpha Q J}{(J - \alpha Q)} \qquad (5)$$

where Θ is the curvature of the light response curve, α is the quantum yield of electron transport and Q is the photosynthetic active photon flux density. In this study Θ was assumed to be 0.90, α was fixed at 0.3 mol electrons mol^{-1} photons and Q was set to 1000 µmol m^{-2} s^{-1}, equivalently to the radiation intensity used during the measurements.

The estimator utility of the model modified by Sharkey *et al*. (2007) was readapted for the current study by Johan Uddling and Göran Wallin. Since the mesophyll conductance was assumed to be negligible, the original value of g_m was replaced by a value of 0 µmol m^{-2} $s^{-1}$$Pa^{-1}$. Another modification concerned the C_i concentration and the dominant factor that limits

photosynthesis. At C_i < 100 photosynthesis was considered to be Rubisco limited, while at C_i > 1000 it was determined to be RuBP regeneration limited.

After having obtained values for the photosynthetic capacities V_{cmax} and J_{max}, predictions of both parameters could be made for any desired measurement temperature. The predictions were calculated in the range of 15 °C up to 40 °C in steps of 0.5 °C using the following equation

$$k_{pred} = k_{opt} \frac{H_d \exp\left(\frac{H_a\left(T_k - T_{opt}\right)}{T_k R T_{opt}}\right)}{H_d - H_a\left(1 - \exp\left(\frac{H_d\left(T_k - T_{opt}\right)}{T_k R T_{opt}}\right)\right)} \qquad (6)$$

where pred k_{pred} is the predicted capacity of photosynthesis (V_{cmax} or J_{max}), k_{opt} is the value of V_{cmax} or J_{max} at T_{opt} (the optimal temperature of the reaction), H_d is the rate of decrease of the function above the optimum, T_k is the measurement temperature in K, R is the universal gas constant (8.314 mol^{-1} K^{-1}) and H_a is the rate of exponential increase of the function below the optimum (Medlyn et al. 2002b). In their review about temperature dependencies of photosynthetic capacities, Medlyn *et al*. suggested values for V_{cmax} and J_{max} to use as initial model guesses. Following their suggestions, certain values for T_{opt}, H_d and H_a, which had been particularly determined for climate chamber-grown *Betula pendula*, were used to primarily estimate V_{cmax} and J_{max}. Using equation 4 again, the sum of squares was minimized by adjusting the values of H_a, k_{opt}, and T_{opt} (H_d was assumed to be constant at 200 kJ mol^{-1}). Thereby, final predictions of V_{cmax} and J_{max}, which are adjusted to the photosynthetic capacities derived from the gas exchange measurements, were obtained.

The final prediction for a measurement temperature (T_k) of 25 °C was used to standardize the obtained values of V_{cmax} and J_{max}, respectively. That is, the observed rates of V_{cmax} and J_{max} were divided by the prediction for 25 °C, resulting in V_{cmax} - and J_{max} normalized. Plotting the normalized values against T_{meas} indicates the temperature response of the birches grown in cold, and those grown in warm temperatures.

Also those standardized rates of V_{cmax} and J_{max} were used to make predictions (normalized) for a temperature range of 15 °C up to 40 °C in steps of 0.5 °C, using equation 6 and 4 equivalently to the procedure described above.

Nitrogen analysis

In order to determine foliar nitrogen content and relate it to photosynthetic capacity, two discs of 2.01 cm^2 each were taken from the leaves immediately after the gas exchange measurements. With major veins being avoided, the discs were punched out exactly from the 6 cm^2 that have been enclosed in the IRGA chamber of the LI-COR. After having dried them in 70 °C for at least three days, the discs were weighed and the leaf mass per area (LMA) could be calculated. The discs were then grinded in a ball mill to fine powder, which was

analyzed with regard to nitrogen content (N%) using an CHNS-O analyzer (model EA1108, Fison Instruments, Italy). By multiplying the percentage of nitrogen by the corresponding LMA, the nitrogen mass per area (NMA) was obtained. Eventually, the nitrogen mass per area could be related to the photosynthetic capacity, which is also expressed on area basis. That is, obtained rates of area-based V_{cmax} and J_{max} were divided by the corresponding values of NMA, resulting in rates for V_{cmax} - and J_{max} N-based.

Lipid extraction and analysis

After the birches have been growing in their respective temperature for 10 weeks, leaves from all eight trees were frozen in liquid nitrogen and stored at -80°C. The leave material was boiled for 5 min in 2-propanol, dried under N_2, weighed and the lipids were extracted by butanol-methanol extraction as described (Lofgren *et al.* 2012). A portion of the total extract was subjected to alkaline transmethylation (Christie 1976) after addition of a known amount of diheptadecanoyl phosphatidylcholine. The fatty acid methyl esters were quantified by gas liquid chromatography as proposed by Andersson *et al.* (2005). Lipid species were determined by LC-MS/MS as described (Yin *et al.* 2012), using multiple reaction monitoring for head group specific fragmentation.

Chlorophyll a fluorometry

In order to measure the functioning of PS II as an indicator of photosynthetic efficiency, chlorophyll *a* fluorescence signals were measured using a portable plant efficiency analyzer termed Pocket Pea (Hansatech Intruments, King's Lynn, Norfolk, UK). This was performed after a growing period of three months. The applied fluorometer automatically calculated and displayed a value for the fluorescence parameter F_v/F_m. In each growth chamber, five leaves per birch were measured, with the average of these five leaves representing the chlorophyll *a* fluorescence of one tree. The measurements were conducted within the climate chambers, implying that the fluorescence signals of the birches were examined at the respective growth temperature. For reliable fluorometry results, the PSII reactions centers are supposed to be fully oxidized. Thus, the leaves were dark-adapted at the measuring temperature for 15 minutes prior to the onset of the chlorophyll fluorescence induction.

Morphology and biomass determination

After the birches have been grown in their respective temperature for three months, several parameters of morphology and biomass were derived. In order to estimate leaf area and leaf thickness for each birch, the average of five leaves per tree was calculated. The criteria for choosing the leaves to measure are stated in the paragraph gas exchange measurements above. Regarding the leaf thickness of one single leaf, it was taken from the blade between the first and the second principal lateral veins. It was measured on both sides of the major vein and the average was generated. Further morphological characteristics, such as height, width and stem

diameter, were measured. Both, width and stem diameter represent the average of two dimensions perpendicular to each other. Immediately after the morphological investigations, the birches were cut according to the desired biomass parameters and dried in 70 °C for five days. Finally the total dry mass of leaves, branches, stem, and roots could be determined and the total biomass could be computed for each birch. As properties of morphology and biomass were not determined before the temperature treatment, the parameters *Height new*, *Stem diameter new* and *New shoots* were introduced. They refer to the decisive biomass that entirely developed under the particular growth temperature. That is, *Height new* and *Stem diameter new* were taken from the main stem that completely developed within the climate chambers. Equivalently, values for *New shoots* were gained by summing up the mass of newly developed shoots including leaves, branches and stem.

Statistical analysis

Correlations between two parameters were analyzed using the regression function in Microsoft Office Excel 2007. The resulting p-value is presented besides the function and R^2-value of a trendline that was fitted to the data. To investigate the significance of differences, the statistical software SPSS 18 (SPSS, Inc., Chicago, IL, USA) was applied using a One-way ANOVA. Regarding the difference between the two growth temperature treatments (T_{growth}), a "between groups" One-way ANOVA, which is similar to a T-test, was applied. When evaluating the difference between the parameters throughout one temperature elevation measurement, besides the growth temperature treatment, the measurement temperature had to be taken into consideration. Hence, a repeated measures One-way ANOVA was used to assess the difference given by T_{growth}, and to test the interactions of T_{growth} and T_{meas} (T_{growth} * T_{meas}) between the growth conditions for a significant difference.

Results

Leaf characteristics

Investigating *Betula pendula* leaves, which were either grown in 15 °C or in 25 °C, a positive correlation between nitrogen mass per area (NMA) and leaf mass per area (LMA) was observed (R^2 = 0.7949). This correlation was significant (p = 0.002). Examining the leaf characteristics with respect to various growth temperatures, both, NMA, as well as LMA were significantly different, with NMA and LMA being approximately twofold larger when the leaf was developed in lower temperatures (NMA: p = 0.001 and LMA: p = 0.002). The difference for nitrogen mass per area was even highly significant. Figure 3A displays the relationship between the two leaf parameters and allows the respective comparison of the temperature treatments. Regarding N%, the birches[15] exhibited a slightly higher nitrogen percentage. However, no significant differences could be observed (data not shown).

