Elevated CO2 effects on nitrogen assimilation and growth of C3 vascular plants are similar regardless of N-form assimilated

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Abstract

Atmospheric carbon dioxide concentration ([CO2]) increased from around 280 ppm in 1750 to 400 ppm in 2016 and is likely to continue to increase throughout this century. It has been argued that wheat, Arabidopsis, and C3 plants in general respond more positively to elevated atmospheric [CO2] under ammonium (NH4+) nutrition than under nitrate (NO3−) nutrition because elevated CO2 inhibits their photo-reduction of NO3− and hence reduces their total plant nitrogen (N) assimilation and ultimately growth. Here, it is argued that the weight of evidence in the literature indicates that elevated atmospheric [CO2] does not inhibit NO3− assimilation and growth of C3 vascular plants. New data for common bean and wheat support this view and indicate that the effects of elevated atmospheric [CO2] on N assimilation and growth of C3 vascular plants will be similar regardless of the form of N assimilated.

Keywords: Ammonium, carbon dioxide, N2 fixation, nitrate, photo-reduction of nitrate, urea.

Introduction

Most vascular plants take up and utilize a range of forms of nitrogen (N) from the soil, with the relative importance of each N form primarily dependent on its availability (Andrews et al., 2013). Nitrate (NO3−) is an important form of N available to, and utilized by, most plants in cultivated or otherwise disturbed (aerated) soils with concentrations usually in the range 0.5–20 mol m−3 depending on the level of disturbance and N fertilizer added (Andrews et al., 2013). However, ammonium (NH4+) can be the main form of N utilized in undisturbed/ unfertilized soils while amino acids and short peptides/ proteins (‘organic’ N) can be important in unimproved soils (Näsholm et al., 2009; Kraiser et al., 2011). Urea (via urine excretion from animals or urea fertilizer) can, temporally at least, occur in the soil at high concentrations and can be taken up directly and assimilated by plants (Kraiser et al., 2011; Witte, 2011). Also, around 70% of legumes and all actinorhizal plants can obtain a substantial amount of their N requirements from N2-fixing symbiotic bacteria (Herridge et al., 2008; Andrews et al., 2011).

Within the plant, NO3− is reduced to nitrite (NO2−) via the enzyme nitrate reductase (NR), then this NO2− is reduced to NH4+ by nitrite reductase (NiR) (Lillo, 2008; Heidari et al., 2011).
The NH$_4^+$ produced is assimilated into amino acids via the glutamine synthetase–glutamate synthase (GS–GOGAT) pathway (Lea and Miflin, 2011). The root or shoot can be the main site of NO$_3^−$ assimilation depending on plant genotype and environmental conditions, especially NO$_3^−$ availability (Andrews, 1986; Andrews et al., 2013). Nitrate reductase is located in the cytosol of root and shoot cells and, for most plant species, uses nicotinamide adenine dinucleotide (NADH) as the reductant (Campbell, 1988). The NiR enzyme is located in the plastids of roots and other non-photosynthetic tissue and the chloroplasts of photosynthetic tissue, and uses ferredoxin (Fd) as a reductant (Sakakibara et al., 2012). Within the plastid/chloroplast, GS then catalyses the adenosine triphosphate (ATP)-dependent conversion of NH$_3$ and glutamate to glutamine while GOGAT catalyses the NADH–Fd-dependent conversion of glutamate and 2-oxoglutarate to form two molecules of glutamate. NADH-dependent GOGAT is located predominantly in non-photosynthesizing cells, where the reductant is initially supplied by the pentose phosphate pathway (Bowsher et al., 2007). Fd-dependent GOGAT activity is much greater than NADH-dependent GOGAT in leaf chloroplasts, where light energy can be used directly for the synthesis of reduced Fd used in NO$_3^−$ reduction and conversion of glutamine and 2-oxoglutarate to glutamate.

