Does Low Stomatal Conductance or Photosynthetic Capacity Enhance Growth at Elevated CO_2 in Arabidopsis?[1]

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The objective of this study was to determine if low stomatal conductance (g) increases growth, nitrate (NO_3^-) assimilation, and nitrogen (N) utilization at elevated CO_2 concentration. Four Arabidopsis (*Arabidopsis thaliana*) near isogenic lines (NILs) differing in g were grown at ambient and elevated CO_2 concentration under low and high NO_3^- supply as the sole source of N. Although g varied by 32% among NILs at elevated CO_2, leaf intercellular CO_2 concentration varied by only 4% and genotype had no effect on shoot NO_3^- concentration in any treatment. Low-g NILs showed the greatest CO_2 growth increase under N limitation but had the lowest CO_2 growth enhancement under N-sufficient conditions. NILs with the highest and lowest g had similar rates of shoot NO_3^- assimilation following N deprivation at elevated CO_2 concentration. After 5 d of N deprivation, the lowest g NIL had 27% lower maximum carboxylation rate and 23% lower photosynthetic electron transport compared with the highest g NIL. These results suggest that increased growth of low-g NILs under N limitation most likely resulted from more conservative N investment in photosynthetic biochemistry rather than from low g.

The availability of water varies in time and space, and plants in a given environment are expected to evolve a stomatal behavior that optimizes the tradeoff of CO_2 uptake for photosynthesis at the cost of transpirational water loss. The resource of CO_2 also varies over time, and plant fossils indicate that stomatal characteristics have changed in response to periods of high and low atmospheric CO_2 over the past 65 million years (Beerling and Chaloner, 1993; Van Der Burgh et al., 1993; Beerling, 1998; Kürschner, 2001; Royer et al., 2001). Relatively low atmospheric CO_2 concentrations (less than 320 μmol mol^{-1}) over the last 23 million years (Pearson and Palmer, 2000) are associated with increased stomatal conductance (g) to avoid CO_2 starvation (Beerling and Chaloner, 1993). Atmospheric CO_2 concentration has risen rapidly from 280 to 400 μmol mol^{-1} since 1800 and has resulted in lower stomatal density (Woodward, 1987; Woodward and Bazzaz, 1988; Lammertsma et al., 2011). At the current atmospheric CO_2 concentration (400 μmol mol^{-1}), further decreases in g reduce water loss but also restrict CO_2 assimilation and, thus, limit the effectiveness of low g in water-stressed environments (Comstock and Ehleringer, 1993; Virgona and Farquhar, 1996). Elevated CO_2 concentration enhances the diffusion gradient for CO_2 into leaves, which allows g to decrease without severely restricting photosynthetic carbon gain (Herrick et al., 2004). Most consider such an improvement in water use efficiency in C_3 plants to be the main driving force for decreased g at elevated CO_2 concentration, especially in dry environments (Woodward, 1987; Beerling and Chaloner, 1993; Brodribb et al., 2009; Franks and Beerling, 2009; Katul et al., 2010).

Water is the most common factor limiting terrestrial plant productivity, but declining stomatal density has also occurred in wetland environments where water stress is uncommon (Wagner et al., 2005). Improved water use efficiency at elevated CO_2 concentration may be shifting the most common factor limiting plant productivity from water to nitrogen (N). In herbarium specimens of 14 species of trees, shrubs, and herbs, leaf N decreased 31% as atmospheric CO_2 increased from about 270 to 400 μmol mol^{-1} since 1750 (Penuelas and Matamala, 1990). Indeed, many studies have shown that N availability limits the stimulation of plant growth at elevated CO_2 concentration (Luo et al., 2004; Dukes et al., 2005; Reich et al., 2006). That most plants at elevated CO_2 concentration exhibit both lower g and greater N limitation suggests a relationship between these factors.

Plants primarily absorb N as nitrate (NO_3^-) in most temperate soils and assimilate a major portion of this NO_3^- in shoots (Epstein and Bloom, 2005). Elevated CO_2 increases the ratio of CO_2 to oxygen in the chloroplast, decreasing photorespiration and improving photosynthetic efficiency (Sharkey, 1988) but inhibiting photorespiration-dependent NO_3^- assimilation (Rachmilevitch et al., 2004; Bloom et al., 2010, 2012; Bloom, 2014). Greater rhizosphere NO_3^- availability tends to enhance root NO_3^- assimilation and decrease the influence of elevated CO_2 concentration on plant organic N accumulation (Kruse et al., 2002, 2003; Bloom et al., 2010).