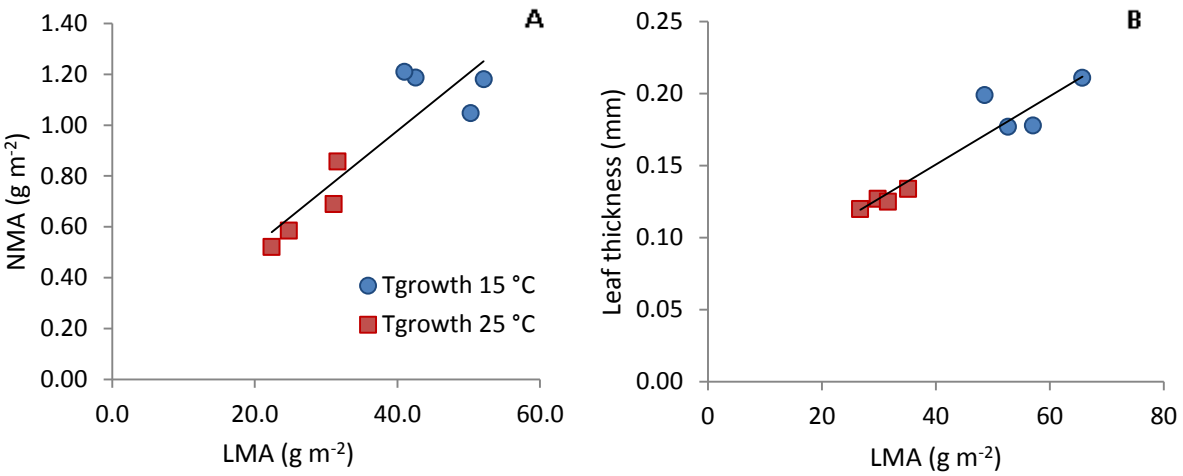


Figure 3. Relationship between nitrogen mass per area (NMA), leaf mass per area (LMA) and leaf thickness derived from *Betula pendula* developed in 15 °C and 25 °C, with the respective difference for each parameter between the temperature treatments. **A** shows the correlation of nitrogen mass per area (NMA) with leaf mass per area (LMA): y = 0.0226x + 0.0727 (R^2 = 0.7949; p = 0.0029). Furthermore, differences in NMA and LMA between leaves grown in 15 °C and 25 °C are illustrated (NMA: p= 0.001; LMA: p = 0.002). **B** shows the correlation of leaf thickness with LMA: y = 0.0024x + 0.0557 (R^2 = 0.8897; p = 0.0004). Equivalently to **A**, differences in leaf thickness and LMA between leaves grown in 15 °C and 25 °C are displayed (leaf thickness: p = 0.0003; LMA: p = 0.001). The data was taken from other leaves than shown in **A**. Each data point in **A** and **B** represents one tree. Both linear regression lines were fitted to all data points disregarding the temperature the leaves were developed in. n = 4 for each growth temperature. Abbreviations and symbols in **B** are the same as those in **A**.

Taking the leaf morphology into account, a general correlation between leaf mass per area and leaf thickness could be observed (R^2 = 0.8897) as displayed in figure 3B. This correlation was positive and highly significant (p = 0.0004). To obtain LMA and leaf thickness, other leaves than shown in Figure 3A were used, which explains the marginal deviation of LMA between the two figures. A linear regression line was fitted to all data points disregarding the temperature the leaves were grown in.. Similarly to Figure 3A, compraisons between the leaves grown in 15 °C and those in 25 °C revealed highly significant differences between the two temperature treatments for both parameters. LMA and leaf thickness were approximately two times higher when the leaf developed in colder climate chambers.

V_{cmax} and J_{max} on area basis

The maximum rate of carboxylation (V_{cmax}), as well as the maximum rate of electron transport (J_{max}) was examined on area basis. Depending on the temperature the leaves were developed in (T_{growth}), the obtained values varied throughout the measurements conducted at 15°, 25 °C, 32.5 °C and 40 °C (T_{meas}). Since the modeled predictions for V_{cmax} and J_{max} were consistent with the observed values in all cases, they accentuate the trends gained in this study (Figure 4).

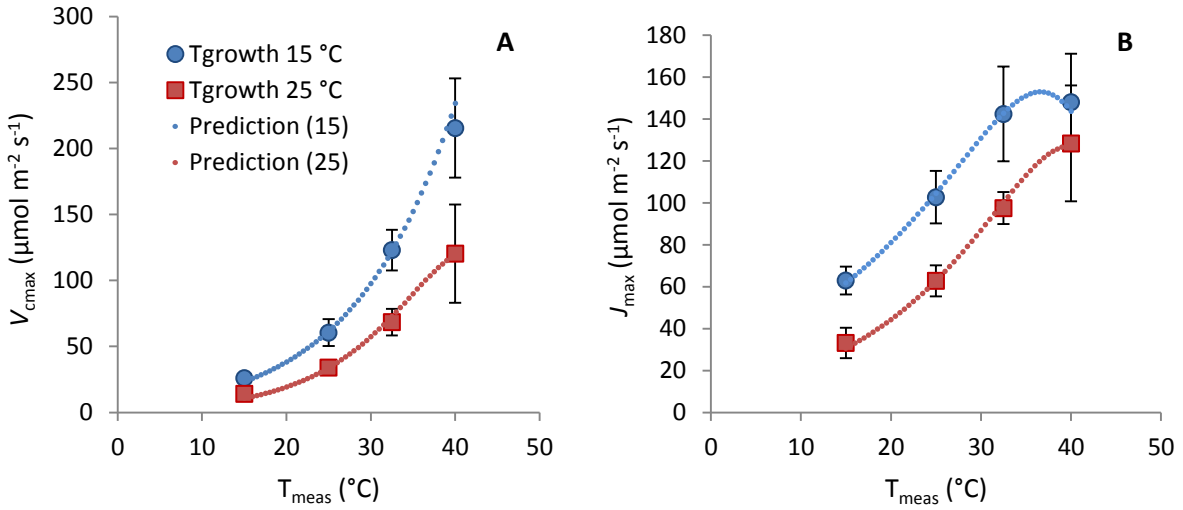


Figure 4. Temperature dependencies of the photosynthetic parameters V_{cmax} and J_{max} on area basis in *Betula pendula*. **A** shows the maximum rate of carboxylation (V_{cmax}) at different measurement temperatures for leaves grown in 15 °C and 25 °C, respectively (T_{growth}: p = 0.008; T_{growth}*T_{meas}: p = 0.065). The dotted line depicts modeled predictions for V_{cmax}. **B** illustrates the maximum rate of electron transport (J_{max}) at different measurement temperatures for both temperature treatments (T_{growth}: p = 0.027; T_{growth}*T_{meas}: p = 0.257). Equivalent to the predictions of V_{cmax}, the predictions for J_{max} were derived from a model and are displayed as a dotted line. Each data point in **A** and **B** represents mean values ± confidence interval (n = 4). Abbreviations and symbols in **B** are the same as those in **A**.

Irrespective of the growth temperature, the maximum rate of Rubisco activity increased progressively with the elevation of measurement temperature (Fig. 3A). However, V_{cmax} was observed to be higher at any given measurement temperature, when the birches were grown in lower temperatures. The difference in V_{cmax} between the birches^{15} and the birches^{25} was statistically significant (p = 0.008) and increased with elevated measurement temperature. Regarding the interaction of T_{growth} and T_{meas}, no significant difference between the birch groups was assessed, nonetheless, the determined p-value (p = 0.065) approaches the level of significance. It is important to note that comparing the values of V_{cmax} between the two growth chambers at a particular measurement temperature, namely the T_{meas} that corresponds to their growth temperature (T_{meas} = 15° and 25 °C), the birches^{15} and birches^{25} exhibit approximately the same maximum rate of carboxylation.

The same pattern was observed for the maximum rate of electron transport (Fig. 3B). The values of J_{max} were similar for the birches^{15} and the birches^{25}, when compared at the particular measurement temperatures 15° and 25 °C corresponding to their growth temperatures. Equivalent to V_{cmax}, the rate of J_{max} is increasing within the measurement range of 15° to 32.5 °C in both temperature treatments. However, unlike the trend seen for V_{cmax}, J_{max} is not increasing to the same extend when coming to the highest measurement temperature of 40 °C. Considering the birches^{25}, the increase of J_{max} seems to be dampened at the highest T_{meas}, whereas for the birches^{15} this effect appears to be even stronger. The prediction of J_{max} for the birches grown in 15 °C depicts a maximum at approximately 35 °C, after which the rate of electron transport bends off. This pattern implies that the difference in J_{max} between the two

temperature treatments, which is present in all T_{meas}, is smaller in higher measurement temperatures. The difference was observed to be significant ($p = 0.027$), whereas the interactions of T_{growth} and T_{meas} showed no significant difference ($p = 0.257$).

V_{cmax} and J_{max} on nitrogen basis

By examining the photosynthetic parameters V_{cmax} and J_{max} on nitrogen basis instead of area basis, different results arose. As illustrated in figure 5A, a progressive increase of V_{cmax} independent on the growth temperature was still observed, however, there was no difference between the two temperature treatments throughout all measurement temperatures. Per nitrogen mass, the birches[15] and the birches[25] exhibited the same maximum rate of carboxylation.