Ammonium taken up from the soil is assimilated into amino acids via the GS–GOGAT pathway, normally in the root (Lea and Miflin, 2011). Within the plant, urea undergoes hydrolysis via the enzyme urease with NH$_3$ and CO$_2$ being produced (Witte, 2011). The NH$_3$ is assimilated into amino acids via the GS–GOGAT pathway. There are few data for the partitioning of urea assimilation between root and shoot of vascular plants, but there is evidence that for common bean (Phaseolus vulgaris) the root is the main site of urea assimilation at 1–2 mol m$^{-3}$ applied urea–N but shoot assimilation increases in importance as the applied urea–N concentration increases from 2 to 10 mol m$^{-3}$ (Hine and Sprent, 1988). Root nodules are the primary site of N$_2$ fixation for legume and actinorhizal plants.

Nitrogen fixation in legume roots is catalysed by the enzyme nitrogenase in rhizobial bacteroids and Frankia endophytes and, generally, this NH$_3$ as NH$_4^+$ is transported to the plant root cells where it enters the GS–GOGAT pathway (Betti et al., 2012; Seabra et al., 2012).

Evidence indicates that atmospheric carbon dioxide concentration ([CO$_2$]) increased from around 280 ppm in 1750 to 400 ppm in 2016, and will continue to increase throughout this century (Intergovernmental Panel on Climate Change, 2014). Bloom and co-workers over a range of research papers and reviews argued that C$_3$ plants respond more positively to elevated [CO$_2$] under NH$_4^+$ nutrition than under NO$_3^−$ nutrition because elevated CO$_2$ inhibits the plant photo-reduction of NO$_3^−$ and hence reduces total plant N assimilation and growth (Bloom, 2015a,b; Rubio-Asensio and Bloom, 2017). The main evidence presented for a specific effect of elevated CO$_2$ on photo-reduction of NO$_3^−$ in C$_3$ plants was the effect on the assimilatory quotient (AQ), the ratio of net CO$_2$ consumption to net O$_2$ evolution in a photosynthetically active tissue. Under controlled environment conditions, the AQ for wheat (Triticum aestivum) and Arabidopsis was greater with NH$_4^+$ than with NO$_3^−$ nutrition at ambient CO$_2$ (Bloom et al., 2002, 2010, 2012; Rachmilevitch et al., 2004; Bloom, 2015a; Rubio-Asensio et al., 2015). Across these studies, it was argued that the lower AQ with NO$_3^−$ was due to transfer of electrons to NO$_3^−$ and NO$_2^−$ during NO$_3^−$ photo-reduction/assimilation, which resulted in O$_2$ evolution from the light-dependent reactions of photosynthesis with little effect on CO$_2$ fixation in the light-independent reactions of photosynthesis. The difference in AQ between plants receiving NH$_4^+$ or NO$_3^−$ was considered to provide an estimate of shoot NO$_3^−$ assimilation as other processes that could result in a reduced AQ, such as increased sulphate assimilation and lipid synthesis were assumed to be similar with the two N forms (Bloom et al., 2002, 2010, 2012; Rachmilevitch et al., 2004; Bloom, 2015a; Rubio-Asensio et al., 2015). Under NO$_3^−$ nutrition, the AQ was consistently greater at 700–760 ppm CO$_2$ (elevated CO$_2$) than at 360–400 ppm CO$_2$ (ambient CO$_2$) and where tested, similar with NO$_3^−$ and NH$_4^+$ at elevated CO$_2$ (Bloom et al., 2002, 2010, 2012; Rachmilevitch et al., 2004; Rubio-Asensio et al., 2015). Bloom and co-workers argued that this increase in AQ (increased CO$_2$ uptake relative to O$_2$ evolution) under elevated CO$_2$ with NO$_3^−$ was associated with a decrease in photo-reduction of NO$_3^−$ that resulted in a decrease in overall plant N (NO$_3^−$) assimilation, reduced total plant N content, and in some cases reduced growth that did not occur under NH$_4^+$ nutrition. Bloom (2015a) proposed a mechanism for elevated CO$_2$ inhibition of NO$_3^−$ assimilation in C$_3$ plants linked to its inhibitory effect on photorespiration. He argued that under ambient CO$_2$, photorespiration stimulates the export of malate from chloroplasts to the cytoplasm and this malate in the cytoplasm generates NADH that drives the reduction of NO$_3^−$ to NO$_2^−$. However, under elevated CO$_2$, photorespiration is inhibited and this will cause a decrease in transport of malate from the chloroplast to the cytoplasm and, consequently, decreased generation of NADH and associated NO$_3^−$ assimilation in the cytoplasm.

