A thesis

Entitled

Effect of CO$_2$ on the response of C and N relations to a heat wave in sunflower and corn

By

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Submitted as partial fulfillment of the requirements for

The Master of Science in Biology

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August 2008
Heat-wave events have been increasing in recent decades, and more frequent, intense, and longer-lasting heat waves are predicted for the high-CO$_2$ warmer world of the future. Little is known about how heat-wave events impact carbon and nitrogen relations in plants under elevated CO$_2$. I investigated the effect of 5-day-long heat wave (10°C above the normal daytime growth temperature) on gas-exchange, growth, and carbon and nitrogen relations in various tissues (i.e., new leaf, mature leaf, old leaf, stem, and root) of sunflower and corn grown under both current and elevated CO$_2$.

- In sunflower, heat wave (~ 4hrs) initially had a negative impact on net photosynthesis ($P_n$) under low, but not high, CO$_2$, highlighting the protective effect of elevated CO$_2$ on $P_n$ during high-temperature events. In corn, however, the heat wave initially stimulated $P_n$ under both CO$_2$ concentrations. The heat
wave increased respiration rate (R) in corn under elevated CO₂, but did not affect 
R under low CO₂ in corn and under both the CO₂ concentrations in sunflower. But 
Pₙ (corn) and R (corn and sunflower) were not affected by the heat wave on day 5 
under both the CO₂ concentrations in both the species.

- The heat wave did not affect total biomass in either species, or root/shoot (R/S) 
ratio in corn, under both the CO₂ concentrations. But in sunflower, the heat wave 
increased R/S under elevated CO₂. These alterations of Pₙ, R and R/S by heat 
wave or CO₂ may alter the C cycling.

- In sunflower, the heat wave tended to increase %N in its tissues under both the 
CO₂ concentrations, but five days after termination of heat wave, %N was 
reduced in tissues of heated plants under high CO₂. In corn, the heat wave 
increased %N in shoots under elevated CO₂ and decreased %N in all the tissues 
except old leaves under low CO₂ by the end of heat wave, but the effects 
disappeared 5 days after termination of the heat wave.

- In sunflower, the heat wave increased %C in roots under both the CO₂ 
concentrations, and decreased %C in roots and mature leaves under elevated CO₂ 
five days after heat-wave termination. In corn, the heat wave increased and 
decreased %C in tissues under low and high CO₂, respectively, by the end of the 
heat wave. Five days after termination of the heat wave, the tissues of heated 
plants had higher %C under both the CO₂ concentrations in corn.

- In sunflower, heat wave tended to decrease C:N ratio in shoots by the end of heat 
wave under both the CO₂ concentrations, but the heat wave tended to increase 
C:N ratio in shoots five days after termination of heat wave under high CO₂. In
corn, heat wave decreased C:N ratio in shoots under high CO₂, but tended to increase C:N ratio in all the plant parts except old leaves under low CO₂ by the end of the heat wave.

- These alterations of %N and C:N ratio in plant tissues by heat wave under both current and elevated CO₂ concentrations may impact plant-herbivore interactions, since herbivores are often affected by N concentration and C:N ratio of the tissues they consumes.
Dedicated to

My parents
Acknowledgements

I am deeply indebted to my advisor, Dr. Scott A Heckathorn, for his assistance and guidance during this study. He always encouraged me think creatively and whetted my interest in doing science.

I highly appreciate the advice received from graduate committee: Dr. John Gray and Dr. Jonathan M Frantz. Their advice has helped me to make this thesis better.

I also acknowledge the help received from Dan Wang, my lab mate, for her help during this work, especially for statistical analysis. Dr. Sasmita Mishra and Kumar Mainali, my other lab mates, also helped me.

My sincere thanks goes to my fellow graduate student, Rachel Henderson, who taught me how to use CHN analyzer.

I am also thankful to Donna Braswell for her help in purchasing necessary materials for this study.

Last but not least, I acknowledge my wife, Anita Sigdel, for her moral support, a huge source of strength to me.
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INTRODUCTION

What is a heat wave?

A typical definition of a “heat wave” is a short (a few days) period of higher-than-normal temperatures that result in human injury or death and damage to vegetation (Hunt, 2007). Heat waves will be associated with future increases in global mean temperatures caused by increases in greenhouse gases (IPCC, 2001). The increased concentration of atmospheric CO$_2$ is the major contributor to this increasing temperature, though other greenhouses gases also have been playing a part. The concentration of CO$_2$ has increased at an unprecedented rate from 280 ppm in pre-industrial times to 379 ppm in 2005. With the increase in greenhouse gases, the global mean surface temperature also increased in the 20$^{th}$ century by 0.6±0.2$^\circ$C (IPCC, 2007). Along with this increase in mean surface temperature, the frequencies and intensities of extreme temperature events like heat waves has increased in the major land masses; e.g., Southern and West Africa (New et al., 2006), Europe (Della-Marta et al., 2007), Central and South Asia (Klein Tank et al., 2006), Northeast United States (Griffiths and Bradley, 2007), and Australia (Alexander et al., 2007). It has been projected that the CO$_2$ concentration may rise to between 540 and 970 ppm by 2100, causing a rise in temperature of 1.4 to over 5$^\circ$C by the end of 21st century. Since a small increase in mean temperature can increase the duration, frequency,
and severity of heat-wave events significantly (Karl et al., 1997), along with this increase in mean temperature, heat waves will be more likely and the rise in temperature will be especially at night (IPCC, 2001, 2007). The most-cited heat-wave events of recent decade are the 2003 heat wave of Europe, which caused thousands of human deaths (WMO, 2003), and the 1995 heat wave of the United States, which caused hundreds of death in the Chicago area (Palecki et al., 2001).

