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Photosynthetic Response to increasing Temperatures - a Comparison between prostrate and erect alpine Shrubs

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MSc in Biology

Submission date: May 2015

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ACKNOWLEDGEMENT

This master thesis was carried out at the department of biology, at the Norwegian University of Science and Technology.

First and foremost I sincerely want to thank my supervisors Richard Strimbeck, Mia Vedel Sørensen, and Bente Jessen Graae (NTNU) for their excellent guidance and inspiration during the entire process, and especially their patience in the face of seemingly endless statistic questions.

My thanks also go to the Norsk Villreinsenter, Dovre, for accommodation during fieldwork. Special thanks to the entire shrub-gang for making the fieldwork enjoyable, and for providing clearly needed cake-breaks all through the semester. For all the fun and good times during my time in Trondheim, I sincerely want to thank my friends and fellow students. Last, but not least, thanks to Anders for keeping the house somewhat together, and making even stressful days better.

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ABBREVIATIONS

A_{mass} : Photosynthetic rate per unit leaf mass

LMA: Leaf mass to area ratio

N_{mass} : Nitrogen content

ES: Erect shrubs

PS: Prostrate shrubs

R_{mass} : Respiration rate per unit leaf mass

PAR: Photosynthetically active radiation

ABSTRACT

As a response to climate change, shrub biomass has increased in alpine and arctic tundra ecosystems. Substantial shrub encroachment can lead to changes in carbon fluxes, due to a shift in community compositions and functional groups. Some shrub species or functional types could have advantages over others at current and future distributions, because of different characteristics such as leaf traits. To gain more knowledge about such differences and what this might mean for future species composition in the alpine environment in Norway, six different alpine shrubs were subjected to temperature regimes of 14, 17 and 22°C, during which photosynthetic and respiration rate was measured. In addition, leaf mass per area and nitrogen content was measured and compared to leaf traits from the Global Plant Trait Network. The results showed that net photosynthetic rate of all species was down-regulated, due to the substantial increase in respiration rate, at temperatures higher than 14°C. The deciduous erect shrubs *Betula nana*, *Salix phylicifolia*, *S. glauca*, and *S. lapponum* had significantly higher net photosynthetic and respiration rates at all temperatures than the prostrate shrubs *Empetrum nigrum* and *Vaccinium vitis-idaea*. However, there was no difference in how much the photosynthetic rate was down-regulated. Thus, prostrate shrubs did not have more of a disadvantage at higher temperatures, and the increase of erect shrubs in experiments observed in artificial warming experiments can therefore not be explained by different temperature responses of net photosynthetic rate alone. Rather, the higher nitrogen content and lower leaf mass per area ratio in erect shrubs could indicate greater potential to increase growth rates under more favorable conditions. In addition, respiration rate and thus net photosynthetic rate can potentially adjust under prolonged warming. The long-term effects of increased temperature on photosynthesis, growth and shrub species composition should therefore be explored further.

SAMMENDRAG

Som en følge av globale klimaforandringer har biomassen til buskvekster økt i økosystemene i alpin og arktisk tundra. Betydelige forandringer i buskenes utbredelse kan føre til endringer i karbon-flukser på grunn av ny sammensetningen av arter og funksjonelle grupper i samfunnene. Noen arter eller funksjonelle grupper kan ha trekk som gir dem fordeler i et varmere klima. For å få mer kunnskap om slike forskjeller, og hva disse kan bety for fremtidige artssammensetninger i alpine miljø i Norge, ble seks ulike alpine buskvekster utsatt for regimer på 14, 17 og 22°C, hvor fotosyntese og respirasjonsrate ble målt. I tillegg ble "leaf mass per area" og nitrogeninnhold målt, og sammenlignet med trekkene samlet inn fra den globale databasen Global Plant Trait Network. Resultatene viste at den fotosyntetiske raten til alle arter ble nedregulert på grunn av betydelig økning i respirasjonsraten ved temperaturer høyere enn 14 C. De løvfellende, oppreiste buskene *Betula nana*, *Salix glauca*, *S. lapponum* og *S. phylicifolia* hadde signifikant høyere netto fotosyntese og respirasjon ved alle temperaturer enn de eviggrønne, krypende buskene *Empetrum nigrum* og *Vaccinium vitis-idaea*. I midlertid var det ingen forskjell mellom gruppene i hvor mye de nedregulerte netto fotosynteseraten. Krypende busker har derfor ikke en større ulempe under varmere temperaturer, og den observerte økte utbredelsen av oppreiste busker kan ikke forklares kun ut i fra fotosyntesens forskjellige temperatur-responser alene. Imidlertid kan det høyere nitrogeninnholdet og lavere "leaf mass per area" i oppreiste busker indikere et større potensial for å øke vekstrater under mer gunstige forhold. I tillegg kan gass-utveksling og dermed netto fotosyntese potensielt justere seg under langvarig oppvarming. De langsiktige effektene av økt temperatur på fotosyntese, vekst, og artssammensetningen i alpine buske-heier burde derfor undersøkes nærmere.

INTRODUCTION

Over the last few decades, alpine and arctic tundra ecosystems have seen a substantial increase in shrub biomass and abundance (Goetz et al. 2011, Myers-Smith et al. 2011), which generally has been interpreted as a response to climate warming and an increase in CO₂ levels (Wookey et al. 2009, Settele et al. 2014). Plant growth in arctic and alpine biomes is largely limited by low temperatures, and these systems thus are especially sensitive to a warming climate. The shrub encroachment can in turn lead to changes in carbon fluxes, due to a change in plant production and ecosystem carbon storage (Mack et al. 2004, Settele et al. 2014), potentially even resulting in a positive feedback to climate warming (Sjogersten and Wookey 2009, Pearson et al. 2013, Parker et al. 2015). In addition, predicted and observed shifts in the distribution of vegetation zones (Thuiller et al. 2005, Engler et al. 2011, Michelsen et al. 2011) suggests that some shrub species may have an advantage in a warmer climate. Knowledge about advantages, such as a higher photosynthetic rate at warmer temperatures, could help predict future species composition in alpine and arctic communities. Deciduous erect shrubs such as birch and willow species are predicted to show the greatest increases at lower altitudes and tundra ecosystems (Sturm et al. 2001, Tape et al. 2006, Wehn et al. 2014). It can therefore be speculated if this group, at their current distribution, will have an advantage as the temperature rises. Smaller shrubs, such as prostrate species, which generally have a slower growth, might be outcompeted in heath tundra zones, where these two groups coexist. Knowledge about the photosynthetic capacity of the encroaching species is therefore important to gain information about possible changes in carbon storage and CO₂ flux as the vegetation types are changing (Cahoon et al. 2012).

Several elements influence the effect that climate change will have on the global vegetation, but generally, higher concentrations of CO₂, changes in precipitation, and higher temperatures seem to be the most prominent factors (Settele et al. 2014). These elements will influence plants differently, depending on their preferred climate and leaf economic traits

(Wright et al. 2004, De Deyn et al. 2008). An increase in atmospheric CO₂ has the potential to enhance net photosynthetic rate in plants, and can be one reason for the observed shrub encroachment (Sage et al. 1989). Plants in arctic and alpine environments, however, are generally restricted by cooler temperatures and shorter growing seasons (Doak and Morris 2010). The increasing temperatures could thus affect the growth and distribution of these species more significantly than CO₂ increase alone. For example Chapin et al. (1995) found a decreased species richness due to a strong dominance of *Betula nana* after experimental warming. Although it has been shown that increased temperature enhances the biomass production of shrubs in general (Walker et al. 2006), Michelsen *et al.* (2011) found a decline in some evergreen prostrate shrubs and understory forbs, along with indications of increase in abundance of deciduous erect shrubs. This suggests that an increase in temperature can reduce the distribution of tundra heath dominated by prostrate shrubs as the taller, erect shrubs become more dominant.

