Photosynthetic Acclimation, Biomass Allocation, and Water Use Efficiency of Garlic in Response to Carbon Dioxide Enrichment and Nitrogen Fertilization

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ABSTRACT. Garlic (Allium sativum) is a commercially and culturally important crop worldwide. Despite the importance of garlic, there have been few studies investigating how garlic growth and development will be affected by the atmospheric enrichment of carbon dioxide (CO₂). A split-plot experiment with CO₂ concentrations as main plot and nitrogen (N) fertilization as subplot was carried out to examine the effects of elevated CO₂ at (mean ± SD) 745 ± 63 μmol mol⁻¹ across three levels of N: high-N (16.0 mmol), mid-N (4.0 mmol), and low-N (1.0 mmol). Three hypotheses were tested: 1) garlic plants will allocate proportionally more biomass to bulb when grown in elevated CO₂ compared with the plants grown in ambient CO₂; 2) plants will sustain improved photosynthesis without downregulation in elevated CO₂, irrespective of N; and 3) elevated CO₂ will improve plant water use efficiency (WUE) across N fertilization levels. We found that proportional biomass allocation to bulb was not significantly enhanced by CO₂ enrichment in garlic. Overall biomass accumulation represented by leaf, stem, and bulb did not respond significantly to CO₂ enrichment but responded strongly to N treatments (P < 0.001). Contrary to our hypothesis, photosynthetic downregulation was apparent for garlic plants grown in elevated CO₂ with a decrease in Rubisco capacity (P < 0.01). Instantaneous leaf WUE improved in response to elevated CO₂ (P < 0.001) and also with increasing N fertilization (P < 0.001). Finally, our results indicate that bulbing ratio is likely to remain unchanged with CO₂ or N levels and may continue to serve as a useful nondestructive metric to estimate harvest timing and bulb size.

Garlic is an important food crop (Food and Agriculture Organization of the United Nations, 2011) that has been incorporated into cuisines around the world. In addition to its culinary contributions, for millennia garlic has been used medicinally as a remedy for a wide variety of medical conditions (Kik et al., 2001; Rivlin, 2006; Tattelman, 2005). Multiple books and reports have been written detailing optimal garlic horticulture (Andrews, 1998; Meredith, 2008) in regard to N fertilization (Bertoni et al., 1992; Buwalda, 1986) and water inputs (Villalobos et al., 2004). For example, Buwalda and Freeman (1987) reported that the N fertilization rate of 120 kg ha⁻¹ yielded highest harvestable bulb yield under a field condition. Despite the economic and cultural importance of garlic, there have been few studies investigating garlic growth and development in future environmental conditions predicted with global environmental change, in particular the atmospheric enrichment of CO₂. One of the most consistent responses to CO₂ enrichment has been an increase in biomass and total nonstructural carbohydrates in plant tissues (Körner, 2000). As we prepare to adapt crop management to predicted elevated CO₂ concentrations (Ziska et al., 2012), it is particularly important to understand how CO₂ enrichment will affect carbon allocation to the belowground storage structures for garlic, and other valued crops that form bulbs, corms, or rhizomes.

Extensive research has demonstrated that atmospheric enrichment of CO₂ will stimulate photosynthetic responses for C₃ plant growth. However, the substantial increases in net photosynthesis immediately after doubling CO₂ are typically unsustainable as a consequence of decreased photosynthetic capacity (Leakey et al., 2009; Sage, 1994; Sage et al., 1989). This unsustainable stimulation of photosynthesis is thought to be an acclimation response to high CO₂ environment as a result of decreased Rubisco activity (Faria et al., 1996; Vu et al., 2008) and is often accompanied with an accumulation of nonstructural carbohydrates and the dilution of N illustrated by a higher C/N ratio (Stitt, 1991; Stitt and Krapp, 1999). This feedback inhibition,
known as the “carbon sink limitation hypothesis,” ascribes the downregulation of photosynthesis to carbohydrate buildup, which is due to an imbalance between carbohydrate quantity and plant sinks mediated by N nutrition (Pollock and Farrar, 1996). Carbon assimilated during photosynthesis is competitively partitioned to active sinks, and the stimulating effects of CO₂ enrichment on photosynthesis will be negated unless a plant has sufficient sinks with which to store the additional carbohydrates (Paul and Foyer, 2001). Carbon sink limitations may not be marked in plants with large carbon sinks such as bulbs and rhizomes (Kinmonth-Schultz and Kim, 2011), or in trees and other perennial species for which long-term storage of carbon is important for their fitness. In optimal conditions, belowground carbohydrate storage structures have been attributed to sustained photosynthetic capacity throughout the plant life cycle (Monje and Bugbee, 1998). However, row crops, like garlic, grow in a dynamic environment with naturally or induced (e.g., targeted drought) suboptimal conditions.