The most important factor regulating chloroplast CO_2 concentration among natural accessions of Arabidopsis

[1] This work was supported by the National Research Initiative Competitive Grants Program (grant no. 2008–0214546).

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www.plantphysiol.org/cgi/doi/10.1104/pp.114.245241

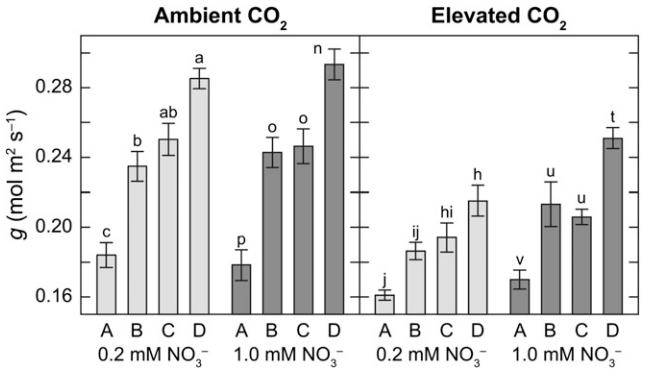

Figure 1. g of four Arabidopsis NILs grown at ambient or elevated CO_2 concentration under 0.2 mM NO_3^- (light-gray bars) or 1 mM NO_3^- (dark-gray bars). Each bar represents the mean of five to six plants ± SE. Means within NO_3^- treatment are significantly different if labeled with different letters.

(*Arabidopsis thaliana*) is g and to a lesser extent mesophyll conductance (Easlon et al., 2014). Low g may decrease the ratio of CO_2 to oxygen in the chloroplast at elevated CO_2 concentration, enhancing photorespiration-dependent NO_3^- assimilation. Alternatively, increasing atmospheric CO_2 may down-regulate the need to synthesize enzymes such as Rubisco to support photosynthesis, which conserves organic N, and g may decline as a by-product of lower photosynthetic capacity (Sage et al., 1989; Moore et al., 1998).

Here, we examined the influence of atmospheric CO_2 concentration and NO_3^- supply on photosynthesis, leaf N, and growth in near isogenic lines (NILs) of Arabidopsis differing in g. Arabidopsis accessions differ in many traits (including g) and likewise differ in DNA sequence at a large percentage of genes across the genome (Cao et al., 2011). Use of these NILs greatly reduces the proportion of the genome that varies and minimizes the influence of variation in other traits that are frequently associated with low g and could limit growth (Arp et al., 1998). We tested the extent to which (1) low g was associated with greater CO_2 growth enhancement at low and high NO_3^- supply; (2) low leaf intercellular CO_2 concentration (C_i) increased shoot NO_3^- assimilation; and (3) low g at elevated CO_2 concentration was associated with altered N utilization in photosynthetic biochemistry.

RESULTS

Differences in g Were Maintained at Elevated CO_2 Concentration

Arabidopsis NILs maintained the same relative statistical ranking independent of CO_2 and NO_3^- treatment (Fig. 1). All the NILs had lower g at elevated than at ambient CO_2, ranging from a 4.7% decrease in NIL A at 1 mM NO_3^- to a 24.5% decrease in NIL D at 0.2 mM NO_3^-. NO_3^- treatment had a significant effect on g at elevated CO_2 concentration ($P < 0.001$) but not at ambient CO_2 ($P = 0.792$). Genotype had a significant effect on g at both ambient and elevated CO_2 concentrations (both $P < 0.001$).

Growth and Gas Exchange at Ambient and Elevated CO_2 Concentrations

All Arabidopsis NILs at elevated CO_2 concentration had 93% to 189% more shoot dry weight at harvest (26 d after planting) than those at ambient CO_2 (Fig. 2). Elevated CO_2 had a similar effect on rosette area (Fig. 2). NO_3^- treatment had a significant effect on shoot dry weight at both ambient and elevated CO_2 concentrations (both $P < 0.001$). There was a significant interaction between NO_3^- and genotype on shoot dry weight at elevated CO_2 concentration ($P < 0.001$). When grown at elevated CO_2 concentration, shoot dry weight and rosette area at harvest were higher in the high-g NIL (D) than in the low-g NIL (A) under 1 mM NO_3^- and lower in the high-g NIL (D) than in the low-g NIL (A) under 0.2 mM NO_3^-.

