

# Quantifying the Impact of Nocturnal Warming on Whole-Plant Water Use Efficiency in *Glycine max*

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## Abstract

As climate change occurs and resource limitations increase, scientists must improve our understanding of agricultural systems and their response to environmental shifts to ensure farmers maximize crop yields while using the least amount of water possible. Water use efficiency (WUE) – the crop biomass gained per unit water lost – is becoming increasingly important as irrigation continues to require greater amounts of the world's limited fresh water supply. In this study, we investigated whether WUE in soybean (*Glycine max*) was altered in response to elevated nighttime temperatures (15°C vs. 19°C). We found no significant difference in WUE between the control and treatment groups, nor were there differences in respiration rate or stomatal conductance to H<sub>2</sub>O between groups. The elevated temperature group did have significantly higher water usage (3645 ± 173 mL/plant) than the control group (3025 ± 105 mL/plant; P = 0.005), but the potential difference in WUE was mitigated by a non-significant trend whereby the warmer-grown plants were generally larger (5.43 ± X. X g) than the control plants (4.51 ± X. X g). Further analysis revealed that the control group exhibited a marginally significant increase in V<sub>cmax</sub> (P = 0.064) when compared with the elevated temperature group, indicating different carbon-gain patterns may have occurred in the two groups that were undetected in other measurements. Additional studies are needed to determine how differences in night temperature impact photosynthetic capacity and plant carbon gain.

## Introduction

### Definitions

- WUE: The crop biomass gained per unit water lost.
- Transpiration: The passage of water to the atmosphere from plant tissues

### Water Use

- In most agricultural systems around the world, water is the limiting factor in plant growth and yield (1, 2).
- Crop irrigation requires approximately 80% of the world's useable water supply (3).

### Interacting Processes

- Crop systems lose water via transpiration and soil evaporation, so decreasing these processes can lead to an increase WUE (3).
- Maximizing the amount of water taken up by plants can decrease evaporative losses, leading to an increase in WUE.
- In C<sub>3</sub> plants like soybean, increased temperatures lead to increased transpiration and photosynthetic rates, but transpiration rates increase faster than photosynthetic rates, leading to a net decrease in WUE (4).

## Methods

Soybeans (*Glycine max*, n=48) were grown in one-gallon pots for 43 days in plant growth chambers (Percival) programmed with a 14-hour day/10-hour night cycle. Growing medium (Metro-Mix 360) was fertilized regularly; a thin layer of gravel was added to the soil surface to minimize soil evaporation after germination. Day temperature (25°C) and light intensity (600 μmol m<sup>-2</sup>s<sup>-1</sup>) were held constant in both chambers. Nighttime temperature was held at 15°C in the control chamber and elevated to 19°C in the treatment chamber. Humidity was adjusted to provide a similar vapor pressure deficit in both darkened chambers. Plants were watered daily with known quantities of water and daily water use was recorded by collecting and measuring pot runoff.

Leaf gas exchange traits, including photosynthetic light response curves, photosynthetic CO<sub>2</sub> response curves, and nocturnal respiration and stomatal conductance were measured using a portable photosynthesis system (LiCor 6400). All plants (except 4B and 7B, which never grew) were harvested at Day 43. One leaf was collected from each of ten plants per chamber (20 leaves total) for leaf area scans using imaging software (ImageJ). Finally, all aboveground and belowground plant materials were dried in an oven at 70°C for 72 hours. The ratio of dried aboveground biomass to total water used by each plant was determined to assess plant water-use efficiency.

Two-sample T-tests were run using Minitab and R statistical software.

## Results

Warm-temperature plants used more water and tended to be larger than control plants.