In suboptimal conditions, there is uncertainty whether assimilated carbon will be allocated to storage, reproduction, or defenses (Bazzaz, 1996). Plants allocate biomass to belowground parts for the acquisition of water and nutrients, and to aboveground parts for the acquisition of light and CO₂. It has been reported that when plants are grown in elevated CO₂, there is a reduction in the relative investment in leaf areas with a concomitant increase in the partitioning of carbon to belowground carbon sinks (Körner, 2000). Rogers et al. (1994) suggested that increasing levels of CO₂ in the earth’s atmosphere will positively affect root dry weight, length, diameter, width, and root:shoot ratio. To date, many studies focus on roots as the representative belowground structure (Pendall et al., 2004; Rogers et al., 1992; Volder et al., 2007; Xu et al., 2007) with limited studies examining bulbs, corms, or rhizomes. However, the current practices of categorizing plant tissues by location (i.e., belowground structures) may be misleading because these structures are morphologically and anatomically distinct, which may result in diverse physiological responses. For instance, fleshy leaf scales are the primary storage tissues for garlic, whereas the root cortex is the primary storage organ for carrot (Daucus carota). Questions remain whether plants will prioritize growth or development of long-term reserves when atmospheric CO₂ supplies are abundant, but other resources such as nutrients or water may be limiting.

Little is known about the link between anatomical and morphological aspects of storage structures and biomass partitioning at the whole plant level under elevated atmospheric CO₂ concentrations (Ziska and Bunce, 2006), and how carbon assimilation and allocation responses in high CO₂ are related to N availability in bulb-producing plants. The objectives of this study were to determine photosynthetic responses, patterns of biomass allocation to bulbs, and WUEs of garlic plants grown in elevated CO₂ across a range of N levels. Specifically, our hypotheses consisted of the following: 1) garlic plants will allocate proportionally more biomass to bulb, a storage structure consisting of modified leaves, when grown in elevated CO₂ compared with the plants grown in ambient CO₂; 2) garlic plants will sustain improved photosynthesis with little down-regulation in elevated CO₂ irrespective of N supply due to increased sink strength represented by large bulb size; and 3) elevated CO₂ will improve plant WUE across N fertilization levels.

Materials and Methods

**Plant materials and facilities.** An Asiatic hardneck garlic (cv. Korean Mountain) purchased from Filaree Garlic Farm (Okanogan, WA) was used for this study. On 13 Jan. 2011, 60 pots (2.54-L tree pots) were filled with unfertilized, sterilized, potting mix (Sunshine Potting Mix #2; Sun Gro Horticulture, Vancouver, BC, Canada), and planted with one clove per pot. The pots were then placed on wire tables in a glasshouse located in Seattle, WA, and watered from a hose by hand. Daylight was supplemented in the glasshouse with high-pressure sodium 400-W single-phase bulbs during 0800 to 2200 HR. The glasshouse uses natural ventilation, exhaust fans, and evaporative cooling pads to prevent excessive temperatures. After 14 d, all cloves had germinated and the pots were organized into experimental treatment groups.