Twenty-five days after planting, rosette photosynthesis per unit of leaf area was 22% to 41% higher at elevated than at ambient CO_2 concentration (Fig. 3). At elevated CO_2 concentrations but not at ambient CO_2 concentration, rosette photosynthesis per leaf area was faster (elevated, $P < 0.001$; ambient, $P = 0.407$), C_i was lower ($P < 0.001$ and $P = 0.166$), and leaf carbon isotope composition ($\delta^{13}C$) was smaller ($P < 0.001$ and $P = 0.438$) under low than high NO_3^-. Genotype had a significant effect on C_i and

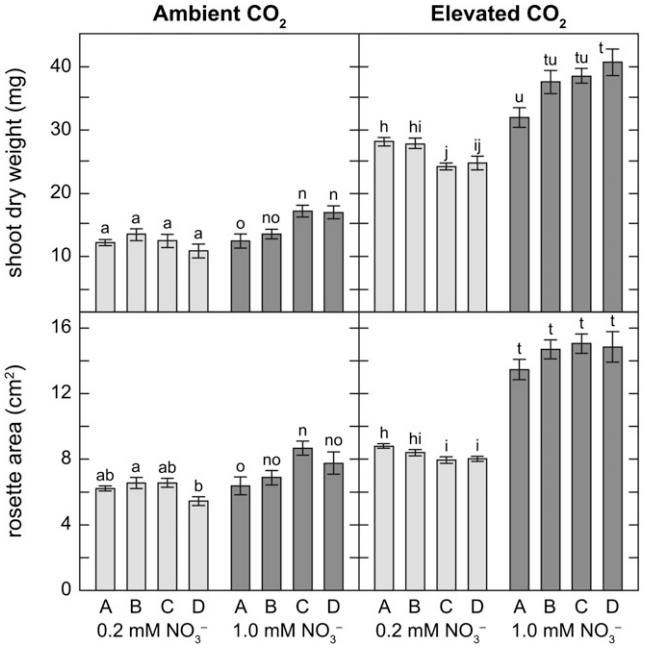

Figure 2. Shoot dry weight and rosette area of four Arabidopsis NILs grown at ambient or elevated CO_2 concentration under 0.2 mM NO_3^- (light-gray bars) or 1 mM NO_3^- (dark-gray bars). Each bar represents the mean of five to six plants ± SE. Means within NO_3^- treatment are significantly different if labeled with different letters.

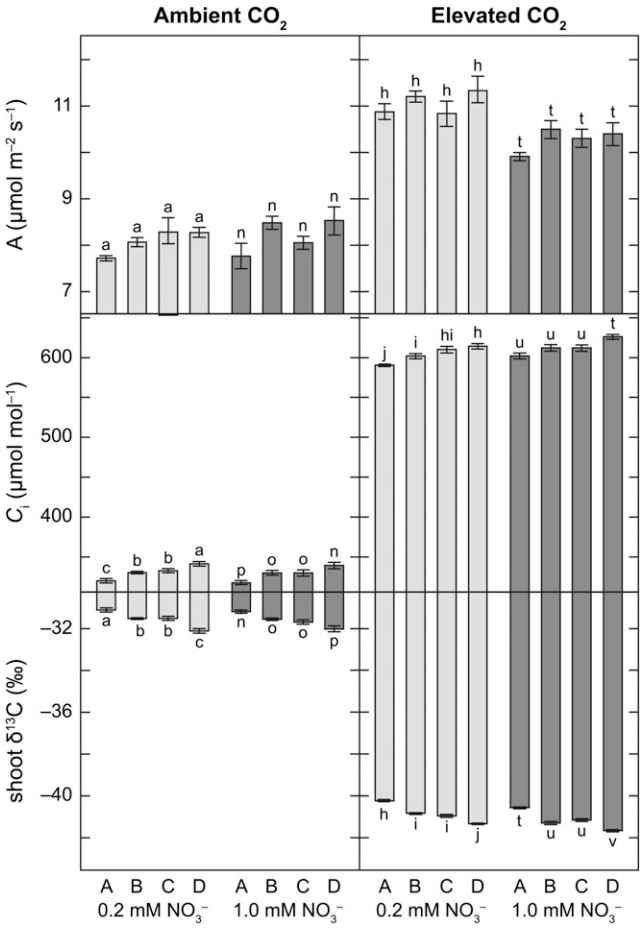

Figure 3. Photosynthesis (A), C_i, and shoot $\delta^{13}C$ of four Arabidopsis NILs grown at ambient or elevated CO_2 concentration under 0.2 mM NO_3^- (light-gray bars) or 1 mM NO_3^- (dark-gray bars). Each bar represents the mean of five to six plants ± SE. Means within NO_3^- treatment are significantly different if labeled with different letters.

$\delta^{13}C$ in all NO_3^- treatments at ambient and elevated CO_2 concentrations (all $P < 0.001$).