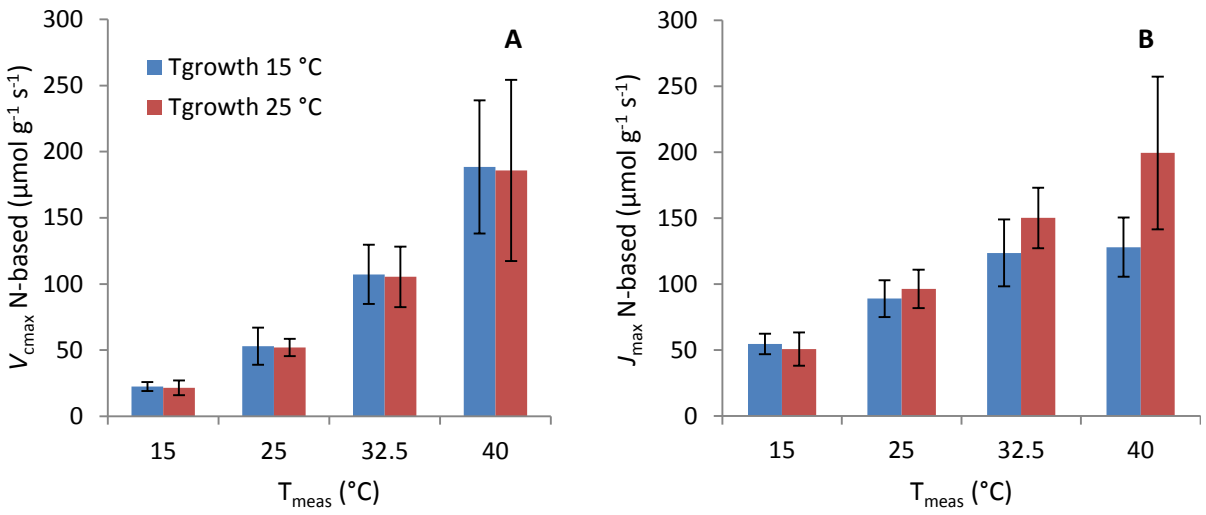


Figure 5. Temperature dependencies of the photosynthetic parameters V_{cmax} and J_{max} on nitrogen basis in *Betula pendula*. **A** shows the maximum rate of carboxylation (V_{cmax}) per nitrogen mass (V_{cmax} N-based) at different measurement temperatures for leaves grown in 15 °C and 25 °C, respectively. **B** illustrates the maximum rate of electron transport (J_{max}) per g nitrogen (J_{max} N-based) at different measurement temperatures for both temperature treatments. Each column in **A** and **B** represents mean values ± confidence interval ($n = 4$). Abbreviations and symbols in **B** are the same as those in **A**.

This also applies to the values of J_{max} derived from the measurements at 15 °C and 25 °C (Fig. 5B). The maximum rates of electron transport of the birches grown in different temperatures approximated each other and increased when going from 15° to 25 °C. However, this was not valid for higher measurement temperatures. In contrast to the birches[25], the birches[15] don't show a further increase in the maximum rate of electron transport with rising measurement temperatures. Equivalent to the trends of J_{max} area based, the series of nitrogen-based J_{max} bends off in high measurement temperatures. Yet, comparing J_{max} N-

based between the growth conditions, the birches grown in cold temperatures even achieve a lower maximum rate of electron transport per nitrogen mass when the exposure temperature is high.

Temperature responses of V_{cmax} and J_{max}

Standardizing the values of V_{cmax} and J_{max} to 25 °C and plotting the resulting V_{cmax} - and J_{max} normalized against T_{meas} indicates the temperature responses of the birches grown in 15 °C and those grown in 25 °C, respectively. The maximum carboxylation rates of the birches[15] and the birches[25] increased to the same extend implying that the birches exhibited the same temperature response independent on the temperature they were grown in (Fig. 6A). In both cases the maximum rate of Rubisco activity was approximately 2 times higher in 32.5 °C and 3.5 times higher in 40 °C compared to 25 °C. When measured at 15 °C it was relatively lower. Similarly to Figure 4, predictions made for the normalized values of V_{cmax} and J_{max} were applicable and underlined the course of the normalized data points.

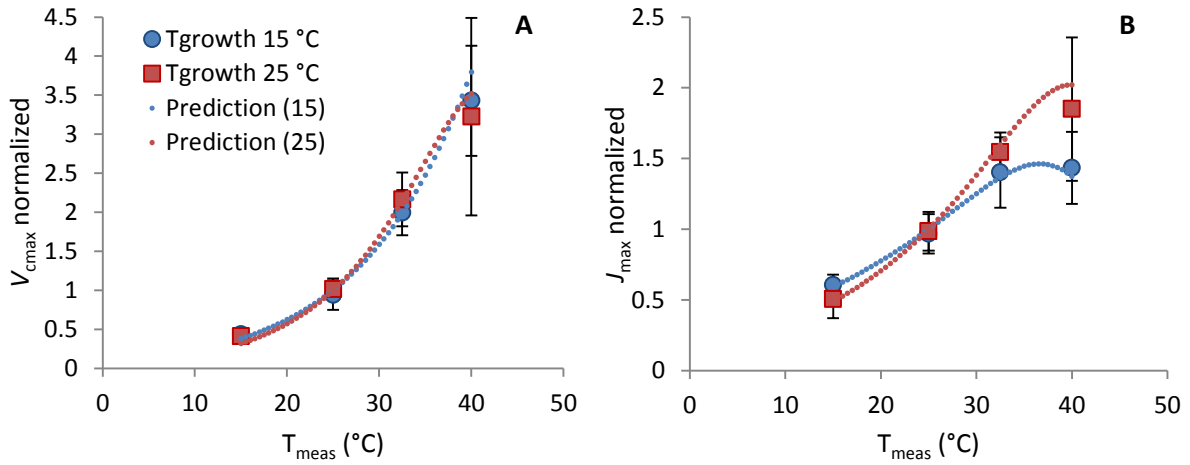


Figure 6. Normalized photosynthetic parameters V_{cmax} and J_{max} illustrating temperature rescponses of V_{cmax} and J_{max}in *Betula pendula*. **A** shows the maximum rate of carboxylation (V_{cmax}) at different measurement temperatures in relation to a V_{cmax} predicted for a measurement temperature of 25 °C (V_{cmax} normalized). The temperature response of V_{cmax} is shown for leaves grown in 15 °C and 25 °C, respectively. The dotted line depicts modeled predictions for V_{cmax} normalized. **B** illustrates the maximum rate of electron transport (J_{max}) at different measurement temperaturesin relation to a J_{max} predicted for a measurement temperature of 25 °C (J_{max} normalized). The temperature response of J_{max} is shown for for both temperature treatments. Equivalent to the predictions of V_{cmax} normalized, The predictions of J_{max} normalized were derived from a model and are displayed as a dotted line. Each data point in **A** and **B** represents mean values ± confidence interval (n = 4). Abbreviations and symbols in **B** are the same as those in **A**.

Regarding the obtained temperature response, no conclusions about a shift in optimum temperature (T_{opt}) for V_{cmax} can be drawn, since V_{cmax} normalized rised progressively and did not reach a maximum within the prevailing measurement range. The trend of J_{max} bending off

at high measurement temperatures described before is accentuated in Figure 6B. Lower maximum rates of electron transport in high measurement temperatures lead to a different overall temperature response of J_{max}. The J_{max} normalized derived from the birches grown in 25 °C tends to reach a maximum around 40 °C, whereas the course of the birches grown in 15 °C bends off after having reached a maximum at 35 °C. No significant difference was observed for modeled optimum temperatures of J_{max} between the birches[15] and the birches[25] (data not shown). Still the temperature response curves indicate a shift of T_{opt} towards lower temperatures, when the trees were grown in a cold environment. Furthermore, when grown in 15 °C, the birches achieve lower maximum electron transport rates than the birches grown in warmer climates.

Ratio of J_{max} to V_{cmax}

The area based ratio of J_{max}/V_{cmax} was observed to decrease when the measurement temperature is raised, with the value derived from the birches grown in 15 °C achieving even lower values in high T_{meas} (Figure 7). For both data series the negative correlation of J_{max}/V_{cmax} with the measurement temperature is highly significant (T_{grwoth} 15 °C: p = 0.0004, T_{growth} 25 °C: p = 0.0007). Comparing the two data series, the ratio of the birches[15] ranges from an approximate value of 2.4 to 0.7, instead of from 2.4 to 1.1, as observed for the birches[25]. The function of the corresponding regression lines are y = -0.0695x + 3.4576 (R^2 = 0.9987) for the birches[15] and y = -0.0509x + 3.1223 (R^2 = 0.9992) for the birches[25]. No significant difference due to various growing temperatures was assessed.

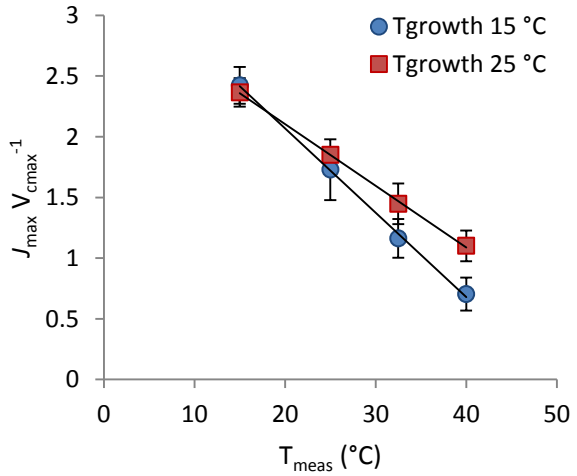


Figure 7. Relationship between the maximum rate of electron transport (J_{max}) and the maximum rate of carboxylation (V_{cmax}) in *Betula pendula*. The data points reperent the ratio J_{max}/V_{cmax} at different measurement temperatures. A linear regression line is fitted to each data series of the two growth temperatures. T_{growth} 15 °C: y = -0.0695x + 3.4576 (R^2 = 0.9987, p = 0.0004); T_{growth} 25 °C: y = -0.0509x + 3.1223 (R^2 = 0.9992, p = 0.0007). Each data point represents mean values ± confidence interval (n = 4).

Chlorophyll a fluorescence

Measuring the chlorophyll fluorescence (F_v/F_m) in the climate chambers, a difference between the birches in warmer and those in colder temperatures was observed. Although both birch groups exhibited similar F_v/F_m values around 0.83, a statistically significant difference was obtained ($p = 0.02$). In contrast to the value of 0.822 (± 0.0074) evaluated from the birches in 15 °C, the birches in the warmer chamber revealed a higher chlorophyll fluorescence of 0.84 (± 0.0086).