**Experimental design.** Three N levels and two CO₂ levels were used as the experimental treatments. First, the 60 pots were randomly split into three N groups of 20 pots each. The treatments were three levels of N delivered as ammonium nitrate delivered as part of a modified Hoagland’s liquid fertilizer solution: full strength [high-N (16.0 mm N, 0.224 g L⁻¹)], 1/4 strength [mid-N (4.0 mm N, 0.056 g L⁻¹)], and 1/16 strength [low-N (1.0 mm N, 0.014 g L⁻¹)]. The macronutrients were provided as follows: (NH₄)₂(NO₃), CaCl₂·2H₂O, K₂SO₄, and KH₂PO₄ (concentrations for K, Ca, Mg, S, and Cl were 6.0, 3.0, 2.0, 3.0, 1.0, and 6.0 mm, respectively). The micronutrients were in the forms of H₃BO₃, MnSO₄·H₂O, ZnSO₄·7H₂O, CuSO₄·5H₂O, H₂MoO₄, and NaFeDTPA (concentrations of B, Mn, Zn, Cu, Mo, and Fe chelate were 25, 2.0, 2.0, 0.5, 0.5, and 18 μm, respectively). Only N levels differed; all other elements were maintained at equal levels between treatments. The treatments were applied when the plants were watered by hand at least once per week for the duration of the experiment. Next, the pots were organized into CO₂ treatment groups, “ambient” and “elevated.” Five pots from each N group were randomly placed in one of four CO₂ controlled sunlit growth chambers that were located inside the glasshouse. The growth chambers (100 × 100 × 200 cm) were closed-topped, polyvinyl chloride-framed units, surrounded with Mylar polyester sheeting (DuPont Teijin Films, Chester, VA). For additional details of the chamber see Kinmonth-Schultz and Kim (2011). In each chamber, temperature/light sensors (HOBO Pendant Temperature/Light sensor; Onset Computer, Bourne, MA) recorded temperature and illuminance (kilolux) every 15 min for the experimental period January to June (Fig. 1). After the experiment concluded, a quantum sensor (LI-190; LI-COR, Lincoln, NE) was installed inside the chambers to measure greenhouse photosynthetic photon flux (PPF (μmol·m⁻²·s⁻¹)). The PPF data from the subsequent 2 years (i.e., 2012 and 2013) are also provided herein to illustrate the expected characteristics of the chamber light environment during the 2011 experiment (Fig. 1).

Fans forced air through ventilation ducts from outside the greenhouse into the chambers. Two of the chambers were assigned “ambient” air and received air directly through the pipes from outside. The remaining two were designated as “elevated” and were supplemented with additional CO₂ that was added to the ventilation ducts through flexible plastic tubing delivered from a 22.70-kg tank (Praxair, Seattle, WA). The elevated CO₂ concentrations were maintained by using...
bubble flow meters (FL-2000; Omega, Stamford, CT). CO2 concentrations were automatically measured, every 15 min, by sampling air from within the chambers through an infrared gas analyzer (IRGA) (CIRAS-1; PP Systems International, Amesbury, MA). The CIRAS-1 IRGA has two channels to detect CO2 concentrations, reference (ref.) and differential (diff.). We were able to continuously monitor both of the “elevated” CO2 chambers by connecting a flexible plastic tubing from the two chambers to the ref. and diff. ports. Once a week, for the duration of the experiment, the CIRAS-1 was taken to an adjacent laboratory and was calibrated against two gas cylinders of certified CO2 concentrations (0 and 700 μmol·mol–1). The “ambient” CO2 was measured, every 15 min, with a sensor (Carbocap GMP242; Vaisala, Vantaa, Finland) placed in one of the “ambient” chambers. The daily mean value with SD for the concentrations recorded within the ambient chambers was 48% of the –8d13C gas from the tanks creating an ambient δ13C grown leaves (at multiple C4 values were estimated to test for photosynthetic downregulation under elevated CO2 conditions (for detailed methods see Kim and Lieth, 2003; Kinmonth-Schultz and Kim, 2011; Sharkey et al., 2007). In addition, A and WUE were compared for all CO2-N treatments. WUE was evaluated with the IRGA by comparing instantaneous WUE (A/E), as well as the C4/C3 ratio. The C4/C3 ratio reflects the balance between net assimilation and stomatal conductance (gs); whereas A/E reflects the amount of carbon gained per water lost. In addition, carbon isotopes (13C, 12C) compositions were analyzed from leaf samples to indicate long-term internal regulation of carbon uptake and water loss.

