

CO₂ adaptation and consequences for transpiration rates in *Betula nana*



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Degree-thesis for the research part of the master
Science Education and Communication

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The photograph on the front page shows *B. nana* in her natural environment in Abisko, Sweden

Abstract

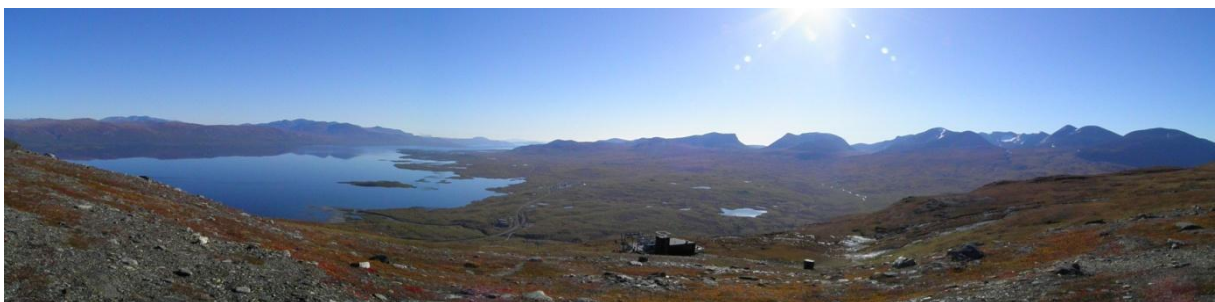
This thesis aims to validate the properties of *Betula nana* leaf cuticles, to use this specie as palaeo-proxy. The first part is about the effect of microhabitats on the stomatal characteristics of *B. nana* from Abisko, Sweden. The undulation index (UI), growing degree days (GDD₅) and the stomatal index (SI) will be examined. The second part will be about the effect of the increasing amount of atmospheric CO₂ on the stomatal properties of *B. nana*, to test and validate the CO₂ responsiveness via stomatal frequency (PL, SD and SI). The third part determines the consequences of CO₂ adaptation for the hydrology of the birch species via stomatal conductance (g_{smax}). The UI and SI are not influenced by micro-habitat. There is a correlation of 0.61 between GDD₅ and UI. The PL increases, while the SD decreases along an increasing amount of CO₂ in the atmosphere. The conduct of the g_{smax} of the *Betula species* is similar to other tree species. Therefore the leaves of *B. nana* can be used as palaeo-proxy. The stomatal characteristics are not influenced by micro-habitat, and they are in line with other species. Interesting fact is that the birch species fall in the area of gymnosperms with their PL and SD, which could be explained by their evolution. This leads to a proposal for further research.



The author between several *B. nana* specimens, the lake Abiskojaure on the background

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View from mount Nuolja (1261m) towards Lake Torneträsk and Abisko

Introduction

The global climate is changing; the term 'global warming' is mostly used to describe this phenomenon. Lots of research is connected to this change in climate, and governments are very interested in predictions about the progress of the climate change. The Intergovernmental Panel on Climate Change (IPCC) has been established to summarize the research about the global climate change. In the most recent assessment of the IPCC they state that there is a rise in temperature; therefore the term 'global warming' applies. Their analysis shows that there has been a global increase in temperature of 0.6°C in the last decade (IPCC, 2007; Houghton *et al.*, 2001). According to other studies, this increase in temperature may be considered the largest period of warming in the last 1000 years (Walter *et al.*, 2002; Houghton *et al.*, 2001).

One of the causes of the increasing temperature is the atmospheric CO₂ concentration (Houghton *et al.*, 2001; IPCC, 2007). Since 1750 there has been an increase of 31% of the atmospheric concentration of CO₂, and the current concentration is probably the highest concentration of CO₂ in the atmosphere in the past 20 million years (Houghton *et al.*, 2001). Especially since the 1980s there is a rise in the atmospheric CO₂ concentration, which is caused by the increase in the industrial CO₂ emissions (Keeling, 1997).

In the northern latitudes, the impact of global warming and CO₂ rise is larger than the global average according to instrumental measurements. The northern latitudes are considered to be very sensitive to climate change (Douglas & Smol, 2001). According to the IPCC assessment, the average arctic temperatures increased at almost twice the global average rate in the last decades (IPCC, 2007). The Arctic Climate Impact Assessment is an international project to evaluate climate in the northern latitudes among others. They predict an increase in temperature of up to 7°C warmer at the end of the 21st century. This is considered to be an enormous rise in temperature (ACIA, 2005).

The changing climate has an effect on the length of the growing season in the northern latitudes. The growing season in the north starts earlier and continues longer, because of the increase in temperature in the (sub)arctic regions (Taylor *et al.*, 2001). Growing season is the period when plants are able to grow; this is indicated by the amount of growing degree days (GDD_x). GDD_x is the cumulative daily mean temperature above a threshold temperature value x. The commonly used threshold value for the northern latitudes is 5°C (Wagner *et al.*, 2010). Besides the increase in temperature, there is a predicted increase for the amount of precipitation (ACIA, 2005). These changes, combined with the global increase of the atmospheric CO₂ concentration, will have a pronounced effect on the vegetation.

The changes of the temperature and the increase of the atmospheric CO₂ concentration will have an effect on vegetation in general. The environmental factors surrounding the plants during their development have an effect on the form and function of the individual plants (Bret-Harte *et al.*, 2001). Leaf characteristics can be used to define the environmental factors during the development of the plant. For instance, it is possible to use the cuticle morphology of leaves to trace a change in

growing season. The Undulation Index (UI) uses the cuticle morphology to calculate their sinuosity (Kürschner *et al.*, 1997). The UI measures the deviation between the cell circumference (CC) with a given cell area (CA) and the circumference of a circle with the same area. Epidermal cell expansion is depending on the time available for leaf growth (Wagner-Cremer *et al.*, 2010). Therefore, the epidermal cell characteristics provide a quantitative estimate for the degree of sinuosity of epidermis cell walls. This method can be used to trace changes in the amount of GDD.

Leave characteristics are also used to quantify changes in the CO₂ concentration in the atmosphere (Wagner *et al.*, 2004). Stomatal density (SD) and stomatal index (SI) are measurements for the stomatal frequency, which is used to detect changes in atmospheric CO₂ concentrations. The SI is given next to the SD, because this index is independent of the variation in epidermal cell size. Therefore SI is more sensitive to detect stomatal frequency response to the change in atmospheric CO₂ concentration (Wagner *et al.*, 1999).

Besides the stomatal frequency, the size of the stomata is determined by certain environmental factors. The size of the stomata in combination with the SD can be used to calculate the maximum stomatal conductance to water vapor (g_{smax}). The g_{smax} is a measurement for the gas-exchange of the leaves (Franks & Beerling, 2009). Plants can also reduce the loss of water by transpiration, by decreasing the g_{smax} . By altering the g_{smax} plants respond to a change in hydrology and climate (Lammertsma *et al.*, 2011).

All these plant characteristics are used in research about climate change. According to the IPCC assessment, the palaeoclimatic record supports that the amount of warming is unusual (IPCC, 2007). The results from past records are used as indicators for the future. This kind of research has been done by the use of instrumental records and palaeo-proxies. Unfortunately the instrumental records are only from the last centuries, and they are not always complete. Therefore it is important to look at the palaeo-proxies, although they have their limitations as well (Jones *et al.*, 2011).

Palaeo-proxies are indirect data, which contain evidence of climate-controlled processes. There are different kinds of proxy data that can be used for climate reconstructions. There are non-organic archives, which consist for instance of ice-cores, terrestrial sediments, and varves. There are also organic archives, like tree rings, fossil pollen, and animal and plant macro-remains (Pfister *et al.*, 2008). This thesis will focus on this last category, the use of leaf remains from the northern latitudes as palaeo-proxy.

The vegetation of the (sub)arctic consists of tundra and boreal forests. Recently, the amount of tundra seems to decrease, while the amount of boreal forest increases. In the last 20 years there is a shift in the tree line; the line shifts towards the north. Because of this shift there will be less tundra in the northern latitudes (Overland & Wang, 2004). The decrease in tundra could result in the extinction of certain arctic species (ACIA, 2005). One of the most common shrub species of the northern latitudes is the birch species *Betula nana*. *B. nana* is a light demanding pioneer species, and is known to have a flexible growth strategy (Bret-Harte *et al.*, 2001). Because the higher arctic have a paucity of higher plants (Douglas & Smol, 2001), it is important to be able to use the plants that do grow there as effectively as possible as palaeo-proxy.