In our opinion, across the experiments of Bloom and co-workers, the effects of elevated CO$_2$ on N assimilation and growth of wheat and Arabidopsis under NO$_3^−$ and NH$_4^+$ nutrition were not consistent and the AQ value did not indicate the proportion of NO$_3^−$ assimilation taking place in the shoot. Thus, the conclusions reached by Bloom and colleagues cannot be generalized. For example, focusing on wheat, which was tested in almost all studies, Bloom et al. (2002) reported that total plant dry weight (DW) was similar with 0.2 mol m$^{-3}$ N as NO$_3^−$ or NH$_4^+$ at ambient CO$_2$. For NO$_3^−$–fed plants, shoot NR and NiR decreased 12 and 27%, respectively, on a protein basis, and 33 and 30%, respectively, on a fresh weight basis with elevated CO$_2$ for 14 d while root NR and NiR were unaffected by CO$_2$ supply. For most plant species, NR and NiR are substrate (NO$_3^−$/NO$_2^−$)-induced enzymes (Bungard et al., 1999; Andrews et al., 2013) and it was concluded that increased CO$_2$ supply inhibited shoot NO$_3^−$ assimilation (Bloom et al., 2002). However, total plant DW was 78% greater at elevated than at ambient CO$_2$ under NH$_4^+$ nutrition and 44% greater at elevated CO$_2$ under NO$_3^−$ nutrition. Shoot and root N concentration changed little with N form or CO$_2$ supply, and thus NH$_4^+$ uptake was greater than NO$_3^−$ uptake at high CO$_2$.
supply but NO$_3^-$ uptake was substantially greater at elevated than at ambient CO$_2$. Also, shoot protein per plant was 73% greater with NH$_4^+$ and 32% greater with NO$_3^-$ at elevated CO$_2$, and thus NO$_3^-$ assimilation was also greater at elevated CO$_2$. Bloom et al. (2012) reported that for wheat 13–24 d after imposing treatments from a uniform starting point, relative growth rate (RGR) on a fresh weight (FW) basis (DW was not reported) was greater with 0.2 mol m$^{-3}$ NO$_3^-$ than with 0.2 mol m$^{-3}$ NH$_4^+$ at ambient CO$_2$. RGR was greater at elevated than at ambient CO$_2$ under NH$_4^+$ but did not change with CO$_2$ supply under NO$_3^-$ nutrition. Nevertheless, highest RGR across all treatments was with NO$_3^-$ at elevated CO$_2$, and Bloom et al. (2012) concluded that there must be an alternative mechanism of NO$_3^-$ assimilation at high CO$_2$, for example enhanced root NO$_3^-$ assimilation, to explain this result. Rubio-Asensio et al. (2015) reported that at 14 d after imposing treatments, total plant DW was greater with 0.2 mol m$^{-3}$ NO$_3^-$ than with 0.2 mol m$^{-3}$ NH$_4^+$ at ambient and elevated CO$_2$, but here growth was greater at elevated than at ambient CO$_2$ for plants supplied NH$_4^+$ or NO$_3^-$ in a related study in which plants were exposed to ambient or elevated CO$_2$ from seedling to maturity, shoot DW and grain yield were unaffected by CO$_2$ supply under 0.2 mol m$^{-3}$ NO$_3^-$ nutrition but decreased with elevated CO$_2$ under NH$_4^+$ nutrition (Carlisle et al., 2012). Thus, across these related studies, the effects of elevated CO$_2$ under NH$_4^+$ or NO$_3^-$ nutrition were not consistent. Also, the NO$_3^-$ and NH$_4^+$ concentrations supplied were very low in comparison with those commonly used in nutrition studies on wheat and other ‘fast growing’ plants (Andrews et al., 1984, 1992, 2006, 2013; Bungard et al., 1999). Plants that exhibited no response to elevated CO$_2$ at 0.2 mol m$^{-3}$ applied NO$_3^-$ may have shown a growth response if additional NO$_3^-$ had been supplied.