**Heat wave and plant performance**

According to the estimation of Ciais et al. (2005), the European heat wave, in combination with drought, caused a 30% reduction in plant productivity and substantially reduced crop yield (e.g., 36% reduction of corn in Italy) over Europe. This event eliminated some types of vegetation, encouraged forest fires (UNEP, 2004), and reduced species richness (Penuelas et al., 2007). This provided evidence that future increases in heat waves will cause severe damage to plants in different parts of the world. Furthermore, heat waves can differentially impact co-occurring plant species in a community, which can cause substantial shifts in the community by altering existing competitive balances between species (Marchand et al., 2006a, 2006b). So, understanding plant responses to heat wave, alone and in combination with other climate change factors (e.g., elevated CO₂, drought), will be critical to set strategies for adaptation and mitigation of climate-change impact.

Many of the past studies that looked at the impact of high temperature on plants have focused on its effect on photosynthesis, since photosynthesis is one of the most heat-sensitive physiological processes (Berry and Björkman, 1980; Weis and Berry, 1988).
Extreme high temperature has a negative impact on photosynthesis in both $C_3$ (Haldiman and Feller, 2004; Crafts-Brandner and Salvucci, 2000; Sharkey et al., 2001) and $C_4$ (Crafts-Brandner and Salvucci, 2002) plants. The effect of high temperature on net photosynthesis may vary within genotype (Camejo et al., 2005). A gradual increase in temperature during heat stress treatment had a less severe impact on net photosynthesis than a rapid increase in temperature in the $C_4$ plant, corn (Crafts-Brandner and Salvucci, 2002), but both gradual and rapid increases in temperature equally impacted *Quercus pubescens* ($C_3$) (Haldiman and Feller, 2004). Elevated CO$_2$ can ameliorate the negative effect of extreme high temperature in $C_3$ plants (Faria et al., 1996; Ferris et al., 1998; Faria et al., 1999; Hamerlynck et al., 2000; Huxman et al., 1998; Taub et al., 2000; Haldiman and Feller, 2004; Wang et al., 2008). But CO$_2$ can have negative impact on photosynthesis of $C_4$ and CAM plants during heat stress (Wang et al., 2008). Also, photosynthetic recovery from heat shock is faster in elevated CO$_2$ in $C_3$ plants (Faria et al., 1996).

Even if heat waves are not longer-lasting, high-temperature events can have a negative impact on plant growth if temperature is high enough to substantially damage physiology. For example, the application of a high-temperature event (23°C higher than the growth temperature) during anthesis reduced biomass by 40% in wheat ($C_3$) (Wollenweber et al., 2003). Similarly, Bassow et al. (1994) reported a significant decrease in biomass in plants heat-treated for only one day at 45°C (gray birch, striped maple, and yellow birch, all $C_3$), when the plants were measured 35 days after heat treatment. Even 105 days after heat treatment, the heated plants were significantly smaller than untreated controls. Shoots seem more sensitive than roots to high temperature, since roots growth was inhibited at a
higher temperature (40°C) than shoot growth (35°C) in C₃ cotton plant (Crafts-Brander and Salvucci, 2004). In the study of Bassow et al. (1994), the damage by the heat treatment was more severe in plants grown under elevated CO₂. However, Roden and Ball (1996) found greater biomass under elevated CO₂ in two species of *Eucalyptus* (C₃) grown at 45°C. The plants in the former study (Bassow et al., 1994), were severely stressed by heat, but in later study (Roden and Ball, 1996), plants were not severely stressed. From the two studies, it can be hypothesized that CO₂ can have a negative impact on plants if heat stress is substantial, but CO₂ can have a positive role in decreasing stress when the temperature is high. Heat shock (14-23°C above growth temperature) significantly retarded growth and reduced total biomass in both C₃ (*Abutilon theophrasti*) and C₄ (*Amaranthus retroflexus*) plants grown at elevated as well as ambient CO₂ condition (Coleman et al., 1991).

Most of the past studies cited above applied short-term (< 4 hr) acute heat stress, and only a few studies have tested the effect of more-moderate longer-term heat stress; *e.g.*, simulating natural multi-day heat wave (9 days by Huxman et. al., 1998 and Hamerlynck et al., 2000; 8 days by Ferris et al., 1998). Also, past studies looking at the impact of heat stress alone (Bassow et al., 1994; Crafts-Brander and Salvucci, 2004), and heat as well as elevated CO₂ (Wollenweber et al., 2003; Coleman et al., 1991), on growth are rare, and these previous studies have looked at the effect of short-term severe heat stress. To my knowledge, no one has studied the impact of long-term moderate heat wave and its interaction with CO₂ on biomass allocation (*e.g.*, between root and shoot). Only one study (Wollenweber et al., 2003) has investigated the impact of high temperature on nitrogen concentration in leaves under ambient CO₂ and they reported that the extreme
temperature event of 35°C, which is 23°C higher than the ambient temperature, reduced leaf [nitrogen] in wheat (C₃). No study has looked at the impact of high temperature events on C or N allocation to different parts (e.g., new leaf, mature leaf, old leaf, stem, and root) of a plant during heat treatment under both present and elevated CO₂ concentration. Whether the impact of heat waves on C and N allocation in various parts of the plant differ between C₃ and C₄ plants is not known. If heat wave changes tissue N concentration, C:N ratio, or alters C and N allocation in the plant, it will affect plant-herbivore interactions, since performance of herbivores is often determined by N concentration and C:N ratio of the plant part consumed (Stiling and Cornelissen, 2007). Also, it is highly likely that any heat-induced change in plant N concentration may persist even in the litter, and this would alter nutrient dynamics, since decomposition rates depend on C:N ratio of degrading tissue.

