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# THE EFFECT OF INCREASED GROWTH TEMPERATURE ON MID-DAY AND MID-NIGHT $CO_2$ FLUXES IN *POPULOUS DELTOIDES*

**by**

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THESIS

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# The effect of increased growth temperature on mid-day and mid-night $CO_2$ fluxes in *Populous deltoides*

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## ABSTRACT

As global temperatures rise, understanding the effects of increased temperature on daily carbon dioxide fluxes is necessary to predict changes to the global carbon cycle. Using tunable diode laser spectroscopy, I measured $CO_2$ fluxes and changes in isotopic composition of root respiration, leaf respiration and photosynthesis over a 12-hour night to day transition. I found that after a 10˚C increase in growth temperature for four weeks, root $CO_2$ fluxes acclimated to the higher temperature (i.e. they were not significantly different from controls). In contrast, both photosynthesis and respiration of leaves were higher at the elevated growth temperature. Though the root fluxes were not significantly different between growth temperatures, two patterns may become significant with greater replication: (1) the difference in rates of mid-day/mid-night root respiration in plants grown at 22˚C may be greater than those grown at 32˚C and (2) variability in the isotopic composition of root respired $CO_2$ is greater during the mid-night period compared to mid-day period. These data show that leaves and roots respond differently to grow temperature and suggest that root respiration during the day may respond to temperature differently from root respiration at night.

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**Introduction:**

Approximately 120 GtC per year are respired from terrestrial systems with half from autotrophic sources (IPCC 2007). Understanding how aboveground and belowground autotrophic carbon dioxide fluxes will change with increased temperature is necessary in determining the effects of increased temperature on the global carbon cycle. Temperature is an important driving force in ecological systems because it impacts all enzymatic processes in a well-characterized manner. As temperature increases, enzymatic activity increases, until a temperature threshold is reached where enzymes begin to deactivate and/or denature. This increase in enzymatic activity causes increases in the rates of physiological processes including respiration and photosynthesis (Berry and Bjorkman 1980). However, there is a difference in the short-term responses of photosynthesis and respiration due to differences in the temperature sensitivity of enzymes in each pathway. Photosynthesis increases with temperature at a slower rate compared to respiration, and photosynthesis tends to reach a threshold at a lower temperature than respiration (Ryan 1991). The lower temperature threshold of photosynthesis is caused by damage and/or deactivation of enzymes at temperatures that do not cause losses of activity for respiratory enzymes (Dewer *et al*. 1999).

Although the enzyme kinetics of temperature responses are well characterized, the regulation of metabolism in response to temperature is not. For example, exposure to a temperature that is high enough to damage photosynthetic but not respiratory enzymes will decrease the net carbon gain of a plant. However, plants may respond to the higher temperature by increasing expression of protective mechanisms to increase the thermostability of photosynthesis and thereby improve net carbon gain (Hanson and

Sharkey 2001, Sharkey *et al*. 2001, Penuelas and Llusia 2002). The regulation of mechanisms controlling thermotolerance is complex and not well characterized, and more data are needed to understand how photosynthesis and respiration are balanced in response to increased temperatures.

Belowground (soil) respiration tends to increase with increased temperature (Widen and Majdi 2001, Lloyd and Taylor 1994). As temperature increases 10˚C, soil respiration increases 1.3-3.3 fold (Reich and Schlesinger 1992). However, we do not fully understand how the individual components of soil respiration (autotrophic and heterotrophic) are regulated as temperature increases and how they interact with the regulatory changes in photosynthesis. It is difficult to partition between autotrophic and heterotrophic respiration because roots are so intimately connected with the soil fauna, and few studies have simultaneously examined shoot and root responses to look for interactions. Along with the challenge of measuring root respiration, there are conflicting data on the effects of belowground respiration in response to increased temperature. Some of this conflict is associated with the time scale of the temperature change.

On an ecosystem scale, Mahecha *et al*. found that the response of respiration to temperature is broken into two time periods: short-term (days to 3 months) and long term (>3 months). These two time periods are defined by the small range of $Q_{10}$ values (the proportional change in the respiration rate over a 10˚C temperature change) found across several ecosystems ($Q_{10}$=1.4±0.1) during periods of less than 3 months (short-term), and the increased variability of ecosystem $Q_{10}$ values after 3 months (Mahecha *et al*. 2010). However, physiologists break root respiration responses to temperature into two different time-scales: short-term (minutes to hours) (Atkin *et al*. 2000b) and long-term (weeks to

years) (Atkin and Lambers 1998). In order to scale-up plant physiological processes to an ecosystem scale, three categories are needed: immediate (minutes to days), short-term (weeks to 3 months), and long-term (>3 months). The immediate responses are governed by enzyme kinetics and regulation of cell metabolism throughout the plant. These immediate responses yield signals that then affect short-term developmental processes such as leaf and fine root development and eventually long-term whole plant to ecosystem response. Ultimately, a mechanistic understanding of the integration of these three time scales will help determine how plants and ecosystems respond to temperature.

Root respiration responds to immediate and short-term changes in temperature; therefore, diel and seasonal temperature changes can affect root respiration rates (Körner and Larcher 1988). Immediate temperature sensitivity, described by a $Q_{10}$ value, varies among plant species (Loveys *et al*. 2003). This variability in $Q_{10}$ results in some plant species having no diel pattern in root respiration and others having strong temperature driven diel patterns (Bekku *et al*. 2009). These immediate temperature responses are important for understanding daily root respiration fluctuations; however, short-term and long-term changes in growth temperature may also affect these patterns.

Immediate temperature changes cause a change in respiration rates; however, short and long-term changes have different effects due to some plants "acclimating" to the new growth temperature. Respiratory acclimation (or respiratory homeostasis) is the process by which plants grown at two different temperatures exhibit the same respiration rates when measured at their growth temperatures (Berry and Bjorkman 1980). Not all plants experience the same levels of respiratory homeostasis, where some plants never reach acclimation, and some reach partial acclimation. Tjoelker *et al*. (1999) found that

warm-grown plants of 5 boreal tree species had root respiration rates of 50-74% of the same species when grown at cold temperatures indicating some acclimation to the new growth temperature; however, Sowell and Spomer (1986) found no acclimation in the roots of two *Picea* species or *Abies lasiocarpa*. This variability of acclimation will greatly affect the impact of increased temperature on $CO_2$ efflux from soils.

Along with affecting the overall diel patterns, short and long-term increased temperature may affect the isotopic composition of respired $CO_2$ ($\delta^{13}C_{respiration}$) (Steinmann *et al*. 2004). According to Werner and Gessler (2011), temperature can explain the changes in $\delta^{13}C_{respiration}$ over a 24-hour period. Werner and Gessler (2011) hypothesize that changes in temperature will lead to changes in $\delta^{13}C_{respiration}$: (1) because of possible differences in growth and maintenance respiration $\delta^{13}C_{respiration}$, where a change in temperature will predominantly effect the isotopic composition of maintenance respiration due to it's higher temperature sensitivity (Ryan 1991); and (2) due to differences in substrates used for respiration since different substrates have different isotopic signatures. For example, transported starch is often $^{13}C$ enriched compared to soluble sugars (Gleixner *et al*. 1998).

Currently, $\delta^{13}C_{respiration}$ is frequently used to differentiate between autotrophic and heterotrophic respiration in soil respiration because there is a difference in isotopic signature (Hanson *et al*. 2000, Cheng 1996). Because $\delta^{13}C_{respiration}$ is used to distinguish autotrophic and heterotrophic respiration, understanding what short and long-term increased temperature will do to the diel isotopic signature will allow for better modeling of future belowground carbon effluxes. Using Equation 7 from Hanson *et al*. (2000), increasing the $\delta^{13}C_{root\ respiration}$ will decrease the estimated root respiration rate from a soil-

partitioning model. For example, a 10% increase in $\delta^{13}C_{root\ respiration}$ would correlate with a decrease in the estimated respiration rate of ~8%. Thus, if increased growth temperature causes a change in the isotopic signature of root respiration, but not in the rate of root respiration (due to roots reaching respiratory homeostasis), current models will underestimate the amount of root respiration at an individual and global scale. Therefore, it is critical to understand the effect of temperature on $\delta^{13}C_{root\ respiration}$.

The effect of increased temperature (at any time-period) on aboveground autotrophic $CO_2$ fixation is better understood compared to belowground autotrophic $CO_2$ production (Atkin *et al*. 2000a). Photosynthesis typically increases with immediate, short, and long-term temperature increases, within the thermal limits of the species (Hikosaka *et al*. 2006) because of the effect of increased temperature on the enzyme Rubisco and photosystem II (reviewed in Allakhverdiev *et al*. 2008). Photosynthetic carbon isotope discrimination ($\Delta$) is also influenced by temperature (Farquhar *et al*. 1989). Discrimination is the net effect of selection against the heavy $^{13}C$ isotope during stomatal diffusion and a preferential choice of Rubisco for $^{12}CO_2$ (Farquhar *et al*. 1989). This discrimination against $^{13}C$ is influenced by several parameters that are affected by temperature, such as: mesophyll conductance, which has an impact on the transfer of $CO_2$ to Rubisco (Bernacchi *et al*. 2002) and stomatal conductance (Lloyd and Farquhar 1994). One key parameter of discrimination that is not effected by temperature is the preferential choice of $^{12}C$ by Rubisco (Christeller and Liang 1976). All of these factors will influence the discrimination against $^{13}C$, and because of this, plant tissues have a different isotopic signature ($\delta^{13}C_{assimilation}$) than the atmosphere (Bender 1971). The $\delta^{13}C$ of assimilated carbon provides insight into the biochemical processes that have modified the captured

$CO_2$ during fixation (Farquhar *et al*. 1980). This assimilated carbon is the substrate for respiration, so the $\delta^{13}C_{assimilation}$ is closely coupled with the $\delta^{13}C_{respiration}$ (Klumpp *et al*. 2005, Gessler *et al*. 2007). If the isotopic composition of substrates change with increased temperature, $\delta^{13}C_{root\ respiration}$ will also change resulting in a need to modify carbon cycle models.

