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Project in plant ecophysiology

# A comparison of species and temperature responses of growth and photosynthesis for four riparian paludiculture plants

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# A comparison of species and temperature responses of growth and photosynthesis for four riparian paludiculture plants


## Abstract

Riparian plants, suitable for peatland cultivation, has been investigated in relation to their ability to produce biomass at different temperatures. The plants investigated were *Salix viminalis, Pennisetum purpureum, Arundo donax and Phragmites australis.* Individuals of each species were kept in climate chambers at either 20 or 30 °C for 21 days, after which chosen physiological traits were measured. Pigment content (chlorophyll and carotenoids) and ratio changed with both species and temperature, with *P. australis* containing the most chlorophyll. $P_{max}$ was highest for *P. australis*, and temperature impacted species differently. *Phragmites australis* and *S. viminalis* acclimated to 20 °C increased their $P_{max}$ drastically when measured at 30 °C. The highest relative growth rate (RGR) was found in *P. australis* and *A. donax* at both temperatures, and temperature appeared to increase the growth rate. A significant relationship was found between RGR, and both chlorophyll content of leaves and $P_{max}$, but no relationship was found between RGR and SLA. In terms of overall suitability for paludiculture, *P. australis* seemed the best, as it had the highest RGR, $P_{max}$ and chlorophyll content, while also exhibiting great adaptability to varying temperatures, a trait that might prove desirable in temperate regions.


## Introduction

The continuous global increase in population size requires more arable land for food production. Because of the growing market for sustainable energy and building materials, the demand for biomass production has become a big competitor for land use (Wichtmann & Joosten, 2007). The lag of suitable land for cultivation has led to exploitation of unreclaimed land, abandoned fields and low production areas such as peatlands (Wichtmann & Joosten, 2007). The drainage of peatlands leads to a fast turnover of organic material, which is problematic from a climatic point of view, because it leads to $CO_2$ emission. Furthermore, the drainage of peatland leads to a loss of biodiversity (Wichtmann & Joosten, 2007). Paludiculture makes use of plants that are able to tolerate high water levels, whilst still having a high productivity. Thereby the peatlands are able to remain wet or become rewetted, while still being effective biomass producers. This ensures better outcomes for biodiversity and lowers $CO_2$ emissions.

Temperature has a major effect on enzymatically catalyzed reactions and membrane processes, and therefore affects photosynthesis and respiration (Lambers et al., 2008, page 61, 127). At lower temperatures, the chemical processes will be slower and therefore reduce the plant's speed at which it converts the photons, harvested by the light harvesting complexes, into sugars. This means that plants grown at 20°C will experience conditions where light is less of a restricting factor, relative to plants grown at 30°C. On this basis the theoretical and expected influence of temperature on some highlighted physiological traits are as shown (Table 1)(Lambers et al., 2008, page 34).

To optimize and ensure a high productivity of paludiculture areas, knowledge about how potential paludiculture plant species respond and perform in different settings is important. This experiment compares the performance of four riparian species: *Phragmites australis, Salix viminalis, Pennisetum purpureum and Arundo donax,* grown at 20 $^{O}$C and 30 $^{O}$C, focusing on measuring photosynthesis and growth. The four species were selected because of their ability to tolerate high water levels and their high productivity, as well as to represent different life forms - trees (*S. viminalis*) and grasses, and different photosynthetic types - C4 (*P. purpureum*) and C3.

It is expected that 1) higher temperatures will result in higher photosynthetic rates, chlorophyll content and growth rate within each species group, 2) higher temperatures will result in a lower chlorophyll a:b ratio, a lower carotenoid:chlorophyll ratio 3) the four species will react differently, due to them having different adaptations and physiology.

## Materials and methods

### Experimental design

The four species were grown vegetatively from a batch of mother plants each, to minimize heterogeneity of the individual plants involved in the experiment. Two to seven plants from the four groups of species were selected for initial growth measurements, and 16 plants were selected for the experimental setup. The plants were put into glas pots covered with black plastic, which contained a fertilizer mix consisting of: Macronutrients (mixture of N, P, K, Mg, S) 1 mL/L, micronutrients (B, Cu, Fe, Mn, Mb, Zn) 0,1 mL/L and ironchelate 0,08 g/L. The pH of the fertilizer mix were adjusted with HCl 3M to a pH about 6,5. The plants were fixated by placing a flamingo disk around the bottom of the stem, and were placed in two different climate chambers. Light intensity was set to 400 µmol $m^{-2}$ $s^{-1}$ and the night/day cycle was 6/18 hours for both chambers. The chambers were set accordingly to 20°C/30°C day-temperature, 17°C and 25°C night-temperature, 60% RH/70% RH day humidity and 80% RH/85% RH night humidity. Plants from each group were haphazardly placed in the chambers for 21 days. The fertilizer mix was changed every 3-4 days to ensure that the plants were not nutrient limited.

In the data from plants in chamber 2 (30°C), one *P. purpureum* and three *S. viminalis* were partially excluded from the dataset, due to stress of these plants and lack of data. For the *P. purpureum,* the result for RGR (relative growth rate) and growth measurement were included but the rest excluded. For *S. viminalis* in chamber 2, three replicates in the chlorophyll and carotenoid measurement were excluded, because these plants were dying and had very small leaves. These leaves weighed less than 5 mg when freeze dried and was therefore insufficient for measuring chlorophyll and carotenoids.