To be able to use *B. nana* cuticle morphology as a palaeo-proxy, it should be known if the microhabitat has an effect on the leaf properties. According to Wagner *et al.* (1998) the SI of *B. pendula* and *B. pubescens* is not influenced by environmental factors. This effect is assumed for *B. nana* as well, but it is not properly examined, yet.

This thesis is divided in three parts. The first part (Chapter 2) examines the effect of microhabitat on the properties of *B. nana*, to be able to use *B. nana* as palaeo-proxy. This part will answer the question: 'Does microhabitat have an effect on the stomatal characteristics of *B. nana*?' The hypothesis is that there will be no response of *B. nana* values on different micro-habitats (geographical distribution, altitudinal range). The second part (Chapter 3) is about the effect of the increasing amount of atmospheric CO₂ on the stomatal properties of *B. nana*. The aim is to test and validate the CO₂ responsiveness via stomatal frequency. The hypothesis is that the stomatal density will decrease, while the pore length will increase along with an increasing concentration of atmospheric CO₂. Part three (Chapter 4) is about the stomatal conductance of different birch species. The aim is to determine the consequences of CO₂ adaptation for the hydrology of the birch species, and it is expected that the g_{smax} will decrease while the atmospheric CO₂ concentration increases.

Effect of microhabitat on *B. nana*

To validate the use of *B. nana* as palaeo-proxy, the effect of microhabitat on the leaves of this species is investigated. The stomatal characteristics will be compared to geographical distribution and altitudinal range, factors inducing various microhabitats.

2.1 Material and methods

B. nana leaves from mature specimens growing at the Lake Torne area around the Abisko Research Station (68°21' N, 18°48' E, Abisko, Sweden, Figure 1) were collected during the first weeks of September 2010. These leaves were growing under different conditions in various microhabitats, including exposure to average and low light conditions, southern slopes to northern slopes, very moist to average and low water availability (see Appendix Table 1 for a more detailed description of the samples and sites). Subfossil leaf fragments were taken from a peat section that was cut from mire surfaces at the Lake Torne area (68°21'50.3" N, 18°48'31.4"E, Figure 1). The leaf fragments were separated from 12 samples of 0.5 cm thickness each. The ages from these samples are between 1989 and 2011. The age determination is based on ²¹⁰Pb.

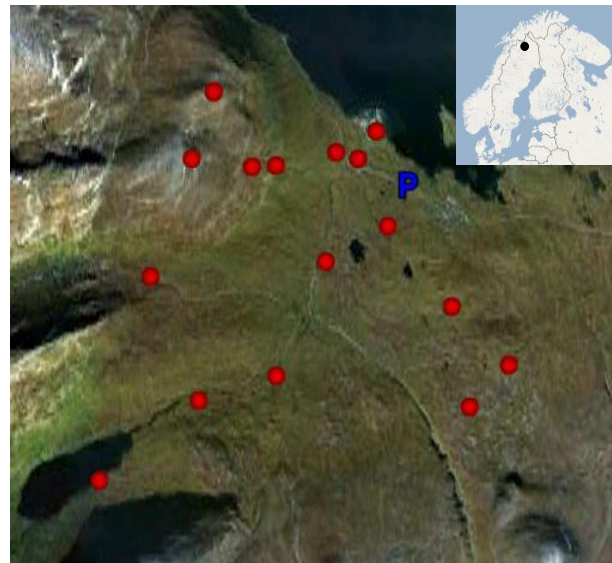


Figure 1 Lake Torne area, Abisko, Sweden: red dots represent the sites of the modern leaf samples; the blue P represents the site of the peat samples.

Microscopic analysis

The central part (~0.25cm²) of up to 6 modern leaves was bleached in 4% sodium hypochlorite. The lower cuticle embedded in glycerine jelly, was used for microscopic analysis. The subfossil leaf fragments of *B. nana* were separated and identified under a binocular microscope by leaf and cuticle characteristics. The number of subfossil leaf fragments used for analysis varies in each sample, because of different cuticle preservation in the sediments (1-3 leaf fragments). The leaf fragments from the peat deposits were bleached in 4% sodium hypochlorite. The standardised, computer-aided analysis of epidermal cell properties was performed on an Olympus ANALYSIS Image Analysis System. The magnification used to analyse the leaf fragments was 500x. The following parameters were analysed: Stomatal density (SD [n/mm²]), epidermal cell density (ED [n/mm²]), stomatal length (SL [μm]) and pore length (PL [μm]).

At least 30 cells per modern leaf and at least 20 cells per subfossil leaf fragment were analysed for the estimation of the mean and standard deviation of epidermal cell areas (CA [μm²]) and epidermal cell circumferences (CC [μm]). The epidermal cell wall undulation index

$$UI [dimensionless] = \frac{CC}{\sqrt{CA/\pi} \cdot 2\pi}$$

was calculated for each leaf (fragment) from the CA (μm^2) and CC (μm^2) (Kürschner, 1997). The Stomatal Index (SI [%]) was also calculated for each leaf according to the following formula (Salisbury, 1927)

$$SI [\%] = \frac{SD}{SD + ED} \times 100$$

where the SI is the ratio of epidermal cells which are differentiated into stomata, this has the advantage that it is an area independent measure for the number of stomata. The SD and ED measurements are based on 4 digital images from each leaf. The SL and PL are measures from 10 stomata per modern leaf and 6 to 10 stomata per subfossil leaf fragment.

2.2 Results and discussion

Undulation Index

To evaluate the dependence of the UI against a latitudinal and an altitudinal gradient leaf material from ranges of 68°17'45.3"N to 68°22'20.8"N and 355 to 1121m have been studied. UI values of modern *B. nana* leaves vary from 1.35 to 1.42 (Figure 2). The UI average is 1.38 with a standard deviation of 0.02. There seems to be no connection between the latitudinal gradient and the UI. There is also no significant correlation (<0.01) between the UI and the altitudinal gradient.

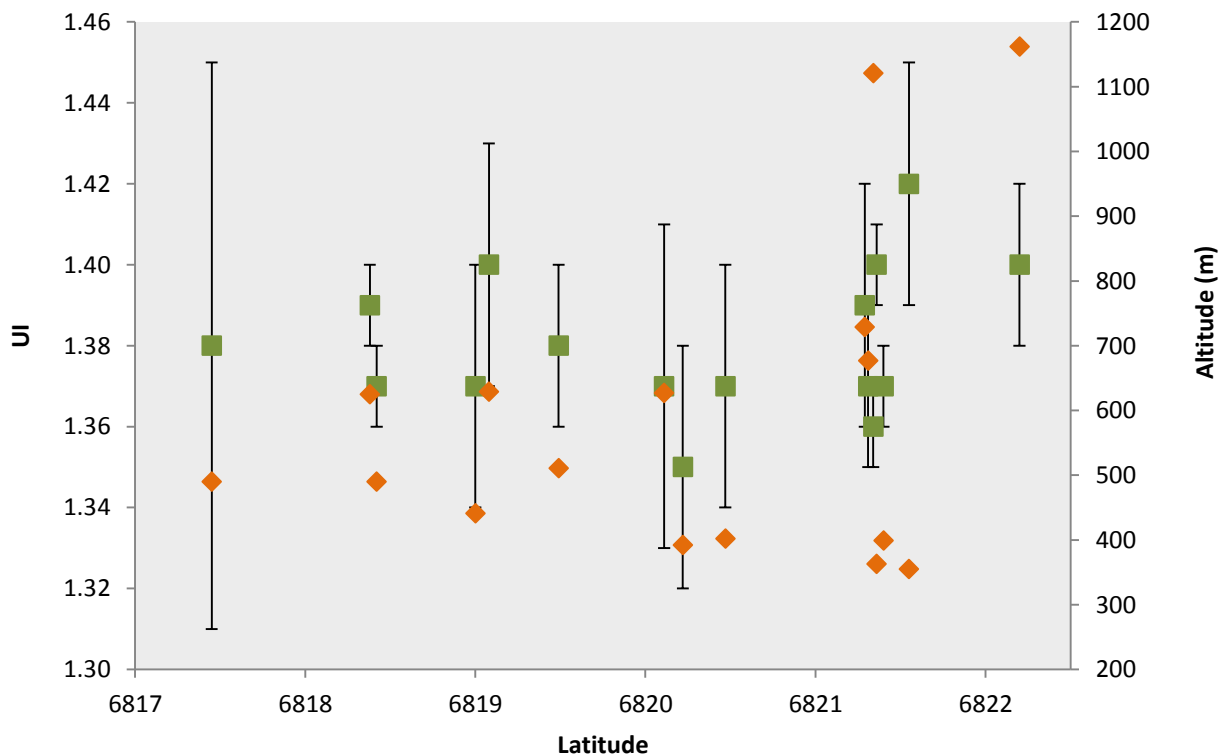


Figure 2 UI (green) versus GDD₅ (orange) of the subfossil leaves from Abisko from 1989 until 2010. The primary y-axis gives the values of the UI (dimensionless). The secondary y-axis gives the altitude in meters above sea level. The correlation between UI and GDD₅ is 0.61 (without sample 2005 the correlation is 0.75). The error bars give the standard error (if applicable).