**Objectives and hypothesis of the present study**

I investigated the impact of CO₂ on carbon and nitrogen relations of representative C₃ and C₄ plants during heat-wave events, analyzing photosynthesis, respiration, biomass, and carbon and nitrogen content of various tissues of sunflower (C₃) and corn (C₄) after a long-term heat wave (5 days, 10°C above normal daytime temperature) of plants grown under both current and elevated CO₂ concentrations. I tested the following hypotheses:

- Heat waves reduce tissue N and increase tissue C concentration, respectively, which increases C:N ratio under both current and elevated CO₂ concentrations (as heat wave impairs the physiology of plants, it is likely that N absorption capacity of the plants decreases causing a decrease in N content).
• The impact of CO₂ on tissue C and N concentration in heat-treated plants will be greater in C₃ plants than in C₄ (as C₃ plants are more responsive to CO₂).

• The elevated CO₂ increases and decreases the thermotolerance of net photosynthesis (Pₙ) in C₃ and C₄ plants, respectively, during long-term moderate heat wave, as found by Wang et al. (2008) in short-term acute heat stress.

• In both C₃ and C₄ plants, heat wave reduces total biomass, as heat negatively impacts Pₙ.

• Root/shoot is increased by heat wave in both C₃ and C₄ plants, as shoot growth is more sensitive than root growth at high temperature (Crafts-Brandner and Salvucci, 2004).
MATERIALS AND METHODS

Plant materials and growing conditions

*Helianthus annus* L. (sunflower) and *Zea mays* L. (corn) were selected in this experiment because both species are warm-season temperate annuals, represent both C₃ and C₄ photosynthetic types (*i.e.*, sunflower, C₃ and corn, C₄), and the species are widely used as models in plant biology. The seeds of these plants were sown in pots filled with top soil, perlite, and sand in the ratio of 7:1:1 (v:v:v). The pots were 15cm (h) x 12.5cm (d) for sunflower and 30cm (h) x 10cm (d) for corn. Plants were grown from seed in growth chambers having temperature, light, and CO₂ control (E-36HO, Percival Scientific, Iowa, USA). Both species were grown at a day and night temperature of 30°C and 25°C, respectively. The light and dark periods were 14 and 10 hrs, and light intensity was 1050-1100 µmol m⁻² s⁻¹ PAR (photosynthetically active radiation) at the height of the top leaf of the plants. For each species, one group of plants was grown under ambient CO₂ (370 ppm) and the other under elevated CO₂ (700 ppm). The plants were irrigated every day to prevent water stress. Ten days after sowing the seeds, each plant was fertilized with 100ml of half-strength Hoagland’s nutrient solution, and fertilized every two days thereafter. To minimize position effects, plants were moved daily in the growth
chambers. To maintain constant light intensity at the top of the plants, the plants were moved to a lower position in the chambers weekly.

**Figure 1.** Sunflower plants, before harvesting at “day 10”. The left two rows of plants were grown under ambient CO₂, whereas the right two rows were grown under elevated CO₂.

**Figure 2.** Corn plants, before harvesting at “day 10”. The left two rows of plants were grown under ambient CO₂, whereas the right two rows were grown under elevated CO₂.
Heat-wave treatments

From each of the CO2 growth conditions (i.e., 370 ppm and 700 ppm of CO2), half of the plants were randomly selected and transferred to separate growth chambers, supplied with their respective CO2 concentration, for a heat-wave treatment. The heat-wave treatment was imposed by increasing air temperature to 40°C during the daytime for five days. For each day of heat wave, plants received seven hrs of higher temperature starting at 8:30 and ending at 16:30 (Figure 3). After the 5-day-long heat wave, the plants were returned to normal growth temperatures and allowed to recover from the treatment for five days. Night-time temperature remained always the same.

Figure 3. Time-course of temperature during normal and heat-wave treatment days.

Gas exchange

Measurements of net photosynthesis ($P_n$), stomatal conductance to water vapor ($g_{st}$), intercellular CO2 concentration ($C_i$) and respiration rate ($R$) were made on leaves using a portable CO2/H2O gas exchange system (Li-6400, LICOR Inc., Lincoln, NE, USA) with
an infra-red gas analyzer (IRGA) and cuvette of 250 mm$^3$ (as in Wang et al., 2008). For the measurements, one plant at a time was taken from the growth chamber and measured immediately, following stabilization of gas fluxes (*i.e.*, within 1 min). Fully-expanded mature leaves were used for the measurements on day 1, and these same leaves were used again on day 5 and day 10 for all the treatments. One hour before the measurements, walls of the growth chambers were sprinkled with water to keep the chamber humid, and the plants watered to ensure that the plants were not water stressed at the time of the measurements. The cuvette temperature was set at 40°C while measuring heat-treated plants during heat-wave treatment (at day 1 and day 5), while other plants were measured with the cuvette temperature at 30°C. All the plants were measured at 30°C during recovery (day 10). Plants were measured at 370 ppm or 700 ppm CO$_2$ corresponding with their growth CO$_2$ concentration. All the measurements except respiration were made under 1500 µmol m$^{-2}$ s$^{-1}$ PAR. Respiration was measured without any light in the cuvette, after keeping the plants in the dark for half an hour, under their respective CO$_2$ and temperature conditions as in measurements of photosynthesis.