## Growth measurements

Dry weight (DW) was measured at day zero (beginning) on initial plants and again in the end of the experiment on all individuals. The first measurements were made on initial plants using two-seven individuals similar to the 16 in the experimental setup for each species. From the total DW in the beginning and end of the experiment a Relative Growth Rate (RGR) was calculated for each plant:

$$RGR = \frac{ln(DW\ preceeding\ measurement) - ln(DW\ new\ measurement)}{days\ bt.\ measurements}$$

At the end of the experiment (harvest of plants), the total area of the leaves on each plant were measured.

## Photosynthesis

Photosynthetic activity was measured using an LI-6400 XT infrared gas analyser (IRGA) (LI-COR Biosciences, Inc., Lincoln, NE, USA). The principle behind the IRGA-equipment is that photosynthesis is determined by measurements of $CO_2$ concentrations, gas flow and other parameters like temperature. The measurement-technique makes it possible to study a plant's response to different environments. The IRGA measurement used in this experiment is based on an 'open system'. Two gas analyzers are placed in the sensor head to determine the absolute $CO_2$ and $H_2O$ concentration in both the reference and the sample, which is a leaf. The difference in concentrations between the chambers is caused by the photosynthesis and the rate is calculated from this difference.

Fully developed non-shaded leaves were chosen for the measurements. This criteria was not always simple and especially for *S. viminalis* this was for some individuals difficult, since the plants grown at 30°C was in a very poor condition. Also, one of the *P. purpureum* had had so little growth that its $P_{max}$ was not measured. The leaf area were measured in the IRGA by measuring the width of the leaf on both sides of the IRGA chamber, which was 3 cm in length, making the maximal leaf area 6 $cm^2$. The stomata ratio was noted to express whether stomata occurs on both sides of the leaf. Stomata ratio is one (for grasses *P. australis, P. purpureum and A. donax*) or only on one side of the leaf, stomata ratio is zero (*S. viminalis).* First full light response curves with PAR set to 2000, 1500, 1000, 750, 500, 250, 120, 60, 30, 15 and 0 $\mu mol*m^{-2}*s^{-1}$ and $CO_2$ level of 400 ppm were measured. The light-response-curves were measured on one plant of every species from each temperature, giving 8 different light-response curves. From these curves, the light intensity of 1500 $\mu mol*m^{-2}*s^{-1}$ for our measurements of $P_{max}$ on all individual replicates were chosen. At this light intensity all plants had reached $P_{max}$ without any of them being photoinhibited.

Each plant were measured at both 20°C and 30°C to get an impression of how the different acclimations affected the performance at the 2 temperatures. First each plant was measured at the temperature which it was acclimated to and was then relocated to the other temperature. Second the plants were allowed to stabilize for 5 minutes before measurements were made in the second chamber. The second measurement of $P_{max}$ at 1500 $\mu mol*m^{-2}*s^{-1}$ were made on the same leaf as the first, and the $P_{max}$ was allowed to

stabilize after the leaf was placed in the IRGA. This process took three to ten minutes, plants grown at 20°C and measured at 30°C being the slowest to stabilize.
When the $P_{max}$ measurements were done, the leaves were cut off and weighed (FW). Then plants were moved back to their original chamber. A picture of them were taken with a scale, for measuring the specific leaf area (SLA), using the "ImageJ" program (https://imagej.nih.gov/ij/download.html). The leaves were then weighed again (DW), and prepared for measurements of chlorophyll.

### Chlorophylls and carotenoids measurements

The content of pigments (Chl a, Chl b and carotenoids) were determined by a whole-pigment extract of leaf tissue by UV-VIS spectroscopy (Lichtenthaler 1987). A part of the leaf measured for photosynthesis was cut from the fresh plant, weighed, and its area was measured. The leaf sample was freeze dried in liquid nitrogen and kept in a freeze drier for 24 hours. Thereafter the dry weight was measured and sample tissue crunched into small pieces. Approximately 5 to 10 mg of the dried sample was added 100 µL Milli-Q water and eight mL 96% ethanol and then mixed on a vortex mixer. All the samples of *S. viminalis* needed to be ground extra fine in a Tenbroeck tissue grinder. All the samples were incubated overnight and then mixed on a vortex mixer again the next day before being centrifuged. After visually confirming that the leaf tissue had no colour left, the absorbance of the extract was measured at 470 nm, 648,6 nm, 664,2 nm, 665 nm and 750 nm, allowing for calculation of the chlorophyll and carotenoid concentrations.

### Statistics

Linear models were fitted with species and acclimated temperature as factorial independent variables, and the highlighted physiological traits - specific leaf area, photosynthetic activity, relative growth rate (RGR), chlorophyll content and ratio, and carotenoid to chlorophyll ratio - as dependant variables. Two-way analysis of variance (ANOVAs) were conducted to test for the impact of species and temperature, and Tukey post-hoc tests were used to determine significant differences among species. Unpaired two-way t-tests were carried out to test for differences between acclimation temperature for each of the factors; for each species. ANOVAs and Tukey tests were carried out in R 3.1.1, and t-tests were carried out in Microsoft Excel.