Shoot N at Ambient and Elevated CO_2 Concentrations

Leaves accumulated substantial NO_3^- in all treatments except for the low-NO_3^- treatment at elevated CO_2 concentration (Fig. 4). Genotype had a significant effect on total organic N under 1 mM NO_3^- in both CO_2 treatments (ambient, $P = 0.006$; elevated, $P = 0.02$). Shoot organic N concentration was 31% to 40% lower under low than high NO_3^- at elevated CO_2 concentration but was similar in both NO_3^- treatments at ambient CO_2 concentration (Fig. 5). The low-*g* NILs (A and B) did not have significantly more organic N than the high-*g* NILs (C and D) under 0.2 mM NO_3^- at either CO_2 concentration (Fig. 4). Shoot organic N concentration, however, was negatively correlated with shoot dry weight at harvest ($r^2 = 0.931$, $P < 0.001$) in low-NO_3^- plants grown at elevated CO_2 concentration but not in any other treatment (Fig. 5).

NO_3^- Assimilation after N Deprivation at Elevated CO_2 Concentration

In the second experiment, multiple harvests following transfer from 1 mM NO_3^- to 0 mM NO_3^- were used to assess the rates of shoot NO_3^- assimilation in the lowest and highest *g* NILs (A and D). After transfer from 1 mM NO_3^- to 0 mM NO_3^-, total shoot NO_3^- declined 49% to 51% in both low- and high-*g* NILs during the first light period, indicating similar NO_3^- assimilation rates in these genotypes (Fig. 6). Root NO_3^- declined to undetectable levels after the first light period but recovered partially during the first night. Shoot NO_3^- content did not change during the first night. Both shoot and root NO_3^- concentrations became negligible by the second day. Shoot growth differences between NILs become apparent as shoot NO_3^- declined, but root growth of the NILs was similar throughout the experiment.

N-Sufficient and N-Limited Photosynthesis at Elevated CO_2 Concentration

Photosynthetic CO_2 response curves of the lowest and highest *g* NILs (A and D) were compared 5 d after transfer to 0 mM NO_3^- or 1 mM NO_3^- nutrient solution (Fig. 7). N limitation had a large effect on maximum carboxylation rate (V_{cmax}) and photosynthetic electron transport (*J*) in both NILs (both $P < 0.001$). At high NO_3^-, V_{cmax} was 14% lower and *J* was 2% lower in NIL A than in NIL D. Under N limitation, genotype differences in photosynthetic parameters became more pronounced, with V_{cmax} 27% lower and *J* 23% lower in NIL A than in NIL D.

DISCUSSION

Our hypothesis that the low-*g* Arabidopsis NILs would grow faster than the high-*g* NILs at elevated CO_2 concentration under low NO_3^- supply was confirmed (Figs. 2 and 6). Plant responses to elevated CO_2 concentration

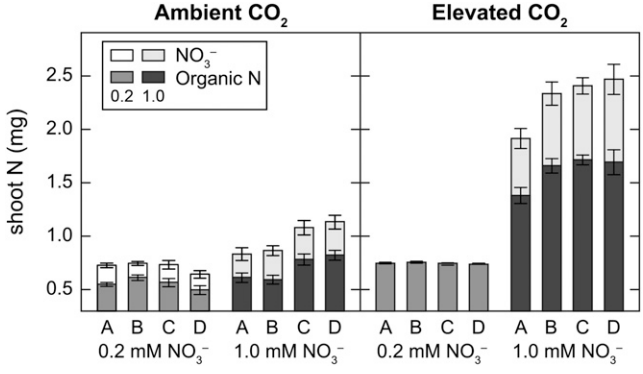

Figure 4. Total shoot NO_3^- and organic N in four Arabidopsis NILs grown at ambient or elevated CO_2 concentration under 0.2 mM NO_3^- or 1 mM NO_3^-. Each bar represents the mean of four to six plants ± SE.

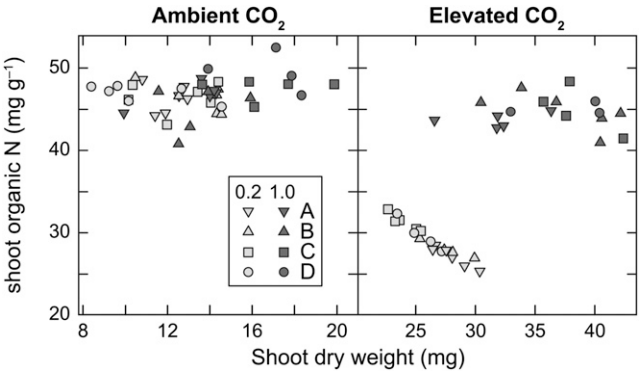

Figure 5. Relationship between shoot organic N concentration and shoot dry weight in four Arabidopsis NILs grown at ambient or elevated CO_2 concentration under 0.2 mM NO_3^- or 1 mM NO_3^-. Symbols represent individual plant values.