The occurrence of a temperature inversion can be a possible explanation for the lack of correlation between the altitude and the UI. This is very likely, because temperature inversions are a common meteorological phenomenon (Phillpot *et al.*, 1970). Usually it's getting colder at an increasing height, but in this area there is a high temperature current in the higher parts of the mountains. Earlier research shows that the UI is highly influenced by temperature, only recently this is verified for *B. nana* from Kevo (Wagner-Cremer *et al.*, 2010). Unfortunately we can't correlate the UI values of one year in the same area with temperature data, because there's no temperature data that accurate.

The latitudinal gradient (68°17'45.3"N to 68°22'20.8"N) is probably too small to show differences in UI, because the sample sites are close to each other. To estimate a correlation between the UI and the latitudinal gradient, the area of the samples should be increased. Therefore further research on this part is encouraged.

The highest UI value (1.42, Figure 2) seems to be an outlier, a possible explanation for this is the fact that this sample is taken where the river Abiskojåkka flows into Lake Torne. It is possible that the growing conditions differ from the growing conditions of the other samples, because there is a bird sanctuary located, which probably results in a larger amount of available nutrients. Especially nitrogen seems to have an effect on the UI, where an increasing amount of nitrogen results in a higher UI (Wagner, 1998). Other research showed that *B. nana* has a large growth response to fertilization (Bret-Harte *et al.*, 2001); therefore this effect should be kept in mind.

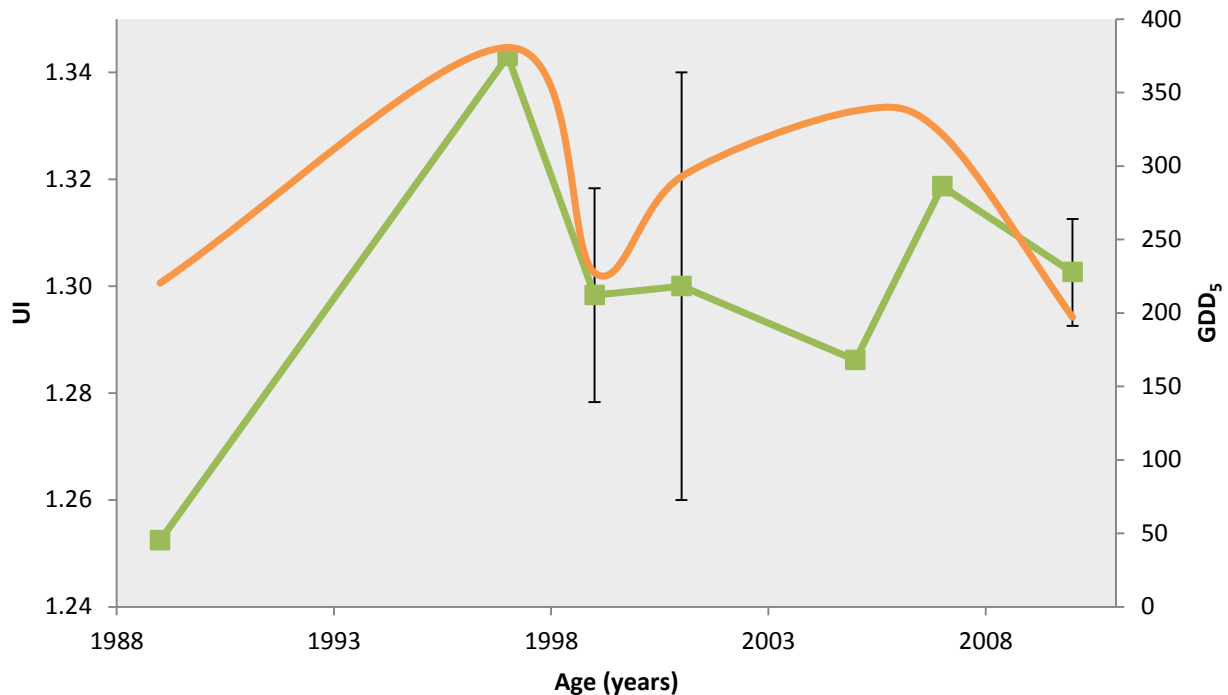


Figure 3 UI values plotted along the latitudinal gradient from 68°17'45.3"N to 68°22'20.8"N in green squares, the orange dots show the altitudinal values from 355 to 1162m. The x-axis gives the latitudes, where 6819.08 stands for N68°19'08. The primary y-axis gives the values of the Undulation Index (dimensionless). The secondary y-axis gives the altitude in meters. The correlation between UI and altitude is <0.01.

The UI of the subfossil leaf fragments is tested against the GDD_5 . Figure 3 shows the conduct of the UI and GDD_5 in time from 1989 to 2011. There is a positive correlation between the UI and the GDD_5 , namely 0.61. Sample 2005 seems to be an outlier, without that sample the correlation is even higher (0.75), nevertheless the first value seems to be a good positive correlation. This means that the subfossil leaf fragments are suitable to be used as palaeoproxy. However, there must be noted that there is a limited number of samples, because of the poor leaf preservation in the peat. For the same reason it is not possible to say something about the stomatal properties from these subfossil leaf fragments. Research about *B. nana* from Kevo demonstrates a positive correlation between UI and GDD_5 of 0.79 (Wagner-Cremer *et al.*, 2010). That correlation is even higher than the correlation from the present study; therefore it is probable that this correlation is valid.

Stomatal Index

The SI of the modern samples varies between 6.03 and 8.31%. These values show now correlation with the latitudinal and altitudinal gradients (Figure 4 A and B). There is a high intrinsic variability of almost 2.5%, unfortunately there is no explanation for a variability that high.

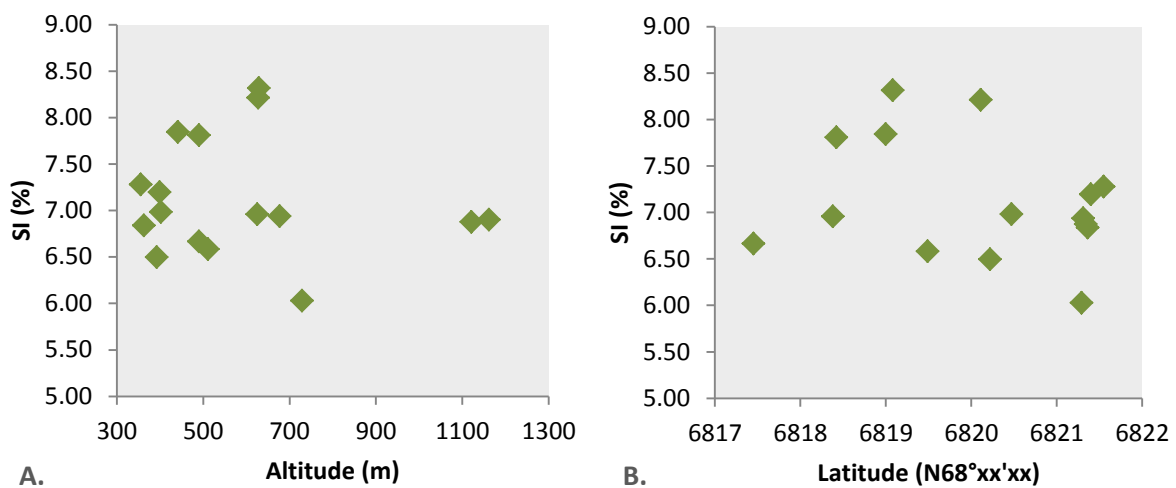


Figure 4 The SI values of the modern *B. nana* leaves plotted against the altitude (A) and the latitude (B). The y-axes from both graphs give the SI in percentages, the x-axis from A. gives the altitude in meters, the x-axis from B. gives the latitude, where 6819,08 stands for N68°19'08.