While the response of aboveground carbon fixation to increased temperatures is fairly consistent, the response of aboveground autotrophic respiration is highly variable (Azćon-Bieto 1992) with no demonstrated linkage between rates of photosynthesis and leaf respiration (Ow *et al*. 2008). In addition, leaf respiration during the day is poorly understood and could be different from leaf respiration at night (Atkin 1997). Daytime leaf respiration is challenging to measure because respiration is producing $CO_2$ and photosynthesis is simultaneously fixing $CO_2$, so dark respiration is often used as an estimate of respiration throughout the day (Atkin *et al*. 1997). Dark respiration is temperature sensitive in the immediate time period (Körner and Larcher 1988), however with short and long-term temperature increases there may be acclimation where an increase in temperature no longer effects respiration to the same extent (Atkin *et al*. 2000b).

Each component of the whole plant $CO_2$ flux may respond to short and long-term temperature increases differently. These differential responses combined will result in a plant that is either a large carbon sink (carbon fixation is much greater than respiration) or a small carbon sink (carbon fixation is almost the same as respiration). Several studies have found that individual plants will remain carbon sinks (Gifford 1995) as temperatures increase. However, if respiration and photosynthesis respond differentially to temperature

increases, there may be a shift in the carbon balance of individual plants. If a large proportion of plants in an ecosystem become smaller sinks, and heterotrophic respiration does not decrease, then there will be a shift in the net ecosystem $CO_2$ balance.

Using *Populus deltoides*, I assessed the impact of a short-term temperature increase on: (1) the diel pattern of root respiration rate, (2) the isotopic composition of root respiration, (3) the sensitivity of respiration to temperature ($Q_{10}$), (4) photosynthesis, (5) the isotopic composition of leaf assimilation and respiration, and (6) leaf respiration. Testing the effects of increased temperature on all of these parameters simultaneously provides a novel look at whole plant $CO_2$ fluxes.

**Methods:**

**Plant growth conditions:** *Populus deltoids* (poplar) cuttings were propagated in soil for 8 weeks. After the roots were washed, the plants were transplanted into a hydroponic drip system with a substrate of Hydroton®. They were supplied a modified 25% strength Hoagland's nutrient solution, which was changed weekly (Hoagland and Arnold 1938). Plants were grown under consistent green house conditions with differing temperature regimes for 10 weeks. Plants were either grown at 22/18±1°C or 32/28±1°C (day/night) for the 10 weeks. Both greenhouses maintained a relative humidity of ~25%. One week prior to measurements, plants were moved from the greenhouse into growth chambers. Growth chambers were set to simulate the greenhouse as closely as possible, with temperatures of 22/18°C or 32/28°C (day/night); a 15-hour light period where the lights were gradually stepped on and off to simulate sunrise and sunset; and humidity set to 25%. All measurements occurred in the growth chambers.

**Measurement System:** A custom, airtight root chamber (Fig.1) was built to fit around the pots that the poplars were grown in so that the entire root system was not disturbed prior to the measurement process. Air (which contained ~1000 ppm $CO_2$) was pumped at a flow rate of 600 μmol $sec^{-1}$ into the base of the pot where it moved through the root system and Hydroton®. Exiting ($root_{sample}$) air was taken from the top of the chamber, to make sure that the entering ($root_{reference}$) air had circulated through the entire root system prior to being sampled. The air was dried and sampled by a tunable diode laser absorption spectrometer (TDL) (TGA100A, Campbell Scientific, Inc., Logan, UT, USA) to assess total $CO_2$ and the amount of $^{12}CO_2$ and $^{13}CO_2$ in the $root_{sample}$ and $root_{reference}$ air.

An individual leaf was also measured during the same time period using a portable photosynthesis system (Li-Cor 6400, Li-Cor, Inc., Lincoln, NE, USA) coupled with the TDL. A custom, clear top leaf chamber (similar to Barbour et al., 2007, Fig. 1) was attached to the leaf, and light in the growth chamber (~1000 PAR during mid-day) illuminated the measured leaf as well as all other leaves on the plant. Air, containing 400 ppm $CO_2$, was supplied to the leaf by the Li-Cor 6400 at a flow rate of 500 μmol $sec^{-1}$. Both the Li-Cor 6400 and the TDL measured the air before entering the chamber ($leaf_{reference}$) as well as the air leaving the chamber ($leaf_{sample}$).

The TDL sampled air from two calibration tanks (473ppm $^{12}CO_2$ with 5.18ppm $^{13}CO_2$ and 243ppm $^{12}CO_2$ with 2.66ppm $^{13}CO_2$). In a 10-minute period, the TDL sampled the each of the air samples: $root_{sample}$, $root_{reference}$, $leaf_{sample}$ and $leaf_{reference}$ twice, and each calibration tank once for 60 seconds each. I calculated a 10-minute mean of the isotopologue concentrations using the last 10 seconds of each air sample.

**Measurements:** Plants were placed in the measurement system in the afternoon, and they remained in the system for 24 hours during which they were watered 9 times. Water filled the pots via an automated dripper system, and excess water drained out of the pots within 15 minutes of the flooding. Electronic water/air-tight valves (Mouse Pneumatic Electronic Valve, Clippard Instrument Laboratory Inc., Cinncinati, OH, USA) were synchronized with the watering, so that when watering/draining was occurring the watering associated valves were open, but when watering/draining was not occurring, the valves were shut maintaining a airtight, closed system. Online gas exchange measurements continued while watering occurred. Plants were dried and weighed immediately after measuring.

**Data Manipulation:** Data that did not represent a steady state was removed from the data set. This included data during/after watering. As well, data at the beginning of the measurement period (~14:00-22:00) has been excluded, because the plant had not equilibrated in the measurement system.

**Leaf Day Respiration:** In a previous study, poplars were grown at 22˚C and 27˚C under the same conditions as the poplars used in this experiment. These poplars were used to determine the day respiration rate of leaves. Using the Laisk method (Yin *et al*. 2011), day leaf respiration was measured then calculated using changes in $CO_2$ levels (from 5 to 200 μmol $mol^{-1}$ $CO_2$) at a constant light intensity (400 PAR). Leaves were then dark adapted (R dark) for at least 20 minutes before dark respiration was measured.

**Nitrogen content and $\delta^{13}C$ of plant tissue:** Measured plant material was dried, powdered, and weighed for analysis of the nitrogen and carbon content as well as the isotopic signature of carbon in the tissues via an elemental analyzer connected to a

continuous flow isotope ratio mass spectrometer (University of New Mexico Stable Isotope Lab). A CONFLO II interface was used to couple a Costech ECS 4010 Elemental Analyzer to a Thermo Finnigan Delta Plus mass spectrometer. A soy standard was used for calibration and the standard was calibrated against NBS 21, NBS22, and USGS 24. Two 0.2mg soy standard samples were used for every six 0.2mg plant samples. The results were reported in delta (δ) notation to follow the isotope ratio notation set as the standard notation by Vienna Pee Dee Belemnite (V-PDB). Nitrogen content is presented as estimated total tissue nitrogen content where the sub sampled nitrogen content was then multiplied by the total mass of the tissue to determine the estimated total tissue nitrogen content.

**Specific leaf area:** The specific leaf area of the plants was calculated using 5 dried leaves from each temperature. Leaves were randomly selected from 3 age classes (young, average, old). The area and weight of the dried leaves was measured.

**Calculations:**

**Discrimination (Δ):** Carbon isotope discrimination was calculated using Evans et al. (1986): $\Delta=(\xi(\delta_o-\delta_e))/(1+\delta_o-\xi(\delta_o-\delta_e))$ (Equation 1) where $\xi=C_e/(C_e-C_o)$. $C_e$ is the concentration of $CO_2$ entering the chamber (reference), $C_o$ is the concentration of $CO_2$ leaving the chamber (sample), $\delta_e$ and $\delta_o$ are the $\delta^{13}C$ of the reference and sample gas, respectively.

**$\delta^{13}C_{respiration}$:** The isotopic composition of respiration ($\delta^{13}C_{root\ respiration}$ and $\delta^{13}C_{leaf\ respiration}$) was calculated relative to the Vienna Pee Dee Belemnite (VPDB) standard following Barbour et al. (2007): $\delta^{13}C_{respiration}=(\delta^{13}C_o-\delta^{13}C_e(1-p))/p$ (Equation 2) where $p=(C_o-C_e)/C_o$.

**$\delta^{13}C_{assimilation}$:** The isotopic composition of assimilated sugars was based on Farquhar et al. (1989): $\delta^{13}C_{assimilation} = (\delta_e - \Delta)/(\Delta+1)$ (Equation 3), where $\delta_e$ was assumed to be -8.625‰ based on current atmospheric estimates (White and Vaughn 2011).