# Results

*A. donax* and *S. viminalis* showed no significant difference in $P_{max}$ at the two temperatures (Figure 1F). *P. australis* showed a significantly higher $P_{max}$ at 30 ºC compared to 20 ºC, with some measurements above 45 µmol $CO_2$ $m^{-2}$ $s^{-1}$, while *P. purpureum* showed the opposite pattern, having lower $P_{max}$ values at 30ºC, compared to 20ºC.

The plants acclimated to 20 ºC showed a tendency towards having $P_{max}$ higher than those acclimated to 30ºC, regardless of measuring temperature (Figure 2). Noteworthy responses to the change in temperature is seen in *P. australis* and *S. viminalis*, specifically the plants acclimated to 20ºC when measured in 30ºC. Both these plants increased their $P_{max}$ markedly in response to the increase in temperature (*P. australis* - p=0.07, n=4 & *S. viminalis* - p=0.04, n=8).

All species, except *P. purpureum*, have a higher total chlorophyll content when grown in 30 ºC, than when grown in 20 ºC (Figure 1C). *Phragmites australis* also exhibited the highest chlorophyll values, with *S. viminalis* exhibiting the lowest.

The Chlorophyll a:b ratio was higher in plants acclimated to 20 ºC, than those acclimated to 30 ºC (Figure 1B). This tendency was significant for *P. purpureum* and *S. viminalis*. The highest chlorophyll a:b ratio was exhibited by *P. purpureum*.

The carotenoid:chlorophyll ratio was significantly higher in 20 ºC for *P. purpureum* and *S. viminalis*, but it should be noted that the difference between temperature acclimations were very small for all species except *S. viminalis* (Figure 1A). All the four species are significantly different from each other, with *P. purpureum* exhibiting the highest ratio, and *P. australis* exhibiting the lowest.

There was no difference found between the specific leaf area (SLA) of the four species (figure 1E), and the only species exhibiting significant response to temperature was *P. australis*.

The relative growth rate (RGR)(Figure 1D), was highest for *P. australis* and *A. donax* and lowest for *S. viminalis*. *Arundo donax* had a higher relative growth rate at 30 °C, and the same tendency is seen in *P. australis* and *P. purpureum*. Mean total DW growth was largest for *P. purpureum* and *S. viminalis* (Table 2).

RGR was found to be significantly correlated with chlorophyll content and $P_{max}$, explaining 54% and 47% of the variation respectively (General linear model)(Figure 4). No correlation was found between RGR and SLA (p=0.22, $R^2$<0.00).

A great degree of clustering was found in the models, with *P. australis* and *A. donax* being fairly similar, whilst *S. viminalis* and *P. purpureum* remain more isolated.

# Discussion

## $P_{max}$

Plants grown and acclimated at 20 °C had a higher $P_{max}$ value than plants grown at 30°C when measured at 20 °C. This fits our prediction, and is caused by the plants having adapted their photosynthetic apparatus to the temperature and light availability. The same pattern is not clear for plants measured at 30 °C though. Here we see very little difference between the temperatures for *A. donax* and *P. purpureum*.

Conspecifics have been shown to have similar $P_{max}$ when measured at their acclimation temperature, even if these temperatures differ (Atkin et al. 2006). The large variance between the temperatures for *P. australis* and *S. viminalis,* and the within species variance seen at 30°C does not correlate with this previous study. This is probably caused by the immediate increase in enzyme activity when plants are moved from 20 °C to 30 °C (Berry and Raison, 1981). The increased enzyme activity will increase photosynthetic activity and thus $P_{max}$ will increase, whereas the enzymatic activity in respiration will increase less. Thus the net $CO_2$ uptake that is measured with $P_{max}$ will increase for plants moved from 20 °C to 30 °C.

*Phragmites australis* consistently had the highest $P_{max}$ at both temperatures with both acclimations (Figure 2). This is interesting because it shows that *P. australis* has a high level of plasticity when it comes to $P_{max}$. Data for *S. viminalis* is based on very little data, since many of the individuals almost died at 30 °C, which makes the plot unreliable.

We expected the highest $P_{max}$ at 30 °C from *P. purpureum*, due to it being the only C4 species, but this was not the case. This can be caused by the relatively low light intensity in the climate chamber, since lower irradiance have been shown to increase leakage of carbon already fixed by PEP carboxylation (Ubierna et al, 2011). Growing at these relatively low light intensities, *P. purpureum* might be light limited and unable to exploit the C4 photosynthetic apparatus. Alternatively it may because respiration is correspondingly higher in the 30 °C treatment. As can be seen from the light response curves (Figure 3), 400 $\mu mol*m^{-2}*s^{-1}$ is at the point of the curve, where the plant is still light limited, whereas 1500 $\mu mol*m^{-2}*s^{-1}$ is at a point where the plant is limited by carboxylation (Lambers et al. 2008, p. 27, figure 8). During growth, *P. purpureum* have not had the need for much Rubisco and other enzymes related to carboxylation but rather light harvesting pigments since light is the limiting factor during photosynthesis. When measured at 1500 $\mu mol*m^{-2}*s^{-1}$ the relatively low Rubisco content could limit the photosynthesis resulting in the low $P_{max}$.