Outside these studies, considerable data from free air carbon dioxide (FACE) trials carried out under conditions (cultivated/quartered soils) in which NO$_3^-$ is likely to be the main form of N available to plants indicate that a wide range of C$_3$ species, including wheat, show increased growth under CO$_2$ enrichment, especially if they receive high applications of N (Ainsworth and Long, 2005; de Graaff et al., 2006; Wang et al., 2013; Han et al., 2015; Cai et al., 2016; Coskun et al., 2016; Fitzgerald et al., 2016; Kimball, 2016). Bloom et al. (2012) proposed that in such studies, application of N increased the availability of soil NH$_4^+$ and this compensated for lower rates of shoot NO$_3^-$ assimilation. However, work carried out on a range of C$_3$ species under controlled environment or glasshouse conditions with NO$_3^-$ as the sole N source indicated that greatest growth across treatments occurred under elevated CO$_2$ with high NO$_3^-$ supply (Hocking and Meyer, 1991; Geiger et al., 1999; Matt et al., 2001; Dong et al., 2017). For wheat grown in sand substrate under glasshouse conditions, dry matter production over 60 d increased with increased NO$_3^-$ supply over the range 0.5–25 mol m$^{-3}$ NO$_3^-$ under ambient and 1500 ppm CO$_2$ (Hocking and Meyer, 1991). Dry matter was greater at 1500 ppm CO$_2$ than at ambient CO$_2$ with all NO$_3^-$ treatments, and thus greatest growth across treatments occurred with the highest NO$_3^-$ supply under elevated CO$_2$. Also, for a given NO$_3^-$ supply, total plant reduced N was greater at elevated CO$_2$ indicating that greater NO$_3^-$ assimilation had occurred at the higher CO$_2$. However, the proportional increase in reduced N was not as great as that for DW and tissue N content per unit DW was lower at elevated CO$_2$.

In our view, from the above discussion, there is no strong evidence in the literature that (i) C$_3$ plants respond more positively to elevated CO$_2$ under NH$_4^+$ nutrition than under NO$_3^-$ nutrition, or (ii) elevated CO$_2$ inhibits NO$_3^-$ assimilation in C$_3$ species. Here we present new data for common bean and wheat that indicate the effects of elevated atmospheric CO$_2$ on growth and N assimilation of C$_3$ vascular plants will be similar regardless of form of N assimilated or the site (root or shoot) of assimilation.

Materials and methods

Experiments were carried out on the C$_3$ species common bean (Phaseolus vulgaris) and wheat (Triticum aestivum). Common bean can show substantial growth with NO$_3^-$, NH$_4^+$, urea, glutamine, or N$_2$ fixation as its main N source and reports are consistent that the leaf is the main site of NO$_3^-$ assimilation at 1–20 mol m$^{-3}$ applied NO$_3^-$ (Andrews, 1986; Andrews et al., 2013). Because of these characteristics, common bean was used as a test plant to assess the importance of form and site of N nutrition in determining the response of a C$_3$ species to elevated CO$_2$. Wheat was used for direct comparison with results from the studies of Bloom and co-workers.

Four experiments were conducted across two Conviron BDW 120 plant growth rooms (Thermo Fisher Scientific, Auckland, New Zealand). The lighting system in both growth rooms consisted of 48 × 400 W metal halide bulbs (Venture Ltd, Mount Maunganui, New Zealand) in combination with 48 soft tone, soft white 100 W incandescent bulbs (Philips, Auckland, New Zealand) mounted behind a Perspex barrier 2.4 m above floor level. The photoperiod was 16 h with a PAR at the pot surface of >950 μmol photons m$^{-2}$ s$^{-1}$ and light levels ramped for 60 min to simulate dawn/dusk. A top-down airflow pattern, with controlled flow of filtered outdoor air, maintained ambient CO$_2$ conditions (~400 ppm CO$_2$) within one growth room. The second growth room had a similar airflow system but was maintained at 760 ppm CO$_2$ with G214 food grade CO$_2$ (BOC, Auckland, New Zealand) mounted behind a Perspex barrier 2.4 m above floor level. The photoperiod was 16 h with a PAR at the pot surface of >950 μmol photons m$^{-2}$ s$^{-1}$ and light levels ramped for 60 min to simulate dawn/dusk. A top-down airflow pattern, with controlled flow of filtered outdoor air, maintained ambient CO$_2$ conditions (~400 ppm CO$_2$) within one growth room. The second growth room had a similar airflow system but was maintained at 760 ppm CO$_2$ with G214 food grade CO$_2$ (BOC, Auckland, New Zealand) added as required. The CO$_2$ levels in the cabinets were measured continuously using PP Systems WMA-4 Gas Analysers (John Morris Scientific, Auckland, New Zealand). Daytime relative humidity was maintained at 65% and night time humidity peaked at 80%.