**$Q_{10}$ Calculation:** A $Q_{10}$ value was determined using a temperature drop that occurred during the measurement of the 22˚C plants. During the 24-hour respiration measurements of the 22˚C plants, there was a malfunction with the temperature control of the growth chamber that resulted in a steep temperature drop (from ~18˚C to ~11˚C) just before dawn. We used this drop in temperature to calculate the $Q_{10}$ values. The logs of the respiration rates were plotted against the measurement temperature and a linear regression model was fit to the data. Using the slope of the regression, a $Q_{10}$ was calculated where $Q_{10}$=10^(10*regression slope) (Equation 4) (Atkin *et al*. 2000a). The $Q_{10}$ was then used to determine the corrected respiration rate at each temperature using: $R_2=R_1*(Q_{10}^{(1/(10/(T_2-T_1)))})$ (Equation 5), where $R_2$ was the calculated respiration rate adjusted to the growth temperature, $R_1$ was the measured respiration rate for the measurement temperature, $T_2$ was the growth temperature, and $T_1$ was the measurement temperature. For plants grown at 22˚C, $T_2$ was 22.

**Statistical Analysis:** Statistical analyses were performed using R (R Foundation for Statistical Computing, Vienna, Austria). A two-way analysis of variance (ANOVA) using an interaction term was used to determine the differences between day/night and growth temperatures in the diel root respiration rates, $\delta^{13}C_{root\ respiration}$, and $Q_{10}$ corrected data. Tukey's Honestly Significant Difference test was used post-hoc to determine which means were significantly different from one another at the 5% level. Student's t-tests were used to determine the differences between growth temperatures for photosynthetic

rates, leaf respiration rates, $\delta^{13}C_{leaf\ respiration}$, $\delta^{13}C_{assimilation}$, discrimination, and whole plant carbon balance.

**Results:**

Poplars grown at 22˚C and 32˚C were measured using online isotopic gas exchange combined with infrared gas exchange to determine carbon dioxide fluxes in both the roots and leaves. Carbon dioxide isotopologoues ($^{12}CO_2$ and $^{13}CO_2$) were measured to determine discrimination and fractionation during photosynthesis and respiration. As well, the effects of increased growth temperature on other parameters (tissue mass, nitrogen content, $^{13}C$ content of different tissues, and the specific leaf area) were assessed. The results presented are the mean ± the stand deviation of 3 plants where 1 hour of stable data was averaged during mid-night and another hour during mid-day was averaged to obtain a single night and day value per plant.

**Effect of growth temperature on root respiration rate:** There was no difference in the root respiration rates between the two growth temperatures either during the day or the night periods (p=0.58 and 0.64, respectively). During the day, respiration rates were 38.5±11.7 μmol $CO_2$ $g^{-1}$ $hr^{-1}$ and 48.2±3.7 μmol $CO_2$ $g^{-1}$ $hr^{-1}$ for plants grown at 22˚C and 32˚C respectively. During the night, respiration rates were 25.0±12.5 μmol $CO_2$ $g^{-1}$ $hr^{-1}$ and 33.9±5.3 μmol $CO_2$ $g^{-1}$ $hr^{-1}$ for plants grown at 22˚C and 32˚C respectively (Fig.2). Including data from a preliminary study with plants grown at 25˚C and 27˚C also showed that there was no difference in the root respiration rates between growth temperatures or between day and night (Fig. 3) (p-values were all greater than 0.05).

**$Q_{10}$:** The $Q_{10}$ value of plants that were grown at 22˚C but measured at a range of temperatures from 11˚C-18˚C during the dark period was 1.3. After applying the $Q_{10}$ correction to account for temperature, there was no significant difference in day and night respiration or between growth temperatures (p=0.82 and 0.92 respectively) (Table 1).

**Day/night pattern in root respiration:** Poplars grown at 22˚C exhibited a difference in mean mid-day and mid-night root respiration rates (13.5 μmol $CO_2$ $g^{-1}$ ) (Fig. 2). In these poplars, mean root respiration was lower during the night period relative to steady state in the late morning; however, due to between plant variation, this difference is not statistically significant (p=0.33). A similar pattern occured in the poplars grown at 32˚C with difference in mid-day and mid-night root respiration of 14.3 μmol $CO_2$ $g^{-1}$ (p=0.29). However, once a $Q_{10}$ correction was applied (Table 1), the difference between mid-day and mid-night in poplars grown at 22˚C was 7.7 μmol $CO_2$ $g^{-1}$ compared to 2.9 μmol $CO_2$ $g^{-1}$ for plants grown at 32˚C (p=0.82).

**Isotopic composition of root respiration:** As seen in Figure 4, the $\delta^{13}C_{root\ respiration}$ is more variable during the dark period in poplars that have grown at 22˚C (-26.8±6.1‰), compared to during the light period (-30.1±1.0‰); however, there is no statistical difference between the two values (p=0.45). There is less variability for plants grown at 32˚C (-29.9±2.0‰ and -30.9±1.4‰ for day and dark respectively), but there is still no significant difference between day and dark $\delta^{13}C_{root\ respiration}$ in plants grown at 32˚C (p=0.52).

**Effect of growth temperature on photosynthetic rate**: There was no difference in the photosynthetic rates between the two growth temperatures in the afternoon (p=0.67). There was a significant difference between the two growth temperatures in the

early morning (61.3±3.0 and 154.4±44.5, for 22˚C and 32˚C grown plants respectively) (p=0.03). Poplars grown at 32˚C exhibited the highest photosynthetic rates early in morning (154.4±44.5 μmol $CO_2$ $g^{-1}$ $hr^{-1}$) and then the photosynthetic rate dropped in the early afternoon (103.4±60.4 μmol $CO_2$ $g^{-1}$ $hr^{-1}$) (Fig. 5). This drop is photosynthesis correlates with a drop in stomatal conductance (Table 2).

**Isotopic composition of assimilation:** The $\delta^{13}C_{assimilation}$ of plants grown at 22˚C was -21.3±2.3‰, and for plants grown at 32˚C, it was -20.3±2.5‰. There was no difference in the $\delta^{13}C_{assimilation}$ between the two growth temperatures (p=0.62) (Fig. 9).

**Discrimination:** There was no significant difference in the carbon isotope discrimination between the two growth temperatures (p=0.30) (Fig. 6). Plants grown at 22˚C discriminated 20.2±1.8‰, while plants grown at 32˚C discriminated 16.3±4.9‰. As well, there was no difference in the slope of discrimination compared to Ci/Ca, and no difference in mesophyll conductance (Fig. 7, Table 2).

**Leaf respiration:** In a preliminary experiment, leaf respiration rates measured during the day in the light did not vary from dark respiration (Fig. 8) (p=0.17 and 0.52 at 22˚C and 27˚C). Day respiration in the previous study plants grown at 22˚C was -1.3±0.3 μmol $CO_2$ $g^{-1}$ $hr^{-1}$ and -1.0±0.3 μmol $CO_2$ $g^{-1}$ $hr^{-1}$ for those grown at 27˚C. Dark respiration for these previous study plants was -1.3±0.2 μmol $CO_2$ $g^{-1}$ $hr^{-1}$ and -1.1±0.4 μmol $CO_2$ $g^{-1}$ $hr^{-1}$ for plants grown at 22˚C and 27˚C respectively. As seen in Figure 5, the respiration rate of poplars grown at 32˚C (blue circles) was higher (-10.5±2.4 μmol $CO_2$ $g^{-1}$ $hr^{-1}$) than the poplars grown at 22˚C (-3.3±2.4 μmol $CO_2$ $g^{-1}$ $hr^{-1}$) (red circles) (p=0.02).

**Isotopic composition of leaf respiration:** The rate of leaf respiration was too low for isotopic analysis for plants grown at 22˚C and 32˚C.

**Effect of growth temperature on other parameters:**

**Isotopic signature of tissues:** The $\delta^{13}C$ content of each plant tissue (leaf, stem, and roots) were measured for plants grown at 22˚C and 32˚C (Table 3). There was no significant difference in the whole plant isotopic composition between growth temperatures (p=0.18). There was a significant difference in the $\delta^{13}C$ of stem tissue of plants grown at 22˚C and 32˚C (-30.6±0.2 and -31.8±0.2 respectively, p=0.002).

**Nitrogen content:** Leaf nitrogen content for plants grown at 22˚C was 0.14±0.06 g and 0.13±0.09 g for plants grown at 32˚C. Stem nitrogen content was 0.09±0.03 g and 0.05±0.01 g for plants grown at 22˚C and 32˚C respectively. Root nitrogen content was 0.24±0.8 g and 0.14±0.03 g for plants grown at 22˚C and 32˚C respectively (Fig. 10). The only significant difference was between the roots and the stems grown at 32˚C (p=0.03).

**Leaf area:** The specific leaf area for plants grown at 22˚C was 1.7 $m^2$ $kg^{-1}$ (mean of 5 leaves taken from the 3 measured plants). The specific leaf area for plants grown at 32˚C was 5.1 $m^2$ $kg^{-1}$ (mean of 5 leaves taken from the 3 measured plants). Using the total canopy leaf dry mass and the specific leaf areas, I estimated canopy leaf area. For plants grown at 22˚C the estimated canopy leaf area was 0.01±0.005 $m^2$, and it was 0.03±0.02 $m^2$ for plants grown at 32˚C.

**Dry plant tissue mass:** There was no difference in dry plant mass between plants grown at different temperatures (Fig. 11). Leaf mass was 5.9±3.3 g and 6.5±4.8 g; stem mass was 10.3±2.7 g and 8.2±2.3 g; and root mass was 14.2±5.0 g and 9.2±1.4 g for plants grown at 22˚C and 32˚C respectively.

**Discussion:**

In this study, I found that leaf metabolism responds to increased growth temperature and that root metabolism does not over the short term (defined here as days to weeks). However, I also found that root metabolism was much more variable between plants and that this variability may be obscuring the root response. If roots are indeed regulating the metabolic response to temperature in a manner fundamentally different to that of the leaves, then this will have important implications for how we understand plant responses to increased growth temperature.