**Electron transport**

PSII efficiency ($F'_v/F'_m$) is very heat sensitive, so it can be a good index of early heat effects on physiology. $F'_v/F'_m$ in light-adapted leaves was measured using a pulse-amplitude-modulated (PAM) fluorometer (Model PAM 101/103, Walz, Germany) as in Wang et al. (2008). Basal chlorophyll fluorescence ($F'_s$) was measured under steady-state illumination of 900 µmol m$^{-2}$ s$^{-1}$ PAR, while maximum fluorescence ($F'_m$) was measured after a 1-second pulse of saturating light (> 5000 µmol m$^{-2}$ s$^{-1}$ PAR). After turning off the
sources of both actinic and flash light, minimum fluorescence ($F_{0}'$) was measured. The $F_{v}'/F_{m}'$ was calculated as in Genty et al. (1989): $F_{v}'/F_{m}' = (F_{m}' - F_{0}')/ F_{m}'$.

A fully-expanded mature leaf from each plant was used for the measurement of electron transport at day 1 (the first day of the heat-wave treatment), day 5 (the fifth day of the heat-wave treatment), and day 10 (5 days after termination of the heat-wave treatment). New developing leaves were measured only at day 10 for both sunflower and corn, to examine delayed effects of heat stress on leaves. New leaves were those leaves which were produced after the beginning of the heat-wave treatment and were still not mature on day 10.

**Plant harvesting**

Five plants from each treatment were harvested at day 0, day 5, and day 10. Above-ground plant parts were harvested and separated into four categories: new expanding leaves, mature fully-expanded leaves, old senescing leaves, and stems. Roots were washed with tap water after harvesting. Harvested plant tissues were dried at 70°C for 3-4 days, and then weighed.

**C and N analysis**

Corn tissue was first ground in a Wiley mill, and then further ground in liquid nitrogen using a mortar and pestle. For sunflower, due to the soft nature of its tissues, grinding in liquid nitrogen alone was sufficient. The ground tissues were homogenized prior to CN analysis. The percent of C and N in the samples was determined using a CHN analyzer (Perkins Elmer, Model 2400), following the manufacturer’s protocol.
Statistical analysis

Using Statistica software, two-way ANOVA, along with Tukey’s honest-significant-difference (HSD) test, was employed to test for significant effects of CO₂, temperature, and their interaction on all the parameters that were measured after beginning of the heat-wave treatment. For the parameters that were measured before heat-wave treatment, a two-sample t-test was applied using Microsoft Excel. All average values were derived from three to six independent replicates. A $P$ value of 0.05 or lower was considered significant. Separate ANOVAs were conducted for each time-point, in order to understand treatment effects for each sampling time.
RESULTS

Photosynthesis and respiration

In mature leaves of sunflower, photosystem II efficiency (Fv'/Fm') decreased during heat treatment, indicating damage to, or down-regulation of, PSII, and these decreases were greater under low CO2 (Figure 4). After 5 days of post-heat recovery (day 10), heat-treated sunflower plants had higher Fv'/Fm' than untreated plants in both mature and developing leaves (no CO2 effect). In mature leaves of corn, heat treatment initially decreased Fv'/Fm' (day 1, low CO2 plants), but then increased Fv'/Fm' (day 5), and high CO2 tended to increase Fv'/Fm' on both days (excluding unheated plants on day 1). During recovery from heat stress in corn, neither CO2 nor heat stress had any effect on Fv'/Fm' in either mature or developing leaves.

In sunflower, the heat wave initially reduced net photosynthesis (Pn) only in low CO2 plants (day 1, after 4 hrs of heat wave) and elevated CO2 increased Pn (only in heated plants), but after 5 days of heat wave, Pn was reduced by both heat wave and elevated CO2 (Figure 5). In corn, both heat wave and elevated CO2 increased Pn on day 1, but neither heat nor CO2 had significant effects on Pn on day 5. In both species, after five days of recovery from heat wave (day 10), the plants that experienced heat wave (both
Figure 4. Effect of heat wave on light-adapted Photosystem-II efficiency ($F_v'/F_m'$) in mature (fully-expanded) and new (developing) leaves of sunflower and corn. Day 1 = the first day of heat wave, day 5 = the fifth day of heat wave, and day 10 = 5 days after termination of heat wave. 370 and 700 are CO$_2$ concentrations in ppm. C = control, H = heat wave. Within each day, significant effects ($P \leq 0.05$) of CO$_2$, heat, and their interaction are indicated by x, xx, and xxx, respectively. For each day, significant differences among treatments are indicated by different letters. The error bars are standard errors of 3-5 replicates.
CO₂ levels) or unheated controls under elevated CO₂ had lower Pₙ than that of the unheated plants under low CO₂.

In sunflower, stomatal conductance (gₛₜₐₜ) was initially decreased by elevated CO₂ and increased by heat wave (day 1), but neither heat nor CO₂ had effects on gₛₜₐₜ on day 5 or 10 (Figure 5). gₛₜₐₜ also decreased rapidly with time in sunflower, as leaves aged. In corn, both heat wave and elevated CO₂ had small but significant effects on gₛₜₐₜ on all days. In particular, heat wave increased gₛₜₐₜ under elevated CO₂ (days 1 and 5), and heat and elevated CO₂ decreased gₛₜₐₜ on day 10.