The carbon isotope discrimination (δ13C) was evaluated from five randomly selected plants from each treatment group. Leaves of these plants were milled through 1-mm mesh screen using a Wiley mini-mill (Thomas Scientific, Swedesboro, NJ). The pulverized leaf samples were then processed at the University of California at Davis stable isotope laboratory with an elemental analyzer (PDZ Europa ANCA-GSL elemental analyzer; Sercon, Irvine, UK) interfaced to a continuous flow isotope ratio mass spectrometer (PDZ Europa 20–20 isotope ratio mass spectrometer, Sercon). Farquhar and Richards (1984) proposed the use of Δ as a measure of the carbon isotope discrimination by the plant as shown below:

$$\Delta(\text{discrimination}) = \frac{\delta_{\text{air}} - \delta_{\text{plant}}}{1 + \delta_{\text{plant}}}$$

The CO2 tanks were filled with gas that was captured during fossil fuel refining, which had been measured (Nackley et al., 2014) as having a different isotopic signature (δ13C, –35.5‰) compared with atmospheric CO2 (δ13C, –8‰). As previously stated, the supplemental gas was used to elevate the CO2 concentration from ambient levels (∼290 μmol·mol–1) to 745 μmol·mol–1. Therefore, the air within the elevated chambers was composed of 52% of the –8‰ δ13C air and 48% of the –35.5‰ δ13C gas from the tanks creating an atmosphere with an isotopic composition of –21.2‰ δ13C chamber. Although the ambient CO2 outside of the greenhouse was measured at ∼290 μmol·mol–1, the average CO2 concentration recorded within the ambient chambers was
437 ± 1 μmol·mol⁻¹. Therefore, the isotopic composition of the ambient chambers was −11.025‰ 813C.

**Biomass growth and allocation.** The N and CO₂ treatments were applied to the garlic plants for 152 d. The experiment concluded on 14 June 2011, at which point all of the plants were harvested. The harvest involved separating the plants into leaves, stem, bulb, and roots; stem part included pseudostem (i.e., sheath) and scape when present. Because of incomplete separation of roots from media in multiple samples, root biomass was not included in the final analysis. The harvested plant parts were bagged in paper and dried for 48 h in a forced air oven heated to 80 °C. The oven-dry biomass was weighed and analyzed to compare the effects of CO₂ and N on biomass development and allocation.

**Leaf N concentrations and C/N ratio.** When the plants were harvested on 14 June 2011, 36 leaf samples were randomly selected for determining leaf C and N concentrations (w/w). These leaves were clipped, bagged, and placed in a forced air oven at 80 °C for 48 h. The dried leaf samples were then ground with a Wiley mini-mill to fit through a 1-mm mesh. The ground leaf samples were transferred into consumable aluminum capsules and combusted in a pure oxygen environment with a CHN analyzer (model-2400; PerkinElmer, Waltham, MA) to determine C and N contents in the leaves.

**Data analysis.** The experiment constituted a split-plot design with CO₂ being the main plot and N being the subplot. Accordingly, CO₂ and N were considered fixed effects and the chambers (blocks) were treated as random effects. Initially, a linear mixed-effects model was used to determine whether significant variability could be attributed to the chambers. When the chamber effects were not found to be significant (P > 0.05), they were removed from further linear models that tested the main effects. Succeeding analyses included two-way analyses of variance, using type III sums of squares, to quantify the main effects and interactions from CO₂ and N for biomass responses, leaf gas exchange, and leaf tissue composition. For leaf gas exchange data analysis, we used the temperature (i.e., 15 or 25 °C) as a blocking variable to account for the variability due to different measurement temperatures although no significant differences were found between the two measurement temperatures in all gas exchange parameters with an exception of the rate of transpiration (E), similar to the findings in a previous study (Kim et al., 2013). All statistical analyses were calculated using SAS (version 9.4; SAS Institute, Cary, NC) or R 3.2.1 statistical software (R Development Core Team 2011). SigmaPlot 12.5 (Systat software, San Jose, CA) was used to plot the figures.