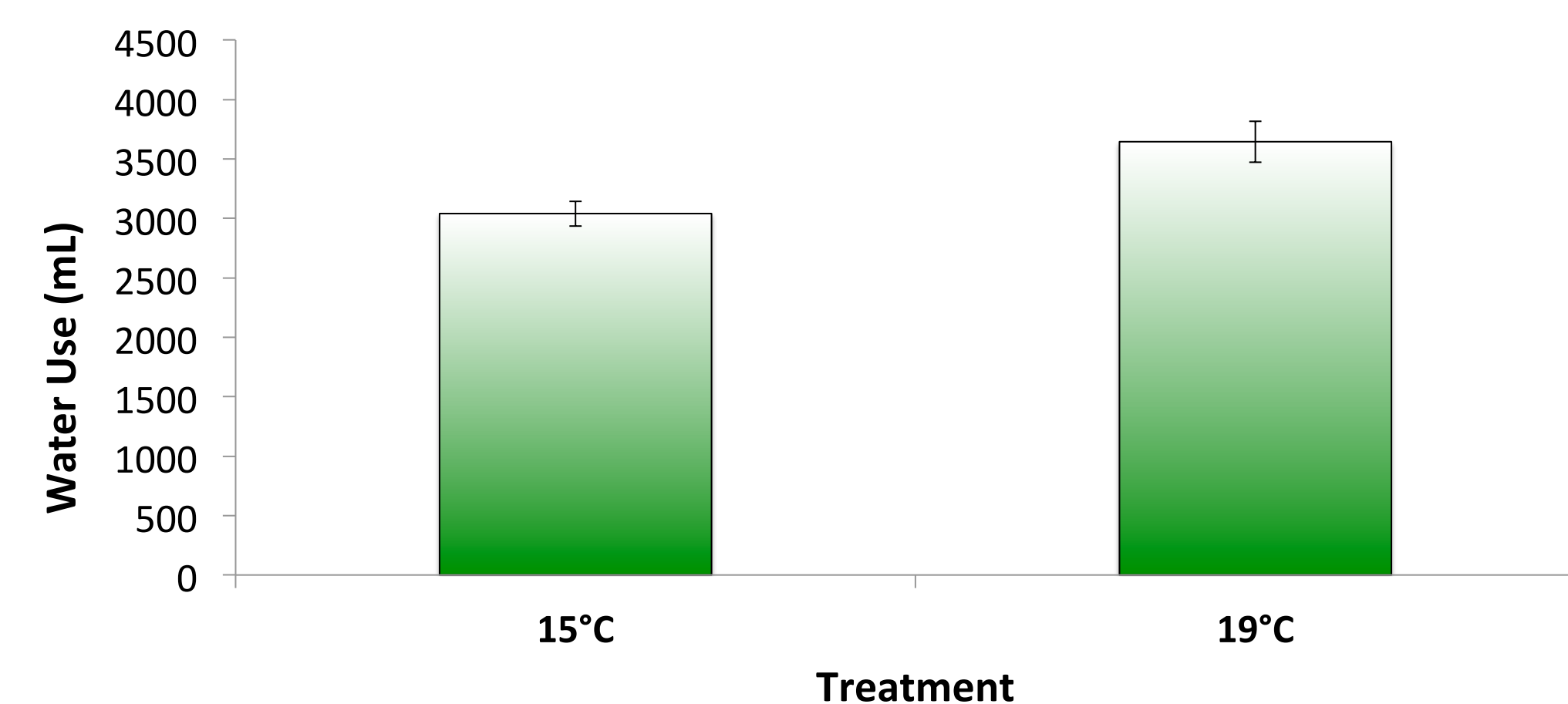


Figure 1. Mean water use of *G. max* plants grown in each treatment chamber. Water use was recorded each day for every individual from time of germination, which was approximately seven DAP (two-sample t-test, t=2.99, p=0.005, df=37, N<sub>15</sub>=22, N<sub>19</sub>=24). Error bars represent 1 SEM.