Lipid analysis

Several fatty acids with various chain lengths and various numbers of inherited double bonds were established in leaves of *Betula pendula*. Those fatty acids exhibited either 16, or 18 carbon atoms. These two groups are subdivided according to their amount of double bonds, which ranged from 0 to 3. Table 1 provides information about the respective occurrence of each of the fatty acids comparing the birches that were grown in 15 °C and those grown in 25 °C. Evaluating the percentages, 16:0 and in particular 18:3 were the most abundant types of fatty acids in both growth conditions. However, the ratio of those two fatty acids differed between the birches that were grown in cold and those grown in warm temperatures. The birches[15] revealed a significantly smaller portion of 16:0 fatty acids (13.3 ± 0.8 mol %, $p = 0.009$) compared to the birches[25] (17.7 ± 1.2 mol %), instead they exhibited a significantly higher percentage of 18:3 fatty acids (70.1 ± 1.6 mol %, $p = 0.027$) than the birches[25] (63.4 ± 3.3 mol %). Dividing the fatty acids with 3 inherent double bonds by the remaining ones lead to a value of 2.5 ± 0.2 for the leaves developed in 15 °C and a value of 1.8 ± 0.3 for the leaves grown in 25 °C. This ratio was significantly different between the temperature treatments ($p = 0.019$).

Table 1. Effect of growth temperature (T_{growth}) on lipid composition.

Fatty acid (mol %)	T_{growth} 15 °C	T_{growth} 25 °C
16:0	13.3 ± 0.8*	17.7 ± 1.2
16:1	0.0 ± 0.0*	0.2 ± 0.0
16:1	0.2 ± 0.0	0.2 ± 0.1
16:1D3-trans	0.9 ± 0.1*	1.4 ± 0.2
16:2	0.1 ± 0.0*	0.3 ± 0.0
16:3	1.5 ± 0.3*	0.7 ± 0.2
18:0	0.9 ± 0.2*	1.5 ± 0.3
18:1	1.8 ± 0.3*	3.4 ± 0.4
18:2	11.1 ± 1.4	11.2 ± 2.1
18:3	70.1 ± 1.6*	63.4 ± 3.3

Notes: all fatty acids derived from *Betula pendula* leaf samples are listed with their respective occurrence for the birches grown in 15 °C and those grown in 25 °C. The amount of every single fatty acid is displayed as a mol % of all fatty acids that were detected for the particular leaf. Data is presented as mean ± SE, *n* = 4 for each growth temperature. * A statistically significant difference between the temperature treatments was observed ($p < 0.05$).

The lipid classes, the fatty acids were derived from are Monogalactosyldiacylglycerol (MGDG), Digalactosyldiacylglycerol (DGDG), Phosphatidylglycerol (PG), Sulfoquinovosyl-diacylglycerol (SQDG), Phosphatidylcholine (PC), Phosphatidylethanolamine (PE), Phophatidylinositol (PI) and Phosphatidic acid (PA). Comparing the birches grown in low to those grown in high temperatures, the relative lipid content stays fairly unchanged for MGDG, DGDG, SQCG, PE and PE, whereas a large decrease appears in PG and PI when the trees developed in 15 °C (Figure 7). The cool-grown birches even revealed a drop of approximately 50% in PG. Besides that, there was a slight shift in the ratio of DGDG over MGDG, with the low-temperature grown trees exhibiting more DGDG per MGDG. A closer look at the two galactolipids MGDG and DGDG revealed changes in unsaturation in accordance with the total fatty acid analysis. Although the relative amount of the phospholipid PG was less in the birches grown in cool temperatures than in those grown in warm temperatures, it was also relatively more desaturated (data not shown).

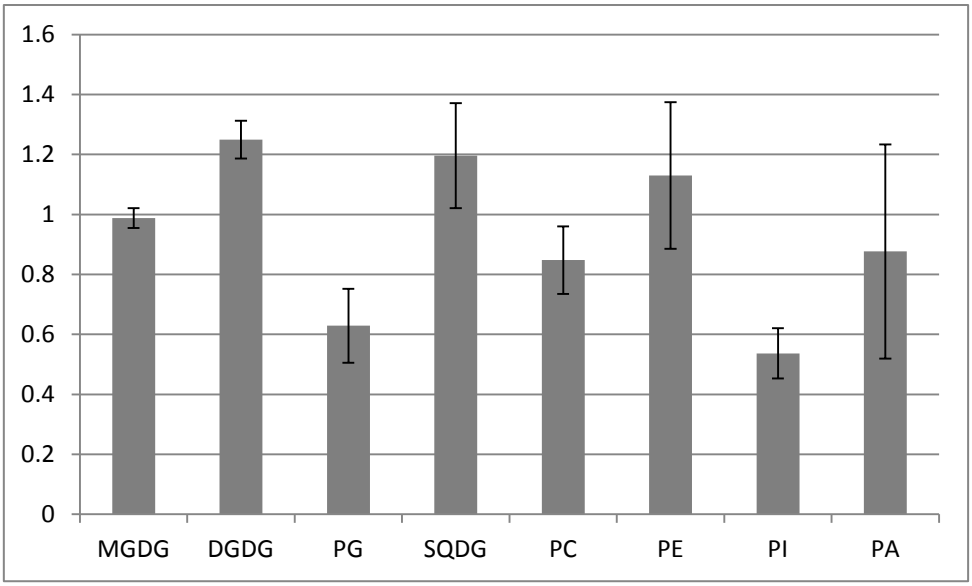


Figure 8. Lipid molecules identified in *Betula pendula* grown in 15 °C and 25 °C, respectively. Each lipid class is presented as a ratio of the lipid content of the low-temperature grown birches (15 °C) over the lipid content of the warm-temperature grown birches (25 °C). Each column represents the mean ratio (mol/mol) on mass basis ± SE, *n* = 4 for each growth temperature.

Morphology and biomass

After a growing period of two months in 15 °C and 25 °C, respectively, the appearance of the birches was clearly different. The phenotypic disparities are displayed in Figure 7. For most of the morphology and biomass data that were derived after a growing time of three months, significant differences were observed between the birch groups, namely in leaf area of single leaves, height, width, total leaf mass and branch mass ($p < 0.05$). Those mentioned parameters were all higher in the birches that grew in warmer climate chambers. Investigating the height of the stems that entirely developed during the temperature treatments (*Height new*), even highly significant differences were obtained ($p < 0.01$). This also applied to the stem diameters of the newly developed stems measured at the bottom (*Stem diameter new*). However, regarding the original stems, the diameters at the very bottom and the stem mass, as well as the mass of the roots did not show any significant differences duo to various growth temperatures. Taking the total biomass into consideration, significant differences occurred comparing the birches grown in 15 °C to those grown in 25 °C, with the warm-grown birches revealing a greater dry mass.

Figure 9. *Betula pendula* grown in 15 °C (left) and 25 °C (right) for two months.
Photo: Martina Lutz

The listed parameter *New shoots* gives an estimation of the total decisive biomass that entirely developed under the particular growth temperature (leaves, branches and stem), illustrating significantly higher values when the birches were grown in warmer climate chambers. Morphology and biomass data are summarized in table 2.

Table 2. Effect of growth temperature on morphology and biomass (dry mass) of *Betula pendula* plants at growth temperatures (T_{growth}) of 15 °C and 25 °C, respectively.

Parameter	**T_{growth} 15 °C**		**T_{growth} 25 °C**	
Morphology				
Leaf area (cm²)	16.5	± 2.3*	47.9	± 18.6
Height (cm)	89	± 7*	130	± 19
Height new (cm)	33	± 5**	85	± 3
Crown width (cm)	48	± 8*	76	± 12
Stem diameter (cm)	3.5	± 0.5	3.8	± 0.3
Stem diameter new (cm)	1.5	± 0.2**	2.5	± 0.3
Biomass				
Leaf mass (g)	7.9	± 1.2*	12.9	± 2.6
Branch mass (g)	0.7	± 1.4*	6.2	± 2.3
Stem mass (g)	8.4	± 2.2	12.6	± 3.8
Root mass (g)	8.1	± 2.9	8.4	± 2.3
New shoots (g)	19.0	± 3.2*	32.9	± 7.3
Total biomass (g)	25.0	± 4.8*	40.0	± 8.2

Notes: All values are averages per plant. Italicized parameters indicate the portion that specifically grew under the controlled temperatures 15 °C and 25 °C, respectively. *Height new* assesses the height of the stem, which grew during the temperature treatment. *Stem diameter new* refers to the stem diameter taken at the bottom of this new stem. *New shoots* integrates all decisive new biomass, which obviously grew in the climate chambers (leaves, brachnes, stem). Data is presented as mean ± SE, n = 4 for each growth temperature. * Statistically significant differences between the temperature treatments were observed ($p < 0.05$). ** the difference was highly significant ($p < 0.001$).

Discussion

The main aim of this study was to investigate the impact of growth temperature on photosynthetic performance, which implies thermal acclimation regarding the modeled parameters V_{cmax} and J_{max}. In order to draw inferences, the results part investigates the maximum rate of carboxylation (V_{cmax}), as well as the potential rate of electron transport (J_{max}) from different angles, such that the nitrogen mass per area (NMA) and various predictions are incorporated. Furthermore, underlying lipid compositions and chlorophyll *a* fluorescences complete the inspections.

Taking the findings of V_{cmax} and J_{max} together, the photosynthetic capacity is generally increased when the exposure temperature is raised, irrespectively of the temperature the birches were grown in. This short time effect is widely known and can be explained by a temperature-induced up-regulation of enzymatic activity and biochemical processes. As the kinetic energy and the movement of molecules, respectively, increases, so does the probability of a substrate to interfere with the active side of an enzyme. The following discussion intends to focus on differences inferred to low (15 °C) and high growth temperatures (25 °C).