vary with the degree to which CO_2 is the primary factor limiting growth. Sustained stimulation of growth in long-term CO_2 enrichment studies generally requires heavy fertilization and irrigation, so neither N nor water is a limiting resource (de Graaff et al., 2006; Newingham et al., 2013). Here, the high-*g* Arabidopsis NILs showed the greatest growth enhancement at elevated CO_2 concentration under high NO_3^- supply (Fig. 2). This agrees with the common view that inherently fast-growing species have the greatest potential for growth enhancement at elevated CO_2 concentration (Poorter and Navas, 2003). Conversely, low *g* is usually associated with nutrient- or water-limited environments that constrain growth enhancement by elevated CO_2 concentration. Adaptation to nutrient- or water-limited environments results in a suite of changes in plant allocation and growth, but our use of Arabidopsis NILs minimized differences in plant performance that did not derive from genetic differences in *g*. Better performance in the low-*g* NILs under N limitation at elevated CO_2 concentration (Fig. 2) indicates a coupling of low *g* and improved N utilization or that improved N utilization lowered *g*.

Effect of Low *g* on C_i and NO_3^- Assimilation at Elevated CO_2 Concentration

Shoot NO_3^- and shoot organic N content at elevated CO_2 concentration suggest that the low-*g* NILs did not have significantly faster shoot NO_3^- assimilation than the high-*g* NILs. At elevated CO_2 concentration, C_i varied by only 24 μmol mol^{-1} on a background of about 610 μmol mol^{-1} between the highest and lowest *g* NILs. Likewise, $\delta^{13}C$, which is affected by both stomatal and mesophyll conductances (Seibt et al., 2008), indicates that chloroplast CO_2 concentrations mirrored C_i estimates (Fig. 3). This relatively small change in C_i would have negligible effects on the rates of photorespiratory NO_3^- assimilation (Bloom et al., 2012). More pronounced decreases in *g* and/or mesophyll conductances would be required to lower chloroplast CO_2 concentration and stimulate rates of photorespiration similar to those observed at 400 μmol mol^{-1} atmospheric CO_2.

If elevated CO_2 concentration suppressed NO_3^- assimilation, shoot NO_3^- accumulation should increase and shoot organic N concentration should decrease. Accordingly, at high NO_3^- supply, total shoot NO_3^- was higher (Fig. 4), and shoot organic N concentration was lower (Fig. 5) at elevated than at ambient CO_2 concentration. Total shoot organic N increased at elevated CO_2 concentration, but this may reflect that elevated CO_2 concentration stimulates root NO_3^- assimilation (Kruse et al., 2002, 2003). Being able to distinguish between shoot and root NO_3^- assimilation would allow us to discern the effects of CO_2 inhibition of photorespiratory NO_3^- assimilation on total shoot organic N content.

Under low N at elevated CO_2 concentration, all four genotypes assimilated all of the available NO_3^-. The large decline in organic N concentration at elevated CO_2 concentration under low NO_3^- supply resulted from an N limitation (Fig. 5). Under N limitation, the low-*g* NILs had higher shoot dry weight and leaf area than the high-*g* NILs (Fig. 2). Shoot dry weight was negatively correlated with shoot organic N concentration at elevated CO_2 concentration under low NO_3^- supply, indicating that improved N utilization may explain the better performance of the low-*g* NILs under N limitation (Fig. 5). This negative correlation was not observed at ambient CO_2 concentration under low NO_3^- supply, most likely because slower growth resulted in a delayed onset of N limitation.

NO_3^- Assimilation following N Deprivation at Elevated CO_2 Concentration

In the second experiment, plants were transferred from high NO_3^- supply to a solution without N. In both the highest and lowest *g* NILs, shoot NO_3^- content declined rapidly during the first day and not at night, indicating that NO_3^- assimilation depended on photosynthesis and occurred at similar rates in the highest and lowest *g* NILs (Fig. 6). Indeed, the low- and high-*g* NILs did not differ much in internal C_i (Fig. 3).