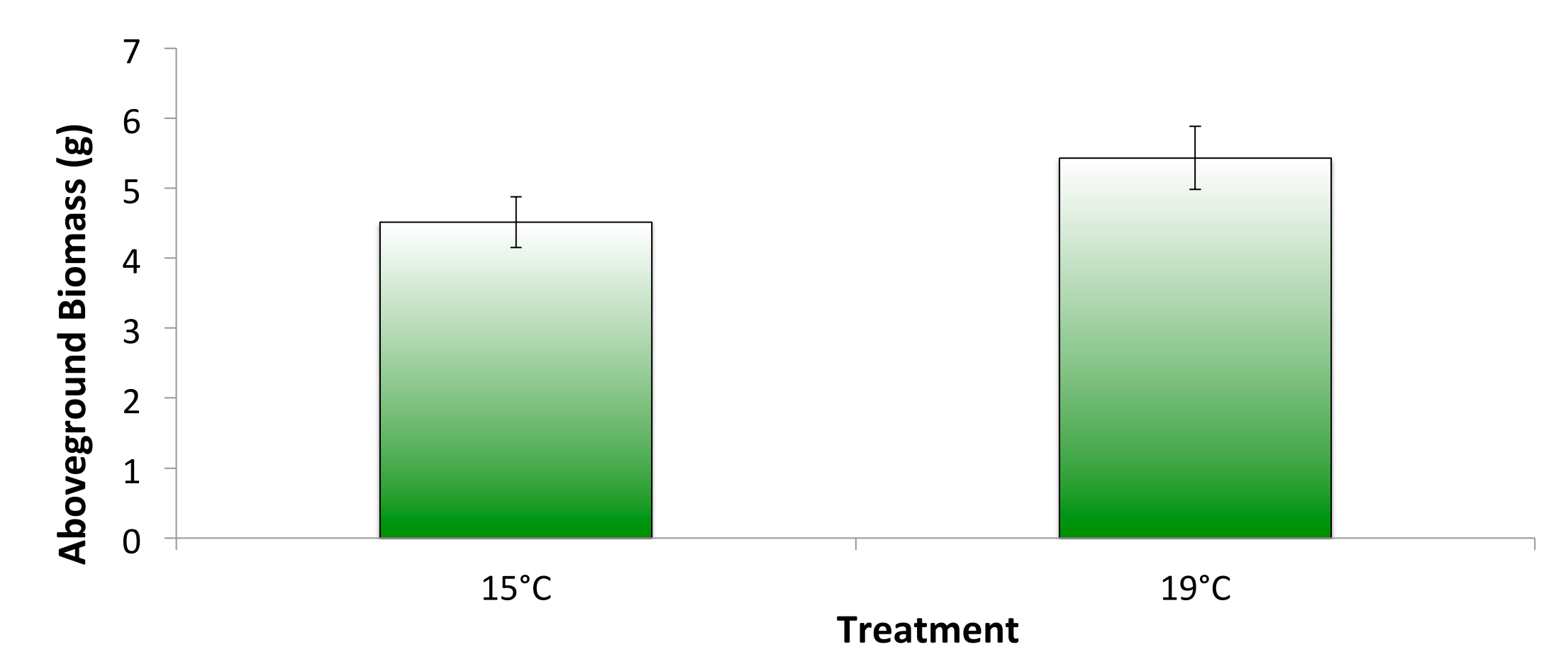


Figure 2. Mean aboveground biomass of soybeans grown in each treatment chamber. Plants were dried in a 70°C oven for 72 hours before weighing (two-sample t-test, t=1.58, p=0.122, df=42, N<sub>15</sub>=22, N<sub>19</sub>=24). Error bars represent 1 SEM.

Treatments showed no significant difference in WUE, but Vcmax did vary between treatment groups.