Growth temperature induces difference in V_{cmax} and J_{max}

The parameters V_{cmax} and J_{max} commonly refer to the maximum rate of carboxylation and electron transport expressed on leaf area basis. Investigating the photosynthetic performance per area, *Betula pendula* grown in low temperatures clearly exhibited higher photosynthetic rates for V_{cmax} and J_{max} at any given exposure temperature, alluding to a potential thermal acclimation. The observation that the birches display the same photosynthetic rate in their respective climate chambers is in agreement with the findings of Hikosaka (2005). Although being exposed to low temperatures, the birches in the cold climate chambers achieve similar rates of maximal RuPP carboxylation and regeneration to those located in the warm chamber, when V_{cmax} and J_{max} are compared per leaf area. This suggests that temperature acclimation is a response with the objective to maintain a certain photosynthetic rate at the growth condition (Hikosaka, 2006) and implies higher values for V_{cmax} and J_{max} throughout all measurements conducted at 15 °C, 25 °C, 32.5 °C and 40 °C. An explanation for the dampened increase of electron transport capacity of the birches^{15} at high exposure temperatures and the associated smaller difference between the birches^{15} and the birches^{25} at 40 °C is given below.

Difference in V_{cmax} and J_{max} due to disparity in NMA

In order to examine the potential temperature acclimation, underlying leaf properties have to be considered, and related to the results derived from gas exchange measurements. In this context, the nitrogen content was taken into consideration, since the amount of foliar nitrogen was found to strongly correlate with photosynthetic capacity (Evans 1989; Medlyn *et al.*

2002a; Hikosaka 2004). The same leaf tissue was used for investigations, gas exchange - and nitrogen content analysis. Therefore, a relation between nitrogen and assimilation capacity can be established. The fact that low temperature grown birches exhibit a higher nitrogen mass per area (NMA) in combination with higher photosynthetic rates per corresponding leaf area compared to the birches[25] already indicates for such a correlation.

Assessing the photosynthetic performance expressed on a basis of nitrogen content, the assumption of a relationship between foliar nitrogen and the photosynthetic parameters is confirmed. V_{cmax} - and J_{max} N-based allow a comparison between the trees grown in different temperatures, assuming the birches exhibited the same amount of nitrogen. More precisely, the nitrogen based parameters provide information about the maximum rate of carboxylation and electron transport per nitrogen mass. Since the low temperature grown birches reveal approximately the same photosynthetic rate per nitrogen mass as the birches grown in high temperatures, it can be concluded, that the difference between the trees seen for V_{cmax} - and J_{max} on area basis can be traced back to their difference in nitrogen content per area. Hence, the present study outlines that birches grown in colder temperatures reveal higher photosynthetic capacities regarding a certain area, which are attributed to an elevated nitrogen mass in that particular area.

Kerkhoff *et al.* (2005) claimed that, globally, leaves from colder biomes appear to have higher photosynthetic nitrogen use efficiencies with the objective of compensating for reduced Rubisco activity due to lower temperatures. Thereby, their carbon uptake would be optimized. Yamori *et al.* (2005) performed gas exchange measurements in combination with biochemical analysis of Rubisco/N and CytF/N amongst others. Their study ascertained that the elevated photosynthetic capacity including RuBP regeneration and carboxylation of leaves grown in low temperatures is associated with a relatively higher amount of photosynthetic enzymes. Hence, Yamori *et al.* conclude that plants grown in low temperatures invest in photosynthetic compounds in order to compensate for low temperatures which decrease enzyme activity. This compensatory response has been reported by other researchers, such as Strand *et al.* (1999) and Hikosaka (2005).

The present study does not provide information about whether the birches grown in 15 °C invested nitrogen relatively more in proteins related to photosynthesis than in other proteins, since no biochemical analysis in terms of protein composition were made. Yet, it is noteworthy, that the accessory amount of nitrogen per area in the leaves grown in 15 °C seems to be allocated to photosynthetic compounds, since correspondingly higher assimilation rates per area were observed. Hence, the additional nitrogen per area is used efficiently for photosynthesis. Besides that, it can be presumed that this accessory nitrogen is portioned equally with regard to the carboxylation capacity and the potential electron transport, since both V_{cmax} and J_{max} appear to be elevated when NMA is increased.

NMA correlated with LMA and leaf thickness

The value of NMA is gained by multiplying LMA by the corresponding nitrogen percentage (N%). Regarding N%, the low-temperature grown birches exhibited a slightly higher nitrogen

percentage compared to the birches grown in high temperatures. However, no significant differences could be observed. Thus, since there were no intrinsic differences in N% between the birches, but highly significant differences in LMA, the obtained difference in nitrogen mass per area between the birches^{15} and birches^{25} can be traced back to their disparity in leaf mass per area. This LMA is highly correlated with leaf thickness, alluding to the fact that accessory leaf mass is rather allocated in leaf thickness, than leaf density. Hence, birches grown in 15 °C exhibited higher photosynthetic capacities per area (in terms of V_{cmax} and J_{max}), due to their generally increased leaf mass per area associated with thicker leaves.

Van de Weg (2011) also found a significant relation between area based V_{cmax}, nitrogen content per area and leaf mass per area, as well as a positive correlation of area based J_{max} with LMA. A recent study by Dumlao *et al.* published in 2012 assessed photosynthetic rates in the combination with LMA and thickness, as well. Similar to the present study, Dumlao *et al.* ascertained up-regulation of maximal intrinsic photosynthetic capacity in thick low-temperature grown leaves. They attributed the increased leaf thickness of cold acclimated leaves to a greater number of chloroplast-rich palisade cell layers implying that the photosynthesis-performing chloroplast volume is packed into a given leaf area. This dense accumulation of biochemical reactions involving heat production might be advantageous in low growth temperatures. Stefanowska *et al*. (1999) reported increased leaf thickness due to cool growth temperatures as well. However, in contrast to the study mentioned above, the cold-induced leaf thickness in their study was inferred to an increased size of both palisade and spongy mesophyll cells in a perpendicular direction to the leaf surface, rather than to an elevated number of cell layers in the mesophyll.

In the present study leaf histology was not investigated, but it is apparent that the leaf thickness is either attributed to a vertical elongation of palisade mesophyll cells, an increased number of palisace cell layers, or both, as demonstrated by Boese and Huner (1990). Equivalent to the findings of this study, Stefanowska *et al*. (1999) depicted decreased leaf surface area with increased leaf blade thickness. Ultimately, the investment in leaf thickness instead of leaf area might imply an optimal ratio of leaf surface to volume, such that the surface, which is exposed to cold prevailing temperatures, is minimized, whereas the volume that can retain heat is maximized. Gray *et al*. (1997), who examined growth temperature impacts on spinach and cereal leaves supposed that the respective leaf morphogenesis is controlled by a redox sensing/signaling system in chloroplasts.

Temperature response and optimum temperature of V_{cmax} and J_{max}

Eventually, to display a potential difference in temperature response between the birches grown in 15 °C and those grown in 25 °C the area based parameters V_{cmax} and J_{max} were standardized to the same temperature (25 °C). It is evident, that V_{cmax} and J_{max} approximate a value of 1 at the measurement temperature of 25 °C, as a prediction for 25 °C was used for normalizing. Regarding the temperature response trends of V_{cmax} exhibited by the birches grown in low and high temperatures, respectively, no differences due to various growth conditions were obtained. The maximum rate of carboxylation of both birch groups overlaps

in its increase with measurement temperature. Since the temperature response trends of V_{cmax} do not display saturation within the measurement range, which is coherent with absent enzymatic deactivation (von Caemmerer 2000), no inferences about optimum temperatures and a possible shift can be drawn. Yet, it can be concluded, that the Calvin cylcle enzymes of the birches grown in low and those of the birches grown in high temperatures exhibit a similar heat tolerance, enabling the trees to maintain the same temperature-induced increase of Rubisco activity. In other words, the results derived from the measurements at 15 °C up to 40 °C indicate, that the birches did not undergo thermal acclimation in terms of carboxylation capacity, which contradicts the findings of Yamori (2005), Kattge and Knorr (2007) and Hikosaka (1999). The absence of a temperature response shift is consistent with the assumption, that kinetic parameters of Rubisco are relatively conserved among species and growth conditions (van Caemmerer 2002).

Among C_3 higher plants, the temperature dependence of carboxylation is thought to be more stable than the dependence of the electron transport rate. Usually, at temperatures greater than 30 °C, the response curve of net photosynthesis displays a steep decline, which is, besides an accompanied increase in respiration, largely attributed to the temperature dependence of the electron transport chain, and thus, J_{max} (von Caemmerer 2000).

This is in good agreement with the present study, since in contrast to the temperature responses of V_{cmax}, the temperature dependencies of J_{max} differ between the birches grown in low with those developed in warm temperatures. Compared to the birches developed in 25 °C, low-temperature grown birches appear to be dampened in their maximum electron transport rate when approaching higher leaf temperatures. The birches^{15} cannot keep pace elevating their rate of to the same extends and achieve lower values for J_{max} at 32.5 °C and 40 °C. Furthermore, as displayed by the prediction curve, their electron transport capacity decreases after having reached a maximum around 35 °C. This maximum represents their optimum temperature for J_{max}, which is shifted towards lower temperatures.