The competition between erect and prostrate shrubs is partially balanced by spatial and temporal differentiation in photosynthetic activities (Karlsson 1987). Spatial and temporal differentiation can be seen as variances in leaf investment strategies. For example, high photosynthetic rates are related to low leaf mass per area (LMA), while high LMA is positively correlated with a longer leaf life span (Wright et al. 2001, Cornelissen et al. 2003). It therefore makes sense to take the leaf traits of the two groups into consideration, when looking at how they respond to increased temperatures. Wright et al. (2004) suggest that a spectrum of leaf economic variation exists that can describe variation in carbon investment and growth strategy (Wright et al. 2004). Variations in growth strategy range from species of short-lived plants that are typically fast growing, to long-lived plants, which grow more slowly because they invest more energy and carbon into long-lived organs. Leaf traits show these different investment strategies; for example LMA is related to photosynthetic capacity per unit leaf mass (A_{mass}) in a way that predicts high A_{mass} in plants with low LMA, while plants with high LMA have low A_{mass} . Such traits can be used to predict how species respond to environmental changes, or give information on the impact some functional types have on ecosystem processes (De Deyn et al. 2008). Deciduous erect shrubs generally have a lower LMA, and higher nitrogen content (N_{mass}), while the opposite

is true for evergreen prostrate shrubs (Reich et al. 1997). It would therefore be interesting to link the traits to A_{mass} to see how the shrubs compare in a global leaf economic spectrum, further explaining differences in growth rates, and see if the traits can predict photosynthetic performance.

Significant positive correlations between mean summer temperature and annual growth of shrubs from the northern hemisphere have been found (Bar et al. 2007, Rozema et al. 2009), and they suggest that warmer seasons can promote growth by changing physiological processes in the plants. It is reasonable to assume that temperature has an effect on the enzymes catalyzing photosynthesis, especially the ratio of the carboxylase to oxygenase reactions of rubisco, and hence has an influence over photosynthesis overall. Therefore, knowledge about temperature optima for photosynthesis in alpine shrubs could give more information about why deciduous erect shrub growth has increased, and why different species may have different preferred temperatures. Myers-Smith et al. (2011) highlight the need to explore the differences in species-specific responses to warming, especially on what will happen in the future with continued increases in temperature. The response of whole tundra plots and communities has been studied comprehensively by the International Tundra Experiment (ITEX), but variation in differences among species can also lead to changes in community structure or biodiversity in these areas. This may further be of interest because coexisting plant species can change growth patterns differently during the same temperature regimes (Niu and Wan 2008).

The aims of this study are to estimate the A_{mass} and the R_{mass} of the encroaching erect shrubs and prostrate shrubs, see how they compare to each other at different temperatures, and determine if one of the groups has a better performance at higher temperatures. In this study, erect shrubs (ES) include the deciduous shrubs *Betula nana*, *Salix phylicifolia*, *Salix glauca*, and *Salix lapponum*, as all of these species functionally are erect shrubs at the study site, Dovrefjell. The prostrate shrubs (PS) include *Empetrum nigrum* and *Vaccinium vitis-idaea*, two evergreen species that dominate the dwarf shrub heath in the area. We will look at how A_{mass} compares to

LMA and N_{mass} in terms of a leaf economic spectrum, and specifically if LMA can be used as a predictor of how the A_{mass} of these two groups will perform in a warmer climate. In detail, our research questions are:

(1) How is net photosynthesis and respiration affected by temperature in the six different species?

(2) Is there a consistent difference in performance in the four erect species compared to the two prostrate shrubs, and are the erect species responding more positive to warmer temperatures?

(3) Where do the species fall in the leaf economics spectrum, and can LMA be used to predict performance in these shrubs?

MATERIALS AND METHODS

SITE DESCRIPTION

The study site was next to Norsk Villreinsenter Nord (62°13' N, 9°32'E), Hjerkin, within the Dovrefjell-Sunndalsfjella National Park in central Norway. It is situated in the lower alpine zone at 1012 m.a.s.l. The climate is characterized by mean temperatures between -11.8°C in January to 10.1°C in July (1961-1990), while in 2014 the warmest measured day was 25.9°C and the mean daytime temperature in July was 14.1 (Norwegian Meteorological Institute, 2015). The mean annual precipitation is 787 mm; 2014 however, was rather dry with an annual precipitation of 440 mm (Norwegian Meteorological Institute, 2015). The vegetation type around the research station is primarily tundra heath, and the deciduous erect shrubs *Betula nana*, *Salix phylicifolia*, *Salix glauca* and *Salix lapponum*, and the evergreen prostrate shrubs *Vaccinium vitis-idaea* and *Empetrum nigrum*, are all common in the area.

GAS EXCHANGE

Photosynthetic gas exchange was measured with a portable photosynthesis system (LI-6400; LI-COR, Inc., Lincoln, USA) with a lighted conifer chamber (6400-22L Opaque Conifer Chamber, LI-COR, INC.), in July and early September. In both months, the measurements were conducted in the course of five days between 0800 h and 1800 h, and the order in which the species were measured was randomized. A single branch of the current seasons' growth was taken indoors, and a smaller twig of about 4-5 cm was recut under water, attached to a water-filled tube, and measured immediately. The airflow through the chamber was maintained at 700 mL*min⁻¹, and reference air was taken from outside and was at 383 ± 5 μmol CO₂ mol⁻¹. Gas exchange was measured in both dark and light at three temperatures: 14, 17 and 22°C. Although it originally was planned to measure at 12°C as the lowest temperature, due to limitations of the measuring system, it was not possible to decrease the temperature below 14°C. However, 14°C is close to the mean daytime temperature during summer, and thus a reasonable estimate of the current temperature in the main growing season. As temperature is predicted to increase ca 4°C on average at high latitudes (Prentice 2001), 17°C represents the mean daytime temperature in possible future growing conditions. 22°C simulates a higher amount of warmer days and heat

waves. The branch was given five minutes for each new temperature in the dark, and three minutes in the light before measurement was started, as this was found to be sufficient adjustment time for the new conditions. Gas exchange was measured for one minute in the dark, and three minutes in the light at each temperature. A table describing the protocol for this can be found in Appendix A.

Light curves were used to assess the photosynthetic activity of the shrubs, over a range of ambient light intensities. Most commonly, they are used to describe the acclimation of the photosynthetic apparatus to different light intensities, by using gas exchange measurements (Ralph and Gademann 2005). The point where more light does not increase the photosynthetic rate, i.e. where the curve has flattened out, gives information about the saturation characteristics of the electron transport of the plant (Ralph and Gademann 2005). Photosynthetically active radiation (PAR) that was closest to this saturation point for all species, was found to be $800 \mu\text{mol m}^{-2} \text{s}^{-1}$ by using the LI-COR standard program "LightCurve2" (LI-COR, INC, Lincoln, USA.), and therefore used for all the gas exchange measurements. Examples of light-curves can be found in Appendix B.

The ratio of intercellular to atmospheric CO_2 (C_i/C_a) can be used to evaluate the condition of a leaf, and for C_3 plants, such as shrubs, the typical ratio is 0.7 ± 0.1 (Bunce 2005). Results differing from this ratio were therefore removed from the final dataset. This resulted in 133 measurements (of originally 162), five to nine replicates per species per temperature.

LEAF TRAITS

The assessment of leaf area, weight, leaf mass per area (LMA), and nitrogen content were conducted as described by Cornelissen et al. (2003). All leafs of the measured branch were detached and scanned, and the leaf areas were found using ImageJ 1.42 software (ImageJ, National Institutes of Health, Washington DC, USA). Subsequently, all leafs were placed in plastic bags with moist paper towels to keep them fresh, and stored in a refrigerator. The fresh weight was measured at most two days after harvesting. All leaves were dried for three days at 60°C , and then measured. LMA was calculated as the leaf dry mass per unit leaf area. The photosynthetic rate was calculated both with the area measurements (A_{area}) and with dry weight

measurements (A_{mass}). The formula used by the system to calculate photosynthetic rate for both A_{area} and A_{mass} , was:

$$A_{\text{Area}} \text{ and } A_{\text{Mass}} = ([CO_2]_{\text{Reference air}} - [CO_2]_{\text{Sample air}} * \frac{1000 - [H_2O]_{\text{Ref. air}}}{1000 - [H_2O]_{\text{S. air}}}) * (\text{Flow} * (\frac{0.001}{\text{Area or Mass}}))$$

Flow is the flow rate in $\mu\text{mol s}^{-1}$, and area or mass can be entered in the equation as cm^2 or decigrams, respectively (LI-COR, Inc., Lincoln, USA). Only A_{mass} was further used in the analysis, as it was considered a more reliable measure for between species comparisons (Wright et al. 2001).