In Experiment 1, common bean cv Top Crop Dwarf (Kings Seeds, Katikati, New Zealand) was grown from seed (350–400 mg in 1 litre pots (one plant per pot) containing a 1:1 volume mix of vermiculite and perlite that was flushed every 2 d (or 1 d on the last week of the experiment) with basal nutrient solution (Andrews et al., 1992) containing the appropriate N treatment. The treatments were 0.5, 1, 2, 3, 4, 6, 8, and 10 mol m$^{-3}$ N supplied as potassium nitrate, ammonium sulphate, urea, or glutamine (all N forms were matched on a N basis) and a 0.5 mol m$^{-3}$ NO$_3^-$ treatment inoculated with Rhizobium leguminosarum var phaselii strain ICMP 2672, the recommended rhizobial inoculant for common bean in New Zealand (International Collection of Microorganisms from Plants, Auckland, New Zealand). Potassium concentration was made equal across all treatments by the addition of potassium sulphate as required, and sulphate was not balanced. The day/night temperatures were 25/20 °C. Plants were harvested at the onset of flowering 33–35 d after sowing, dried at 60 °C for 7 d and their shoot and root DW determined. Shoot and root dry material from all NO$_3^-$ treatments, the rhizobia-inoculated treatment, and plants supplied 3 and 4 mol m$^{-3}$ NH$_4^+$ and 4 and 6 mol m$^{-3}$ N as urea or glutamine were ground and 200 mg samples analysed for N in an Elementar Vario-Max CN analyser (Hanau, Germany). Also, NO$_3^-$ concentration was determined in aqueous extracts of 200 mg samples from roots and shoots of all NO$_3^-$-fed plants (Andrews et al., 1992). In this and all other experiments, values given for tissue N content reflect
reduced N (total N–NO₃⁻–N) for NO₃⁻-fed plants but total N for all other treatments. Experiment 1 was repeated as described except that the cabinets that received the ambient and elevated CO₂ treatments were reversed to test for a possible ‘cabinet’ effect and, for the NO₃⁻ treatments, N and NO₃⁻ were only measured in roots and shoots of plants supplied 4 and 6 mol m⁻³ NO₃⁻.

In Experiment 2, wheat cv. Spring Batten (Plant and Food Research, Lincoln, New Zealand) was grown from seed (40–46 mg) in 1 litre pots (three plants per pot) containing a 1:1 volume mix of vermiculite and perlite that was flushed through with basal nutrient solution containing 0.5, 1, 2, 3, 4, 6, 8, or 10 mol m⁻³ NO₃⁻ as potassium nitrate every 1 to 2 d. The day/night temperatures were 20/15 °C. Plants were harvested 33–35 d after sowing and shoot and root DW, and N and NO₃⁻ content measured in all treatments as in Experiment 1. In Experiment 3, seed (40–46 mg) of wheat cv. Winter Batten (Plant and Food Research, Lincoln, New Zealand) was germinated in vermiculite/perlite containing 4 mol m⁻³ NO₃⁻ (potassium nitrate) in the ambient CO₂ growth room. Seedlings of uniform size were selected and grown under ambient and elevated CO₂ in complete nutrient medium containing 4 mol m⁻³ NO₃⁻ in liquid culture that was renewed every 2 d (Andrews et al., 2006). Plants were harvested 35 d after transfer to liquid culture, in vivo nitrate reductase activity (NRA) was measured in fresh lamina tissue as described by Andrews et al. (1984), then shoot and root DW, and N and NO₃⁻ content were determined.