**Effect of growth temperature on root respiration rate:** In this study, we did not find an increase in overall root respiration rate at an increased growth temperature. This goes against the common theory that respiration is closely tied to temperature (Weger and Guy 1991). Acclimation may account for the similarity in respiration rates despite a 10˚C increase in growth temperature. In our study, the poplars reached respiratory homeostasis, where plants grown at different temperatures exhibit the same respiration rates when measured at their respective growth temperatures (Atkin *et al*. 2000b).

**Day-dark pattern of root respiration:** While several studies have found a diel pattern in root respiration (Huck *et al*. 1962, Bekku *et al*. 2009), the pattern is usually

temperature-dependent. Nighttime respiration is only lower because soil temperatures are cooler. In our study, we found that applying a $Q_{10}$ correction of 1.3 to normalize all of the data to a constant 22˚C did not eliminate the differences in means between day and night respiration. The mean corrected difference between night (27.1±14.9 μmol $CO_2$ $g^{-1}$ $hr^{-1}$) and day (34.6±10.0 μmol $CO_2$ $g^{-1}$ $hr^{-1}$) (p=0.82) was only cut in half (from 13.5 to 7.7 μmol $CO_2$ $g^{-1}$ $hr^{-1}$ Table 1). Thus an unusually high $Q_{10}$ of around 2.6 would be required to explain the day nigh difference based solely on a $Q_{10}$ response. We also found that the same $Q_{10}$ correction of 1.3 eliminated 80% of the difference between day and night root respiration (Table 1). This suggests that the plants grown at 32˚C had a smaller $Q_{10}$, possibly because they were closer to a maximum rate of respiration at night, and they could not increase the rate during the day. Based on the small day/dark variation in the temperature corrected data for the 32˚C grown poplars (Fig. 2), they may have reached homeostasis near the upper limit of respiration. The mechanisms of respiratory homeostasis are not well understood (Atkin and Tjoelker 2003); however at increased temperatures there might be an insufficient substrate supply needed to maintain an increase in respiration (Lambers *et al.* 1996). Because photosynthesis did not increase with temperature in the afternoon, there would not have been the extra substrates needed to increase respiration throughout the afternoon period. As well, Atkin *et al*. (2000a) found that temperature sensitivity of respiration decreases with very high temperatures, which may also account for the lack of a day/dark pattern in the 32˚C grown plants.

**Effect of growth temperature on photosynthesis:** Increased growth temperature resulted in a higher rate of photosynthesis in the early morning (p=0.03). However, temperature had no significant effect on photosynthetic rate in the afternoon (Fig. 5). This

may be due to acclimation of the high temperature grown poplars, but based on the decrease in photosynthesis and conductance (Table 2) during the early afternoon, it is more likely that the plants grown at 32˚C were stressed during afternoon measurements. Decreased stomatal conductance is one indicator of the plants being water limited (Tezara et al, 1999). Because the plants grown at 32˚C had larger total leaf area (0.03±0.02 $m^2$ compared to 0.01±0.05 $m^2$), there was a greater evaporative surface, such that the plants grown at 32˚C transpired more, even if the water content of the air was similar.

**Leaf respiration:** There is a difference in leaf respiration rate between the two growth temperatures (Fig. 5, p=0.02). Unlike root respiration, poplars grown at a higher temperature have a higher leaf respiration rate. This follows the theory that increased temperatures will increase respiration rates (Körner and Larcher 1988). While root respiration had acclimated (similar respiration rates at different growth temperatures) within the growth period, it does not appear that leaf respiration has acclimated. Loveys et al. (2003) found that root and leaf respiration reach respiratory acclimation during the same time period in several species. One factor that may account for the difference in acclimation between leaves and roots in poplars is the difference in available substrate supply in the roots versus the leaves, such as increased soluble carbohydrates in the leaves (Atkin *et al*. 200b). As well, differences in the energy demands of the roots compared to the leaves may be different, such that the roots may be putting more energy into growth, which is less temperature dependent (Ryan 1991), and therefore more likely to give similar respiration rates independent of temperature. The differences in temperature response of leaf and root respiration to long-term temperature increases may have large implications that ecosystem models currently do not account for.

**Isotopic composition of respiration and assimilation:** To understand what the isotopic composition of respiration is, we must also look at the isotopic composition of assimilation. Increased growth temperature had no significant effect on carbon isotope discrimination (Δ) during fixation (Fig. 6), which may be explained by no significant change in mesophyll or stomatal conductances (Table 2). Based on Bernacchi *et al*. (2002), a 2.2 fold increase in mesophyll conductance is expected for a 10˚C increase in temperature, which was not seen in these poplars (Table 2). As well, fractionation is reduced in growth chambers (Berry and Troughton 1974), which would have also dampened the effect of temperature on discrimination.

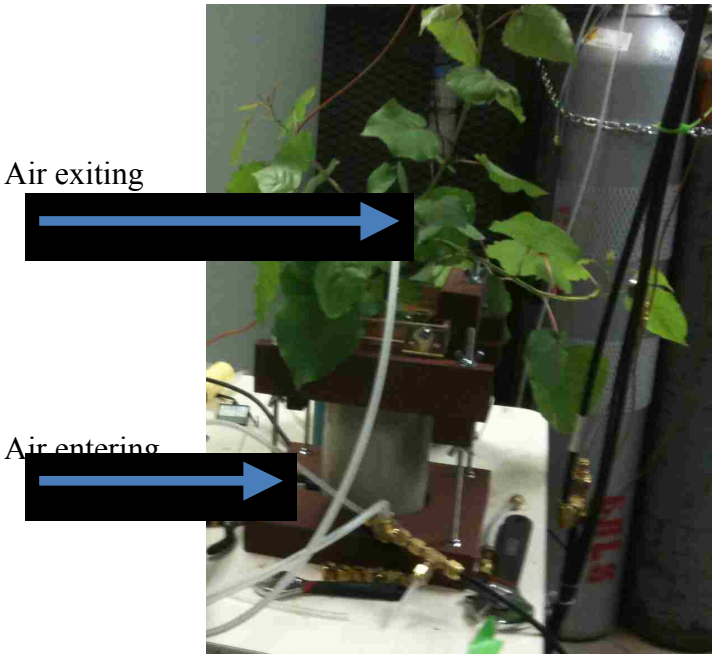
Because there was no change in discrimination, there was also no effect of growth temperature on the $\delta^{13}C_{assimilation}$. There was very little variation in the $\delta^{13}C_{assimilation}$ (Fig. 9). There was more variation in $\delta^{13}C_{root\ respiration}$ during the night compared to the day (Fig. 4). This variation in the isotopic composition of root respiration but not the assimilated carbon may indicate a switch of substrate usage (Brandes *et al*. 2006). The increase in variation during the night should be further explored to see if substrate consumption does account for the variation.

**Conclusion:**

This study highlights some general patterns that warrant further investigation. First, root respiration reached respiratory homeostasis, which is consistent with the idea that the poplars are respiring near their maximum at the 32˚C, but not at 22˚C. Second, the diel pattern of root respiration at 22˚C, but not 32˚C, also indicates that respiration may be near maximum capacity at the higher growth temperature. Third, photosynthesis

did not acclimate in the morning, but it did taper off in the afternoon in the 32˚C grown poplars, resulting in similar photosynthetic rates between the two growth temperatures in the afternoon. Fourth, leaf respiration did not reach acclimation; however, more research needs to be done on the length of time until respiratory homeostasis in different plant organs, if respiratory acclimation occurs in the species. Lastly, the increase in variation in night isotopic composition of root respiration also deserves further exploration to see if substrate supply is actually changing or if another factor is responsible for the increase in variation. Better understanding of the immediate, short-term, and long-term effects of temperature on $CO_2$ fluxes, including shifts in isotopic composition, is essential for predicting future global $CO_2$ fluxes.

Figure 1: Custom root chamber (top) and custom leaf chamber (bottom). A custom-made air-tight root chamber was fabricated to allow gas exchange of the entire root system, without disturbing any of the roots. A custom clear-top leaf chamber allowed for the light of the growth chamber to illuminate the measured leaf along with all other leaves on the plant.



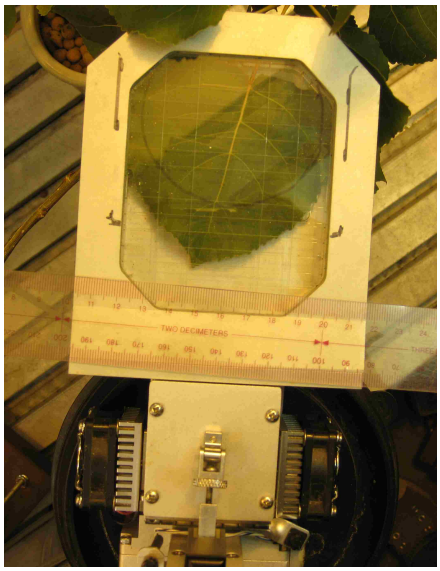



Figure 2: Root respiration rates. Averages of the root respiration rate of plants grown and measured at 22˚C (red circles) and 32˚C (blue triangles). Error bars indicate the standard deviations. n=3 for both temperatures. Note: Filled circles indicate a drop in root temperature due to a growth chamber malfunction. Root respiration data during this period has been removed since it is not representative of steady state.