Leaf nitrogen data from the Hjerkin area was provided by Mia Vedel Sørensen (Department of Biology, NTNU), and included measurements for *Vaccinium vitis-idaea*, *Empetrum nigrum*, *Salix lapponum*, and *Salix glauca*.

GLOBAL LEAF ECONOMIC SPECTRUM

To understand how the leaf traits gathered in this study compare with leaf traits from plants globally, the Global Plant Trait Network (Gloptnet) database was used (Wright et al. 2004). This database covers 2548 species from 175 sites, representing a wide range of different vegetation types, and has been used to study leaf trait relationships (Wright et al. 2004).

LMA and leaf nitrogen content (N_{mass}) of the shrubs was plotted against their A_{mass} and R_{mass} from the temperature resulting in the highest A_{mass} for each species (17°C for *Salix glauca*, and 14°C for all other species) of all temperature trials. This was done because A_{mass} and R_{mass} from Gloptnet was measured at the maximum photosynthetic rate of the species. The data was transformed to the same units, and plotted in the same graph as the measurements gathered by Gloptnet: $\text{Log } [R_{\text{mass}} (\text{nmol g}^{-1} \text{s}^{-1}), A_{\text{mass}} (\text{nmol g}^{-1} \text{s}^{-1}), \text{LMA} (\text{g m}^2), \text{ and } N_{\text{mass}} (\%)]$.

STATISTICAL ANALYSIS

All statistical analyses were performed with the R software, version 2.13.2 (R Development Core Team, 2011).

Relationships between A_{mass} , species and temperature were analyzed by analysis of variance (ANOVA), while the relationship between R_{mass} , species and temperature was analyzed by analysis of covariance (ANCOVA). Species, temperature and the interaction between them were included as main effects to test for different temperature responses among species. Data was subjected to Shapiro test for normal distribution and Bartlett test for variance homogeneity before analysis. Because some photosynthetic temperature responses were non-linear for A_{mass} , temperature was treated as a categorical variable, with "14", "17" and "22" as the categories, rather than using a more complex polynomial model. A main orthogonal comparison between ES and PS was done to see if there was a significant difference in how the two groups reacted to rising temperatures.

Differences in A_{mass} and R_{mass} between the species and the temperatures were analyzed using multiple comparisons with a Tukey's Honestly Significant Differences (HSD) test.

RESULTS

TEMPERATURE EFFECT ON THE PHOTOSYNTHETIC ASSIMILATION RATE

The mean net photosynthetic assimilation rate per kg dry leaf mass (A_{mass}) of the six shrubs ranged from $40 \mu\text{mol kg}^{-1} \text{s}^{-1}$ to $70 \mu\text{mol kg}^{-1} \text{s}^{-1}$ at 14°C (Figure 1), but there were no significant differences between the species at this temperature. Increase in ambient temperature under saturating light intensity ($800 \mu\text{mol m}^{-2} \text{s}^{-1}$) resulted in a consistent decrease in net CO_2 assimilation in all species except *Salix glauca* (Figure 1), and from the analysis of variance there was found a significant effect of temperature (Table 1). At the warmest treatment of 22°C , A_{mass} of *Betula nana* was significantly greater ($p < 0.05$) than *Salix lapponum* and *Empetrum nigrum* (Appendix C). *Betula nana* and *Salix glauca* had greater photosynthetic rates than the other species at the warmest temperature, while *Empetrum nigrum* markedly had the lowest (Figure 1). See Appendix C the other between species comparison. The analysis of variance showed that species had a significant effect on A_{mass} , while there was no significant interaction between species and temperature (Table 1). Further, the analysis also showed that there was a significant difference in overall rate between erect shrubs (ES) and prostrate shrubs (PS). However, there was no significant interaction term, indicating that the two groups generally responded to temperature in a similar way (Table 1).

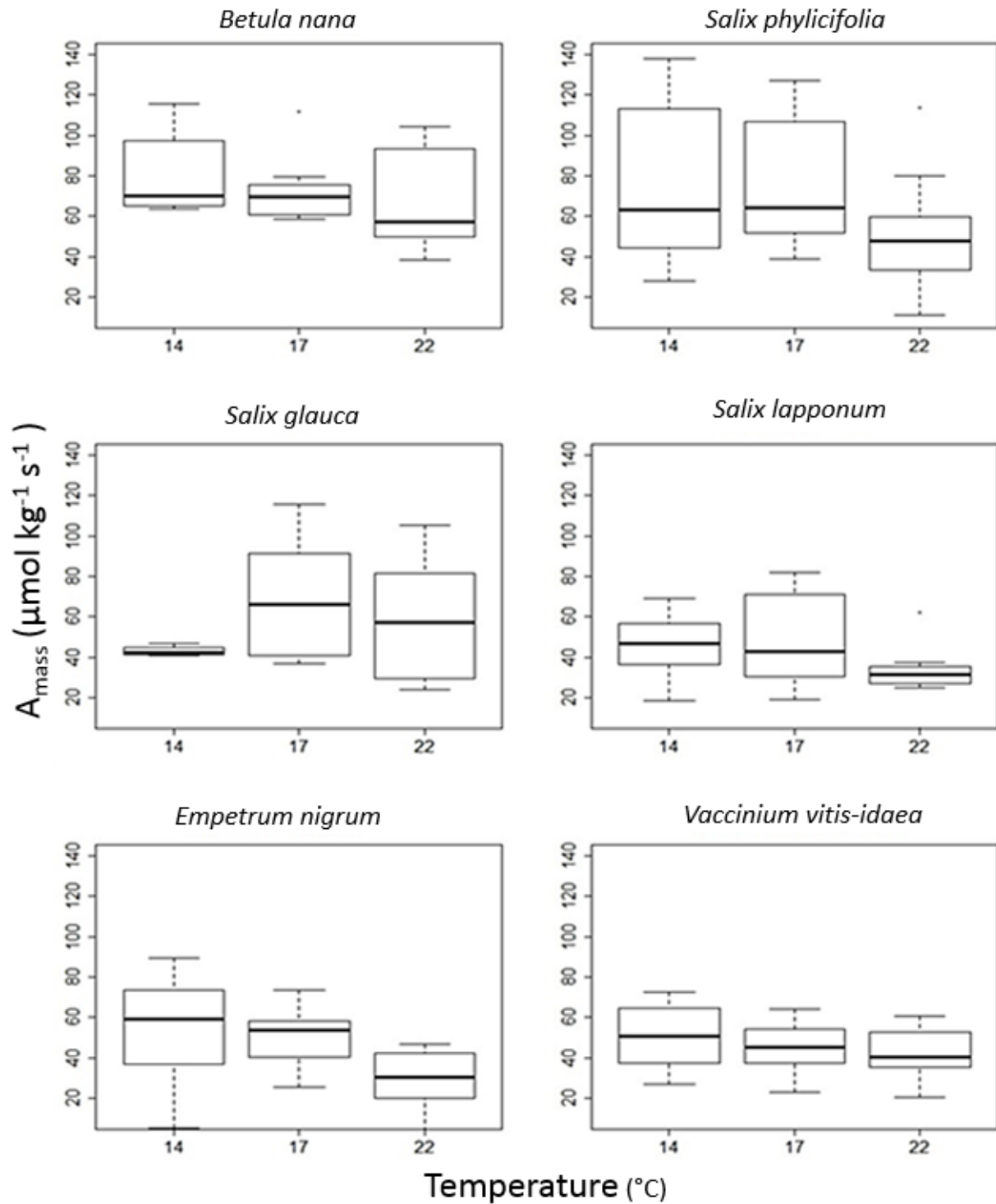


Figure 1: Net photosynthetic assimilation rate of CO_2 per kg dry leaf mass (A_{mass}) of six species of alpine shrubs measured at three different temperatures ($n=5-9$). Expressed as net influx.