In Experiment 4, wheat cv Winter Batten was grown from seed (40–46 mg) in 1 litre pots (three plants per pot) containing a 1:1 volume mix of vermiculite and perlite that was flushed through with basal nutrient solution containing 0.1, 0.5, 1, 2, 3, and 6 mol m⁻³ N as NO₃⁻ (potassium nitrate) or NH₄⁺ (ammonium sulphate) every 1 or 2 d. The day/night temperatures were 20/15 °C. Shoots (but not roots) of plants were harvested 35 d after sowing. In vivo NRA was measured in fresh lamina tissue of plants supplied 0.5–6 mol m⁻³ NO₃⁻ shoot DW and N content were determined for all treatments, and shoot NO₃⁻ content determined for NO₃⁻-fed plants. The cabinets that received the ambient and elevated CO₂ in Experiment 2 were reversed for Experiments 3 and 4.

All experiments had a completely randomized design. There were three replicate pots for all treatments in Experiments 1, 2 and 4 and six replicate pots for the two treatments in Experiment 3. A one-way or two-way analysis of variance (ANOVA) was carried out on data from each experiment as appropriate, with CO₂ level and N treatment considered as categorical factors. Fisher’s least significant difference (LSD, P<0.01) was calculated from the results of the ANOVA and all effects described as significant have a probability (P) value <0.01. Regression analysis was carried out on data from Experiments 1, 2, 3 and 4 and straight line and quadratic models were tested. Model choice was based on the R² values. Variability given in the text and in tables is the treatment standard deviation.

Results and discussion

At both ambient and elevated CO₂ in Experiment 1, total plant DW of common bean increased with increased NO₃⁻ or NH₄⁺ supply from 0.5 to 3–6 mol m⁻³, then decreased with increased concentration thereafter (Fig. 1A, B). In contrast, total plant DW increased with increased urea or glutamine concentration over the entire range used (Fig. 1C, D). Similar responses to the different N forms have been reported previously for common bean at ambient CO₂ (Andrews et al., 2013). Maximum DW achieved decreased with N form in the order glutamine > NO₃⁻ = urea > NH₄⁺. DW was greater with elevated CO₂ for almost all concentrations of all N forms and for rhizobia-inoculated plants (Table 1). Rhizobia-inoculated plants (supplied 0.5 mol m⁻³ NO₃⁻) were nodulated and their total DW was approximately 50% greater than that for uninoculated plants supplied 0.5 mol m⁻³ NO₃⁻ indicating that they had been fixing N₂. At maximum total plant DW for the different N forms, the proportional increase in total plant DW under elevated CO₂ was as great under NO₃⁻ nutrition as with the other N forms and greater with NO₃⁻ than NH₄⁺.

At limiting and optimal NO₃⁻ supply, shoot N content (mg N g⁻¹ DW) was lower at elevated than ambient CO₂ but...
Effects of elevated CO2 on nitrogen assimilation

Total plant N was greater at elevated CO2 (Fig. 2A, B). Similar effects were obtained for plants supplied 3 or 4 mol m−3 N as NH4+, 4 or 6 mol m−3 N as urea or glutamine, or Rhizobium-inoculated plants (Table 1). Thus, for C3 common bean, which has the leaf as its main site of NO3− assimilation, elevated CO2 gave increased total plant DW, decreased shoot N content but increased total plant N with NO3−, NH4+, urea, glutamine, or N2 fixation as its main form of N nutrition. Similar results were obtained in the repeat experiment (Supplementary Fig. S1; Supplementary Table S1). Common bean did not respond more positively to elevated [CO2] under NH4+ than NO3− nutrition.