In both sunflower and corn on all sampling days, leaf intercellular CO₂ concentration (Cᵢ) was increased under elevated CO₂, and heat wave effects were small except in corn grown at high CO₂ (wherein heat wave increased Cᵢ) (Figure 5).

Leaf respiration rate (R) in sunflower was not affected by the heat wave on any sampling day, but was substantially increased by elevated CO₂ on day 5 (Figure 5). In corn, R was also largely unaffected by heat wave, but was increased by elevated CO₂ on days 1 and 5, and decreased by elevated CO₂ on day 10.
Figure 5. Effect of heat wave on net photosynthesis ($P_n$), stomatal conductance ($g_{st}$), intercellular CO$_2$ concentration ($C_i$), and dark respiration ($R$) in mature (fully-expanded) leaves of sunflower and corn. Day 1 = the first day of heat wave, day 5 = the fifth day of heat wave, and day 10 = 5 days after termination of heat wave. 370 and 700 are CO$_2$ concentrations in ppm. C = control, H = heat wave. Within each day, significant effects ($P \leq 0.05$) of CO$_2$, heat, and their interaction are indicated by x, xx, and xxx, respectively. For each day, significant differences among treatments are indicated by different letters. The error bars are standard errors of 3-5 replicates.
Plant growth

As expected, sunflower had greater total, shoot, and root biomass under the elevated CO₂ than under ambient CO₂ concentration (Figure 6), at the beginning and end of the heat wave and after five days of post-heat recovery. In contrast, in corn, elevated CO₂ increased total and shoot mass only at the end of the heat wave and the recovery period. The heat wave had no effect on total biomass in either species under both of the CO₂ concentrations. In sunflower, shoot mass was decreased slightly by heat wave, and root mass was increased by heat in high CO₂. Heat had no effect on shoot or root mass in corn. In sunflower, the heat wave tended to increase root/shoot ratio (R/S) (at both day 5 and day 10), and elevated CO₂ increased R/S initially. There was no effect of heat wave on root/shoot ratio in corn, but elevated CO₂ decreased R/S.
Figure 6. Effect of heat wave on plant growth in sunflower and corn. Day 0 = 1 day before heat wave, day 5 = the fifth day of heat wave, and day 10 = 5 days after termination of heat wave. 370 and 700 are CO₂ concentrations in ppm. C = control, H = heat wave. Within each day, significant effects ($P \leq 0.05$) of CO₂, heat, and their interaction are indicated by x, xx, and xxx, respectively. For each day, significant differences among treatments are indicated by different letters. The error bars are standard errors of 5-6 replicates.
**Tissue nitrogen**

In general, in sunflower, elevated CO$_2$ decreased %N of leaves (especially), stems, and roots, and heat had little or small effects on %N (Figure 7). Between days 5 and 10, decreases in %N were especially pronounced in heat-stressed sunflower plants under elevated CO$_2$ (excluding old leaves). In corn, elevated CO$_2$ initially increased %N (day 1), but generally had small or no effects on %N on days 5 and 10, excluding large decreases in %N in unstressed plants at elevated CO$_2$ from day 1 to day 5. In sunflower, nitrogen allocation to roots tended to increase with elevated CO$_2$ and time, elevated CO$_2$ decreased N allocation to stems, and heat had little effect on N allocation (Figure 8). In corn, elevated CO$_2$ had little effect on N allocation on days 5 and 10, but decreased root N and increased new-leaf N on day 1. On day 5, heat decreased root N and increased N in stems and old leaves, but heat had little effect on N allocation on day 10.
Figure 7. Effect of heat wave on nitrogen concentration (dry mass basis) in various parts of sunflower and corn. Day 0 = 1 day before heat wave, day 5 = the fifth day of heat wave, and day 10 = 5 days after termination of heat wave. 370 and 700 are CO\textsubscript{2} concentrations in ppm. C = control, H = heat wave. Within each day, significant effects ($P \leq 0.05$) of CO\textsubscript{2}, heat, and their interaction are indicated by x, xx, and xxx, respectively. For each day, significant differences among treatments are indicated by different letters. The error bars are standard errors of 3-5 replicates.
**Figure 8.** Effect of heat wave on nitrogen allocation (% of total plant N) in various parts of sunflower and corn. Day 0 = 1 day before heat wave, day 5 = the fifth day of heat wave, and day 10 = 5 days after termination of heat wave. 370 and 700 are CO\(_2\) concentrations in ppm. C = control, H = heat wave. Starting from black and moving anticlockwise, each segment represents new leaf, mature leaf, old leaf, stem, and root, respectively.
Tissue carbon

In general, CO$_2$ and heat had biologically small effects on $\%C$ (Figure 9). In sunflower, elevated CO$_2$ often slightly increased leaf or stem $\%C$ on days 1 and 5, and heat increased $\%C$ in roots on days 5 and decreased $\%C$ in high-CO$_2$ plants in roots and mature leaves on day 10. In corn, heat and CO$_2$ had interactive effects on $\%C$ on day 5, with heat increasing $\%C$ in shoots at low CO$_2$, but decreasing $\%C$ at high CO$_2$. On day 10, heat increased $\%C$ in corn shoots and roots.
**Figure 9.** Effect of heat wave on carbon concentration (dry mass basis) in various parts of sunflower and corn. Day 0 = 1 day before heat wave, day 5 = the fifth day of heat wave, and day 10 = 5 days after termination of heat wave. 370 and 700 are CO₂ concentrations in ppm. C = control, H = heat wave. Within each day, significant effects ($P \leq 0.05$) of CO₂, heat, and their interaction are indicated by x, xx, and xxx, respectively. For each day, significant differences among treatments are indicated by different letters. The error bars are standard errors of 3-5 replicates.
C:N ratio