Table 1: The results from the analysis of variance showing the effects of temperature and species on net photosynthetic rate (A_{mass}) of different alpine shrubs, as well as orthogonal comparison between erect shrubs (ES) and prostrate shrubs (PS). Statistically significant p -values are shown in bold.

Factor	Sum of squares	Degrees of freedom	Mean Square	F	p
Temperature	6803	2	3401	4.752	0.010
Species	21824	5	4365	6.097	<0.001
ES vs. PS	-	1	-	-	0.002
Interaction					
Temp.*species	5566	10	557	0.778	0.650
Temp.*(ES vs.PS)	-	2	-	-	0.766
Error	82323	115	716		

An interaction plot (Figure 2) showed that ES had greater A_{mass} than the PS at all temperatures. ES also had the highest A_{mass} at 17 °C and the lowest at 22 °C, while the A_{mass} of PS was always decreasing with increasing temperature (Figure 2).

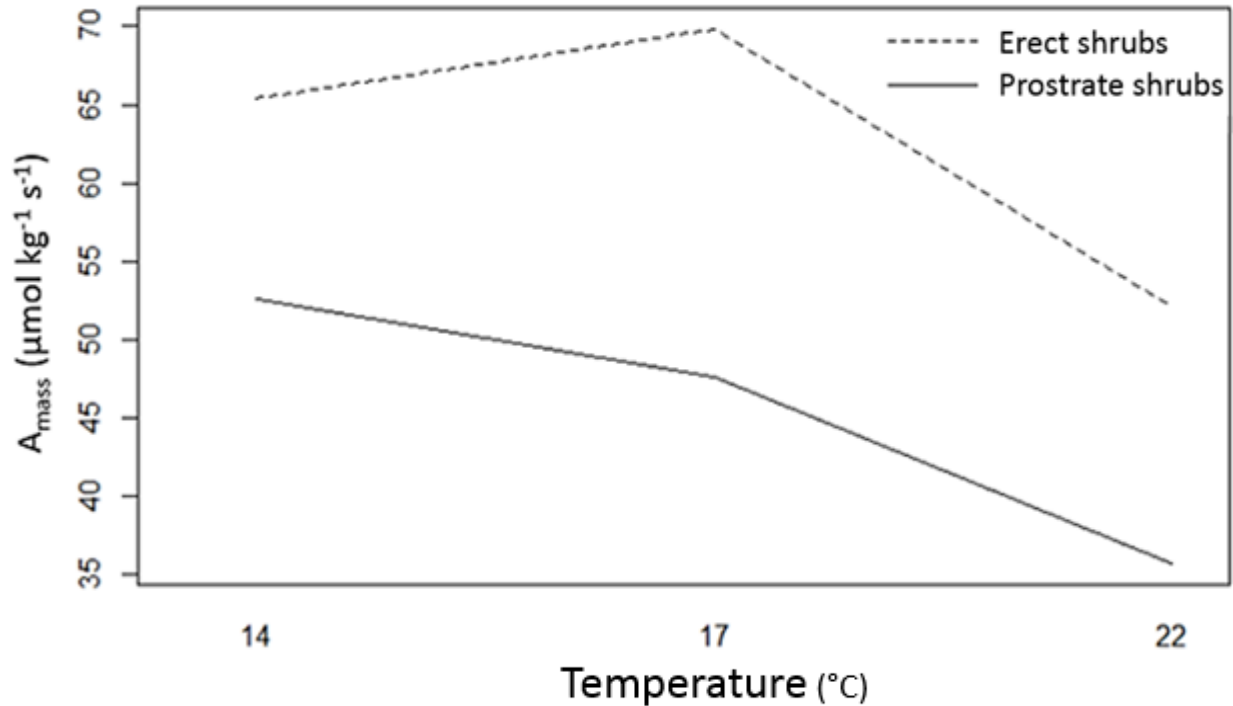


Figure 2: Interaction plot of Net photosynthetic assimilation rate of CO_2 per kg dry leaf mass (A_{mass}) of two prostrate shrubs (*Vaccinium vitis-idaea* and *Empetrum nigrum*, $n=5-9$) and four erect shrubs (*Salix lapponum*, *Salix glauca*, *Salix phylicifolia*, and *Betula nana*, $n=6-9$), at three different temperatures ($^{\circ}\text{C}$).

TEMPERATURE EFFECT ON RESPIRATION

The means of the respiration rate per kg dry leaf mass (R_{mass}) of the six shrubs ranged from $-3 \mu\text{mol kg}^{-1} \text{s}^{-1}$ (*Empetrum nigrum*) to $12 \mu\text{mol kg}^{-1} \text{s}^{-1}$ (*Betula nana*) at 14°C (Figure 3.1). The negative respiration rate in *Empetrum nigrum* and *S. lapponum* is most likely a measurement error due to high humidity in the chamber at low temperatures. This can slightly affect the results in samples with low gas exchange rates (LI-COR 2011). Increase in ambient temperature resulted in a consistent and nearly linear increase of R_{mass} , and all species consistently had a significantly higher respiration rate at 22°C than at 14°C ($p < 0.05$) (see Figure 3). An analysis of covariance showed that species and temperature had significant effects on R_{mass} , and that there was an interaction between temperature and species (Table 2). Testing for differences between ES and PS showed that R_{mass} was significantly different between these two groups ($p < 0.01$) but they

responded similarly to changes in temperature (Table 2, Figure 4). ES had higher respiration rates at all temperatures (Figure 4).

Net photosynthesis is the gross photosynthesis minus the rate of CO₂ simultaneously lost during respiration, and so adding R_{mass} and A_{mass} results in the gross photosynthetic rate of the six species at all temperatures, and can be found in Appendix D. The constant increase in respiration rate seems to be higher than the decrease in A_{mass} , because the gross photosynthetic rate is slightly (although not significantly, $p > 0.1$) increasing for most species.