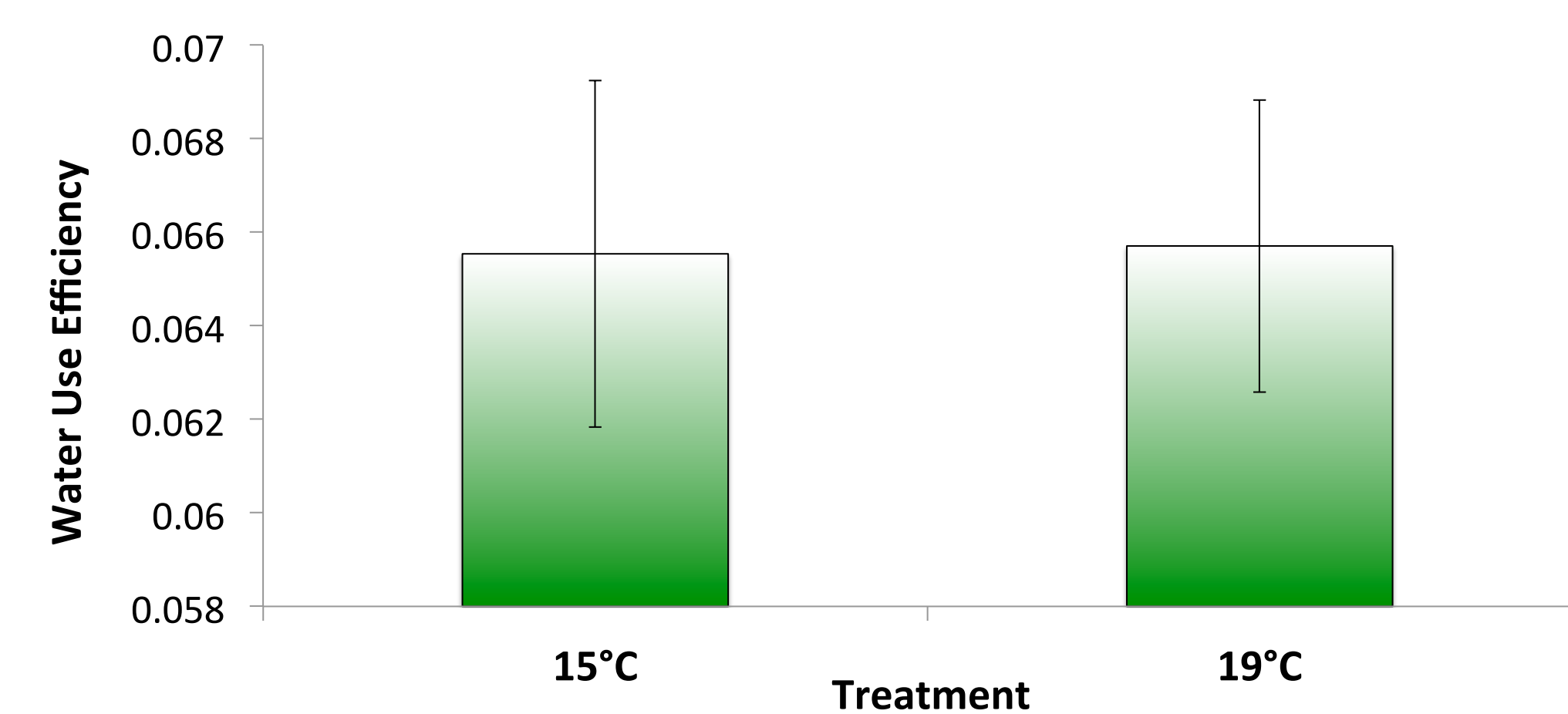


Figure 3. Average water use efficiency (WUE) of soybeans grown in each treatment chamber. WUE was calculated based on aboveground biomass of the plant, area of growing medium, and amount of water used throughout the treatment post-germination (two-sample t-test, t=0.43, p=0.672, df=38, N<sub>15</sub>=22, N<sub>19</sub>=24). Error bars represent 1 SEM.