Lower optimal temperatures for J_{max} in low-temperature grown *Betula pendula* trees were also found by Medlyn *et al.* (2002b). The overall shift in optimum temperature is analog, however, regarding exact values for T_{opt}, no straightforward comparisons between the derived T_{opt} of Medlyn *et al.* (2002b) and the present study can be made. In the study published in 2002, the two growth conditions refer to a greenhouse breeding in 17 °C (GH) and an open-top chamber treatment in Finland (OTC), with the corresponding growth temperature being defined as the mean temperature in the month, in which the measurements were conducted (14 °C). In contrast, this study is based on two temperature treatments of 15 °C and 25 °C. Yet, the optimum temperatures for J_{max} of 35°C (GH) and 20°C (OTC), which are both derived from *Betula pendula* grown in a temperatures close to 15 °C, fit to the T_{opt} prediction of this study, where the birches grown in 15 °C reveal an optimum temperature for J_{max} at 35 °C. Unlike the present study, Medlyn *et al.* (2002b) found an optimum temperature shift for V_{cmax} as well.

This difference in J_{max} between the birches^{15} and the birches^{25} at high measurement temperatures cannot be explained by their disparity in NMA or LMA, since they reveal similar temperature responses at both exposure temperatures. A tremendous mismatch only

appears when the temperature is elevated. Therefore, thermal acclimation of J_{max} underlying the T_{opt} shift can be claimed. Acclimation of the thylakoid membrane in respect of growth temperature has been reported by several studies and involves more fluid membranes in case of low growing temperatures (Berry and Björkman 1980).

Since the short-term temperature response of the entire electron transport chain, being embedded in the thylakoid membrane is closely related to properties of the membrane such as lipid composition and membrane viscosity (von Caemmerer 2000), the observations of this study become evident. As the temperature rises, so does the fluidity of the membrane. In case the membrane is already viscose due to thermal acclimation, integrity of the membrane is no longer provided at high measurement temperatures and the electron transport process is negatively affected. Hence, the temperature response trend of J_{max} bending off at higher temperatures and the associated downwards shift in T_{opt} of the low-temperature grown birches can be attributed to certain membrane properties that are responsible for a J_{max} limitation in high leaf temperatures.

On the other hand the persisting increase in J_{max} of the birches grown in warmer conditions is consistent with the study of Armond *et al.* (1978), who, using *in vitro* methods, clearly demonstrated a changing heat-stability of the photosynthetic apparatus for desert shrubs. Also Yamasaki *et al*. (2002) examined enhanced heat-stability of PSII photochemistry, in case winter wheat was grown in high temperatures.

Summary for V_{cmax} and J_{max}

To sum up the discussion of V_{cmax} and J_{max}, the findings of the present study are in agreement with the hypothesis that the rate of electron transport is more sensitive than the rate of carboxylation when exposed to high temperatures. Considering heat-induced changes in membrane fluidity and altered stability of protein complexes e.g. PSII, the hypothesis implies that membrane bound reactions, such as the electron transport chain are more affected by elevated temperatures than reactions in free solution, such as Rubisco carboxylation (Gundersson *et al.* 2010).

Lipid composition explains temperature response and T_{opt} shift of J_{max}

The assumption, that the temperature response of the electron transport capacity is altered due to changes in the thylakoid membrane was confirmed by the results of the lipid analysis. Low-temperature grown birches revealed relatively more unsaturated fatty acids than the birches grown in high temperatures. By incorporating unsaturated instead of saturated fatty acids into their lipid bilayer, organisms can increase the membrane's viscosity, which is an important mechanism of adjusting to low temperatures that decrease the dynamic properties of lipids in membrane bilayers. A temperature-appropriate proportion of saturated to unsaturated fatty acids is crucial, since the leaf must be sufficiently stiff to resist the force of gravity and provide a solar panel, but on the other hand it should be sufficiently flexible to minimize

wind-induced damages (Beerling 2007). Thus, low plant growth temperatures led to the expression of cold-inducible genes that encode proteins involved in the desaturation of membrane lipids (Mikami and Murata 2003). Consequently, the birches grown in 15 °C exhibited relatively more double bond inheriting fatty acids, in order to maintain certain membrane fluidities and compensate cold growth temperatures.

Regarding photosynthetic capacities of electron transport, the first four lipid classes shown in Figure 7, namely Monogalactosyldiacylglycerol (MGDG), Digalactosyldiacylglycerol (DGDG), Phosphatidylglycerol (PG) and Sulfoquinovosyldiacylglycerol (SQDG) have to be taken into account, since they are lipids of the thylakoid membrane, where the electron transport chain is embedded. Here, the two galactolipids are of special interest, representing the predominant constituents of thylakoid membranes. Comparing the birches grown in low with those grown in high temperatures, there was no difference in their relative quantity, but they revealed differences in unsaturation in accordance with the total fatty acid analysis. Therefore, it can be concluded that the altered temperature response of J_{max} in the cool-grown birches is attributed to a relatively more fluid thylokoid membrane. It is not surprising that the maximum rate of electron transport is negatively influenced when the leaf is exposed to high measurement temperatures. In case the membrane is already fluid, high temperatures increase the molecular motion of lipid molecules even more, such that the maximum rate of electron transport within the membrane is limited and J_{max} displays a dampened increase, as well as a lower optimum temperature, when compared to the trend of J_{max} derived from warm-grown trees. The temperature response of whole electron transport being closely linked to the viscosity and lipid composition of the thylakoid membrane was found by Nolan and Smilie (1976).

Phosphatidylglycerol (PG) is the only pholpholipid present in the thylakoids. Together with the sulfolipid Sulfoquinovosyldiacylglycerol (SQDG) it is a minor component of the thylakoid membrane. While there was no difference between the birches regarding the amounts of SQDG, the birches grown in low temperatures revealed a tremendous drop of 50% in PG, when expressed on mass basis. PG being present in PSII, PSI, as well as in the light-harvesting complex, has been proved to be essential for the electron transport and the development of thylakoid membranes (Wada and Murata 2007). However, the amount of PG cannot be directly linked to photosynthetic capacity, since the latter is expressed on area basis, whereas the LC/MS results are mass based. In other words, though the 15 °C samples reveal relatively less PG per mass, it has to be considered that they exhibited generally more leaf mass per area. Nevertheless, PG was relatively more desaturated in the low-temperature grown trees, which is consistent with the rates of J_{max} being dampened in high measurement temperatures.

The drop of PI in cool-grown birches is not of special interest with respect to photosynthetic performance, as it is usually not attributed to the thylakoid membrane, but rather other membranes in the cell. Nonetheless, it is interesting that PI carries negative charge at neutral pH. Together with the relative decrease of the similarly charged PG, one could safely say that the 15 °C temperature treatment resulted in membranes with less dense negative charge.

Chlorophyll a fluorescence and a vague hypothesis

The chlorophyll fluorescence of the birches grown in 15 °C and those grown in 25 °C reveal a ratio of 0.822 (± 0.0074) and 0.84 (± 0.0086), respectively. As both ratios approximate a value of 0.83, it can be concluded that the birches are healthy and were not exposed to any stress associated with F_v/F_m being lower that 0.83 (Maxwell and Johnson 2000). Still, a significant difference was observed, with the birches[25] exhibiting higher ratios than the birches[15]. According to Maxwell and Johnson, the lower ratios of the birches[15] allude to a decreased functioning of PSII, compared to the birches[25]. However, it has to be considered that the measurements were conducted in the respective greenhouse and thus at different temperatures. Therefore, the observed difference in F_v/F_m is either attributed to the fact that PSII generally shows decreased efficiency in lower temperatures, or the birches[15] show lower values for F_v/F_m due to a lower PSII functionality. At first sight, the complex of PSII being less efficient in the low-temperature grown birches is contradictory to the gas exchange results showing similar values of J_{max} at the respective temperature the birches were grown in. In order to explain such an apparent contradiction, a closer look at the technique of chlorophyll a fluorometry has to be taken.

During the light-dependent reaction of photosynthesis, antenna pigments such as Chlorophyll *a* and *b* collect solar energy and induce an electron transport chain within the thylakoid membrane. Chlorophyll molecules are arranged in and around the protein complex photosystem II. The vast majority of Chlorophyll functions as an antenna, which transfers the absorbed energy via resonance to a specific chlorophyll pair in the reaction center of PSII. In the reaction center, proceeding redox-reactions are triggered involving the reduction of electron acceptors, notably plastoquinone (Q_a, Q_b) resulting in the split of water and the synthesis of chemical compounds (ATP and NADPH). This transformation of solar energy into chemical energy is termed photochemistry. It appears to be evident that the flow of electrons through PSII is indicative of the overall rate of photosynthesis. Thus, in order to investigate the photosynthetic performance of a leaf, chlorophyll fluorescence analysis and the maximum yield of PSII, respectively, is commonly applied.

Light energy absorbed by chlorophyll molecules can undergo three different fates. It can be used to drive photosynthesis (photochemistry), energy can be dissipated in form of heat or it can be re-emitted as light, namely chlorophyll fluorescence. As these three processes occur in competition, measurements of chlorophyll fluorescence allow estimations of the amount of energy that can be dedicated to heat formation and photosynthesis. Fluorescence yield is quantified by illuminating the leaf with light of a particular wavelength and measuring the amount of re-emitted light of longer wavelengths. As the dissipation of energy in form of heat is negligible, it is not taken into consideration in this discussion.