In both sunflower and corn, heat wave and CO$_2$ had few biologically-important effects on tissue C:N ([Figure 10](#)). In sunflower, elevated CO$_2$ tended to increase C:N, especially in old leaves and in leaves and stems of heat-stressed plants on day 10. In corn, elevated CO$_2$ decreased C:N of leaves on day 1, increased C:N in shoots of unheated controls, and had no effects on day 10.
Figure 10. Effect of heat wave on C:N ratio in various parts of sunflower and corn. Day 0 = 1 day before heat wave, day 5 = the fifth day of heat wave, and day 10 = 5 days after termination of heat wave. 370 and 700 are CO2 concentrations in ppm. C = control, H = heat wave. Within each day, significant effects ($P \leq 0.05$) of CO2, heat, and their interaction are indicated by x, xx, and xxx, respectively. For each day, significant differences among treatments are indicated by different letters. The error bars are standard errors of 3-5 replicates.
DISCUSSION

The present study investigates the effect of simulated natural long-term heat wave on gas-exchange, growth, and carbon and nitrogen relations in sunflower (C₃) and corn (C₄) under the current atmospheric CO₂ concentration (370 ppm) and the predicted CO₂ concentration for the end of 21ˢᵗ century (700 ppm) (IPCC, 2007). In an ecosystem, many processes, such as herbivory and nutrient cycling, are largely influenced by biomass allocation and the nitrogen concentration and C:N ratio of plant tissue, so understanding the impact of long-term heat waves on growth and carbon and nitrogen content in plant tissue is important for an understanding of plant and ecosystem responses to heat waves and CO₂. I hypothesized that tissue N and C concentrations are decreased and increased, respectively, by heat wave under both current and elevated CO₂ concentrations, and this leads to increases in C:N ratio. Also, C₃ plants being more responsive to CO₂, heat wave will have a greater impact on C and N relations in C₃ plants than in C₄. In addition, I also hypothesized that elevated CO₂ increases thermotolerance of Pₙ in C₃ plants, but reduces thermotolerance of Pₙ in C₄. Heat negatively impacts Pₙ, so total biomass should decrease and root-shoot ratio should increase with heat wave.

My hypotheses turn out to be either partially true or altogether incorrect. In line with the hypothesis, heat wave reduced tissue N under elevated CO₂ five days after termination of
heat wave in sunflower, and in some tissues (new leaf, mature leaf, and root) in corn under low CO₂ at the end of five days of heat wave. However, in corn, tissue N concentration was increased by the heat wave under elevated CO₂ at the end of five days of heat wave, but N concentration was not affected by heat five days after termination of heat wave under both CO₂ concentrations. Likewise, C concentration was increase by heat only in roots of sunflower at the end of five days of heat wave under both CO₂ concentrations, but heat increased and reduced C concentration under low and high CO₂, respectively, in corn at the end of five days heat wave. Five days after termination of heat, the heat wave increased C concentration under both CO₂ concentrations in corn, but affected %C only in mature leaves and roots of high-CO₂ sunflower, reducing C concentration in them. As the hypotheses regarding N and C concentration became partially true, the hypothesis regarding C:N concentration also became partial accurate. Under elevated CO₂, heat wave had a greater impact on N and C concentration in corn than in sunflower at the end of five days of heat wave, but five days after heat wave termination, the heat had greater impact on N and C concentration in sunflower under elevated CO₂, partially supporting another hypothesis. My other hypothesis was CO₂ increases Pₙ thermotolerance in C₃ plants, but reduces it in C₄ plants. The present study showed that CO₂ increases thermotolerance in both C₃ and C₄ plants during short-term moderate heat wave (< 4hrs) and during recovery time (5 days after termination of heat wave), but CO₂ had no effect on thermotolerance of Pₙ at the end of the five-day heat wave. Opposite to my hypothesis, heat wave did not reduce total biomass in either species and did not affect R/S in corn. However, heat increased R/S in sunflower, confirming the hypothesis.
**Plant growth**

The five-day-long heat wave did not have significant effects on total biomass when measured at the last day of the heat wave (day 5) or five days after termination of the heat wave (day 10) in both sunflower and corn under both ambient (370 ppm) and elevated (700 ppm) CO$_2$. A large CO$_2$ fertilization effect was evident in sunflower, since the total biomass was higher under elevated CO$_2$ than in ambient CO$_2$ concentration, but CO$_2$ fertilization effect was small and appeared only by the end of heat wave (day 5) in corn, since C$_4$ plants are less responsive to elevated CO$_2$ than C$_3$ plants. Wand *et al.* (1999), in their meta-analysis of wild C$_3$ and C$_4$ plants of Poaceae, reported a 44% increase in biomass in C$_3$ plants under elevated CO$_2$, but the increase in biomass was only 33% in C$_4$ plants due to elevated CO$_2$. Even though, there was no significant impact of heat wave on total biomass in our study, the heat wave influenced root:shoot ratio in sunflower at day 5 and day 10 under elevated CO$_2$. The increase in root:shoot ratio by the heat wave in sunflower under elevated CO$_2$ was due to an increase in root biomass and a decrease in shoot biomass. The increase in root biomass might be associated with decreased N concentration by the heat wave in the various tissues of sunflower under elevated CO$_2$ at day 10. As indicated by root biomass, the plant was producing more roots and increasing N absorbing area so that the plant could ameliorate the decrease in %N induced by heat wave at day 10 under elevated CO$_2$. However, the increase in root:shoot ratio by the heat wave at day 5 under elevated CO$_2$ might not be associated with %N in tissues of sunflower, since there was no reduction of tissue N concentration. The increase in root:shoot ratio by the heat wave in sunflower at day 5 under elevated CO$_2$ might be because of the stimulation of root growth by high CO$_2$. 
Plant functions