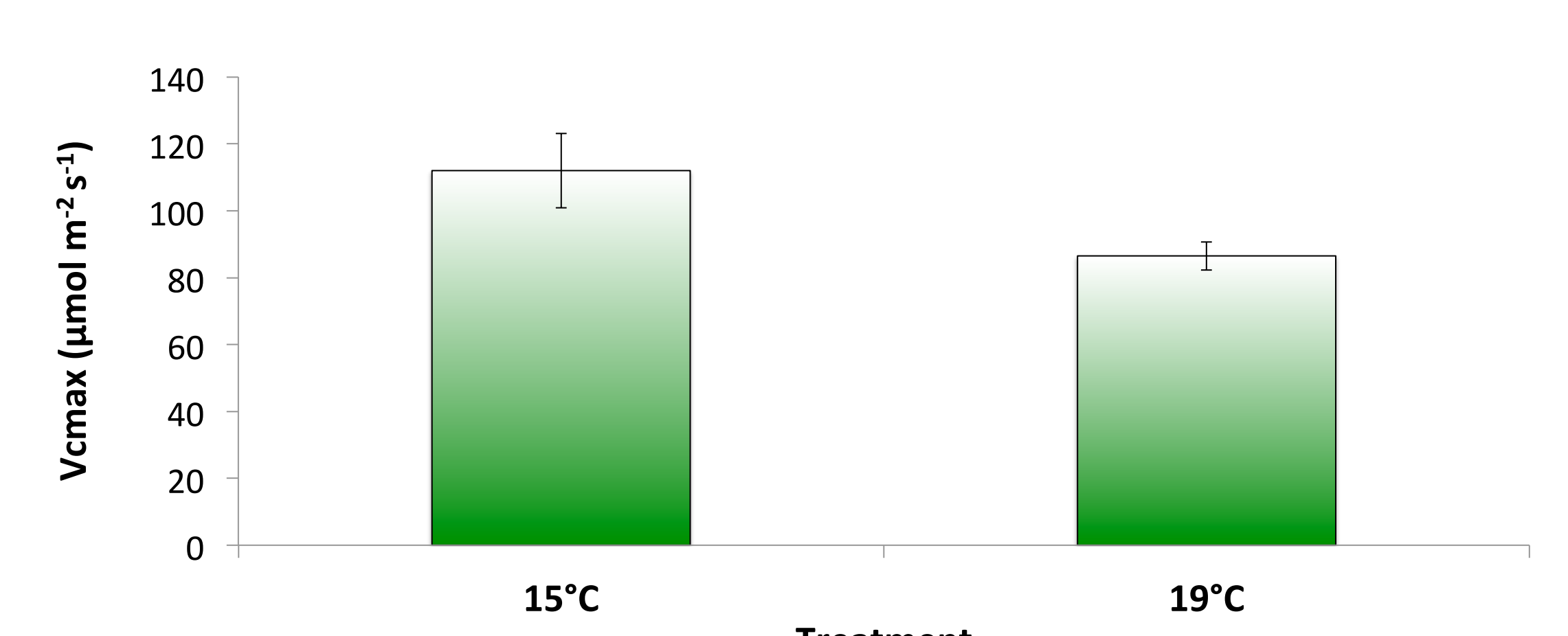


Figure 4. Average Vcmax of soybeans grown in each treatment chamber. Vcmax was calculated using an Aci curve fitting tool after Aci data were collected during the final week of plant growth (two-sample t-test, t=2.14, p=0.064, df=8, N<sub>15</sub>=8, N<sub>19</sub>=8). Instantaneous measurements of net photosynthetic rate were not different between groups (P = 0.56). Error bars represent 1 SEM.

When averages for each plant were used, respiration rate and stomatal conductance did not vary by treatment group.

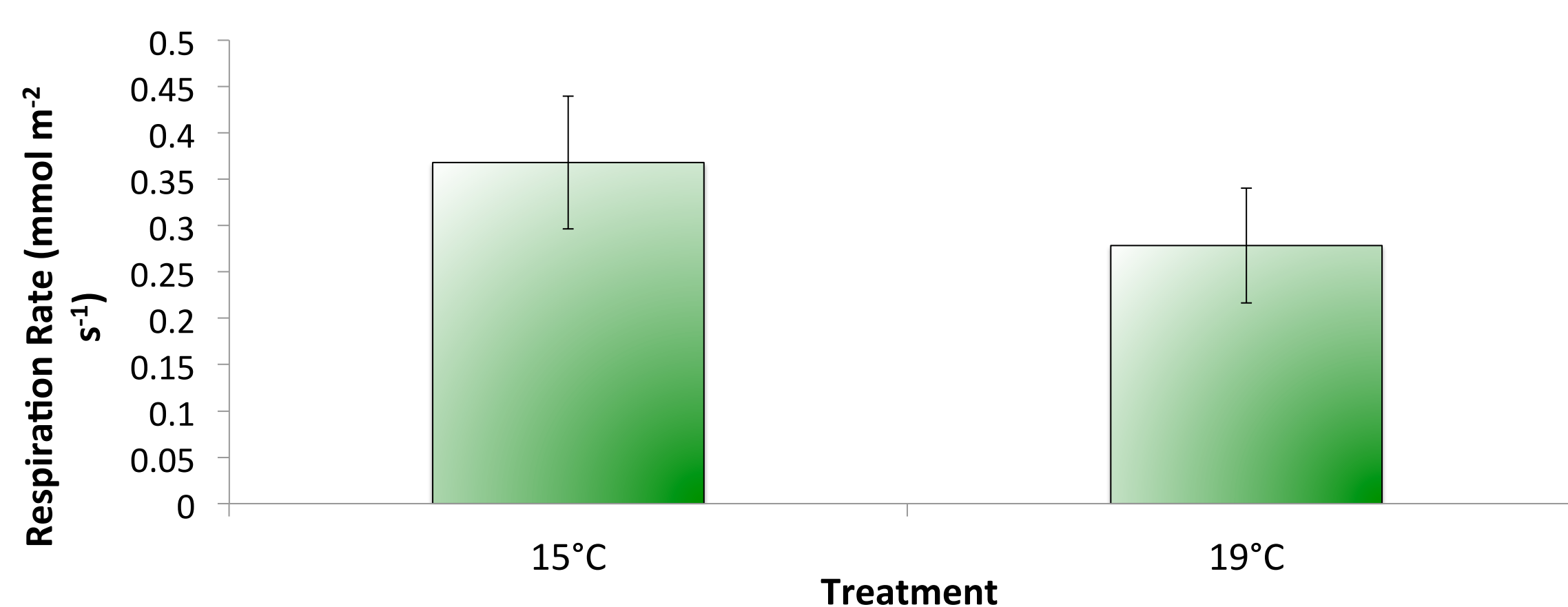


Figure 5. Average respiration of soybeans grown in each treatment chamber. Respiration data was collected on two consecutive nights during the final week of plant growth (two-sample t-test, t=0.946, p=0.36, df=19, N<sub>15</sub>=11, N<sub>19</sub>=11). Error bars represent 1 SEM.