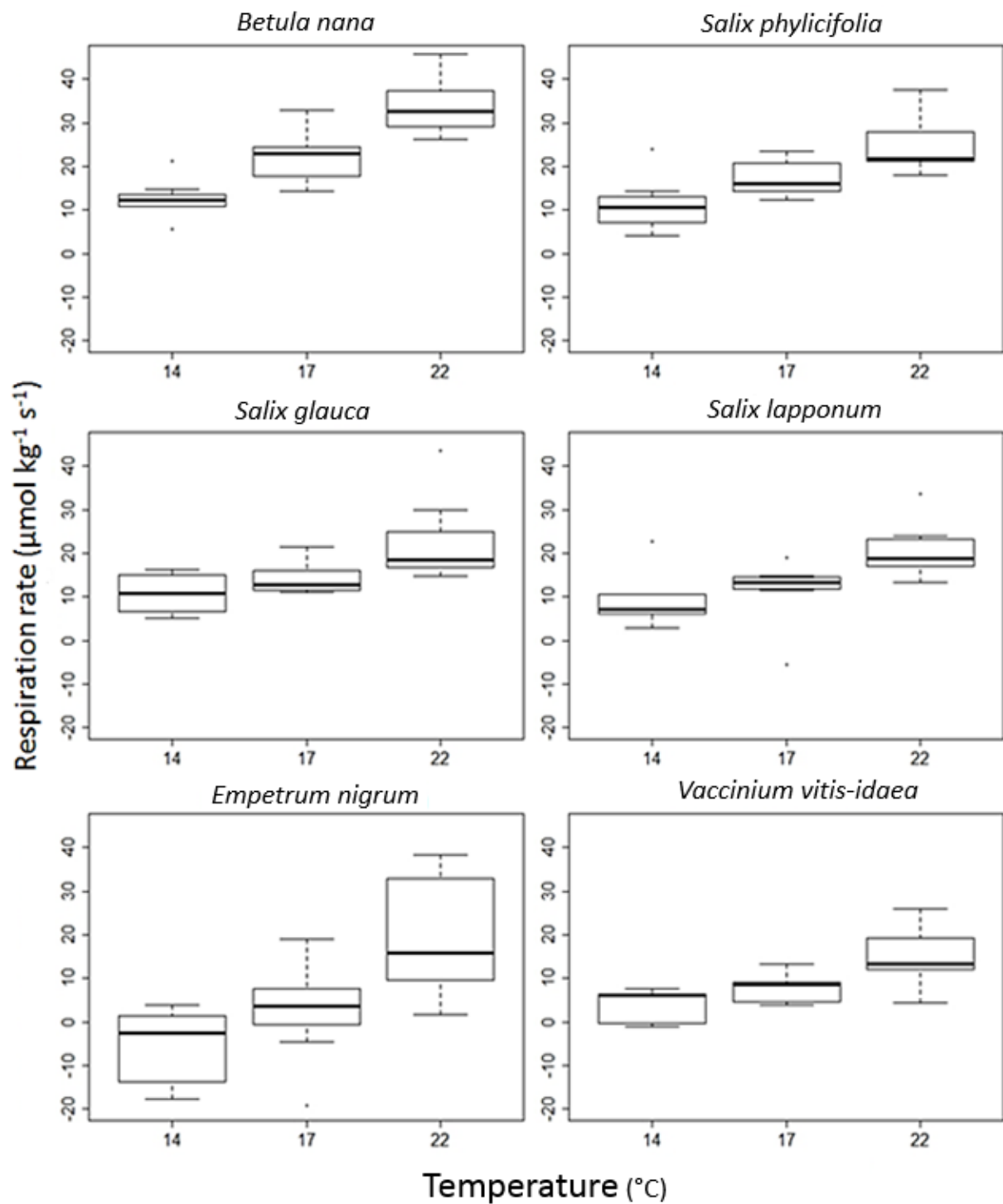


Figure 3: Net respiration rate per kg dry leaf mass (R_{mass}) of six species of alpine shrubs measured at three different temperatures ($n=5-9$). Expressed as net efflux.

Table 2: Results from the analysis of covariance, showing effects of temperature and species on respiration rate of different alpine shrubs, as well as orthogonal comparison between erect shrubs (ES) and prostrate shrubs (PS). Statistically significant p-values are shown in bold.

Factor	Sum of squares	Degrees of freedom	Mean Square	F	Significance
Temperature	6883	1	6883	112.236	$2 \cdot 10^{-16}$
Species	4967	5	993	16.198	$3.47 \cdot 10^{-12}$
ES vs.PS	-	1	-	-	0.00191
Interaction					
Temp.*species	848	5	170	2.765	0.02
Temp.*(ES vs.PS)	-	1	-	-	0.189
Error	82323	115	716	-	-

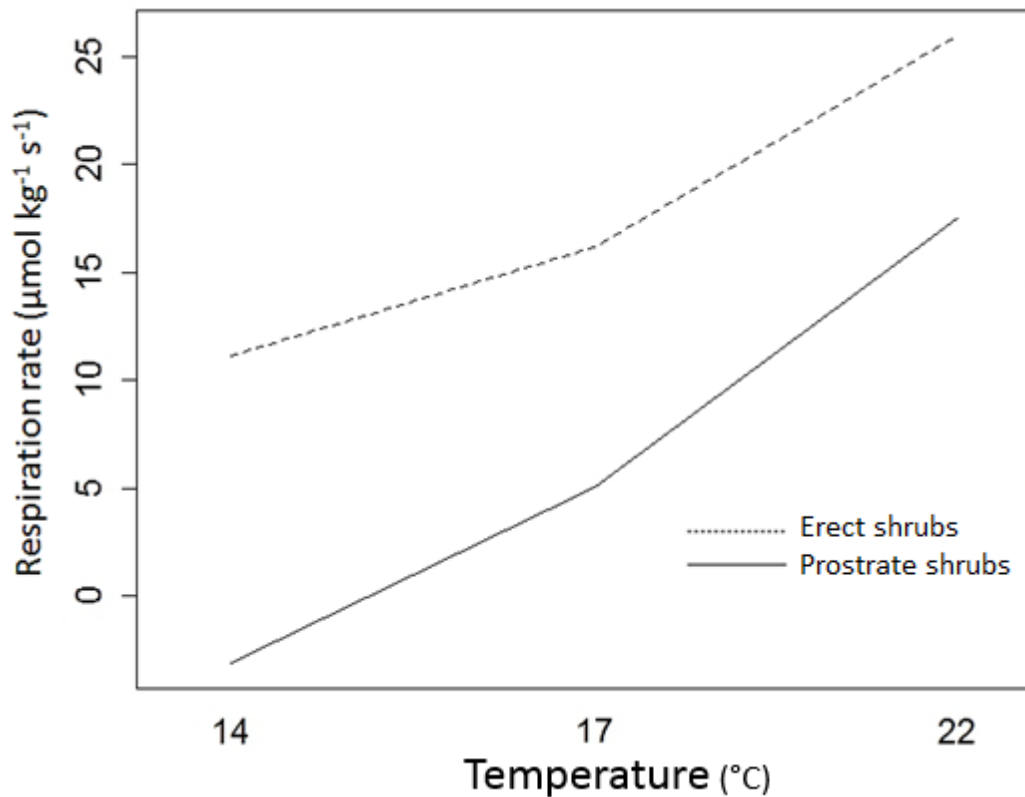


Figure 4: Interaction plot of respiration rate per kg dry leaf mass (R_{mass}) of two prostrate shrubs (*Vaccinium vitis-idaea* and *Empetrum nigrum*, $n=5-9$) and four erect shrubs (*Salix lapponum*, *Salix glauca*, *Salix phylicifolia*, and *Betula nana*, $n=6-9$), at three different temperatures (°C). Expressed as net efflux.

THE GLOBAL LEAF ECONOMIC SPECTRUM

ES had significantly lower LMA ($p < 0.01$) compared to the PS (Figure 4). Nitrogen content (N_{mass}) was significantly higher in the ES ($p < 0.05$) (Figure 4). A_{mass} of both groups was mid-centered, and R_{mass} was at the lower end of the scale for PS compared to the Glopnet species.