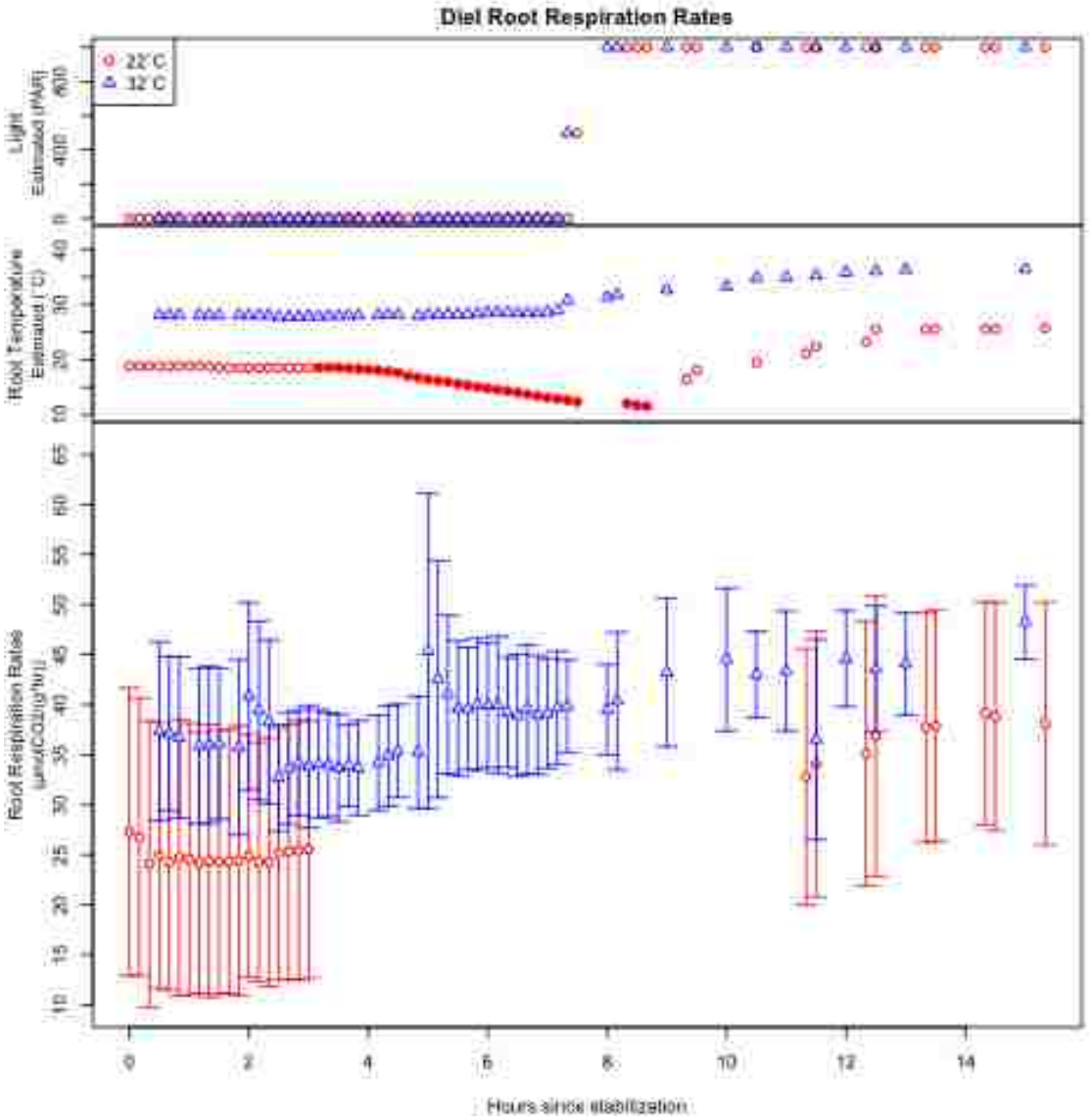


Figure 3: Root respiration rates. Day (open) and night (filled) root respiration rates of plants grown and measured at 22˚C (circles), 25˚C (squares), 27˚C (diamonds), and 32˚C (triangles).

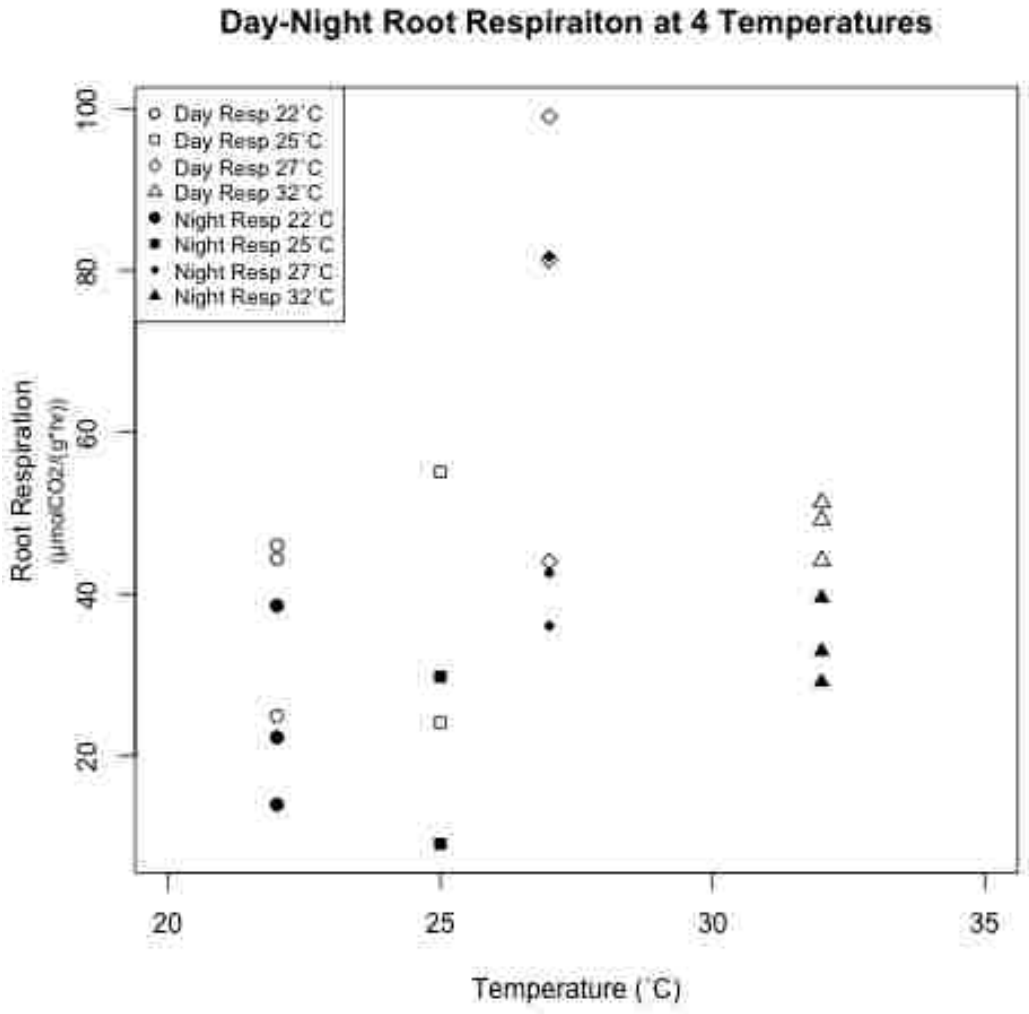


Figure 4: Root $\delta^{13}C$ of respiration. Averages of the $\delta^{13}C$ of plants grown and measured at 22˚C (red circles) and 32˚C (blue triangles). Error bars indicate the standard deviations. n=3 for both temperatures.