2.3 Conclusion

The Abisko data set gives an estimate about the range of values of the UI according to a variability test. The samples are taken from various local habitats, and the UI values are not influenced by these habitats. There can be concluded that the SI is not dependent of altitudinal and latitudinal gradients, so the microhabitats do not have an effect on the SI as well. Besides those outcomes, there is a good positive correlation between GDD_5 and UI. These results lead to the conclusion that leaves of *B. nana* are fit to be used as palaeo-proxy for past GDD_5 estimates. It should be kept in mind though, that there could be a slight effect of the available nutrients to the stomatal characteristics.

Stomatal properties and CO₂ responsiveness

The stomatal properties of plants are affected by a change in the concentration of atmospheric CO₂. (Schlyter *et al.*, 1993; Royer, 2001; Bonan & Shugart, 2011). This is also known for the birch species *B. pubescens* and *B. pendula* (Rey & Jarvis, 1998; Wagner-Cremer *et al.*, 1999; Wagner-Cremer *et al.*, 2002). This chapter will look at the changes in PL and SD of several birch species over the historical CO₂ increase. The hypothesis is that the stomatal density of *B. nana* will decrease, while the pore length will increase along with an increasing concentration of atmospheric CO₂ (Wagner *et al.*, 1996; Lammertsma *et al.*, 2011). If so, it is possible to use (sub)fossil *B. nana* to clarify the amount of atmospheric CO₂ from pre-instrumental records. Also, these stomatal properties and those of some other birch species will be compared to earlier conducted research. This will be done to verify those results.

3.1 Material and methods

An additional dataset is added to the Abisko dataset, because of the poor leaf preservation in the peat. The added dataset comes from Kevo, (69°45' N, 27° E, Utsjoki, Finnish Lapland; Finsinger & Wagner, 2009). The values from these measurements are compared to the modern samples from the Abisko data set. Figure 5 shows the SI values of the Kevo data set and the mean SI from the modern leaves of the Abisko data set. The Abisko mean value fits well into the response line of *B. nana* from Kevo. Therefore it is possible to include the mean *B. nana* values from the modern leaves of the Abisko dataset into the dataset from *B. nana* from Kevo.

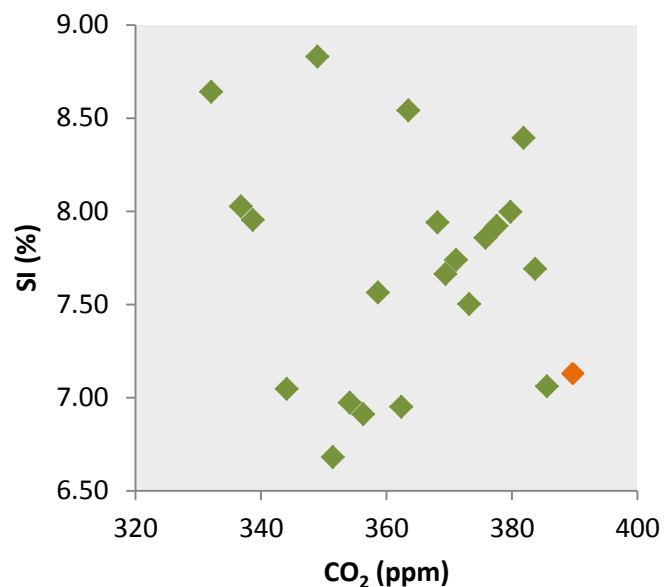


Figure 5 The SI values of the *B. nana* dataset from Kevo (green dots) and the mean SI value of the modern leaves of the Abisko (orange dot). The y-axis gives the SI in %, the x-axis gives the amount of atmospheric CO₂ in ppm.

3.2 Results and discussion

CO₂ responsiveness of *B. nana*

To further examine the stomatal characteristics, the Abisko dataset is still combined with the Kevo dataset in Figure 6 A and B. These graphs show that the PL of *B. nana* slightly increases along the increase of atmospheric CO₂, while the SD decreases. This indicates that the larger pores compensate for the lesser amounts of stomata in the leaves, because the leaves will take up the same amount of CO₂. Earlier research shows that the SD of several plants species is inversely correlated to the amount of CO₂ in the atmosphere (Royer, 2001; Hetherington & Woodward, 2003; Finsinger & Wagner 2009).

It is also known that plants have a mechanism to increase the stomatal pore area, and therefore be able to increase the CO₂ uptake of their leaves (Wagner *et al.*, 1996; Kürschner *et al.*, 1998). This effect has been shown in birch species as well. Wagner *et al.* (1996) conducted research about *B. pendula*. This research showed that the SD decreases and the PL increases along with the increasing concentration of atmospheric CO₂.

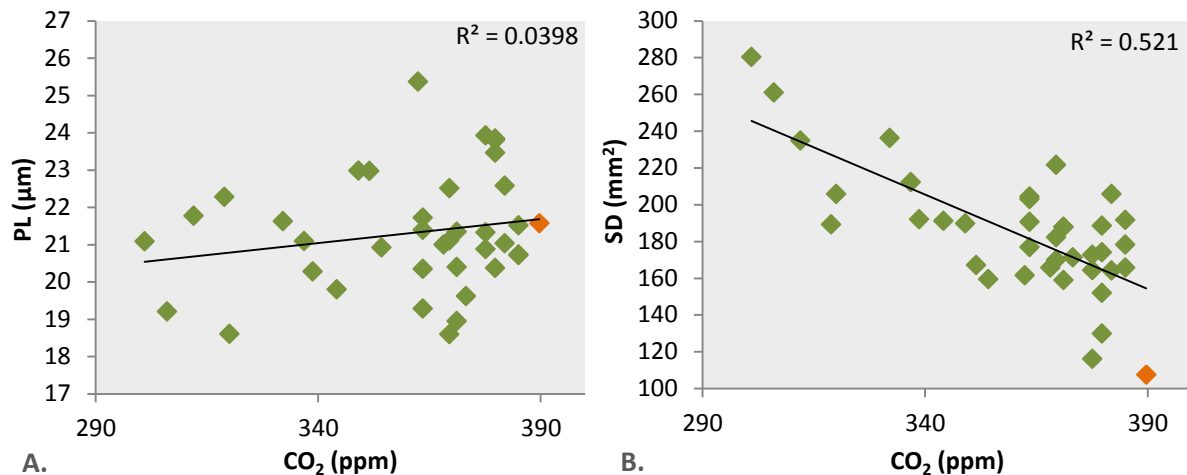


Figure 6 The stomatal characteristics of *B. nana* from Kevo and Abisko plotted against the atmospheric CO₂ in ppm. The y-axis from A. gives the PL in µm, from B. the SD in mm².

Stomatal properties verified

The SD is known to have a strong correlation to the size of the stomata. Earlier research showed that there is a long-time relationship between the SD and the guard cell width of the stomata (Hetherington & Woodward, 2003). More recently, research shows a relationship between the size of the fully opened stomata (a_{max}) and SD (de Boer *et al.*, 2011; Lammertsma *et al.*, 2011). The research shows the stomatal characteristics of various gymnosperm and angiosperm species from Florida (United States of America). The a_{max} is derived from the PL; as a result there is also a connection between the PL and the SD (Lammertsma *et al.*, 2011). This connection shows a same kind of conduct as the relationship between guard cell width and the SD (Hetherington & Woodward, 2003).

To verify the outcomes of the birch species, the results are compared to the results of Lammertsma *et al.*, (2011). Figure 7 shows the PL and the SD of the different species from Florida in grey, and several birch species in green. The first two birch datasets are the ones from the modern leaves from Abisko and Kevo, the *Betula pubescens* comes from Kevo as well, and the last birch species is the *Betula pendula* from the Mariapeel National Nature Reserve (56°99' N, 70°40' E, The Netherlands). *B. pubescens* and *B. pendula* are included, because it is important to see if the conduct of *B. nana* is an artefact of that birch species only, or if there are more species which show the same conduct. *B. pendula* is added for an extra reason as well. This is because *B. nana* is an arctic birch species, while the Florida species are all subtropical. *B. pendula* from The Netherlands has a more temperate environment, which makes the environment slightly more comparable to the climate conditions of the Florida species.