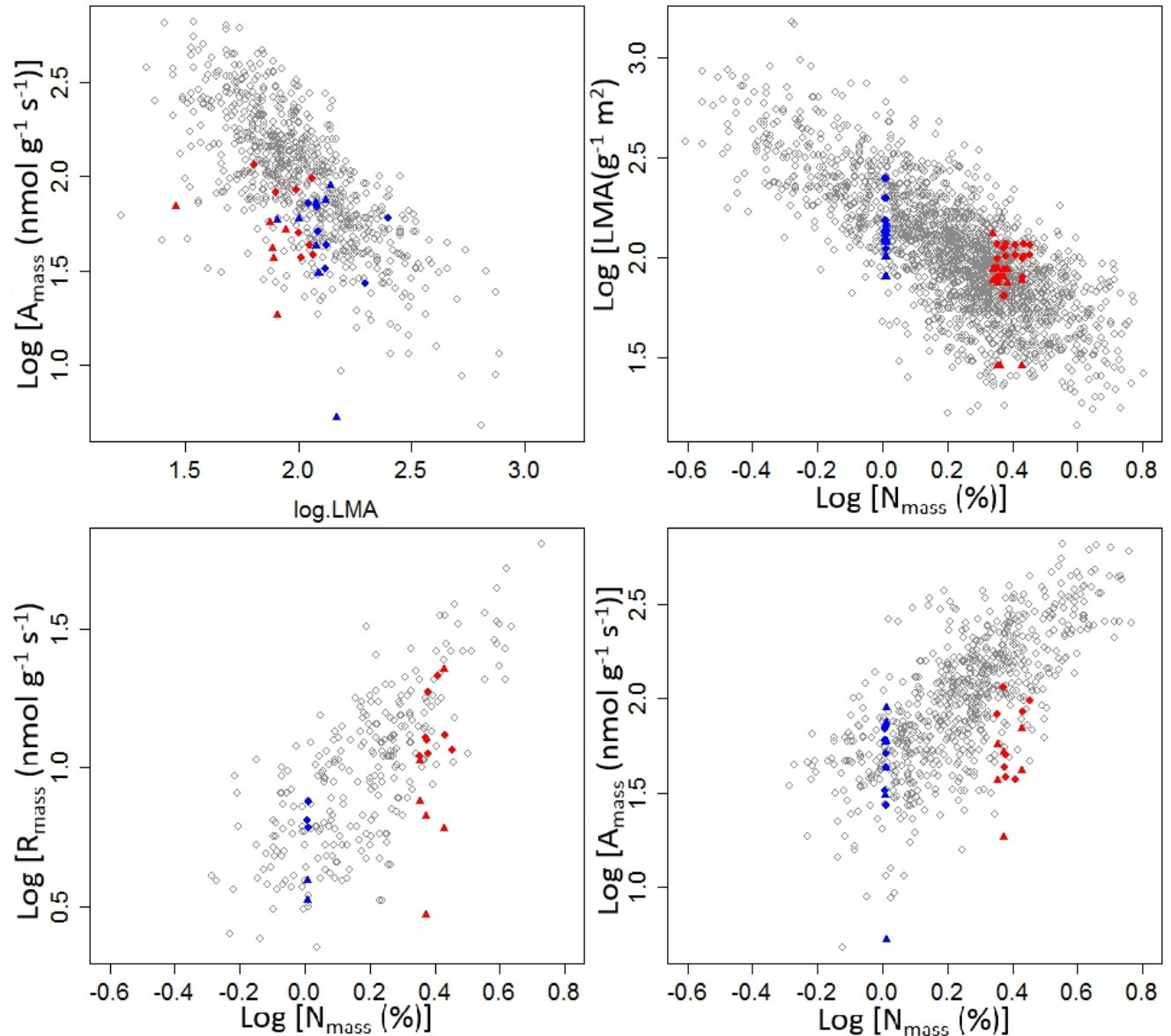


Figure 4: Comparison of leaf mass per area (LMA), respiration rate (R_{mass}), photosynthetic rate (A_{mass}) at the temperature that yielded the highest rate for each species, and nitrogen content (N_{mass}) from different plant species. The white circles represent data from the Global Plant Trait Network (Glopnet), while the colors represent our own data. Blue colors represent the prostrate shrubs *Vaccinium vitis-idaea* (circles) and *Empetrum nigrum* (triangles), and red represents the erect shrub species *Salix glauca* (circles) and *S. lapponum* (triangles).

DISCUSSION

OVERALL EFFECT OF TEMPERATURE ON PHOTOSYNTHESIS AND RESPIRATION

The analyses of variance found that temperature had a significant effect on both net photosynthetic assimilation rate and respiration rate per kg dry leaf mass (A_{mass} and R_{mass} , respectively). Except for *Salix glauca*, all species consistently had slightly lower rates of A_{mass} and higher rates of R_{mass} at the warmest temperature. It is thus plausible that further increase in temperature would result in a more substantial down-regulation of carbon uptake. *Betula nana* and *Salix glauca* had greater net photosynthetic rates at all temperatures, while *Empetrum nigrum* had the lowest (Figure 1).

A decrease in A_{mass} is not necessarily unexpected, as the temperature optimum for photosynthesis in arctic plants generally is about 15°C (Chapin 1983). While temperatures below optimum still maintain a normal photosynthetic rate, higher temperatures can constrain the photosynthetic apparatus somewhat (Berry and Bjorkman 1980). However, shrubs overall have shown a general increase in biomass (Walker et al. 2006, Myers-Smith et al. 2011) and an acclimation of A_{mass} to long-term warming would partially explain this. The positive effect on biomass could also be explained by longer growing seasons, which might counteract the down-regulated net photosynthetic rate of some species. Other studies did not find a down-regulation of net photosynthetic rate with increased temperature. Hobbie and Chapin (1998) found no effect on net ecosystem CO₂ flux in warmed plots, except for a stimulation of respiration and photosynthesis early in the growing season. Yet, the study also found that increased temperature considerably increased the biomass of *Betula nana*, which could be related to the results found here, where *Betula nana* had the highest photosynthetic assimilation rate at all temperatures. Bret-Harte et al. (2001) found that, in addition to improved growth, under higher temperatures, *Betula nana* produced more long shoots and increased the number of branches. This led to a denser canopy, which in turn limited access to light for other species. However, in our study, *Betula nana* had the highest A_{mass} of all species at all temperatures, and thus the effect of warming on photosynthetic rate alone cannot explain the observed encroachment and dominance of this species. As the effect of temperature on gas exchange here was only

monitored over a relatively short time, it is possible that photosynthesis adjusts over time. Another possibility is that the positive effect on photosynthesis early in the growing season observed by Chapin (1998), is used more efficiently by the encroaching shrubs for a short growing-boost.

The respiration rate of all species was significantly higher at the warmest temperature compared to the coldest. This is not surprising, as a well-known effect of elevated temperature in plants in general is a rise in dark respiration, as well as in photorespiration (Wahid et al. 2007). It is most likely driven by an increase in the demand for cellular maintenance associated with greater protein turnover, as well as membrane leakage at warmer temperatures (Amthor 1984). In addition, alpine and other cold-adapted plants have comparatively high respiration rates in the cold, which has been found to likely be due to a higher concentration of mitochondria (Miroslavov and Kravkina 1991). Cold-adapted plants may potentially gain a particularly high respiration rate when exposed to higher temperatures (Larigauderie and Korner 1995). It would be interesting to see how long-term warming affects the respiration in these shrubs. Especially since the gross photosynthetic rate to a high degree is affected by respiration. The gross photosynthetic rate in this study was slightly increasing for most species (except for *Salix phylicifolia*) in warmer temperatures, but this was highly non-significant, so in effect the gross photosynthetic rate was not changing. The increases in R_{mass} mask this effect, resulting in a down-regulation of net photosynthetic rate overall. If respiration adjusts, and is reduced at warmer temperatures, but gross photosynthetic rate stays constant, then more energy could be invested in growth. There are several studies looking at the thermal acclimation of respiration in cold-adapted plants, and the results vary from partial acclimation of respiration (Larigauderie and Korner 1995), to full acclimation in new leaves grown at elevated temperatures (Slot and Kitajima 2015), to acclimation if supplementary nutrients were provided (Heskel et al. 2014). Overall the meta-analysis of Slot and Kitajima (2015) concludes that leaf respiration of most plants can acclimate to an increase in temperature in the long term. At the ecosystem level, experimental warming has shown that ecosystem respiration responds more than photosynthesis, releasing more carbon from the system, so the longevity of this effect should be investigated (Rustad et al. 2001).

ERECT SHRUBS COMPARED TO PROSTRATE SHRUBS

At all temperatures, erect shrubs (ES) had significant higher A_{mass} and R_{mass} than prostrate shrubs (PS), but the change in photosynthetic-, and respiration rates across temperatures did not differ substantially. However, there was a significant interaction between temperature and species on R_{mass} . ES had a slightly higher photosynthetic rate at 17°C than at 14°C, while the PS had their optimum at the lowest temperature (Figure 2). Although this is mainly due to the high A_{mass} of *Salix glauca* at 17°C, the other encroaching species had very little or no change in A_{mass} from 14 to 17°C (Figure 1). This could indicate that the encroaching species have a slightly higher range of temperature optimum, and therefore perhaps also a slightly warmer growth optimum. There is strong evidence that erect shrubs constitute a big part of the observed shrub expansion (Fraser et al. 2014), and so it may be that small differences in photosynthetic rate optimum accumulate and reflect in the growth of these plants.

The fact that the A_{mass} of PS species always was lower is most likely due to leaf structure and investment strategy. Both species are evergreen with a long leaf life span and higher leaf toughness (Kudo et al. 2001), which has been shown to come at the expense of photosynthetic rate (Reich et al. 1991, Wright et al. 2004). These small advantages in photosynthetic rate may contribute to explain how the ES outcompete the smaller, evergreen shrubs, and would especially be true during a prolonged growing season, where the leaf lifespan of deciduous shrubs may be somewhat extended (Miller et al. 1976, Chapin 1983). Even though the erect shrubs had a higher A_{mass} , they also consistently had a higher respiration rate. Considering that gross photosynthetic rate did not increase at the highest temperature, a larger proportion of the produced energy was used for respiration during 22°C than at 14°C. If this was mainly for maintenance purposes, less energy will be available for growth. However, since ES maintained higher levels of A_{mass} than PS, their generally higher gross photosynthetic rate is apparently enough to compensate for the higher respiration rate at warmer temperatures.