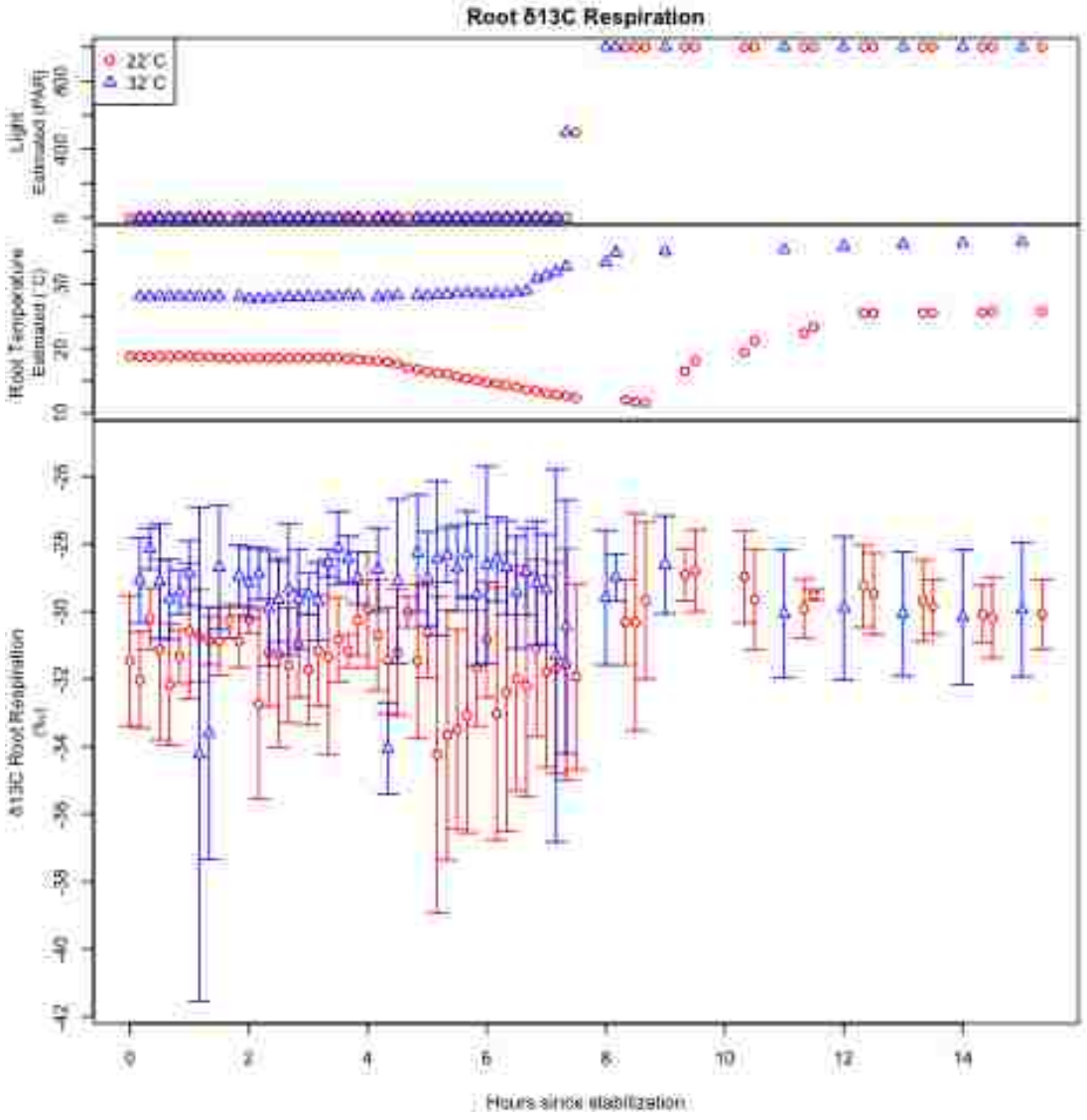


Figure 5: Photosynthetic and leaf respiration rates over 16 hours. Averages of the photosynthetic rate/leaf respiration rate of plants grown and measured at 22˚C (red) and 32˚C (blue). Error bars indicate the standard deviations. n=3 for both temperatures.

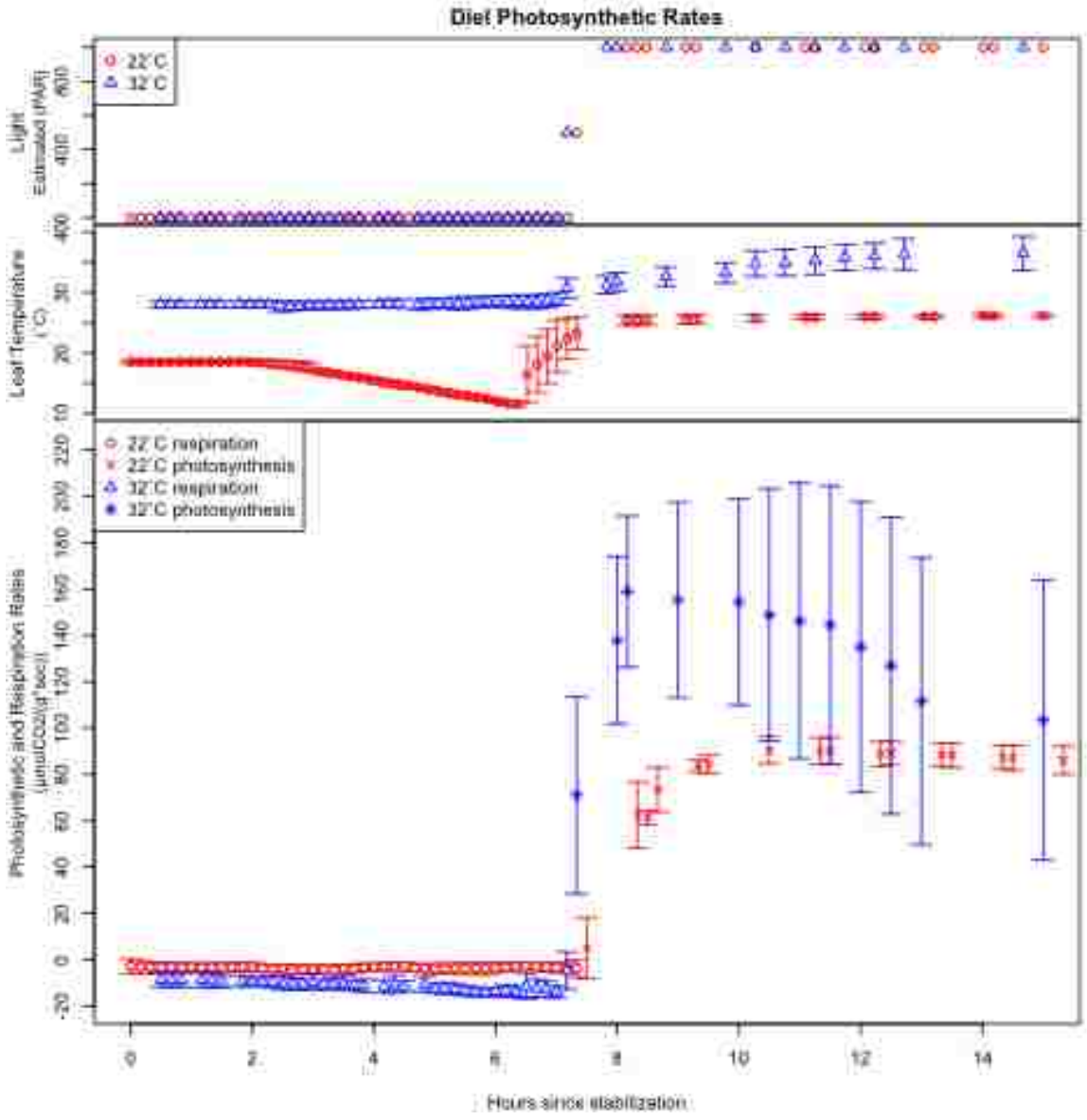


Figure 6: Photosynthetic discrimination. Averages of carbon isotope discrimination of plants grown and measured at 22˚C (red circles) and 32˚C (blue triangles). Error bars indicate the standard deviations. n=3 for both temperatures.

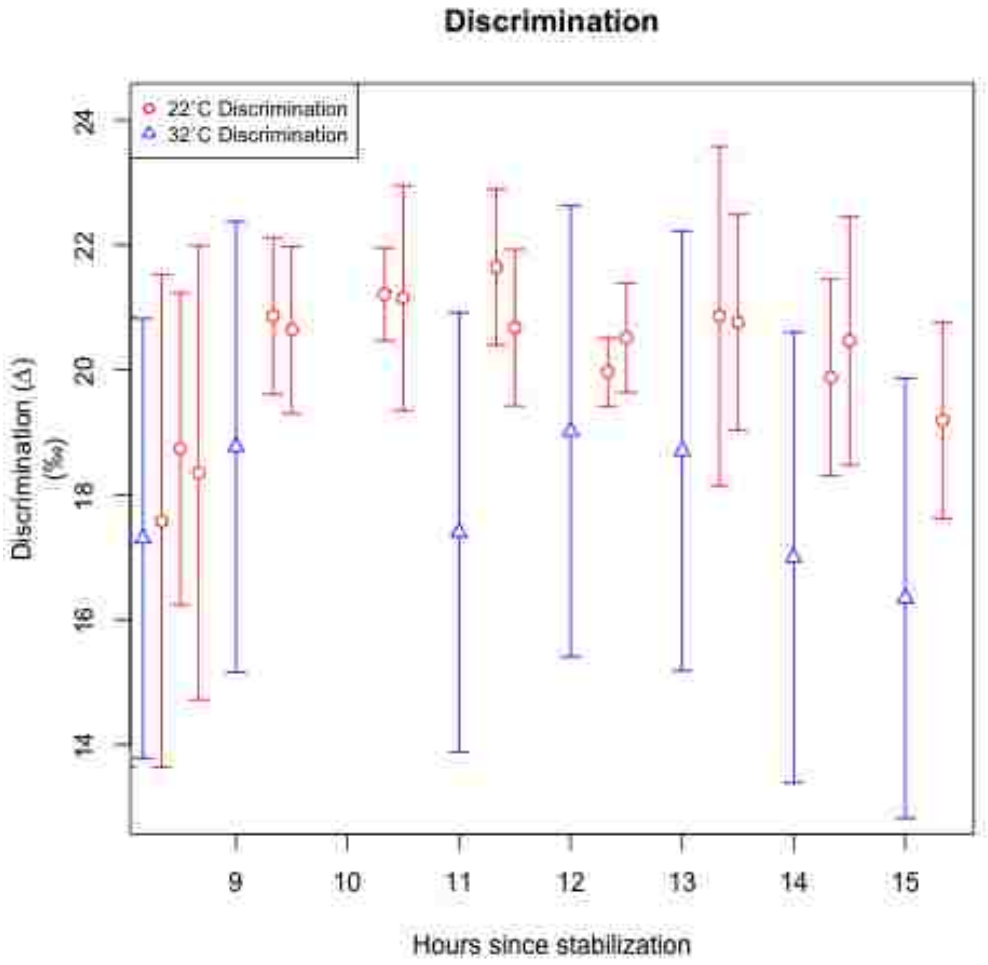


Figure 7: Comparison of discrimination to the ratio of intercellular $CO_2$ and atmospheric $CO_2$. Plants were grown and measured at 22˚C (circles) and 32˚C (triangles).