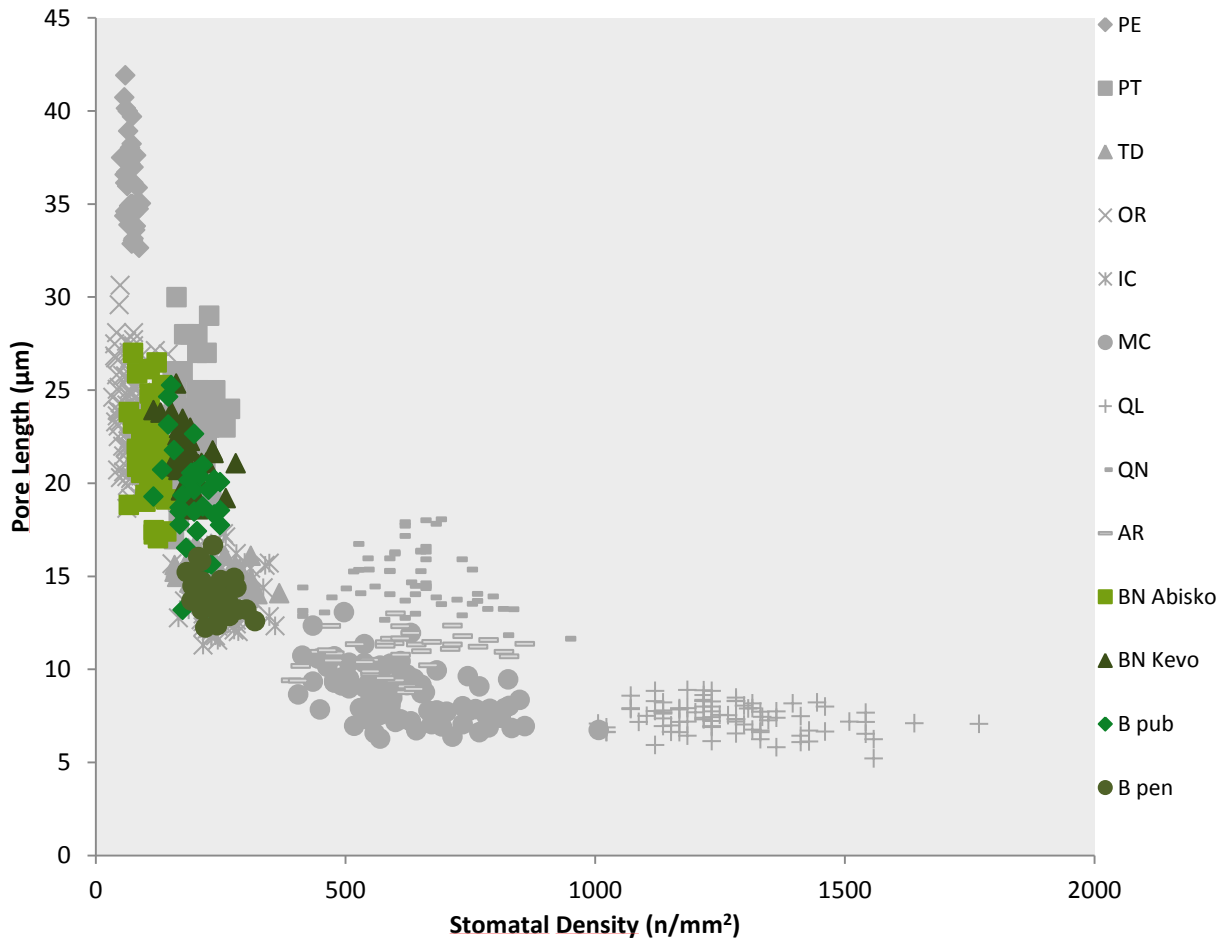


Figure 7 The PL in μm is plotted against the SD (n/mm^2). The left side of the graph includes the gymnosperm species from Florida in grey, *Pinus eliotti* (PE), *Pinus taeda* (PT), *Osmunda regalis* (OR), *Taxodium distichum* (TD) and the angiosperm species, *Ilex cassine* (IC), *Myrica cerifera* (MC), *Quercus laurifolia* (QL), *Quercus nigra* (QN) and *Acer rubrum* (AR). In green the Birch species are included, *B. nana* (BN) from Abisko and from Kevo, *B. pubescens* (B pub) from Kevo, and *B. pendula* (B pen) from The Netherlands.

The three different arctic datasets are all in the same part of the graph, therefore the chance that it is a coincidence is small. The PL and SD means are $21.58 \mu\text{m}$, 106.78mm^2 (*B. nana* from Abisko); $21.30 \mu\text{m}$, 186.46mm^2 (*B. nana* from Kevo) and $18.83 \mu\text{m}$, 196.93mm^2 (*B. pubescens*) respectively. The mean PL and SD of *B. pendula* are $13.90 \mu\text{m}$ and 236.34mm^2 respectively, these values are slightly lower in the graph, but the difference is still small.

In the article from Lammertsma *et al.* (2011) is stated that the angiosperms display large variation in SD, while the conifers and fern display a large variation in a_{max} , which is derived from the PL (Lammertsma *et al.*, 2011). Figure 7 shows that this conclusion can't be generalized (see Appendix Figure 10 for the graph with a_{max} plotted against SD), because birch is angiosperm species, and they show a small variation in SD and a large variation in PL. The arctic birches are in the same part of the graph where the gymnosperm species *Pinus taeda* and *Osmunda regalis* are plotted, while *B. pendula* lays in the transition zone between the gymnosperm and angiosperm species. This birch species is plotted in the part of the graph where the gymnosperm *Taxodium distichum* and the angiosperm *Ilex cassine* are. It should be very interesting to look at other angiosperms from the subarctic and maybe even more interesting where the gymnosperms from the subarctic will be plotted in this graph. Therefore further research is advised.

3.3 Conclusion

With the increasing amount of CO₂ in the atmosphere, the PL of *B. nana* is increasing along with it, while the SD decreases. Most likely larger pores are compensating for the decreasing amount of stomata in the leaves. The stomatal properties fit in the conduct of the results from Lammertsma *et al.* (2011). However, there is extra research advised about the PL and SD measurements of the angiosperm and gymnosperm species, because the angiosperm *Betulas* fit in the part of the graph where mostly the gymnosperms of the Florida species are.

Stomatal conductance

Another response of plants to the increasing amounts of CO₂ in the atmosphere is to reduce water loss from transpiration. This can be done to decrease the stomatal conductance and increase the assimilation rates (Royer, 2001; Hetherington & Woodward, 2003). This chapter will determine the consequences of CO₂ adaptation for the hydrology of several birch species. The expectation is that the g_{smax} will decrease while the atmospheric CO₂ concentration increases. These results will be validated against the Florida dataset of Lammertsma *et al.* (2011).

4.1 Material and methods

The maximum stomatal conductance to water vapour (g_{smax} [mol/m²/s]) of fully opened stomata

$$g_{smax} \text{ [mol/m}^2\text{/s]} = \frac{\frac{dw}{v} \times SD \times a_{max}}{l + \frac{\pi}{2} \sqrt{a_{max}/\pi}}$$

was calculated according to the SD (n/m²). The other determining variables for this calculation are the size of the fully opened stomata (a_{max} [m²]), which is derived from the PL (m), and depth of the stomatal tube (l [m]). The remaining values are constants: the diffusivity of water vapour (d_w [m²/s]) and the molar volume of air (v [m³/mol]). (Franks & Farquhar, 2001)

4.2 Results and discussion

Figure 8 shows the response of the g_{smax} from *B. nana* in Kevo and Abisko. There is a significant decrease in g_{smax} while the atmospheric CO₂ concentration increases. Earlier research shows a similar trend, where the g_{smax} is declining in time or along the increasing atmospheric CO₂ concentration (Polley *et al.*, 1993; De Boer *et al.*, 2011; Gagen *et al.*, 2011). It is predicted that this decrease in g_{smax} will continue, but it is possible that it will be a nonlinear response (De Boer *et al.*, 2011; Gagen *et al.*, 2011).