If ES displace PS in areas where they currently coexist, the heath tundra as a vegetation type will change. This in turn has implications for the entire alpine ecosystem, especially on CO₂ fluxes. Sjögersten and Wookey (2009) for example found that mountain birch forest had higher

rates of organic matter decomposition than tundra heath, most likely due to a higher production of litter, thus contributing to more CO₂ release than a tundra heath system would produce. Further, *Empetrum nigrum* dominated tundra has a slower decomposition rate of litter due to high leaf phenolic content (Tybirk et al. 2000). A change to a vegetation dominated by deciduous shrubs which have faster decomposition rates, will thus release more CO₂ to the atmosphere, and change the nutrition stage of the soil (Veen et al. 2015). In addition, decreases or increases in photosynthetic rates will change the carbon-nitrogen relationship, and can in turn have an impact on the litter decomposition overall.

LEAF TRAITS AND THE GLOBAL LEAF ECONOMIC SPECTRUM

Comparing Glopnet, a global dataset covering many different species and biomes, to the alpine shrubs in this dataset, we expect A_{mass} and R_{mass} to be specific places on the spectra. For A_{mass} , R_{mass} and LMA, this was found to be fairly mid-centered. Nitrogen content however, was at the higher end of the spectra for ES. Further, the ES have a relatively low A_{mass} per amount of nitrogen, suggesting that the nitrogen is not used as efficiently in photosynthesis as for other Glopnet species. As R_{mass} and A_{mass} are positively correlated with N_{mass} , this could indicate a potential to further increase photosynthetic activity, and might be interesting for further analysis.

The prostrate species had significantly higher LMA than the erect shrubs, and lower nitrogen-content, respiration and A_{mass} than ES. Higher LMA is associated with a longer leaf lifespan, representing an investment into durable leaves typical for evergreen species (Poorter et al. 2009). It was therefore expected that *Vaccinium vitis-idaea* and *Empetrum nigrum* should have higher LMA than the deciduous shrubs. Wright et al. (2004) found that LMA correlates negatively with A_{mass} and R_{mass} , as was confirmed for the shrubs in this study, where the PS had significantly lower rates (see Table 1 and 2, and also the interaction plots in Figure 2 and 4). This suggests that LMA can be used as an indicator for performance in alpine shrubs. Species with low LMA tend to have a fast metabolism with high rates of photosynthesis and respiration, but also a shorter leaf life span (Poorter et al. 2009). Consequently, they also have high relative growth rates during optimal growth-conditions (Poorter et al. 2009). The results of this study show that ES have lower LMA and higher A_{mass} than PS even at elevated temperatures, indicating that they indeed have

an advantage that could transmit to growth rate. High LMA species such as PS have tougher leaves, with longer leaf life spans, higher cold resistance, and lower risk of herbivory, at the expense of a reduced growth rate (Tybirk et al. 2000, Wright et al. 2004, Poorter et al. 2009). In regions where these two types of shrubs coexist, the climate may be such that the advantage ES have in the main growing period is cancelled by the advantage PS have in being active earlier and longer in the season. In a warmer climate, this balance could be altered in favor for ES, letting these shrubs dominate such areas.

CONCLUSION

In summary, exposing six alpine shrub species to three different temperature regimes showed that net photosynthetic rate of all species was down-regulated, while respiration rate was up-regulated at elevated temperatures. The overall gross photosynthetic rate was only slightly higher at the warmest temperature, suggesting that the decrease of net photosynthetic rate was mainly due to the significant increase in respiration. This is in accordance with theory, especially for respiration rate, which is expected to increase due to a higher demand for cellular maintenance. Since increased respiration can release more carbon from the ecosystem to the atmosphere, the endurance of this temperature response should be investigated for these species.

Here we have recorded the response of photosynthesis to temperatures in a range of shrub species, which adds more information to consequences of climate change. The deciduous erect shrubs *Betula nana*, *Salix phylicifolia*, *S. glauca*, and *S. lapponum* had significantly higher photosynthetic and respiration rates at all temperatures than the prostrate shrubs *Empetrum nigrum* and *Vaccinium vitis-idaea*. This advantage could lead to erect shrubs outcompeting prostrate shrubs in areas where they currently co-occur. However, the down-regulation response to increased temperatures was linear, and the difference between the groups was as big at the lowest temperature as at the highest; thus, the effect of warming on net photosynthetic rate alone cannot explain a potential dominance of erect shrubs in a warmer climate.

Compared to a global dataset, LMA, photosynthetic-, and respiration rate of the alpine species studied here are in the middle of the spectrum. Erect shrubs had a high nitrogen content, but relative low photosynthetic rate per amount of nitrogen, compared to other species from the dataset. Erect shrubs had a lower leaf mass per area than prostrate shrubs, which positively correlates with high relative growth rates. If growing conditions become more favorable for erect species, for example longer growing seasons, such a greater growth-potential could lead to erect shrubs dominating prostrate shrubs in areas where they currently coexist, changing the vegetation composition. Potential consequences of this are changes in CO₂ fluxes with a possibly greater CO₂ release from the vegetation. The long-term effects of increased temperature on CO₂ flux and shrub species composition should therefore be explored further.

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APPENDIX A

A protocol for measuring gas exchange in alpine shrubs at three different temperatures.

Table A: Programing of the LI-COR conifer chamber, which measured photosynthetic rates at different temperatures in the dark, and in the light.

17°C				14°C				22°C			
Dark		Light 800 PAR		Dark		Light 800 PAR		Dark		Light 800 PAR	
Adjust	Measure	Adjust	Measure	Adjust	Measure	Adjust	Measure	Adjust	Measure	Adjust	Measure
5 min	1 min	3 min	3 min	5 min	1 min	3 min	3 min	5 min	1 min	3 min	3 min

APPENDIX B

Light Curves of six alpine shrubs.