The net photosynthesis ($P_n$) was stimulated by short-term heat wave (4 hr long, day 1) in corn under both the ambient and the elevated CO$_2$ concentrations. But in sunflower, $P_n$ was negatively impacted under ambient CO$_2$ but not affected in elevated CO$_2$ by the short-term heat wave. In contrast to this study, Wang et al. (2008) reported decreases in $P_n$ under both ambient and elevated CO$_2$ conditions during a 4-hr heat treatment in three C$_4$ (corn, sorghum and pigweed) and six C$_3$ (pea, chenopodium, wheat, soybean, sunflower and tomato) species. High CO$_2$ decreased $P_n$ during the heat stress in the C$_4$ plants, but high CO$_2$ increased photosynthetic thermotolerance in the C$_3$ species. The differences between this study and the study of Wang et al. (2008) might have arisen by differences in the applied heat-wave intensity. Wang et al. (2008) applied more intense heat, raising the temperature by 15°C above the ambient growth temperature, but I only raised the temperature 10°C above the ambient growth temperature. In my study, however, the stimulatory and the inhibitory effects of heat wave on $P_n$ observed at day 1 disappeared at day 5 (the fifth day of heat-wave treatment), suggesting that $P_n$ acclimated as the heat wave progressed. More importantly, at day 5, there was no significant alteration of total biomass by the reduction (in sunflower) and the stimulation (in corn) of $P_n$ by heat wave at day 1, indicating that short-term effect of heat on $P_n$ did not remain for five days or the effects were not large enough to affect total biomass within five days of heat-wave treatment. Likewise, the short-term heat wave (4 hr long, day 1) in corn and long-term heat wave (5 day long) in sunflower had a negative impact on PSII efficiency ($F_v'/F_m'$) under the ambient CO$_2$. However, the reduction in $F_v'/F_m'$ by the heat wave did not limit $P_n$, since $P_n$ was stimulated by heat at day 1 in corn and not affected by heat in
sunflower at day 5 under ambient CO₂. At day 10 (five days after heat-wave termination), Pₙ remained significantly higher under the ambient CO₂ and temperature than that of Pₙ under the elevated CO₂ or/and temperature in both the species. This might be due to accelerated aging of the measured leaves under elevated CO₂ or/and temperature. Even if these leaves began senescing by day 10, the other leaves that developed above the measured leaf might have contributed significantly to whole-plant photosynthesis, since there was no impact of the heat wave on total biomass of both the species under both the CO₂ concentrations at day 10.

**Tissue nitrogen and carbon**

The five-day heat wave either increased (new leaf, mature leaf, old leaf and stem) or did not change (root) N concentration under elevated CO₂ in corn by the end of the heat wave (day 5). However, under ambient CO₂, heat wave either decreased (new leaf, mature leaf, and root) or did not change (old leaf and stem) %N plant at day 5. These two findings suggest that N status of corn could be increased and decreased under elevated and ambient CO₂, respectively, by the heat wave. This difference in N status of corn might have been achieved by alteration of N absorption capacity of roots by the heat wave. Probably, the heat wave increased N absorption efficiency of roots under elevated CO₂, which increased N status in the plant. But, under ambient CO₂, it is likely that the N absorption efficiency of roots was decreased by heat, which might be responsible for the decrease in tissue %N in corn. Alternatively, heat wave promoted N availability in the soil under elevated CO₂ but retarded N availability in ambient CO₂. Obviously, a plant can potentially absorb more N from the soil if there is more available N, and this can
increase the N status of the plant, such as by increasing N concentration of its tissue. Thus, the heat wave altered N status of corn under elevated and ambient CO₂ concentration in opposite directions. Similar to our finding, Wollenweber et al. (2003) reported a decrease in %N in wheat leaves by high temperature under ambient CO₂.

In sunflower, there was no significant effect of the five-day heat wave on %N in various parts of the plant at day 5. However, there was an overall trend of increasing %N in all the tissues (new leaf, mature leaf, old leaf, stem, and root) by the heat wave at day 5 under both the CO₂ concentrations. The decrease in %N in all tissues except old leaves at day 10 under elevated CO₂ in sunflower suggested that the heat wave, which was terminated five days earlier, either reduced the nitrogen absorption capacity of roots or it reduced nitrogen availability in soil, resulting in reduced N status in the plant.