## Pigments and SLA

The lower chlorophyll a:b ratio in plants grown at 30ºC seen in *S. viminalis* and *P. purpureum*, fits well with the expectation that chemical processes are less of a restricting factor at 30ºC, therefore making the plants better able to use the energy harvested. This makes them produce more light harvesting pigment, leading to the relative content of chlorophyll b being higher compared to the core pigments (Hopkins et al. 2009, p. 116)

The light intensities were set to 400 $\mu mol*m^{-2}*s^{-1}$ in both chambers, and according to the light response curves made, the plants should be limited by light at this point. Therefore the carotenoid:chlorophyll ratio was expected to be statistically the same in both chambers, which we saw in both *A. donax* and *P. australis*. For *P. purpureum* and *S. viminalis* however there was a significant difference, although not very convincing. The lower carotenoid:chlorophyll ratio found at 30 ºC could be explained in much the same way as with chlorophyll a:b ratio. The faster chemical processes makes the plants at 30ºC better able to use the energy harvested by the chlorophyll pigments, which makes them less vulnerable to photon damage. Therefore plants grown at 30 ºC need less carotenoid pigment, which protects against such damage through the xanthophyll cycle (Lambers et al. 2008, p. 239). *Salix viminalis* was highly stressed at 30 ºC, so the difference in carotenoid content could also be result of efforts made to overcome this stress by *S. viminalis* plants (Hopkins et al.

2009, p. 242 - 14.2) Finally the difference in carotenoid pigment could be explained by a rise in total chlorophyll content, which could be a possible scope for future research.

The higher chlorophyll content in all species at 30 °C except *P. purpureum* is likely explained by light being less of a restricting factor. Therefore it would be necessary to invest in more light harvesting pigment. The pattern seen in *P. purpureum*, no significant difference, could mean that light was not restricting in this case, but a more likely explanation is that *P. purpureum* was limited by its chemical processes, because the C4 apparatus was underdeveloped. The idea that the development of C4 enzymes was incomplete, is also indicated by the lower $P_{max}$ at 30 ºC, which indicates higher respiration rates. An incomplete C4 apparatus might be caused by *P. purpureum* lacking the energy needed due to the low irradiance in the chambers.

The SLA of *P. australis* was higher at 30 ºC, compared to 20 ºC, in accordance with our expectations. The higher SLA is caused by the larger need for light due to the increased enzymatic activity. That this is not the case for all species could be because all plants are exposed to the same light intensity and we therefore only see a difference in pigment content and composition as a response to temperature but no difference in SLA.

## RGR

Because of increased enzymatic activity, the plants at 30 ºC were expected to have the highest RGR, which were the significant pattern seen in *A. donax*. The difference for *A. donax* for the two temperatures is very small in mean RGR and though the difference is statistically significant, the biologic importance is questionable. There was a tendency but no significance for the same pattern in *P. australis* and *P. purpureum*. A tendency to have a higher RGR at 20 ºC was seen at *S. viminalis* which might be explained by the fact that *S. viminalis* showed high levels of stress at 30 ºC.

The highest RGR was seen for *A. donax* and *P. australis*. This is surprising since *P. purpureum* had the highest increase in DW. Since RGR is relative to initial weight it can be explained by looking at the initial DW of the plants. Here *P. purpureum* weighed an average of more than 8 times that of *A. donax* and *P. australis*. Another reason *P. purpureum* was expected to have at higher RGR at 30 ºC is the fact that this is a C4 species. At high temperatures, C4 species should have a higher photosynthetic rate since they have a very small and almost non existing photorespiration. Though C4 photosynthesis is more respiratorily costly, this might be advantageous since the rate of photorespiration increases with temperature for C3 plants. Also *P. purpureum* might be limited by light as discussed earlier which might have limited the growth of this species.

In relation to paludiculture, a high RGR is desirable, but should not be the only deciding factor when determining which species to use. RGR is likely to change throughout a plant's life cycle, and cannot solely describe growth.

## Factors related to RGR

Both $P_{max}$ and Chlorophyll content was able to explain a high amount of variance, when plotted against RGR. This is not surprising since chlorophyll content and $P_{max}$ are probably highly correlated, and because $CO_2$ fixation assimilation to sugars is one of the main limiting factors when it comes to growth. Another point worth noting from the scatter plots, is the

clear case of clustering. It is apparent that *S. viminalis* and *P. purpureum* have characteristic ranges of RGR, which further showcases the difference in growth among the species. This clustering also confirms the same pattern as was found by the ANOVA performed on RGR, that *P. australis* and *A. donax*, the C3 monocots, does not differ significantly, both having a higher growth rate than *S. viminalis* and *P. purpureum*.