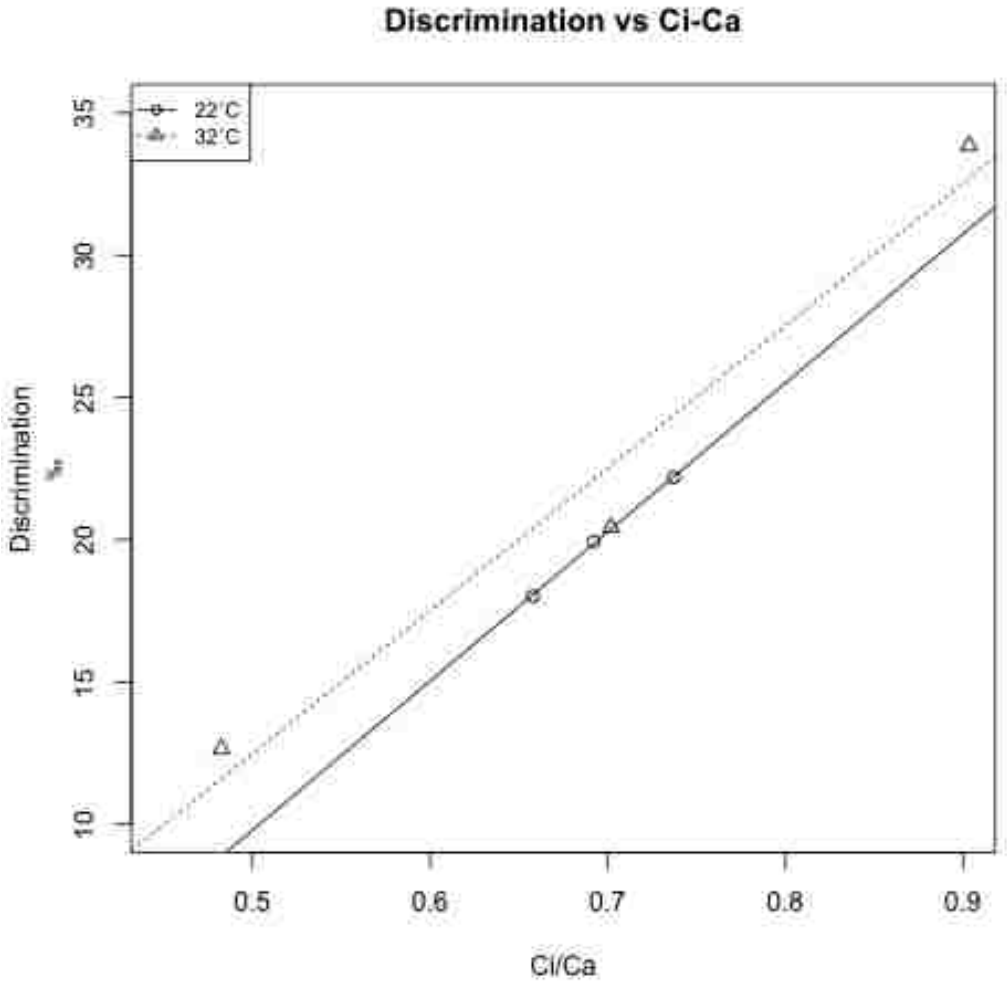


Figure 8: Leaf day respiration rates. Averages of leaf respiration rates of plants grown and measured at 22˚C (red) and 27˚C (blue) using the Laisk method and the R dark method. Error bars indicate the standard deviations. n=3 for both temperatures.

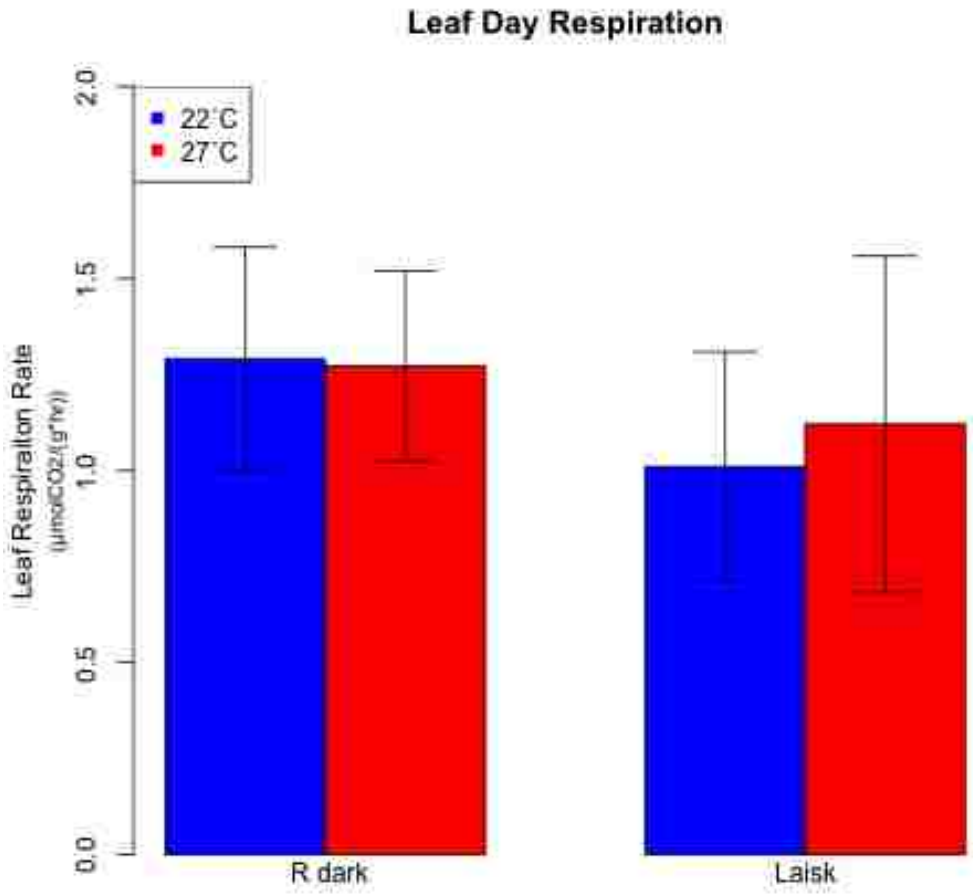


Figure 9: $\delta^{13}C$ of assimilation. Averages of the $\delta^{13}C$ of assimilation of plants grown and measured at 22˚C (red circles) and 32˚C (blue triangles). Error bars indicate the standard deviations. n=3 for both temperatures.

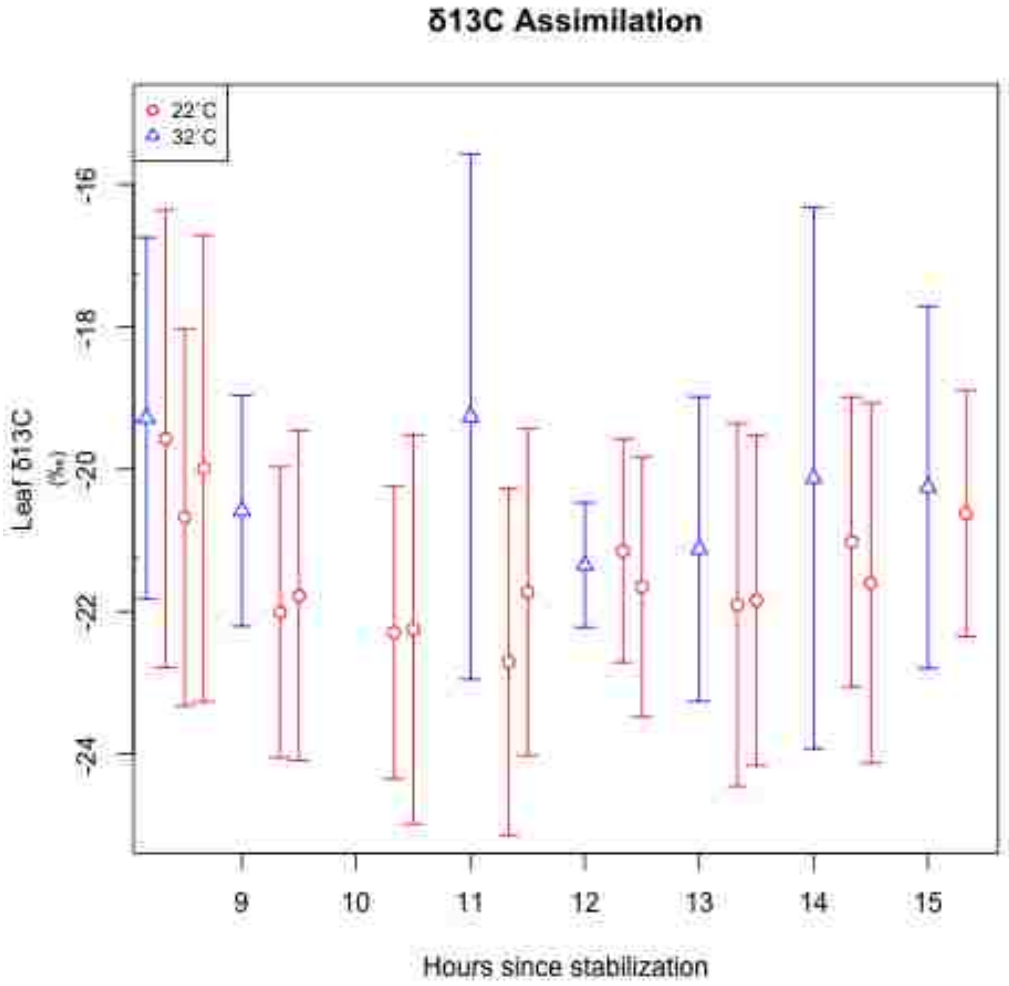


Figure 10: Plant tissue nitrogen content. Averages of nitrogen content of plants grown and measured at 22˚C and 32˚C. Error bars indicate the standard deviations. n=3 for both temperatures. The * indicates a significant difference between stems and roots for plants grown at 32˚C (p=0.03).