At day 5, the last day of the heat-wave treatment, %C was increased by the heat wave in all tissues of corn under ambient CO₂, but the heat wave decreased %C in all tissues of the plant except mature leaf (marginally significant decrease even in mature leaf, \( P=.069 \)) under elevated CO₂. This alteration of %C under the two CO₂ concentrations in two directions might have arisen by the alteration of proportion of structural and non-structural carbohydrates in the tissues of the plant by the heat wave. Structural carbohydrates contain greater carbon than nonstructural carbohydrate. So, as the proportion of non-structural carbohydrate increases in a plant tissue, the %C of that tissue decreases. Probably, the formation of structural carbohydrate from non-structural carbohydrate was slowed by heat wave under elevated CO₂ that favors the accumulation of non-structural carbohydrate in the tissues of the corn exhibiting decreased %C. The
opposite might be true in corn under ambient CO₂ at day 5. Similarly, five days after the end of heat wave (day 10), the heat wave might have stimulated the conversion of non-structural carbohydrate into structural carbohydrate, reducing the proportion of non-structural carbohydrate in various parts of corn under both the CO₂ concentrations resulting in higher %C in corn which received heat wave. After termination of the heat wave, under elevated CO₂, root growth was accelerated (evident from greater biomass of the root at day 10) to ameliorate the reduced %N in various tissues of sunflower. Probably, more carbohydrate was moving to the root for its growth, so that the proportion of non-structural carbohydrate was increasing in the root, which decreased root %C at day 10 in sunflower that received heat wave under elevated CO₂.

The alteration of tissue carbon and nitrogen concentrations by the heat wave in different parts of the sunflower and the corn (as observed above), did alter tissue C:N ratio in these species under either CO₂ concentrations. At day 5, in all the above-ground tissues of corn, the ratio was decreased by the heat wave under elevated CO₂ and the C:N ratio reached the level found under ambient CO₂ and temperature. On the same day, the N dilution effect seen in elevated CO₂ totally disappeared in corn that received heat wave. Likewise, the trend in sunflower was of increases in %N and decreases in C:N ratio by heat wave under both CO₂ concentrations. These findings suggest that nutrient quality of various plant tissues could be improved by heat wave by increasing %N and decreasing C:N ratio under elevated CO₂. This alteration of nutrient quality might modify plant herbivore interaction observed under elevated CO₂ alone. Under elevated CO₂, herbivore performance can be reduced because of a decrease in %N and an increase in C:N ratio and the percentage of secondary metabolites (e.g., phenolics) in tissues of the herbivore’s
host plant (Stiling and Cornelissen, 2007). The present study suggests that the negative effect of reduced %N and increased C:N ratio on herbivores under elevated CO₂ can be offset partly in C₃, and fully in C₄, plants by the heat wave, at least for a few days, until the positive effect of heat wave on nutrient quality (i.e., due to increase in %N and reduction in C:N ratio) in various parts of the plant disappear. The positive impact of heat wave on nutrient quality of the C₄ plant (corn) disappeared five days after the heat wave termination under elevated CO₂. However, the nutrient quality of the C₃ plant became even worse five days after the heat wave termination under elevated CO₂. Thus, it seems that the reduction of %N and the increase in C:N ratio in C₃ plant five days after the heat-wave termination under elevated CO₂ might further exacerbate the performance of those herbivores which preferably consume C₃ plants.

Heat wave can affect plant-herbivore interactions under the present CO₂ concentration as well, because of alteration in %N and C:N ratio in various parts of the plant. At day 5, the last day of the five-day heat wave, the %N of new and mature leaves (which are more vulnerable to herbivory because of being softer than old leaves and stem) was decreased by heat wave in C₄ plant, corn, making the tissue less nutritious to the herbivores under ambient CO₂ concentration. The quality of the new leaves was further reduced by increasing C:N ratio under ambient CO₂. In sunflower, the heat wave did not significantly alter the %N and C:N ratio in various plant parts under ambient CO₂ concentration at the end of the five-day heat wave (day 5) and five days after the heat wave termination (day 10). However, at both day 5 and the day 10, there was a trend for increases in %N and decreases in C:N ratio by the heat wave, in plant parts that are more vulnerable to herbivory (e.g., new leaf and mature leaf due to their softness); i.e., the heat wave
increased the %N in leaves of all ages and decreased C:N in new leaves and mature leaves.

In conclusion, even if a heat wave does not alter the total biomass of a plant, it can alter tissue quality by altering %N and C:N ratio in various tissues of plants under both ambient and elevated CO₂ concentrations. However, the direction and magnitude of the effect may vary with CO₂ concentration, plant functional group (C₃ vs. C₄) and measurement time (immediately after heat-wave termination vs. five days after heat-wave termination). Under elevated CO₂, tissue quality was improved by the heat wave in both the plant groups (C₃ and C₄) by the end of the heat wave, by increases in %N and decreases in C:N ratio in the various parts of the plants, but the improvement was higher in C₄ plants. The improved tissue quality disappeared five days after the heat-wave termination in the C₄ plants. But, five days after the heat-wave termination (day 10), tissue quality became worse under elevated CO₂ in the C₃ plant. However, under ambient CO₂ concentration, the five-day heat wave increased the tissue quality in the C₃ plants, but decreased tissue quality in C₄ plants by the end of the heat wave. The negative effect of heat wave on tissue quality observed at the end of heat wave disappeared in C₄ plant five days after the heat-wave termination, but the positive effect of heat wave on tissue quality still remained in the C₃ plant even 5 days after heat-wave termination. This alteration of tissue quality by altering the %N and C:N ratio in various parts of the plants by the heat wave can impact plant-herbivore interactions under both the present CO₂ concentration (ambient CO₂) and the CO₂ concentration predicted for the end of 21st century (elevated CO₂), since herbivore performance is altered by the quality of tissue it consumes.
REFERENCES