The reason why the $R^2$-values are not higher, could be that the energy produced by photosynthesis does not all go to growth, but also maintenance and nutrient uptake through respiration (Lambers et al. 2008, page 134). Poorter reports that the rate of shoot and root respiration per unit dry weight correlated with relative growth rate so the respiration rate could be an area for further study in relation to this subject.

In Poorter & Remkes (1990) it is reported that there is often a correlation between SLA and RGR. The study thereon was conducted for herbaceous C3 species while this rapport investigated non-herbaceous species, and found no such correlation.

## Conclusion

Results show no clear pattern between temperature and photosynthetic rates. For *P. australis* an increase in temperature resulted in increased photosynthetic rates, whereas the opposite was seen for *P. purpureum*. Plants acclimated to 20 °C generally had higher photosynthetic rates than those acclimated to 30 °C, even when measured at 30 °C. The overall pattern showed that RGR did not change significantly with temperature. Chlorophyll content increased significantly for plants grown at 30 °C, except for *P. purpureum*, which had the same content at both temperatures. Carotenoid:chlorophyll ratios increased in *P. purpureum* and *S. viminalis* when grown at 30 °C, but was the same in *P. australis* and *A. donax*. Lastly the chlorophyll a:b ratio decreased with temperature for *P. purpureum* and *S. viminalis*. Most of these changes could be explained by increased enzymatic activity in plants grown at 30 °C, because of light being more of a restricting factor under these conditions.

Significant relationships were found between relative growth rate (RGR) and both chlorophyll content of leaves and $P_{max}$ but no relationship between RGR and SLA. This shows that both $P_{max}$ and chlorophyll content explains about 50% of the variation in the RGR. Earlier studies have shown that respiration might be better correlated with RGR. This could be scope for further research.

From our results, *P. australis* appears to be the best plant for paludiculture, since it has both a high $P_{max}$, RGR and chlorophyll content at both temperatures. This makes *P. australis* adaptable to many different climates. Additionally, *A. donax* showed a high RGR but did not perform as well as *P. australis* on other parameters.

In southern Europe *S. viminalis* is not suitable for cultivation since many of the plants died by a temperature of 30 °C. It was seen that *P. purpureum* had low $P_{max}$ and chlorophyll content at 30 °C, which could be caused by the C4 apparatus being incomplete. Also the low light intensities might have made it disadvantageous to use C4 photosynthesis. So higher light intensities and more time would be needed to say something about its potential in warm, light saturated environments

Another perspective is the risk of a species to become invasive. Therefore the origin of the species should also be taken into consideration when choosing a plant for paludiculture. In Denmark, *P. australis* is therefore the best choice for paludiculture since both *A. donax* and *P. purpureum* are not native to danish wetlands.

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# Appendix

*Table 1: Expected relations of highlighted factors at the 2 temperatures*

| | 20 °C | 30 °C |
|---|---|---|
| *Maximum photosynthetic activity* | Low | High |
| *Relative growth rate* | Low | High |
| *Specific leaf area* | Low | High |
| *Chlorofyl a+b* | Low | High |
| *Chlorofyl a:b ratio* | High | Low |
| *Carotenoid:Chlorofyl ratio* | High | Low |

**Table 1**. Expected relations of highlighted factors at the two temperatures relative to each other

*Table 2: Mean total DW of all species before and after the experiment, measure in grams.*

| | Start weight | End weight 20 °C | End weight 30 °C |
|---|---|---|---|
| *A. Donax* | 0,45 ± 0,23 | 4,54 ± 1,00 | 7,12 ± 1,01 |
| *P. Australis* | 0,17 ± 0,05 | 2,80 ± 0,79 | 4,60 ± 1,31 |
| *P. Purpureum* | 3,27 ± 0,95 | 11,41 ± 1,86 | 13,26 ± 4,70 |
| *S. Viminalis* | 17,60 ± 4,09 | 26,06 ± 6,34 | 19,56 ± 8,20 |

**Table 2.** The mean DW of the initial plants for each species, along with the mean DW of each species at both temperatures at the end of the experiment, ± 1 standard deviation.