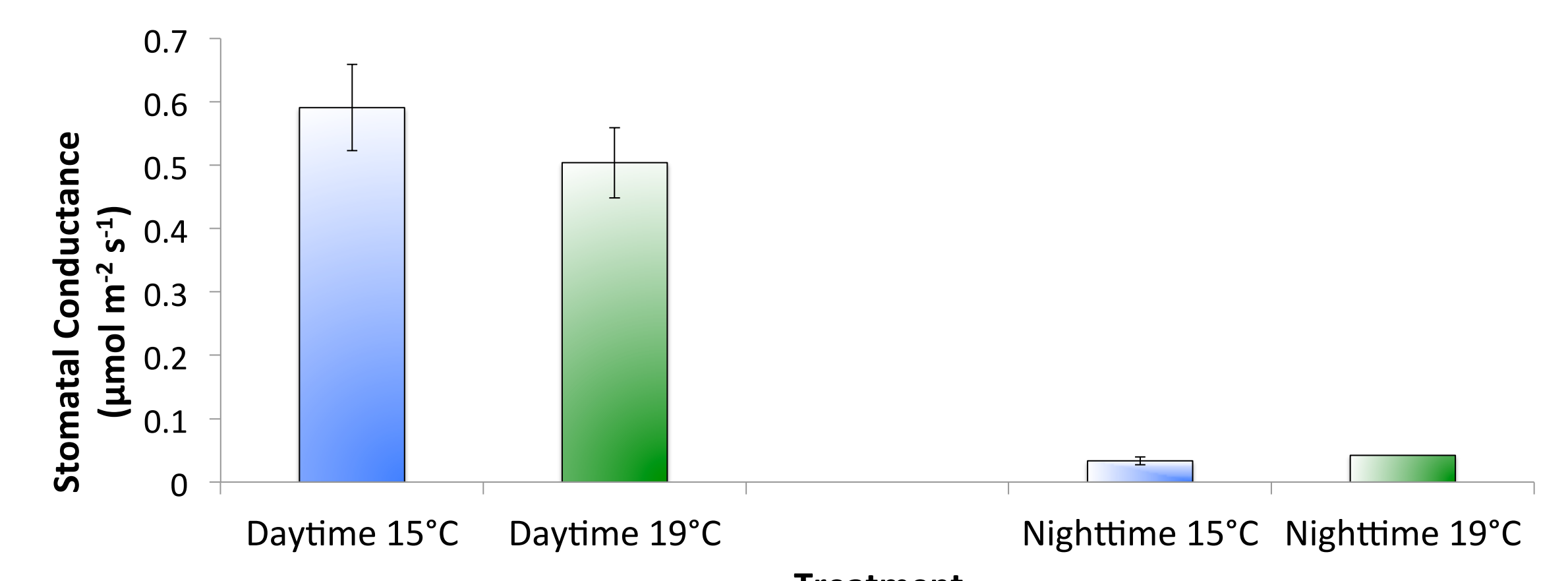


Figure 6. Average daytime and nighttime stomatal conductance of soybeans in each chamber. Data were collected on two consecutive days and nights during the final week of plant growth (two-sample t-test, t<sub>day</sub>=0.99, t<sub>night</sub>=0.667, p<sub>day</sub>=0.33, p<sub>night</sub>=0.51, df<sub>day</sub>=29, df<sub>night</sub>=15, N<sub>15</sub>=27, N<sub>19</sub>=27). Error bars represent 1 SEM.

## Conclusions

- Elevated nocturnal temperature did not have a significant effect on water use efficiency. Warm chamber plants tended to have greater aboveground biomass, but they also used more water than the control plants. These two increases cancelled each other out in terms of overall water use efficiency.
- Vcmax differed significantly between treatment groups, indicating varying photosynthetic capacity between groups. Differences in CO<sub>2</sub> concentrations in the two chambers may have impacted the Vcmax of the plants.
- There were no significant differences in respiratory carbon losses between the two groups, despite relatively large sample sizes.
- Both daytime and nighttime measurements revealed no significant difference in stomatal conductance between treatment groups. Because stomatal conductance regulates water loss, these results align with the observed trend in water-use efficiency.

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