**Results**

**Biomass allocation and leaf N status.** Nitrogen treatments resulted in significant differences in leaf, bulb, and leaf-stem-bulb (LSB) biomass [P < 0.05 (Fig. 2)], whereas elevated CO₂ produced marginally greater stem biomass (P = 0.08). With little response of bulb biomass to elevated CO₂, none of the biomass ratios involving bulb (i.e., bulb/leaf, bulb/stem, bulb/LSB) were significant (Figs. 3 and 4); this result suggests that garlic plants did not allocate preferentially more biomass to the bulb when grown in elevated CO₂. Similarly, the bulb biomass ratio to LSB or other plant parts did not change in response to N levels (Figs. 3 and 4). On the other hand, bulb diameter increased with N level (P < 0.001) but bulbing ratio (bulb diameter/stem diameter) did not change in response to CO₂ or N (Fig. 3). Biomass allocation to leaf increased with N treatment levels [P < 0.05 (Fig. 2)].

Leaf N concentration increased (P < 0.001) with N supply and decreased in response to CO₂ enrichment [P < 0.05 (Fig. 4B)]. Reduction in leaf N under elevated CO₂ was greater when N supply was the highest as evidenced by a significant interaction [P < 0.05 (Fig. 4A)]. Leaf C/N ratios decreased with increasing N supply (Fig. 4B) but did not change in response to elevated CO₂ treatment.

**CO₂ assimilation and photosynthetic downregulation.** As expected for a C₃ plant, the leaf net CO₂ assimilation rate (A) increased with increasing CO₂ concentrations (P < 0.05) and also with N levels [P < 0.05 (Table 1)]. When the LI-6400 leaf chamber was set to CO₂ concentrations near the growth CO₂ concentrations (i.e., 400 or 700 μmol·mol⁻¹), A increased by 23% on average in plants grown in elevated CO₂ compared with
those from the ambient CO2 treatment (Table 1). Similarly, A increased 39% and 40% under mid- and high-N treatments, respectively, compared with low-N treatment (Table 1). This increase in A under elevated CO2 with increasing N levels is likely to have contributed to a marginal increase in biomass responses (Fig. 2).

The $V_{\text{cmax}}$ was significantly affected by both CO2 and N treatments ($P < 0.01$). CO2 enrichment significantly decreased $V_{\text{cmax}}$ rates with the average ambient rates being $1/3$ greater than the Rubisco capacity of the plants grown in elevated CO2 (Table 1). Nitrogen fertilization had a positive relationship with $V_{\text{cmax}}$. The low-N treatment had significantly lower rates than the mid- and high-N treatments at either CO2 treatment level (Table 1). The CO2 effect on $J_{\text{max}}$ was marginal ($P = 0.08$), whereas the N was found to play a significant role ($P < 0.01$). At either CO2, $J_{\text{max}}$ was found to be much lower for the low-N treatment compared with the mid- and high-N treatments (Table 1). The $A_{\text{Elev}}/A_{\text{ambi}}$ ratios stayed below one in most cases indicating apparent photosynthetic downregulations especially at low intercellular CO2 concentrations in mid- and high-N. The values were more variable when N supply was low (Fig. 5). Together with a lower $V_{\text{cmax}}$, this result suggests that biochemical downregulation associated with carboxylation efficiency at low CO2 was highly likely present.

**INSTANTANEON AND LONG-TERM WUE.** The leaf gas exchange indicated enhanced instantaneous WUE ($A/E$) in response to increasing CO2 ($P < 0.001$ (Table 1)). The positive relationship between N fertilization and WUE (Table 1) is also evidenced by the significant difference in $A/E$ ($P < 0.001$) between N treatments. In both cases, the improvements in WUE were more attributable to gains by $A$, which were significantly improved by N and CO2 enrichment, than by the limited differences in $E$, which did not respond significantly to either CO2 or N treatments (data not shown). The interaction between CO2 and N on WUE was significant ($P < 0.05$) indicating that instantaneous WUE increased more with N levels in elevated CO2 than in ambient CO2 conditions. Similar to the instantaneous WUE, the $\Delta^{13}$C results were also significantly related to the CO2 treatments ($P < 0.001$). $\Delta^{13}$C results did not indicate an effect from the N treatment or an interaction between CO2 and N.