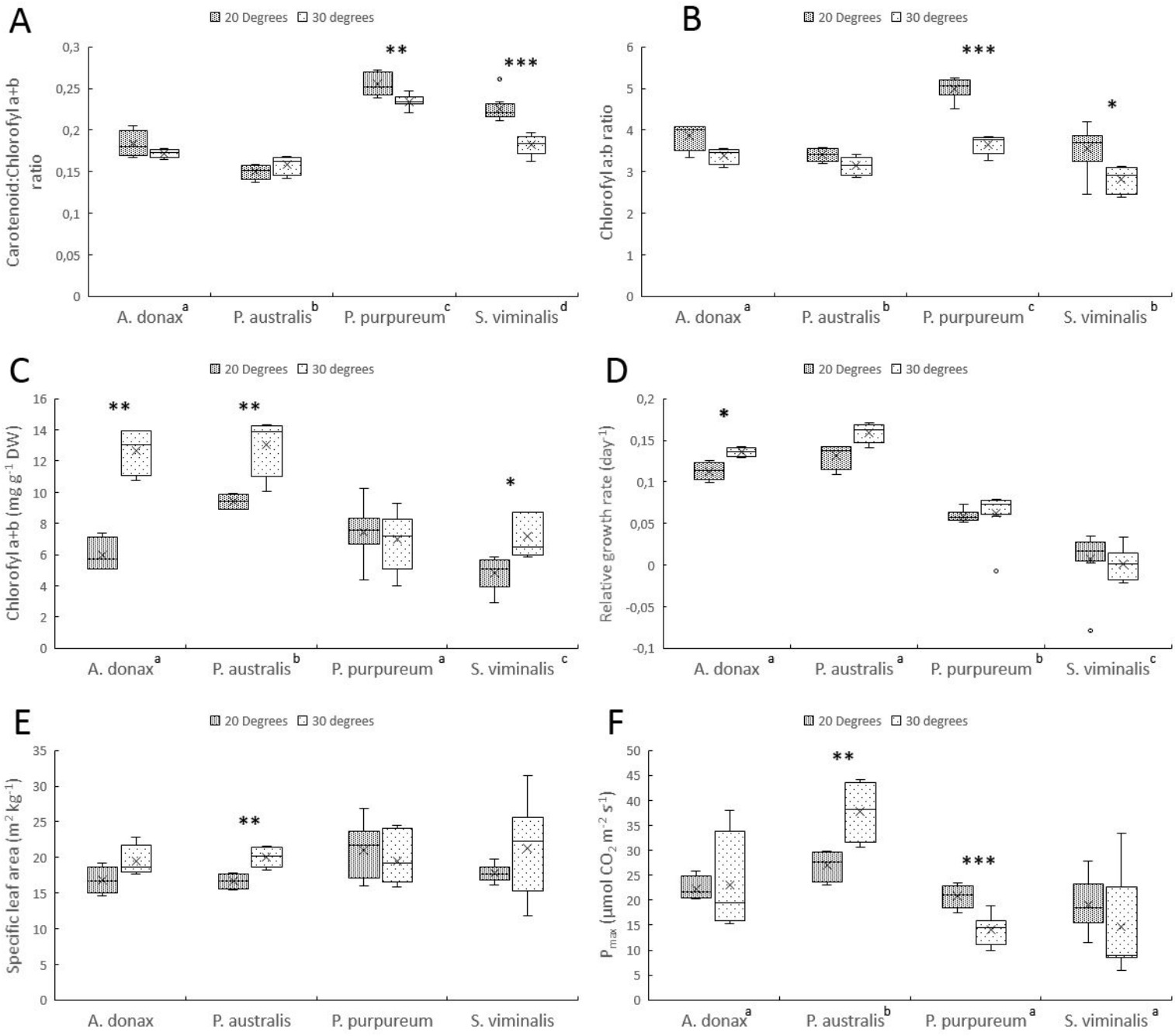


**Figure 1.** Boxplots of various factors for each of the species/temperature combinations. Boxes indicate upper- and lower quadrant along with median, and whiskers indicate upper and lower range of values. Letters by the species name indicates significance groups based on a Tukey-test with a significance value of p=0,05. Asterisks indicate significant differences among temperature treatments within species ('*' = 0.05, '**' = 0.01, '***' = 0,001)