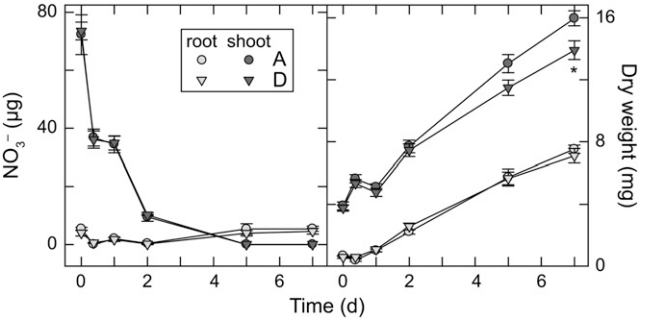

Figure 6. Shoot and root NO_3^- and dry weight for two NILs grown at elevated CO_2 concentration 0 to 7 d after N deprivation. Each symbol represents the mean of five to six plants for each NIL ± SE. The asterisk indicates a statistically significant difference ($P < 0.05$).

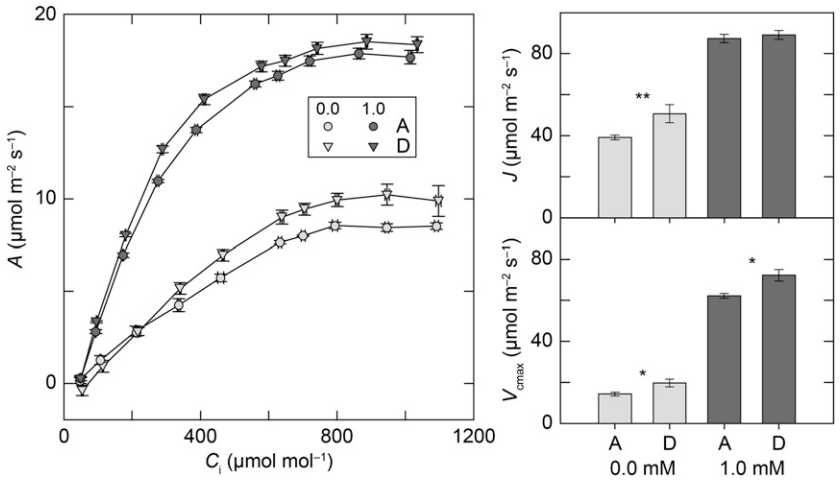

Figure 7. Photosynthetic response curves (A) for two NILs grown at elevated CO_2 concentration 5 d after N deprivation or 1 mM NO_3^- control treatment. V_{cmax} and J were obtained from the photosynthetic CO_2 response curves. Each symbol or bar represents the mean of six plants ± SE. Asterisks represent levels of statistical significance within NO_3^- treatment (*$P < 0.05$ and **$P < 0.01$).

Photoassimilatory NO_3^- assimilation is relatively insensitive to small changes in C_i at elevated CO_2 concentration (Bloom et al., 2012), so more pronounced differences in g and/or mesophyll conductance may be required to observe differences in the rates of shoot NO_3^- assimilation at elevated CO_2 concentration.

N-Sufficient and N-Limited Photosynthesis at Elevated CO_2 Concentration

At elevated CO_2 concentrations but not ambient CO_2 concentration, rosette photosynthesis per leaf area was faster under low than high NO_3^- (Fig. 3). Higher photosynthesis per leaf area under NO_3^- may result from lower specific leaf area under N limitation (Fig. 2); however, shoot organic N concentration was also lower under low than high NO_3^- (Fig. 5). This appears to be in opposition to the well-documented relationship between assimilation and leaf organic N concentration (Field and Mooney, 1986; Evans, 1989). In the second experiment, photosynthesis was higher under high than low NO_3^- (Fig. 7). Differences in photosynthesis between these two experiments may result from gradual versus sudden imposition of N limitation and growth level (350 μmol m^{-2} s^{-1}) versus saturating (1,000 μmol m^{-2} s^{-1}) photosynthetic photon flux density (PPFD) during gas-exchange measurements. Photosynthetic capacity may have been higher under N-sufficient growth in the first experiment, but higher respiration associated with higher leaf organic N concentration may have resulted in lower photosynthesis under nonsaturating PPFD.