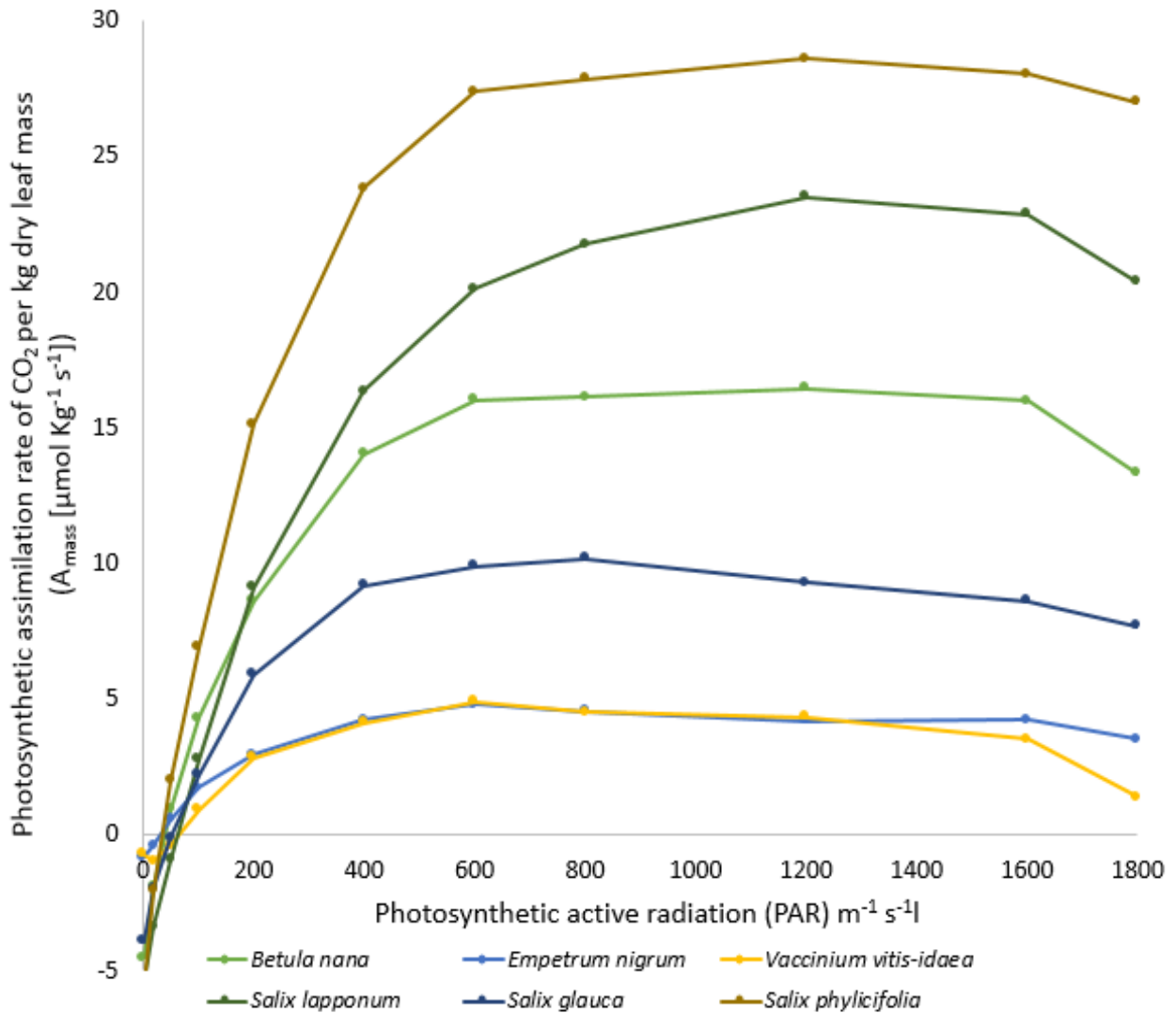


Figure A: Photosynthetic assimilation rate of CO_2 per kg dry leaf mass at different photosynthetic active radiation (PAR), for six different species of alpine shrubs. Photosynthetic gas exchange of adjusted leafs ($C_i/C_a = 0.7 \pm 0.1$) was measured with a portable photosynthesis system (LI-6400; LI-COR, Inc., Lincoln, USA) with a lighted conifer chamber, and conducted at 20°C .

APPENDIX C

Multiple comparison between net photosynthetic rates of different species at different temperatures.

Table A.1: Comparison of Net photosynthetic assimilation rate of CO₂ per gram dry leaf mass (A_{mass}) of six species of alpine shrubs measured at three different temperatures. P-values created by a Tukey's Honestly Significant Differences (HSD) test are given, and significant values are shown in bold.

Temperature	14°C	17°C
17°C	0.947	X
22°C	0.008	0.005

Table A.2: Comparison of Net photosynthetic assimilation rate of CO₂ per gram dry leaf mass (A_{mass}) of six species of alpine shrubs. P-values created by a Tukey's Honestly Significant Differences (HSD) test are given, and significant values are shown in bold.

Species	<i>B. nana</i>	<i>S. glauca</i>	<i>S. lapponum</i>	<i>S. phylicifolia</i>	<i>V. vitis-idaea</i>
<i>S. glauca</i>	0.503	X	0.313	0.800	0.631
<i>S. lapponum</i>	0.002	0.313	X	0.009	0.995
<i>S. phylicifolia</i>	0.994	0.800	0.009	X	0.045
<i>V. vitis-idaea</i>	0.012	0.631	0.995	0.045	X
<i>E. nigrum</i>	0.004	0.469	0.999	0.019	0.999

Table A.3: Comparison of Net photosynthetic assimilation rate of CO₂ per gram dry leaf mass (A_{mass}) of six species of alpine shrubs measured at three different temperatures. P-values of differences between A_{mass} at different temperatures are given by a Tukey's Honestly Significant Differences (HSD) test.

Species	p-value of difference in photosynthetic rate between two temperatures		
	14°C VS 17°C	17°C VS 22°C	14°C VS 22°C
<i>S. glauca</i>	0.316	0.735	0.658
<i>S. lapponum</i>	0.938	0.231	0.423
<i>S. phylicifolia</i>	0.893	0.225	0.442
<i>V. vitis-idaea</i>	0.795	0.889	0.496
<i>E. nigrum</i>	0.775	0.952	0.596
<i>S. glauca</i>	0.916	0.154	0.062

APPENDIX D

Temperature effect on the gross photosynthetic assimilation rate of six alpine shrubs was measured at 14, 17 and 22°C

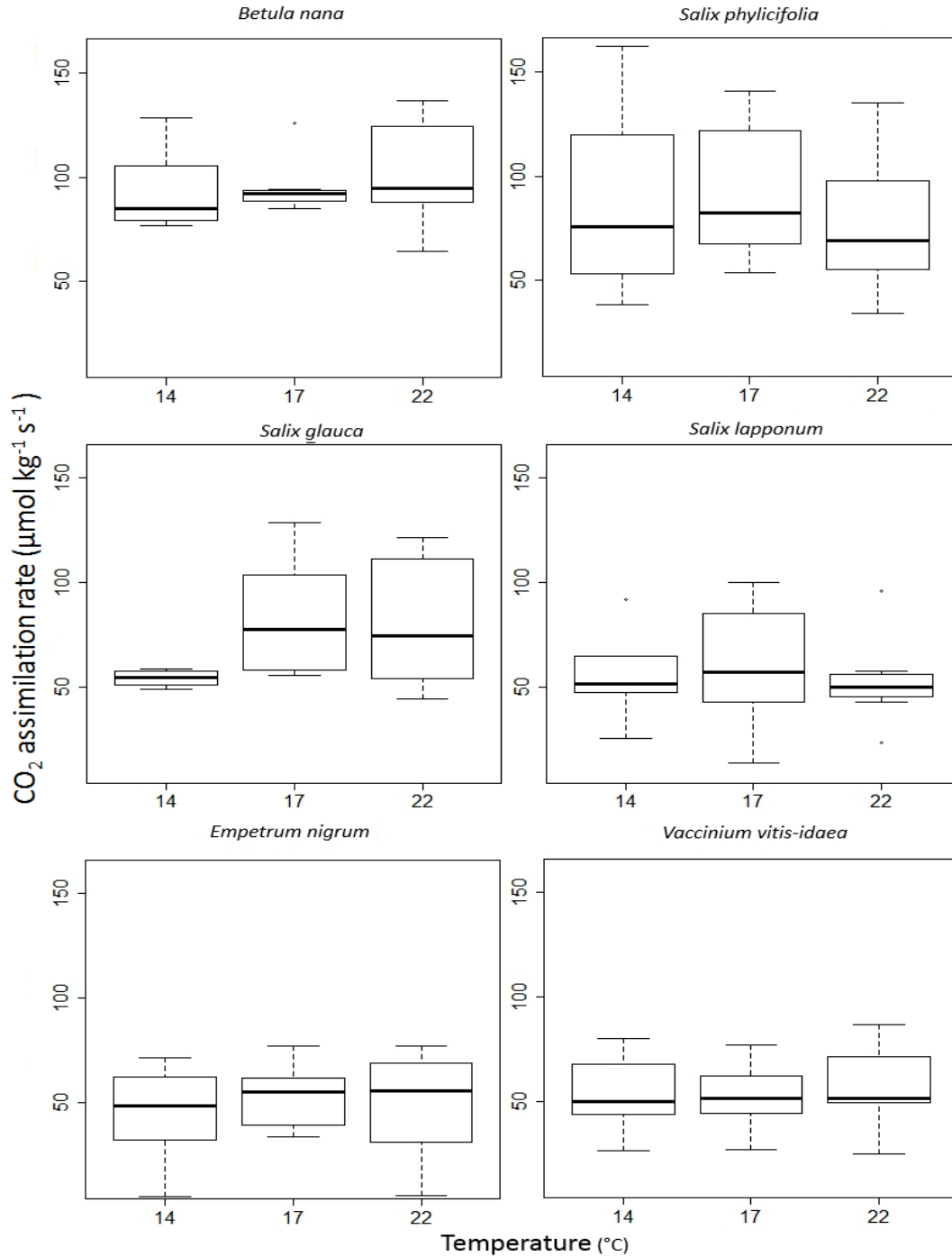


Figure A: Gross photosynthetic assimilation rate of CO₂ (net photosynthetic rate plus respiration rate) per kg dry leaf mass of six species of alpine shrubs measured at 800 PAR at three different temperatures (n=5-9). Expressed as net influx.