**Discussion**

Garlic, like other C3 crops, exhibited beneficial responses to both CO2 and N enrichment. However, the results were not uniform for all plant parts and processes. Specifically, our results did not support our first hypothesis that garlic plants would allocate proportionally more biomass to bulb in response to CO2 enrichment. Although the results indicated that net CO2 assimilation rate ($A$) was enhanced by CO2 enrichment (Table 1) neither bulb nor leaf biomass responded significantly to elevated CO2. Overall, garlic plant biomass did not increase significantly in response to CO2 ($P = 0.11$) with the stem biomass being marginally responsive ($P = 0.08$) (Fig. 2). Studies that investigated the effects of CO2 enrichment on the growth and biomass of crop species with underground storage organs [e.g., potato (Solanum tuberosum), onion (Allium cepa), carrot, and radish (Raphanus sativus)] reported highly variable biomass accumulation and allocation responses (Jasoni et al.,

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**Table 1.** Leaf gas-exchange parameters ($n = 6–8$) and $^{13}$C stable isotope ratios [$\Delta^{13}$C in $\% (n = 5)$] of garlic plants in response to carbon dioxide (CO2) and nitrogen (N) treatments. Leaf gas-exchange parameters include net CO2 assimilation rates ($A$), instantaneous leaf water use efficiency (WUE), Rubisco capacity ($V_{\text{cmax}}$), and potential electron transport rate ($J_{\text{max}}$).

| Growth CO2 | N  | Cuvette $[\text{CO2}]$ | $A$ (µmol·m$^{-2}$·s$^{-1}$) | WUE (µmol·mmol$^{-1}$) | $C/C_{\text{a}}$ ratio$^a$ | $V_{\text{cmax}}$ (µmol·m$^{-2}$·s$^{-1}$) | $J_{\text{max}}$ (µmol·m$^{-2}$·s$^{-1}$) | $\Delta^{13}$C$^b$ (mean ± se) |
|-------------|----|-----------------------|-----------------------------|------------------------|----------------------------|---------------------------------|---------------------------------|-------------------------------|
| Ambient     | Low| 400                   | 8.7 ± 1.4                   | 5.6 ± 0.7              | 0.73 ± 0.02               | 34.7 ± 4.6                    | 97.0 ± 9.3                     | 24.4 ± 1.3                    |
|             | Mid| 400                   | 12.1 ± 1.5                  | 6.2 ± 0.7              | 0.71 ± 0.03               | 47.8 ± 4.3                    | 113.1 ± 8.9                    | 27.4 ± 2.5                    |
|             | High| 400                  | 12.8 ± 1.5                  | 6.8 ± 0.7              | 0.69 ± 0.03               | 53.9 ± 4.3                    | 114.3 ± 8.9                    | 26.9 ± 2.6                    |
| Elevated    | Low| 700                   | 11.1 ± 1.3                  | 6.7 ± 0.7              | 0.79 ± 0.02               | 28.7 ± 4.1                    | 73.2 ± 8.5                     | 15.2 ± 2.6                    |
|             | Mid| 700                   | 15.5 ± 1.7                  | 10.1 ± 0.8             | 0.74 ± 0.03               | 39.5 ± 4.3                    | 107.6 ± 8.5                    | 15.8 ± 3.2                    |
|             | High| 700                  | 14.9 ± 1.5                  | 11.4 ± 0.7             | 0.68 ± 0.03               | 37.8 ± 5.1                    | 103.9 ± 10.5                   | 16.4 ± 3.6                    |
| $P > F$     | CO2| 0.034                 | <0.001                      | 0.20                   | 0.007                     | 0.08                           | <0.001                         |                               |
|             | N  | 0.012                 | <0.001                      | 0.011                  | 0.005                     | 0.01                           | 0.67                           |                               |
|             | CO2 × N | 0.90         | 0.037                      | 0.39                   | 0.52                      | 0.57                           | 0.89                           |                               |

$^a$[CO2] of the air inside leaf cuvette ($C_{\text{a}}$) matching daytime growth [CO2].

$^b$Instantaneous leaf WUE determined as $A$ divided by the transpiration rate.