In the first experiment, the high- and low-g NILs had similar rates of carbon assimilation, photosynthesis at elevated CO_2 concentration (Fig. 3), but in the second experiment, the two genotypes exhibited differences in their biochemical parameters of photosynthesis. At elevated CO_2 concentration, the low-g NIL experienced more of a decrease in V_{cmax} and J under N deprivation than the high-g NIL (Fig. 7). Lower V_{cmax} (the maximum rate of ribulose 1,5-bisphosphate carboxylation) indicates a lower Rubisco content or less Rubisco activation, whereas lower J indicates a lower rate of electron transport driving ribulose 1,5-bisphosphate regeneration. The low-g NIL also had greater growth (Fig. 6) and lower organic N concentration (Fig. 5) than the high-g NIL. Lower V_{cmax} and lower organic N concentration support the interpretation that under N limitation, the low-g NIL invested less N in Rubisco than the high-g NIL and thus was able to continue to grow with a lower leaf organic N concentration. Long-term exposure to elevated atmospheric CO_2 frequently results in a decline in leaf Rubisco content (Sage et al., 1989; Moore et al., 1998). Because Rubisco can constitute as much as 30% of leaf N (Evans, 1989), reallocating N from Rubisco to other components of leaf photochemistry may improve N utilization (Sage et al., 1989; Moore et al., 1998). High rates of Rubisco inactivation at elevated CO_2 concentration suggest that many C_3 plants, even after photosynthetic acclimation to elevated CO_2 concentration, overinvest in Rubisco (Sage et al., 1989). While high investment in photosynthetic biochemistry is beneficial for N-sufficient growth, it may also explain the slower growth of the high-g NIL relative to the low-g NIL under N limitation observed in both experiments (Figs. 2 and 6). Higher photosynthesis at elevated CO_2 concentration can only improve growth if other nutrients are available so that additional carbon fixed can be converted into useful plant tissue (Kirschbaum 2011).

In summary, a 32% lower g in these four Arabidopsis NILs resulted in only a 4% decrease in C_i (Fig. 3). This small change in C_i, particularly at elevated CO_2 concentration,

Table I. *Arabidopsis NILs*

Arabidopsis NILs were generated from introgression of quantitative trait loci for g and leaf $\delta^{13}C$ from Kas-1 into a Tsu-1 genetic background.

NIL	Quantitative Trait Locus	Kas-1 Introgression
A	$\delta^{13}C$	Chromosome 4, 0.17 to 5.6 Mb
B	g	Chromosome 1, 17.5 to 30.2 Mb
C	g	Chromosome 1, 25.9 to 30.2 Mb
D	$\delta^{13}C$	Chromosome 5, 19.6 to 26.9 Mb

should not and did not have a large effect on shoot NO_3^- assimilation. The low-*g* strategy, however, provided an advantage under N limitation, most likely as a result of more conservative investment in photosynthetic biochemistry. Low photosynthetic capacity rather than low *g* is important for N-limited growth at elevated CO_2 concentration in Arabidopsis.

MATERIALS AND METHODS

Plant Material

Natural accessions of Arabidopsis (*Arabidopsis thaliana*) vary in *g* and $\delta^{13}C$, a time-integrated measure of chloroplast CO_2-atmospheric CO_2 ratio (McKay et al., 2003, 2008; Juenger et al., 2005, 2010; Christman et al., 2008; Monda et al., 2011; Des Marais et al., 2012; Lasky et al., 2012; Easlon et al., 2014). A sample of accessions will also differ in many other traits and harbor hundreds of thousands of polymorphisms in coding and noncoding regions (Cao et al., 2011). To minimize the number of genome-wide differences, we selected four NILs of Arabidopsis varying in $\delta^{13}C$. NILs were selected from the NIL library described by Fletcher et al. (2013) based on chromosomal introgressions at quantitative trait loci for *g* or $\delta^{13}C$ from the Kas-1 (CS903) accession in a Tsu-1 (CS1640) accession background (Table I). Kas-1 is a winter accession with high water use efficiency, low *g* and photosynthesis, and long flowering time. Tsu-1 is a spring accession with low water use efficiency, high *g* and photosynthesis, and short flowering time (Easlon et al., 2014).

Hydroponic Growth

Seeds were germinated in GA-7 Magenta vessels on sand saturated with a nutrient solution that contained 1 mM $CaSO_4$, 0.75 mM K_2HPO_4, 0.25 mM KH_2PO_4, 0.75 mM $MgSO_4$, and 0.04 g L^{-1} iron-diethylene triamine pentaacetic acid and micronutrients at 20% (v/v) strength of a modified Hoagland solution (Epstein and Bloom, 2005) with 0.2 mM KNO_3 as the sole source of N. Plants were grown in controlled-environment chambers (Conviron) set at 23°C/20°C light/dark and 50% to 60% relative humidity with 9 h of light per day at 350 μmol m^{-2} s^{-1} PPFD at plant height. After 7 d, seedlings were transferred to 300-mL opaque polyethylene bottles containing the above nutrient solution with 0.2 or 1 mM KNO_3 as the sole source of N. Seedling hypocotyls were placed in a foam rubber plug, and roots were inserted into a hydroponics solution through a hole drilled in the lid.