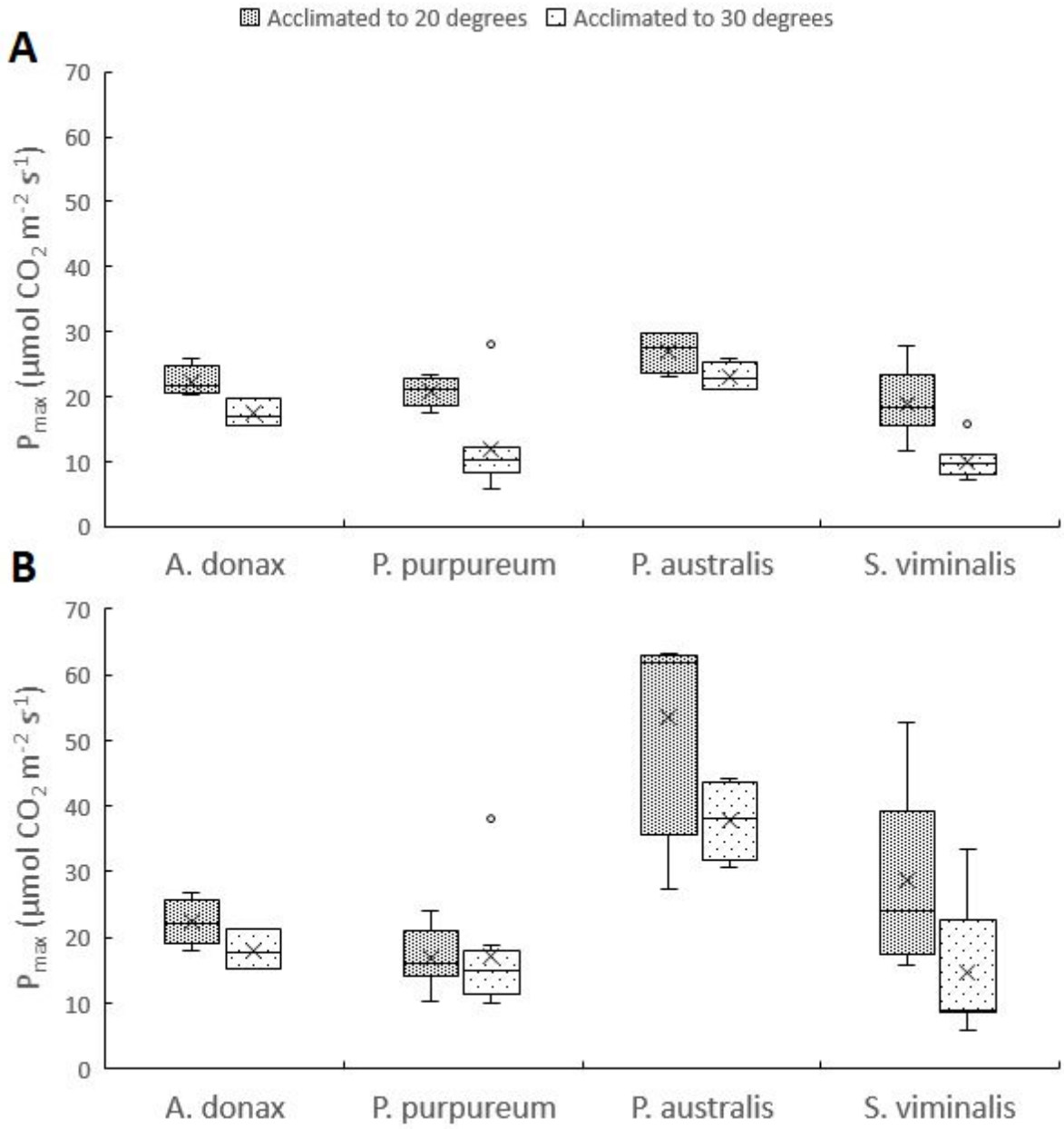


**Figure 2.** Boxplot of $P_{max}$ against the four different species investigated. Figure A plants measured at 20 °C and figure B plants measured at 30 °C. Boxes indicate upper- and lower quadrant along with median, and whiskers indicate upper and lower range of values.