The same trend has been shown by Lammertsma *et al.* (2011). They also looked at the reaction of stomatal conductance of several plant species in

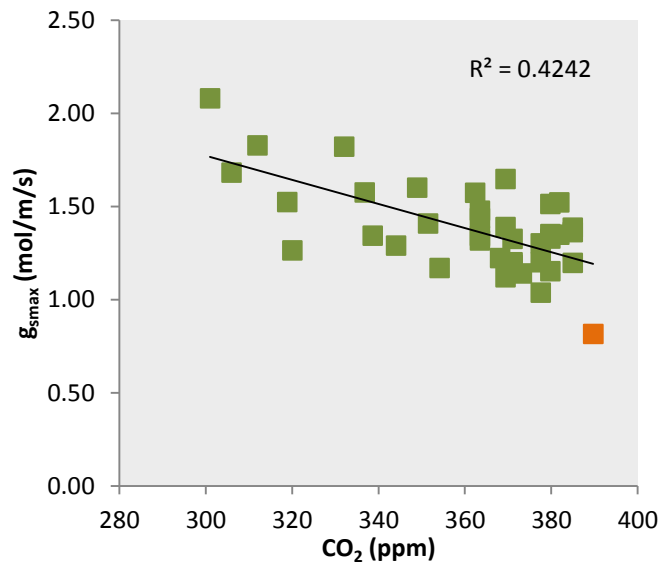


Figure 8 The conduct of the g_{smax} (mol/m²/s) plotted against the atmospheric CO₂ concentration (ppm). The green squares give the values of the Kevo dataset, while the orange square gives the mean value of the Abisko dataset from *B. nana*.

Florida to changes in CO₂ concentrations in the atmosphere. Figure 9 shows the trend lines of several species from Florida and the results from the *B. nana* and *B. pendula* datasets. The birches show a comparable response to the atmospheric CO₂ concentration as the Florida species. The average decrease in g_{smax} of the Florida results is 34%, while the decrease of *B. nana* is 31.4%. The birch species are plotted between the fern species *Osmunda regalis*, the conifers *Pinus eliottii* and *Pinus taeda*, and the angiosperms *Ilex cassine*, *Taxodium distichum* and *Myrica cerifera*. In this case it is not possible to make a clear distinction between the angiosperms and the gymnosperms. Therefore it is valid to say that the birches can be compared to the Florida species, and that their outcomes are quite similar.

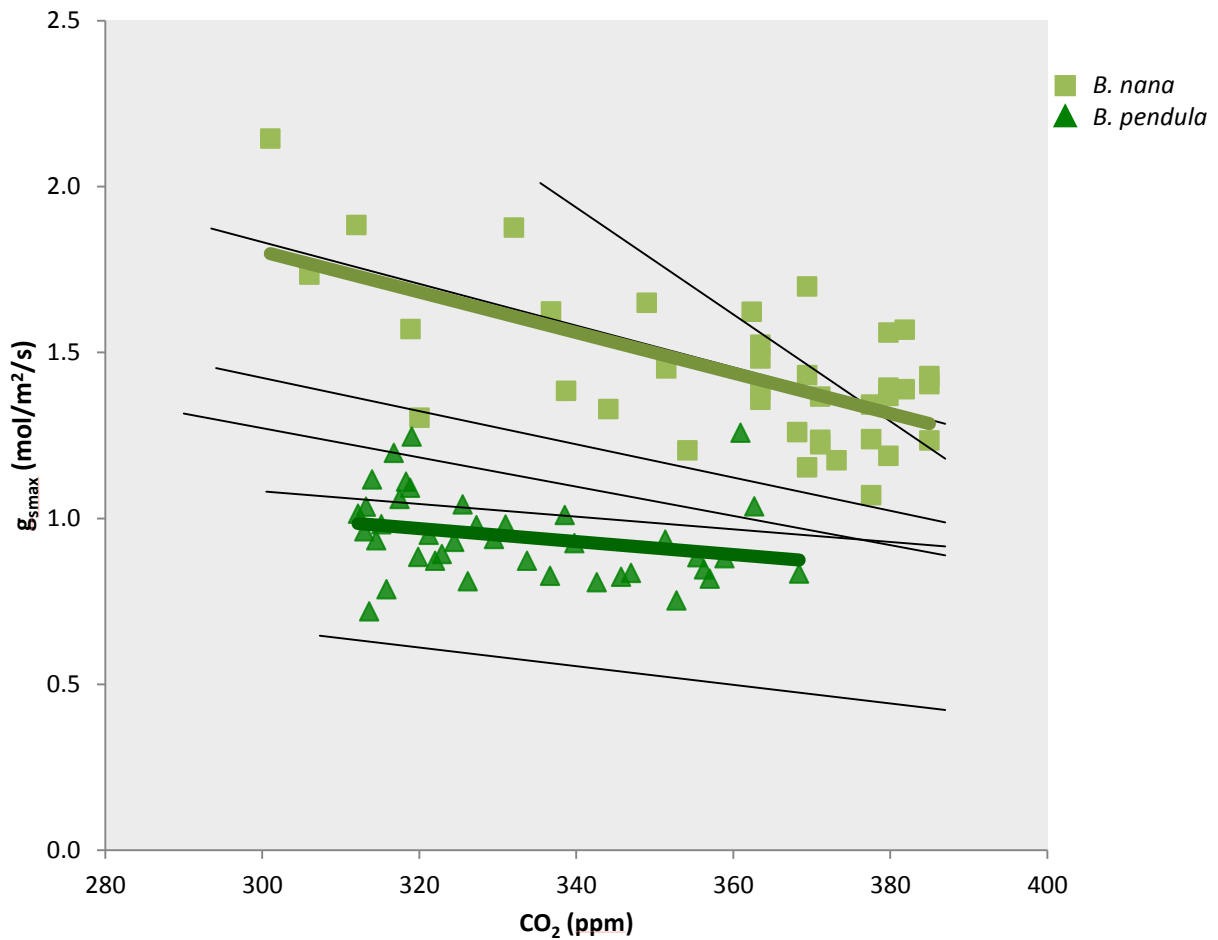


Figure 9 The conduct of the g_{smax} (mol/m²/s) plotted against the atmospheric CO₂ concentration (ppm). The black lines give the trend lines from some of the Florida species, from down to up: *Osmunda regalis*, *Pinus eliottii*, *Ilex cassine*, *Taxodium distichum*, *Myrica cerifera* and *Pinus taeda*.

There is some overlap between the gymnosperms and the birches in this graph as well. A possible explanation could lay in the origin of the species. Lammertsma *et al.* (2011) place the g_{smax} values against an evolutionary background. The conifers and ferns evolved in an environment with a higher atmospheric CO₂ concentration than present. The angiosperms came later, when there was a decrease in atmospheric CO₂ concentration (Lammertsma *et al.*, 2011). Birches are angiosperm species which appeared early in the evolution, and are associated with evergreens (Axelrod, 1966). There could have been some overlap in the environmental conditions, where *Betula* was growing in a high atmospheric CO₂ concentration, which could explain the conduct of the PL, SD and g_{smax} .

4.3 Conclusion

The birch species *B. nana* and *B. pendula* decrease the amount of g_{smax} with a smaller amount of CO_2 in the atmosphere. This is consistent with other research, which means that water loss in birches is reduced by means of a decrease in stomatal conductance. A possible explanation for the place of the Birches in the graphs could lay in the early evolution of the birches. Further research is advised, to find out where the gymnosperm species from the (sub)arctic regions will be in the graphs.

Conclusion

The Abisko data set gives an estimate about the range of values of the UI according to a variability test, from 1.35 to 1.42 (Figure 2). The samples are taken from various local habitats, and the UI is not influenced by the diversity in these micro-habitats. The SI does not depend on the altitudinal and latitudinal gradients. Therefore it can be concluded that the microhabitats do not have an effect on the SI as well. This indicates a positive conclusion about the use of *B. nana* as palaeo-proxy. Also, there is a good positive correlation between GDD₅ and UI.