CO_2 × NO_3^- Study

In the first experiment, four NILs were grown in chambers at either ambient CO_2 (405 ± 25 μmol mol^{-1}) or elevated CO_2 (720 ± 20 μmol mol^{-1}) with either 0.2 or 1 mM KNO_3 as the sole source of N. Six plants per NIL × NO_3^- combination were grown in each chamber in a complete randomized design (48 plants per CO_2 treatment). At 19 d after transfer to hydroponics, rosettes were photographed for leaf area and plants were harvested. This growth duration was selected to avoid flowering, because flowering has significant effects on gas exchange and N partitioning in Arabidopsis.

N Deprivation at Elevated CO_2 Concentration

The NILs (A and D) that had the lowest and highest *g* according to the first experiment were grown at elevated CO_2 concentration in nutrient solution under 1 mM KNO_3 for the first 12 d in hydroponics. At dawn the next day, plants were transferred to a nutrient solution containing 0 or 1 mM KNO_3. Six N-deprived plants per genotype were harvested 0 h (right after solution change), 9 h (dusk), 1 d, 2 d, 5 d, and 7 d after the solution change. Six control 1 mM KNO_3 plants per genotype were harvested on the last harvest date (six replicates × six harvests × two NILs + six replicates × two NILs = 84 plants).

N Analyses

At each harvest, rosettes were photographed for leaf area, plants were divided into shoots and roots, and roots were rinsed in deionized water. Shoots and roots were oven dried at 55°C for 48 h and weighed. Whole shoots and roots were ground to a fine powder in centrifuge tubes with ball bearings to determine NO_3^- and total N. Subsamples (2–5 mg) were extracted in 1.5 mL of 10 mM $CaSO_4$ and clarified by centrifugation. Aliquots were analyzed for NO_3^- using the Griess reaction (Miranda et al., 2001). In the first experiment, total N and $\delta^{13}C$ was determined at the University of California-Davis Stable Isotope Facility (http://stableisotopefacility.ucdavis.edu/). Differences in $\delta^{13}C$ between CO_2 treatments must be viewed with caution, as the ^{13}C composition of chamber CO_2, especially in the elevated CO_2 concentration chamber, was variable. Organic N was estimated by subtracting NO_3^--N from total N. In the NO_3^- deprivation experiment, plants were only analyzed for NO_3^- because there was not sufficient material to measure total N and $\delta^{13}C$.

Gas Exchange

Whole-canopy gas exchange was measured using a LI-6400 device with a 6400-17 whole-shoot Arabidopsis chamber (Li-Cor). In the first experiment, gas exchange was measured on all plants 18 d after transfer to hydroponics. The Arabidopsis chamber was maintained at 350 μmol m^{-2} s^{-1} PPFD, leaf temperature was maintained at 23°C, and relative humidity was maintained at 60%. CO_2 was maintained at either 400 or 720 μmol mol^{-1} to match environmental chamber conditions. In the NO_3^- deprivation experiment, photosynthetic response curves were measured on six plants of each genotype from the N-deprived (0 mM NO_3^-) and control (1 mM NO_3^-) treatments 17 d after transfer to hydroponics (5 d after N deprivation). Arabidopsis chamber conditions were the same as above, but PPFD was at saturating (1,000 μmol m^{-2} s^{-1}) light and CO_2 was adjusted in a stepwise fashion to obtain photosynthetic response curves. Following gas-exchange measurements for each plant, leaf area was determined from digital photographs of plant rosettes using Easy Leaf Area software (Easlon and Bloom, 2014). Because of large CO_2 gradients between the chamber and outside, empty chamber leak corrections were applied to data. V_{cmax} and *J* were calculated using a least-squares iterative curve-fitting procedure (Sharkey et al., 2007) to fit the Farquhar biochemical model for photosynthesis (Farquhar et al., 1980).

Statistical Analyses

We conducted ANOVA using PROC GLM in SAS (SAS 9.3). Mean separations were determined using Tukey's tests ($P < 0.05$ was considered statistically significant). All data except shoot organic N and NO_3^- content satisfied the ANOVA assumptions of normality and homogeneity of variances. One-way ANOVA within each NO_3^- treatment (0.2 versus 1 mM) met the assumptions of ANOVA for N data, so N data were analyzed via one-way ANOVA to test for genotypic effects. We estimated correlations among physiological traits as the standard Pearson product-moment correlation.

ACKNOWLEDGMENTS

We thank Madeline Perez for assistance in the growth chambers and laboratory, Rich Fletcher for help in selecting NILs, and the editor and reviewers for insightful comments.

Received June 15, 2014; accepted January 9, 2015; published January 12, 2015.

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