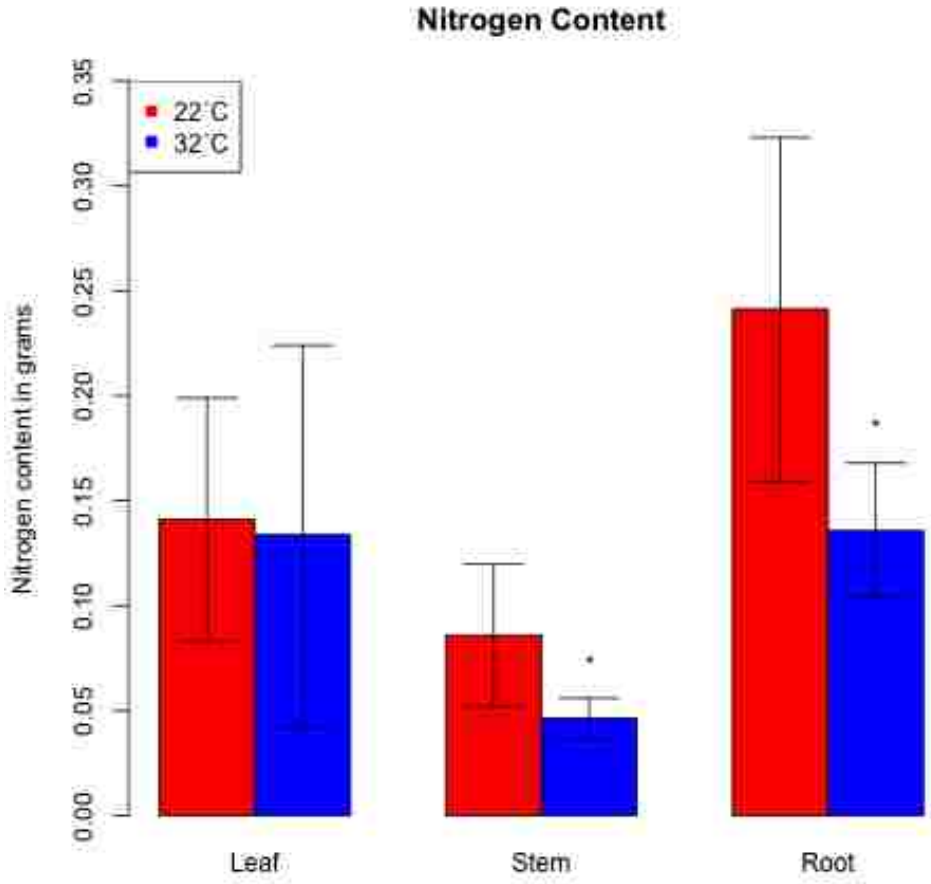


Figure 11: Plant tissue mass. Averages of dry weight of plants grown and measured at 22˚C and 32˚C. Error bars indicate the standard deviations. n=3 for both temperatures.

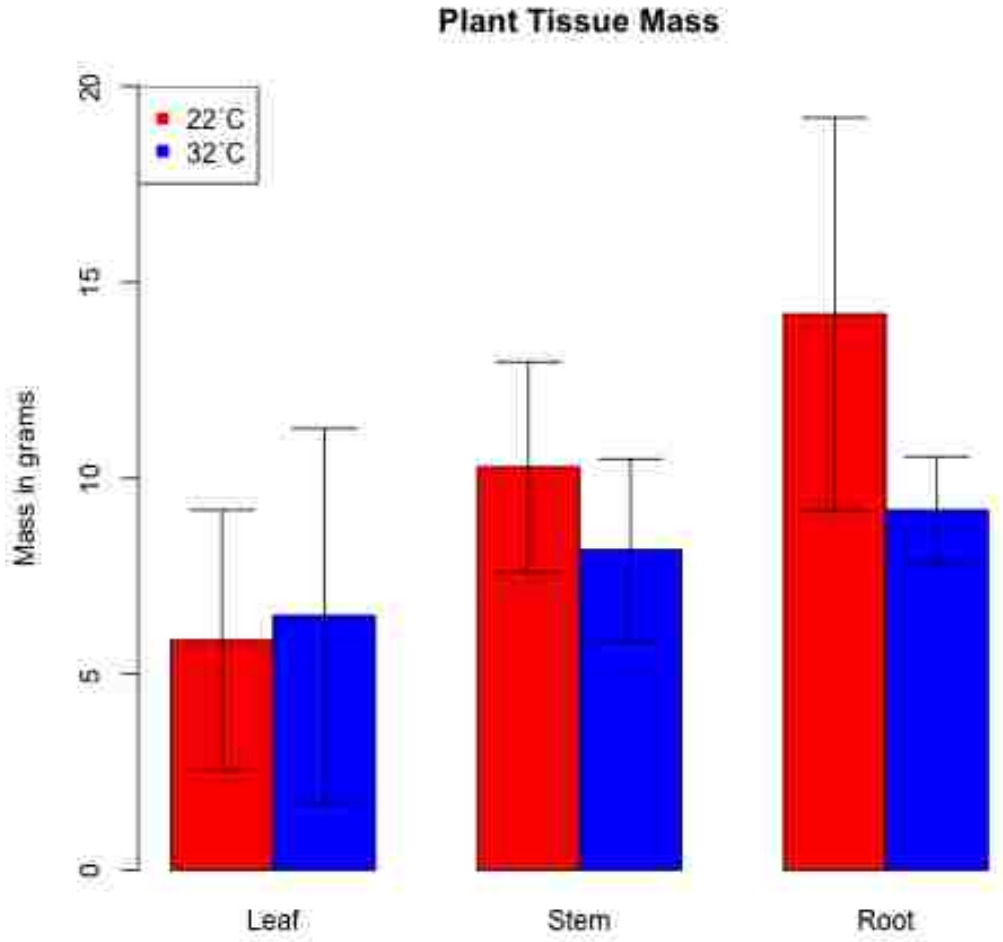


Table 1: Effect of $Q_{10}$ temperature correction. A $Q_{10}$ correction of 1.3 was applied to the data to normalize the respiration rates to their growth temperature (22˚C or 32˚C). Data shown are the means ± the standard deviation (n=3).

| | Raw Root Respiration Rate ($\mu$mol $CO_2$ $g^{-1}$ $hr^{-1}$) | $Q_{10}$ Corrected Rate ($\mu$mol $CO_2$ $g^{-1}$ $hr^{-1}$) |
|---|---|---|
| 22˚C Day | 38.5±11.7 | 34.6±10.0 |
| 22˚C Night | 25.0±12.5 | 26.9±14.9 |
| 32˚C Day | 48.2±3.7 | 42.7±4.6 |
| 32˚C Night | 33.9±5.3 | 39.8±9.4 |

Table 2: Photosynthesis, Conductance and Mesophyll Conductance. Data shown are mean±sd (n=3). An * indicates a significance of $p<0.05$. Note: the units for photosynthesis are both per area and per gram. The higher rate at 22˚C for the per area measurement is due to differences in specific leaf area.

| | Photosynthesis ($\mu$mol $CO_2$ $g^{-1}$ $hr^{-1}$) | Photosynthesis ($\mu$mol $CO_2$ $m^{-2}$ $sec^{-1}$) | Conductance ($\mu$mol $CO_2$ $m^{-2}$ $sec^{-1}$) | Mesophyll Conductance ($\mu$mol $CO_2$ $m^{-2}$ $sec^{-1}$ $Pa^{-1}$) | Specific Leaf Area ($m^2$ $kg^{-1}$) |
|---|---|---|---|---|---|
| 22˚C Morning | 61.3±3.0* | 13.9±0.2* | 0.3±0.09 | 6.6±4.5 | 1.7 |
| 22˚C Afternoon | 86.6±5.7 | 14.5±1.0** | 0.2±0.04* | 7.3±3.6 | -- |
| 32˚C Morning | 154.4±44.5* | 8.4±2.3* | 0.2±0.09 | 2.5±0.3 | 5.1 |
| 32˚C Afternoon | 103.4±60.4 | 5.6±3.3** | 0.09±0.06* | 1.9±1.3 | -- |
| p-value | 0.03 | *0.05; **0.03 | 0.04 | -- | -- |

Table 3: Isotopic Signature/Composition. The $^{13}C$ content of plants grown at 22˚C and 32˚C was measured. * indicates a statistically significant difference between temperatures.

| | Leaf Assimilation (‰) | Leaf $\delta^{13}C$ (‰) | Stem $\delta^{13}C$ (‰) | Root $\delta^{13}C$ (‰) | Root Respiration (‰) |
|---|---|---|---|---|---|
| 22˚C Day | -21.31±2.28 | -31.48±0.15 | -30.57±0.21* | -30.30±0.83 | -30.12±0.95 |
| 22˚C Night | NA | NA | NA | NA | -26.83±6.12 |
| 32˚C Day | -20.26±2.54 | -31.58±0.97 | -31.76±0.18* | -30.85±0.76 | -29.94±1.98 |
| 32˚C Night | NA | NA | NA | NA | -30.93±1.36 |
| p-value | 0.99 | 0.87 | 0.002 | 0.44 | >0.5 |

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