Exposing the leaf to a high intensity flash light induces a transient saturation of the electron transport, such that Q_a, which has accepted an electron, is not able to accept another one until it is re-oxidized and has passed the first electron onto the following electron carrier Q_b. During this state, the functioning PSII reaction centers are said to be "closed". Since the excess energy is not used to drive photosynthesis, a corresponding increase in the amount of fluorescence can be observed. Depending on how many reaction centers are functioning, light

energy is re-emitted with the electron acceptor Q_b being fully reduced. The appendant value of F_m refers to this maximum fluorescence attained in the absence of photochemistry. However, F_m does not represent the intrinsic amount of energy that can be channeled through the photosynthetic apparatus, since it includes re-emission of energy that was used to excite antenna pigments, as well as emissions caused by non-functioning PSII reaction centers. Therefore, another fluorescence signal, F_0 is estimated to provide information about the fluorescence attributed to chlorophyll molecules and non-functioning reaction centers. It is taken at a time base 0 to reveal minimal emissions, implying that the chlorophyll molecules are excited, but the first electron acceptor *Q*a remains oxidized. This is the case when the leaf is sufficiently adapted to darkness and all antenna pigment complexes are assumed to be open. Eventually, the variable fluorescence F_v is obtained by subtracting F_0 from F_m. It relates to the maximum capacity of photochemical quenching, meaning how much energy could be utilized by PSII to drive photosynthesis, accounting for the number of capable reactions centers. The parameter F_v/F_m is widely used to indicate the potential quantum yield and functionality of PSII. It is presented as a ratio of variable fluorescence over the maximum fluorescence.

Coming back to the results of this study, F_v/F_m depicting a ratio only provides information about the functionality of PSII, but no knowledge about the number of chlorophyll *a* and the associated PSII reaction centers is gained. Hence, it can be assumed that although the birches[15] reveal lower ratios and thus decreased functionality of PSII, they exhibit more foliar nitrogen per area (see discussion above) that can be invested in a larger number of PSII related proteins, allowing them to compensate for low temperatures and the accompanied increased membrane fluidity. However, such a hypothesis is vague, since the chlorophyll fluorescence has to be measured at the same exposure temperature. In order to establish this assumption further research is required, possibly involving fluorometry at all gas exchange measurement temperatures.

Balance between V_{cmax} and J_{max}

The area based ratio of J_{max} to V_{cmax} provides information about the temperature dependent parameters in relation to each other. The value of J_{max}/V_{cmax} being constant throughout all measurement temperatures would suggest that the maximum rate of carboxylation and the maximum electron transport rate are changing simultaneously with elevated leaf temperature. However, the results show that, when the temperature is raised, the two photosynthetic rates are changing to a different extend. The increase of V_{cmax} exceeds the increase of J_{max} resulting in a negative slope of the linear regression lines, that were laid underneath the values of J_{max}/V_{cmax} derived from the birches[15] and the birches[25], respectively.

A strongly declining ratio of J_{max} over V_{cmax} with leaf temperature was also found by Medlyn *et al.* (2002b) in all ascertained 19 species, with *Betula pendula* being amongst them. The observation that the ratio of cool-grown birches decreases to a higher extend compared to the birches[25] in elevated measurement temperatures can be traced back to their dampened increase of J_{max} in high exposure temperatures, which was examined in the previously. The

average ratio of 1.67 at 25 °C presented by Medlyn *et al.* is consistent with the ratio gained in this study, reaching a value of 1.7 (birches^{15}) and 1.8 (birches^{25}) at a measurement temperature of 25 °C. The present study did not show any significant differences between the trees grown in cold and warm temperatures regarding J_{max} / V_{cmax}.

The hypothesis put forward by Hikosaka (1997) that temperature acclimation implies re-allocation of nitrogen between carboxylation and electron transport processes to realize co-limitation at the growth temperature, was associated with a change in the ratio of J_{max} to V_{cmax}, with lower ratios for plants grown in colder conditions. This assumption cannot be confirmed by the present study, which is in agreement with other studies questioning such a shift in the context of thermal acclimation (Bunce 2000; Medlyn 2002a). In contrast to Hikosaka's hypothesis published in 1997, Kattge and Knorr (2007) and Yamori *et al.* (2005) reported that the ratio J_{max} / V_{cmax} at 25 °C rather decreases with increasing growth temperature. Considering these discrepancies, further research on the balance between the two partial processes is needed. Nevertheless, in this study, the fact that there were no significant differences in J_{max} over V_{cmax} between the temperature treatments is plausible, considering that both J_{max} and V_{cmax} were elevated in the birches^{15} compared to the birches^{25}, which is associated with an equal partitioning of accessory nitrogen, as discussed above. A correlation between the ratio of J_{max} over V_{cmax} and nitrogen portioning among electron transport chain and carboxylation was shown by Yamori *et al.* (2005).

Differences in morphology and biomass

Even though this research was not primarily dedicated to characteristics of biomass and morphology, several parameters were derived from the trees, in order to outline the tremendous impact of long-term temperature treatment on growth. Especially since this study was conducted in the context of climate change associated with rising carbon dioxide concentrations in the atmosphere, it is relevant to look at the temperature response of the entire tree in terms of potential carbon sequestration. Scaling back from the leaf, growth of *Betula pendula* was clearly enhanced when the birches developed in elevated temperatures, such that the total height, the total width, as well as the total biomass of the birches were significantly greater in the warmer climate chambers. Since there were no data available about the mass of the birches before being exposed to the respective growth temperature, the parameters *Height new*, *Stem diameter new* and mass of newly developed shoots including leaves, branches and stem (*New shoots*) are introduced. As already stated in the results part, these parameters refer to the decisive biomass that entirely developed under the particular growth temperature. Hence, they provide estimation of the difference between the total biomass of the trees, even though the increase of stem diameters and root tissue are not taken into account. This estimation is particularly plausible, since the birches grown in 15 °C and those grown in 25 °C did not show any significant differences regarding stem diameter and root dry mass. In summary, the formation of biomass was clearly accelerated when *Betula pendula* was grown in higher temperatures.

These findings are consistent with the review published by Way and Oren (2010), who also observed enhanced growth in deciduous tree species when the temperature is elevated. According to the review, higher temperatures induce a process with positive feedbacks. Once the temperature is elevated, enzymatic activity is enhanced, leading to accelerated formation of leaf tissue, which is acclimated and thus able to perform photosynthesis more efficiently.

Furthermore, they not only report of an accelerated growth, but also of a certain developmental pattern which was followed by the respective trees. Warmed trees displayed increased allocation of biomass to leaves instead of roots and grew taller instead of investing in stem diameter. This is in very good agreement with the present study, showing that the leaf area and leaf mass are both significantly higher in the tall warm-temperature grown birches, whereas the root mass and the stem diameter did not change with elevated temperatures. A possible explanation for a shift in the allometric relationship of root-to-shoot ratios is given by Way and Oren (2010), namely that due to the buffering of temperature by soils, roots do not experience the same degree of temperature exposure as aboveground components. However, this assumption is not strongly applicable to the present study, since the pots were located within the climate chambers and probably exhibited adjusted temperatures. Here it was rather the small pot size that might have limited root expansion. How a plant exhibiting relatively little root tissue in combination with large leaf surface maintain the supply of water demanding leaf material, is a possible research question, bearing in mind that acclimation of the hydraulic xylem conductivity might occur. According to the review of Way and Oren (2010), little information about this topic is available.

Conclusion

The current study illustrated the impact of growth temperature on photosynthesis in *Betula pendula* and revealed the underlying anatomical and biochemical leaf properties, which were altered according to the particular temperature treatment. The main findings of this study were that low-temperature grown birches exhibited increased photosynthetic capacities per leaf area, which was attributed to their greater amount of leaf mass and nitrogen in this area. That is, trees exposed to cool growth temperatures rather invested in leaf thickness than leaf area, which might optimize the volume to surface ratio and thus allow the retention of heat. In contrast, warm-temperature grown trees appeared to invest in larger solar panels, reducing their photosynthetic performance per area but providing an enlarged area to collect light energy and perform photosynthesis. While growth temperature did not influence the process of carbon fixation, it clearly affected the temperature response of the electron transport chain. The thylakoid membrane being acclimated to warm temperatures allowed the birches to maintain an increase in electron transport capacity even at high leaf temperatures, implying an optimum temperature shift for the electron transport chain. Besides that, chlorophyll *a* fluorescence signals indicated a better functioning of PSII in the warmer growth chamber.

Finally, when grown in elevated temperatures, birches generally displayed enhanced growth, which resulted in greater carbon allocation.