The PL of *B. nana* increases along with the amount of atmospheric CO₂. The SD of *B. nana* decreases with the same increase of the atmospheric CO₂ concentration. This means that the larger pores are compensating for the decreasing amount of stomata in the leaves. The stomatal properties fit in the conduct of the results from Lammertsma *et al.* (2011). However, there is extra research advised about the measurements of angiosperm and gymnosperm species, which do not fit exactly when plotted (Figure 6). The birch results contradict the statement of Lammertsma *et al.* (2011) that angiosperms have numerous small stomata and high g_{smax} , while conifers and fern have few large stomata and lower g_{smax} . The *Betulas*, have few large stomata and lower g_{smax} like the gymnosperms from Florida. The stomatal properties of several birch species do fit in the conduct of the graph of Lammertsma *et al.* (2011), but not on the expected place. In birch species *B. nana* and *B. pendula* the amount of g_{smax} decreases while the atmospheric CO₂ concentration decreases. This means that in birches water loss is reduced by means of decreasing the stomatal conductance. This is consistent with the results of Lammertsma *et al.* (2011), but there seems to be some overlap between the gymnosperms and the birches here as well (Figure 7). There could be an explanation for this in the place of the species in the evolutionary background. Also, the plant species used by Lammertsma *et al.* (2011) are subtropical species, while the birch species are arctic and temperate species.

These results together lead to the conclusion that leaves of *B. nana* are fit to be used as palaeo-proxy. The stomatal characteristics are not influenced by micro-habitat, and they fit in the conduct of other species. There is some discrepancy about the overlap between gymnosperms and the birches. Therefore it is recommended to examine multiple different (sub)arctic species, to see if there is some overlap, or more differences, between the subtropical species used by Lammertsma *et al.* (2011) and the arctic species from this research.

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References

ACIA, 2005. Arctic Climate Impact Assessment, Cambridge University Press, 1042p.

AXELROD, D.I., 1966. Origin of deciduous and evergreen habits in temperate forest. *Evolution*, **20**(1), 1-15.

BOER, H.J. de, LAMMERTSMA, E.I., WAGNER-CREMER, F., DILCHER, D.L., WASSEN, M.J. and DEKKER, S.C., 2011. Climate forcing due to optimization of maximal leaf conductance in subtropical vegetation under rising CO₂. *Proceedings of the National Academy of Sciences of the United States of America (PNAS)*, early edition.

BONAN, G.B. and SHUGART, H.H., 1989. Environmental factors and ecological processes in boreal forests. *Annual Review of Ecology and Systematics*, **20**, 1-28.

BRET-HARTE, M.S., SHAVER, G.R., ZOERNER, J.P., JOHNSTONE, J.F., WAGNER, J.L., CHAVEZ, A.S., GUNKELMAN IV, R.F., LIPPERT, S.C. and LAUNDRE, J.A., 2001. Developmental plasticity allows *Betula nana* to dominate tundra subjected to an altered environment. *Ecology*, **82**(1), 18-32.

DOUGLAS, M.S.V. and SMOL, J.P., 2001. Freshwater diatoms as indicators of environmental change in the High Arctic. *The diatoms: applications for the environmental and earth sciences*, 227-245. Cambridge University Press, 469p.

FRANKS, P.J., and BEERLING, D.J., 2009. Maximum leaf conductance driven by CO₂ effects on stomatal size and density over geologic time. *Proceedings of the National Academy of Sciences of the United States of America (PNAS)*, **106**(25), 10343-10347.

FRANKS, P.J., and FARQUHAR, G.D., 2001. The effect of exogenous abscisic acid on stomatal development, stomatal mechanics, and leaf gas exchange in *Tradescantia virginiana*. *Plant Physiology*, **125**, 935-942.

GAGEN, M., FINSINGER, W., WAGNER-CREMER, F., MCCARROLL, D., LOADER, N.J., ROBERTSON, I., JALKANEN, R., YOUNG, G., and KIRCHHEFER, A., 2011. Evidence of changing intrinsic water-use efficiency under rising atmospheric CO₂ concentration in Boreal Fennoscandia from subfossil leaves and tree ring $\delta^{13}\text{C}$ ratios. *Global Change Biology*, **17**, 1064-1072.

HEATHERINGTON, A.M. and WOODWARD, F.I., 2003. The role of stomata in sensing and driving environmental change. *Nature*, **424**, 901-908.

HOUGHTON, J.T., DING, Y., GRIGGS, D.J., NOGUER, M., LINDEN, P.J. van der, DAI, X., MASKELL, K. and JOHNSON, C.A., 2001. Climate change 2001: The scientific basis. Cambridge University Press.

IPCC, 2007. Climate Change 2007. Fourth Assessment Report of the Intergovernmental Panel on Climate Change.

- JONES, P.D., BRIFFA, K.R., BARNETT, T.P. and TETT, S.F.B., 1998. High-resolution palaeoclimatic records for the last millennium: interpretation, integration and comparison with General Circulation Model control-run temperatures. *The Holocene*, **8**(4), 455-471.
- KEELING, C.D., 1997. Climate change and carbon dioxide: An introduction. *PNAS*, **94**(16), 8273-8274
- KÜRSCHNER, W.M., 1997. The anatomical diversity of recent and fossil leaves of the durmast oak (*Quercus petraea* Lieblein/*Q. pseudocastanea* Goepfert) – implications for their use as biosensors of palaeoatmospheric CO₂ levels. *Review of palaeobotany and palynology*, **96**(1-2), 1-30.
- KÜRSCHNER, W.M, STULEN, I., WAGNER, F. and KUIPER, P.J.C., 1998. Comparison of palaeobotanical observations with experimental data on the leaf anatomy of the durmast oak [*Quercus petraea* (Fagaceae)] in response to environmental change. *Annals of Botany*, **81**, 657-664.
- LAMMERTSMA, E.I., BOER, H.J. de, DEKKER, S.C., DILCHER, D.L., LOTTER, A.F. and WAGNER-CREMER, F., 2011. Global CO₂ rise leads to reduced maximum stomatal conductance in Florida vegetation. *Proceedings of the National Academy of Sciences of the United States of America (PNAS)*, early edition.
- OVERLAND, J.E. and WANG, M., 2004. Detecting Arctic climate change using Köppen climate classification. *Climatic Change*, **67**(1), 43-62.
- PFISTER, C., LUTERBACHER, J., WANNER, H., WHEELER, D., BRÁZDIL, R., GE, Q., HAO, Z., MOBERG, A., GRAB, S. and PRETO, M. R. del, 2008. Documentary evidence as climate proxies. *Proxy Uncertainty Workshop*, 1-10.
- PHILLPOT, H.R. and ZILLMAN, J.W., 1970. The surface temperature inversion over the Antarctic continent. *Journal of Geophysical Research*, **75**(21), 4161-4169.
- POLLEY, H.W., JOHNSON, H.B., MAYEUX, H.S. and MALONE, S.R., 1993. Physiology and growth of wheat across a subambient carbon dioxide gradient. *Annals of Botany*, **71**, 347-356.
- REY, A. and JARVIS, P.G., 1998. Long-term photosynthetic acclimation to increased atmospheric CO₂ concentration in young birch (*Betula pendula*) trees. *Tree Physiology*, **18**, 441-450.
- ROYER, D.L., 2001. Stomatal density and stomatal index as indicators of paleoatmospheric CO₂ concentration. *Review of Palaeobotany and Palynology*, **114**, 1-28.
- SALISBURY, E.J., 1928. On the causes and ecological significance of stomatal frequency, with special reference to the woodland flora. *Philosophical Transactions of the Royal Society of London*, **216**, 1-65.
- SCHLYTER, P., JÖNSSON, P., NYBERG, R., PERSSON, P., RAPP, A., JONASSON, C. and REHN, J., 2011. Geomorphic process studies related to climate change in Kärkevagge, Northern Sweden. *Physical Geography*, **75**(1-2), 55-60.

TAYLOR, M., TUCKER, C., SLAYBACK, D., PINZON, J., LOS, S and MYNENI, R., 2001. Higher northern latitude normalized difference vegetation index and growing season trends from 1982 to 1999. *International Journal of Biometeorology*, **45**(4), 184-190.

WAGNER, F., 1998. The influence of environment on the stomatal frequency in *Betula*. *LPP Contributions Series*, NSG Publication, 980801.

WAGNER-CREMER, F., AABY, B. and VISSCHER, H., 2002. Rapid atmospheric CO₂ changes associated with the 8,200-years-B.P. cooling event. *Proceedings of the National Academy of Sciences of the United States of America (PNAS)*, **99**(19), 12011-12014.