$^c$The ratio of internal [CO2] ($C_{\text{i}}$) over leaf cuvette [CO2] ($C_{\text{a}}$).
N. Hough-Snee, and S.-H. Kim, unpublished data). Yet, elevated CO₂ values in the low-N treatment were likely caused by limited availability of the N-dependent molecules in the light reactions of photosynthesis [e.g., chlorophyll, light harvesting complex, electron transport components, and coupling factor (Evans, 1989)]. Our results on $V_{\text{cmax}}$ and $J_{\text{max}}$ suggest that elevated CO₂ was likely to have reduced the Rubisco capacity in association with photosynthetic downregulation in garlic (Table 1); this was further corroborated by the reduced $A_{\text{elev}}/A_{\text{ambi}}$ ratios (Fig. 5). It has been pointed out that the biochemical signals of photosynthetic downregulation in response to CO₂ enrichment may be influenced by pot-size limitations (Poorter et al., 2012; Sage, 1994). Our plants were not root bound at harvest suggesting that pot size was unlikely to limit belowground sink capacity in our experiment. As discussed by Wullschleger (1993), leaf responses do not always relate to whole plant responses; and it is important to recognize the photosynthesis model parameters (i.e., $V_{\text{cmax}}$ and $J_{\text{max}}$) describe the initial fixation of CO₂ in the chloroplast by Rubisco, and that the subsequent translocation and allocation of this carbon for the growth of organs is a separate question.

Both leaf gas exchange and ¹³C stable isotope data supported the hypothesis that elevated CO₂ improved WUE of garlic plants across N treatments (Table 1). The combination of short-term (gas exchange) and long-term (Δ¹³C) metrics provides us with insights about the mechanics of WUE. The results show that of the WUE parameters, $A/E$ and Δ¹³C were significantly affected by the CO₂ treatment, whereas $E$ and $g_s$ alone were not ($P > 0.05$). For many other plants, improved WUE at elevated levels of CO₂ has been attributed to partial stomatal closure (Field et al., 1995; Nackley et al., 2014) and subsequently reduced stomatal water loss. In our study, the instantaneous WUE (i.e., $A/E$) increased as a result of an increase in photosynthesis rather than a decrease in transpiration (Table 1). The measured conductance and transpiration rates remained unchanged in elevated CO₂. Overtime, this increase in $A/E$ is likely to have resulted in an increase in the long-term WUE represented by the decreased Δ¹³C (Table 1). Indication of short- and long-term improvements in WUE suggest that garlic plants will likely gain more carbon per unit water used in elevated CO₂ irrespective of soil N fertility. The importance of gaining more carbon per unit water loss is especially relevant for garlic agriculture in arid and semiarid climates (i.e., California and other Mediterranean climate zones) where climate change has been predicted to increase summer temperatures and decrease fresh water supplies (Giorgi and Lionello, 2008) exacerbating growing season drought (Seager et al., 2013).

Despite the treatment effects on growth and N content, the bulbing ratio remained consistent (Fig. 3). This result is important considering that the bulbing ratio is a common allometric relationship used by garlic farmers as a nondestructive indicator of harvest time. Our results suggest that the bulbing ratio can continue to be a robust proxy across a range of soil fertility and in light of climate change, because the...
relationship was unaffected by either N or CO₂ treatments (Fig. 3). Research shows that optimal garlic harvests are recommended when the foliage collapses and starts to senesce, and when the bulb elongation ratio is about four or five (Takagi, 1990). When these phenological stages occur, the garlic plants allocate photosynthate to storage carbohydrates including fructans and other metabolites in the bulb. Our values are slightly lower than the recommended bulb elongation ratio for harvest. This is likely because our final destructive harvest was conducted before the bulbs were completely mature to compare biomass of other plant parts in addition to bulbs.

In summary, our results did not support our first two hypotheses. That is, 1) garlic plants did not allocate proportionally more biomass to bulb when grown in elevated CO₂ compared with the plants grown in ambient CO₂, whereas other growth and physiological parameters such as stem biomass and A have been increased in elevated CO₂ and 2) the $V_{\text{c,max}}$ and $A_{\text{elev}}/A_{\text{ambi}}$ ratios decreased in response to elevated CO₂ irrespective of N. This result is a strong indicator of photosynthetic downregulations in response to CO₂, irrespective of N. Our third hypothesis was supported as elevated CO₂ increased instantaneous WUE and reduced $\Delta^{13}$C across N levels. On the other hand, almost all growth and physiological parameters we tested responded strongly to N treatments. Our result also indicate that bulbing ratio is likely to remain unchanged with CO₂ or N levels and may continue to serve as a useful metric to determine harvest timing in a changing climate.

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