Regarding carbon sequestration in the context of climate change, it is important to keep in mind that the trees grew in a CO_2 concentration of approximately 600 ppm instead of 400 ppm prevailing in our contemporary atmosphere (IPCC 2007). The concentration of carbon dioxide interacts with temperature, determining net photosynthetic rates. Increased CO_2 concentrations are known to shift the optimal temperature for photosynthesis towards higher temperatures, as high amounts of CO_2 compensate for the elevation of photorespiration in warm temperatures (Newman *et al.* 2011). This would imply an increased carbon gain in the warmer climate chambers and thus greater potential carbon sequestration. Furthermore, the birches grown in climate chambers were well-watered and fertilized regularly, providing optimal conditions for growth. In contrast, natural environments are apparently associated with biotic and abiotic stress factors limiting plant growth. Especially with respect to climate change such limiting aspects gain in importance. Although higher global mean temperatures and the "CO_2-fertilizing effect" may have a positive impact on net primary production of deciduous trees, as seen in this study, a changing climate is coherent with altered precipitation and nutrient deposition, a higher frequency of disturbance events and insect infestations, an increase of toxic ground-level ozone and so forth, which are factors limiting plant growth (Newman *et al.* 2011). Considering all these variables interacting with each other, it is evident that this is just an attempt to explore a few examples that complicate the prediction of a long-term impact of climate change on carbon sequestration, and *vice versa*, the impact of vegetation on a changing climate. If we are to make any predictions, natural complexity, if possible at all, has to be broken down and interwoven factors have to be explored apart from each other. Hence, this study focused on temperature impacts on the scale of photosynthesis being at the bases of carbon allocation. It could be shown that the deciduous trees *Betula pendula* profited from an acclimation to elevated growth temperatures when the exposure temperature is increased. The underlying heat-stability of the electron transport chain and of the carboxylation related enzymes can be accounted for, when predicting plant responses in warmer environmental conditions. That is, models might incorporate thermal acclimation of photosynthesis, implying that both major photosynthetic processes do not appear to be limited in higher temperatures, in case the plant is acclimated to a warmer climate. Thereby, warmer global mean temperatures might support growth in deciduous forests, counteracting the human-attributed elevation of CO_2 concentrations in our atmosphere. Considering the cycling of carbon between atmosphere and biosphere, the present study depicts the tremendous impact temperature can have on photosynthesis and the allocation of carbon, eventually accelerating the buildup of plant biomass, which retains "solar energy" and therefore represents the lowest rank of our food chain on earth.

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References

Andersson M. X., Larsson K. E., Tjellstrom H., Liljenberg C., Sandelius, A.S. (2005) *Phosphate-limited oat: The plasma membrane and the tonoplast as major targets for phospholipid-to-glycolipid replacement and stimulation of phospholipases in the plasma membrane.* Journal of Biological Chemistry **280,** 27578-27586.

Armond P.A., Schreiber U.S., Björkman O. (1978) *Photosynthetic acclimation to temperature in the desert shrub, Larrea divaricata. II. Light-harvesting efficiency and electron transport.* Plant Physiology **61,** 411–415.

Arneth A., Harrison S. P., Zaehle S. (2010) *Terrestrial biogeochemical feedbacks in the climate system.* Nature Geoscience **3,** 525-532.

Beerling D. (2007) *The Emerald Planet.* Oxford University Press, New York, USA, 288 p.

Bernacchi C. J., Pimentel C., Long. S. P. (2003) *In vivo temperature response functions of parameters required to model RuBP-limited photosynthesis*. Plant Cell and Environment **26,** 1419-1430.

Bernacchi C. J., Singsaas E. L., Pimentel C., Portis A. R., Long S. P. (2001) *Improved temperature response functions for models of Rubisco-limited photosynthesis.* Plant, Cell and Environment **24,** 253-259.

Berry J., Björkman O. (1980) *Photosynthetic response and adaptation to temperature in higher plants.* Annual Review Plant Physiology **31,** 491-543.

Boese S. R., Huner N. P. A. (1990) *Effect of Growth Temperature and Temperature Shifts on Spinach Leaf Morphology and Photosynthesis.* Plant Physiology **94,** 1830-1836.

Bonan G. B. (2008) *Forest and Climate Change: Forcings, Feedbacks, and the Climate Benefits of Forests*. Science **320,** 1444-1449.

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

Von Caemmerer S. (2000) *Biochemical Models of Leaf Photosynthesis.* CSIRO Publishing, Collingwood, Australia, pp. 1–165.

Von Caemmerer S., Farquhar G. D. (1981) *Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves.* Planta **153,** 376-387.

Christie W. W. (1976) *Lipid analysis.* Oxford, Pergamon Press.

Cunningham S. C., Read J. (2002) *Comparison of temperate and tropical rainforest tree species: photosynthetic responses to growth temperature*. Oecologia **133,** 112–119.

Dumlao M. R., Darehshouri A., Cohu C. M., Muller O., Mathias J., Adams III W. W., Demmig-Adams B. (2012) *Low temperature acclimation of photosynthetic capacity and leaf morphology in the context of phloem loading type.* Photosynthesis Research **113,** 181-189.

Edwards G. E., Baker N. R. (1993) *Can CO_2 assimilation in maize leaves be predicted accurately from chlorophyll fluorescence analysis?* Photosynthesis Research **37,** 89-102.

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

Farquhar G. D., von Caemmerer S., Berry J. A. (1980) *A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species*. Planta **149,** 78-90.

Farquhar G. D., von Caemmerer S., Berry J. A. (1982) *Modelling of Photosynthetic Response to Environmental Conditions.* Encyclopedia of Plant Physiology **12,** 549-587.

Gray G. R., Chauvin L.-P., Sarhan F., Huner N. P. A. (1997) *Cold acclimation and freezing tolerance.*

A complex interaction of light an temperature. Plant Physiology. **114,** 46-474.

Gundersson G. R., O'Hara K. H., Campion C. M., Walker A. V., Edwards N. T. (2010) *Thermal plasticity of photosynthesis: the role of acclimation in forest responses to a warming climate.* Global Change Biology **16,** 2272-2286.

Gurevitch J., Scheiner S. M., Fox G. A. (2006) *The Ecology of Plants.* Sinauer Associates, Sunderland, Massachusetts, USA, 574 p.

Hikosaka K. (2004) *Difference in the photosynthesis–nitrogen relationship: patterns, physiological causes, and ecological importance*. Journal of Plant Research **117,** 481–494.

Hikosaka K. (2005) *Nitrogen portioning in the photosynthetic apparatus of Plantago asiatica leaves grown at different temperature and light conditions: similarities and differences between temperature and light acclimation.* Plant and Cell Physiology **46,** 1283-1290.

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

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

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

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

Krause G. H., Weis E. (1991) *Chlorophyll Fluorescence and Photosynthesis: The Basics.* Annual Review of Plant Physiology and Plant Molecular Biology **42,** 313-349.

Lofgren L., Stahlman M., Forsberg G. B., Saarinen S., Nilsson R., Hansson G. I. (2012) *The BUME method: a novel automated chloroform-free 96-well total lipid extraction method for blood plasma.* Journal of Lipid Research **53,** 1690-1700.

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

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

Mikami K., Murata N. (2003) *Membrane fluidity and the perception of environmental signals in cyanobacteria and plants.* Progress in Lipid Research **42,** 527-543.

Nicotra A. B., Atkin O. K., Bonser S. P., Davidson A. M., Finnegan E. J., Mathesius U., Poot P., Purugganan M. D., Richards C. L., Valladares F., Kleunen M. van (2010) *Plant phenotypic plasticity in a changing climate.* Trends in Plant Science **15,** 1360-1385.

Newman J. A., Anand M., Henry H. A. L., Hunt S., Gedalof Z. (2011) *Climate Change Biology*. CAB International, Massachusetts, USA, 289 p.

Nolan W. G., Smillie R. M. (1976) *Multi-temperature effects on Hill reaction activity of barley chloroplasts.* Biochimica et Biophysica Acta (BBA) – Bioenergetics **440,** 461-475.

Sharkey, T. D., Bernacchi, C. J., Farquar G. D., Singsaa, E. L. (2007) *Fitting photosynthetic carbon dioxide response curves for C 3 leaves.* Plant, Cell and Environment **30,** 1035–1040.

Smith N. G. and Dukes J. (2013) *Plant respiration and photosynthesis in global-scale models: incorporating acclimation to temperature and* CO_2. Global Change Biology **19,** pp. 45-63.

Stefanowska M., Mieczyslaw K., Kubacka-Zebalska M., Kakperska A. (1999) *Low Temperature Affects Pattern of Leaf Growth and Structure of Cell Walls in Winter Oilseed Rape (Brassica napus L., var. oleifera L.).* Annals of Botany **84,** 313-319.

Strand A., Hurry V., Henkes S., Huner N., Gustafsson P., Gardeström P., Stitt M. (1999) *Acclimation of Arabidopsis Leaves Developing at Low Temperatures. Increasing Cytoplasmic Volume*

Accompanies Increased Activities of Enzymes in the Calvin Cycle and in the Sucrose-Biosynthesis Pathway. Plant Physiology **199,** 1387-1397.

The Intergovernmental Panel on Climate Change, IPCC (2007) Climate Change 2007:the physical science basis. *Contributions of Working Group I Fourth Assessment Report*. Intergov. Panel Clim. Change, Cambridge, UK

Van de Weg M. J., Meir P., Grace J., Ramos G. D. (2011) *Photosynthetic parameters, dark respiration and leaf traits in the canopy of a Peruvian tropical montane cloud forest.* Oecologia **168,** 23-34.

Vu J. C. V. (2005) *Acclimation of peanut (Arachis hypogaea L.) leaf photosynthesis to elevated growth CO_2 and temperature*. Environmental and Experimental Botany **53,** 85-95.

Wada H., Murata N. (2007) *The essential role of phosphatidylglycerol in photosynthesis.* Photosynthetic Research **92,** 205-215.

Way D. A., Oren R. (2010) *Differential responses to changes in growth temperature between trees from different functional groups and biomes: a review and synthesis of data.* Tree Physiology **30,** 669-688.

Yamasaki T., Yamakawa T., Yamane Y., Koike H., Satoh K., Katoh S. (2002) *Temperature Acclimation of Photosynthesis and Related Changes in Photosystem II Electron Transport in Winter Wheat.* Plant Physiology **128,** 1087-1097.

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

Yin L., Fristedt R., Herdean A., Solymosi K., Bertrand M., Andersson M. X., Mamedov F., Vener A. V., Schoefs B., Spetea C. (2012) *Photosystem II Function and Dynamics in Three Widely Used Arabidopsis thaliana Accessions.* PLoS ONE **7**.