WAGNER-CREMER, F., BELOW, R., KLERK, P. de, DILCHER, D.L., JOOSTEN, H., KÜRSCHNER, W.M. and VISSCHER, H., 1996. A natural experiment on plant acclimation: Lifetime stomatal frequency response of an individual tree to annual atmospheric CO₂ increase. *Ecology*, **93**, 11705-11708.

WAGNER-CREMER, F., BOHNKE, S.J.P., DILCHER, D.L., KÜRSCHNER, W.M., GEEL, B. van, and VISSCHER, H., 1999. Century-scale shifts in early Holocene atmospheric CO₂ concentration. *Science*, **284**, 1971-1973.

WAGNER-CREMER, F., FINSINGER, W. and MOBERG, A., 2010. Tracing growing degree-day changes in the cuticle morphology of *Betula nana* leaves: a new micro-phenological palaeo-proxy. *Journal of Quaternary Science*, Wiley InterScience.

WAGNER, F., KOUWENBERG, L.L.R., HOOF, T.B. van and VISSCHER, H., 2004. Reproducibility of Holocene atmospheric CO₂ records based on stomatal frequency. *Quaternary Science Reviews*, **23**, 1947-1954.

WAGNER-CREMER, F., NEUVONEN, S., KURSCHNER, W.M. and VISSCHER, H., 2000. The influence of hybridization on epidermal properties of birch species and the consequences for palaeoclimatic interpretations. *Plant Ecology*, **148**(1), 61-69.

WALTHER, G., POST, E. and CONVEY, P., 2002. Ecological responses to recent climate change. *Nature*, **416**(6879), 389-396

Appendix

Table 1 Samples and their date of harvesting (Date), GPS coordinates, elevation in meters and the habitat description from the field notes of the author.

Sample	Date	GPS	Elevation	Habitat description
ANS3	9/2/2010	N68°19'08.2" E18°51'54.7"	629	On Puddas, Birch grow in patches close to each other. Not many other plants available, much lichens, mosses and <i>Vaccinium</i>
ANS15	9/2/2010	N68°19'49.8" E18°50'02.2"	511	Swampy, much willows
ANS18	9/3/2010	N68°21'29.7" E18°43'16.9"	729	Fields with <i>B. nana</i> , few berries, willows, pine and some lonely <i>B. pubescens</i>
ANS21	9/3/2010	N68°21'31.1" E18°44'05.3"	677	Fields with <i>B. nana</i> , few berries, willows, pine and some lonely <i>B. pubescens</i>
ANS37	9/4/2010	N68°21'55.9" E18°47'30.6"	355	<i>B. pubescens</i> canopy with <i>B. nana</i> underneath them (picture 53)
ANS40	9/4/2010	N68°21'36.0" E18°46'53.2"	363	Fields with <i>B. nana</i> , few berries and some other small undergrowth and some lonely <i>B. pubescens</i>
ANS57	9/5/2010	N68°20'47.5" E18°47'53.4"	402	Patches from <i>B. nana</i> and <i>B. pubescens</i> , moisty at places, very much space in between them
ANS70	9/6/2010	N68°20'22.0" E18°45'46.6"	392	Open nana field, <i>B. nana</i> is large, <i>B. pub</i> in patches, stream nearby. <i>Vaccinium</i> , many willows and grasses
ANS78	9/6/2010	N68°18'38.5" E18°50'36.8"	625	Patches <i>B. nana</i> , between mosses, some berries and some grass
ANS97	9/8/2010	N68°19'00.5" E18°44'06.5"	441	Large <i>B. pub</i> , canopy, much pine, further berries and grasses, one <i>B. nana</i> patch
ANS99	9/8/2010	N68°18'42.8" E18°41'29.5"	490	<i>B. pub</i> canopy, <i>B. nana</i> patches, berries and lichens, little moisty
ANS103	9/8/2010	N68°17'45.3" E18°38'07.5"	490	Fields of <i>B. nana</i> , <i>B. pub</i> patch in it, berries
ANS104	9/9/2010	N68°21'40.7" E18°46'08.2"	399	<i>B. pub</i> patches, <i>B. nana</i> cover ground, much berries
ANS107	9/9/2010	N68°21'34.3" E18°41'16.7"	1121	Very small <i>B. nana</i> , berries, gras, moss, lichens

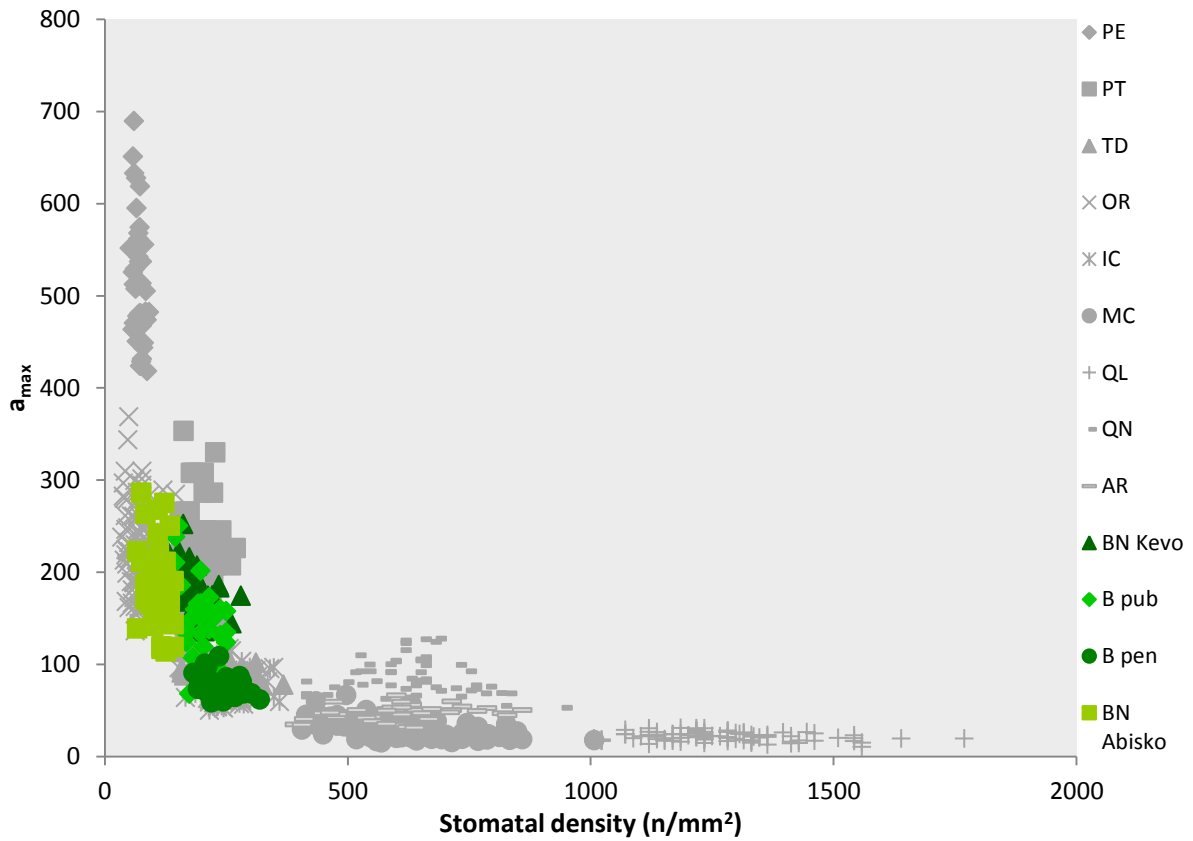


Figure 10 Graphs shows the a_{max} plotted against the SD (n/mm^2). The left side of the graph includes the gymnosperm species from Florida in grey, *Pinus eliotti* (PE), *Pinus taeda* (PT), *Osmunda regalis* (OR), *Taxodium distichum* (TD) and the angiosperm species, *Ilex cassine* (IC), *Myrica cerifera* (MC), *Quercus laurifolia* (QL), *Quercus nigra* (QN) and *Acer rubrum* (AR). In green the Birch species are included, *B. nana* (BN) from Abisko and from Kevo, *B. pubescens* (B pub) from Kevo, and *B. pendula* (B pen) from The Netherlands. The results from the species from Florida come from research of Lammertsma *et al.* 2011.