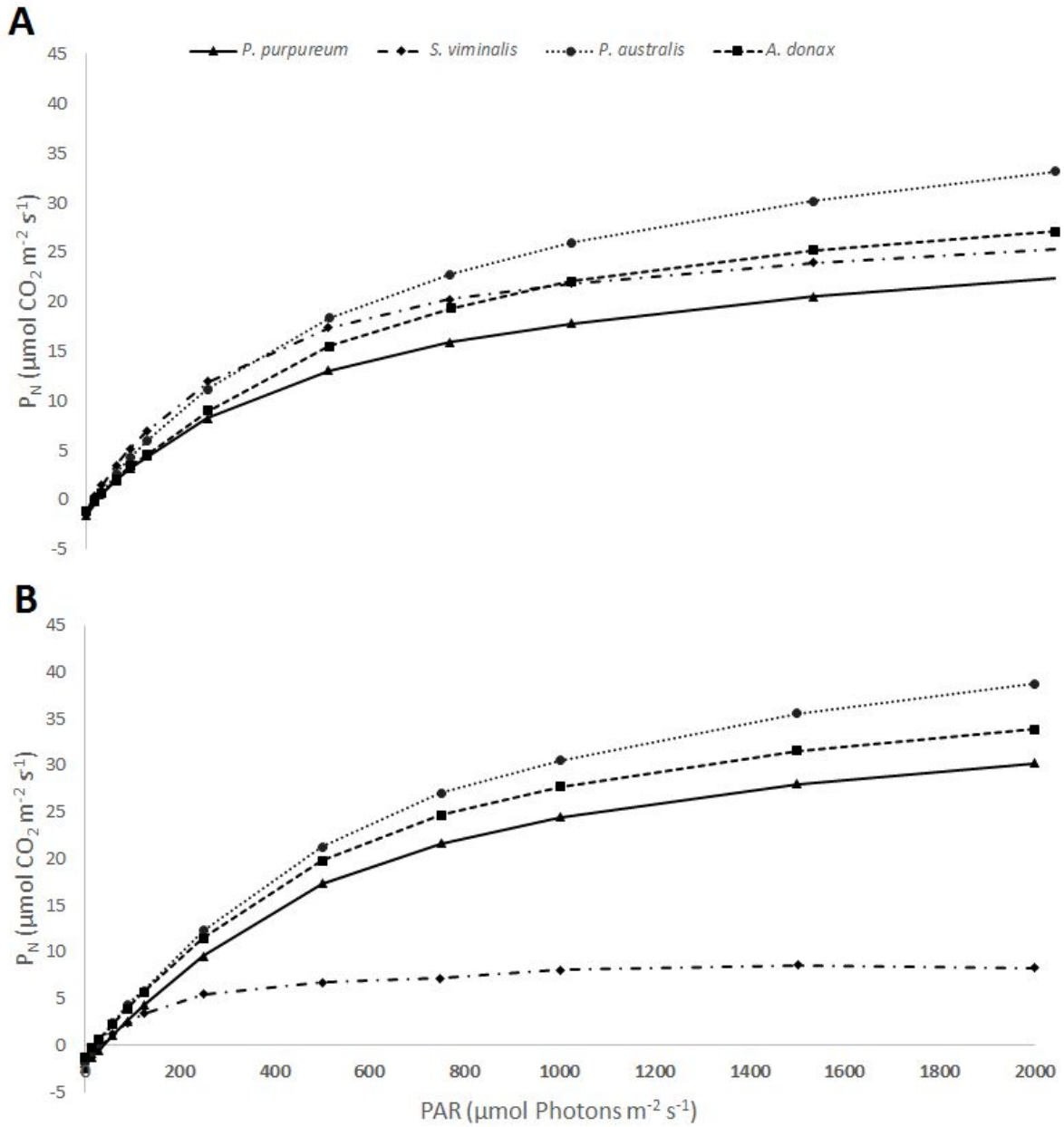


**Figure 3.** Light response curve for the four different species of plants grown at two different temperatures (20 °C and 30°C).

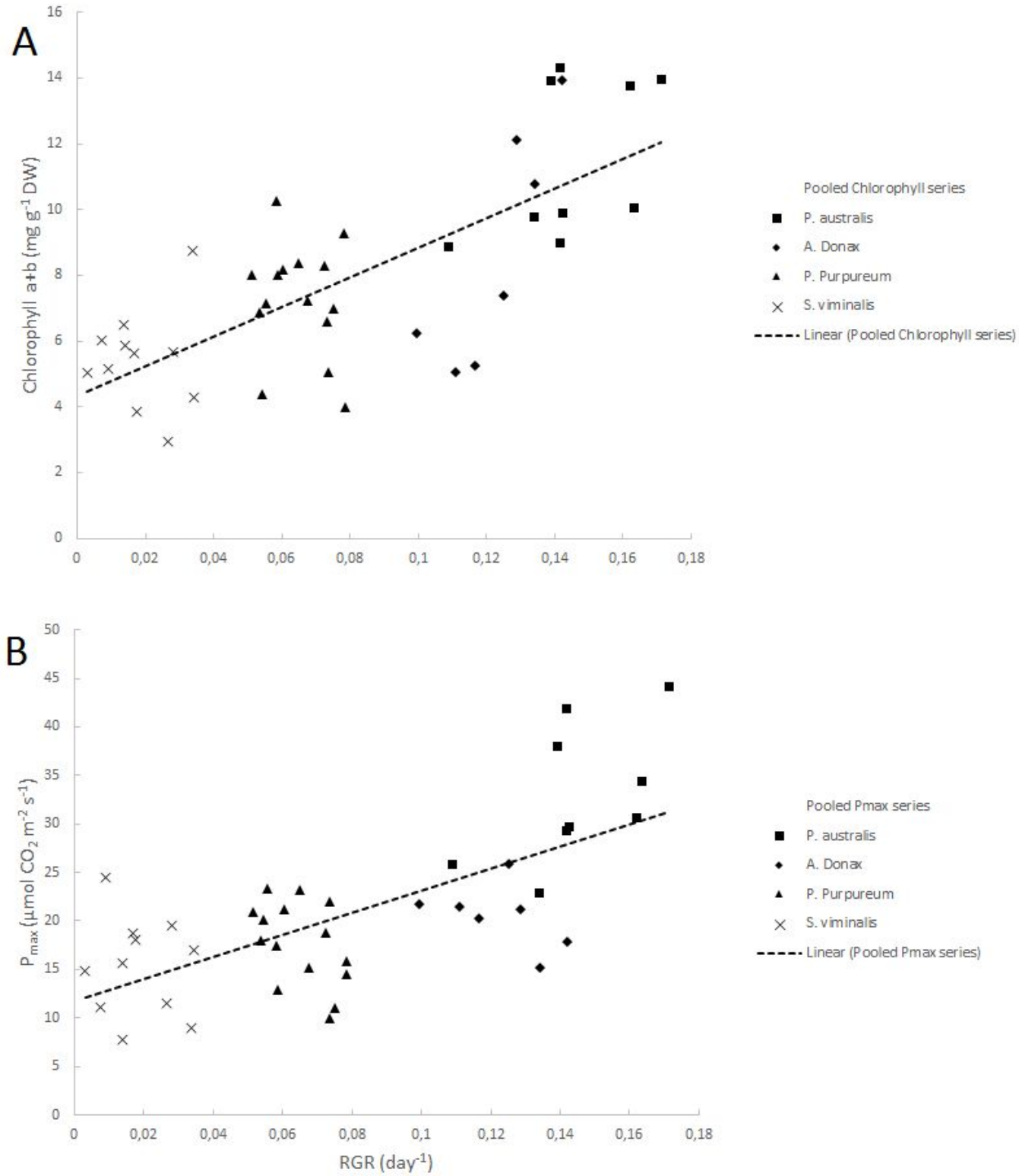


**Figure 4. A**: Total chlorophyll content of leaves as a function of relative growth rate (RGR)($p<0.000$, $R^2=0,54$) **B**: $P_{max}$ as a function of RGR ($p<0.001$, $R^2=0,47$).