For wheat at both ambient and elevated CO2 in Experiment 2, total plant DW increased with increased NO3− supply from 0.5 to 8 mol m−3 then changed little with additional NO3− to 10 mol m−3 (Fig. 3A). Total plant DW was 30–40% greater at elevated CO2 at most NO3− concentrations. As for common bean, shoot N content (NB reduced N g−1 DW) was greater at ambient CO2 but total plant N was greater at elevated CO2 (Fig. 3B, C). Shifting from the solid substrate used in Experiment 2 to the liquid culture used in Experiment 3 did not change the response of NO3−-fed wheat to elevated CO2 (Table 2). Again total plant DW and N were greater at elevated than at ambient CO2 while shoot N content per unit DW was lower. Results for shoots of wheat supplied NO3− in Experiment 4, were consistent with those for whole plants in Experiments 2 and 3 (Figs 2–4). Shoot DW at both ambient and elevated CO2 increased with increased NO3− supply from 0.1 to 6 mol m−3 with values greater with elevated CO2 at all concentrations (Fig. 4A). Also, shoot N content was greater at ambient than at elevated CO2 but shoot total N was greater at elevated CO2 (Fig. 4B, C). The effects of elevated CO2 on total plant and shoot DW and N, and shoot N content for NO3−-fed wheat in Experiments 2–4 are similar to those reported for wheat by Hocking and Meyer (1991).

Previously, it was reported that the root was the main site of NO3− assimilation for wheat at 1 mol m−3 applied NO3−, and shoot assimilation increased in importance as applied NO3− concentration increased to 20 mol m−3 (Andrews et al., 1992).

### Table 1.

Effect of different concentrations of applied nitrate (NO3−), ammonium (NH4+), urea, and glutamine (Gln) and Rhizobium inoculum (N2) on total plant dry weight (DW), shoot nitrogen (N) content and total plant N of common bean grown under ambient (400 ppm) and elevated (760 ppm) atmospheric CO2 (Experiment 1)

<table>
<thead>
<tr>
<th>Applied N (mol m−3)</th>
<th>Total plant DW (g)</th>
<th>Shoot N content (mg N g−1 DW)</th>
<th>Total plant N (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amb. CO2</td>
<td>Elevated CO2</td>
<td>Amb. CO2</td>
</tr>
<tr>
<td>NO3− (3)</td>
<td>6.87 ± 0.63</td>
<td>8.73 ± 0.32</td>
<td>40.4 ± 2.2</td>
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<tr>
<td></td>
<td>(4)</td>
<td></td>
<td>(6)</td>
</tr>
<tr>
<td></td>
<td>6.15 ± 0.54</td>
<td>10.11 ± 0.72</td>
<td>39.8 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>11.20 ± 0.93</td>
<td>37.1 ± 2.5</td>
</tr>
<tr>
<td>NH4+ (3)</td>
<td>5.28 ± 0.17</td>
<td>6.55 ± 0.28</td>
<td>47.9 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td></td>
<td>43.5 ± 4.9</td>
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<tr>
<td></td>
<td>(6)</td>
<td>6.44 ± 0.26</td>
<td>41.8 ± 1.3</td>
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<tr>
<td>Urea (4)</td>
<td>5.88 ± 0.63</td>
<td>6.49 ± 0.56</td>
<td>45.8 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>8.47 ± 0.65</td>
<td>38.8 ± 1.2</td>
</tr>
<tr>
<td>Gln (4)</td>
<td>9.00 ± 0.37</td>
<td>28.1 ± 1.10</td>
<td>40.0 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>12.6 ± 0.58</td>
<td>39.5 ± 4.1</td>
</tr>
<tr>
<td>N2 (4)</td>
<td>6.62 ± 0.09</td>
<td>5.77 ± 0.10</td>
<td>383.4 ± 22.1</td>
</tr>
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</table>

Variability quoted is standard deviation; n = 3.
If elevated $[\text{CO}_2]$ inhibits photoreduction of $\text{NO}_3^-$, and as a result overall plant $\text{NO}_3^-$ assimilation is reduced, then the inhibitory effects on total plant $\text{NO}_3^-$ assimilation in wheat should increase with increased $\text{NO}_3^-$ supply as the contribution of shoot to total plant $\text{NO}_3^-$ assimilation increases. This did not occur; indeed, the stimulatory effect of elevated CO$_2$ on total plant and shoot reduced N and DW was relatively greater at higher $\text{NO}_3^-$ supply (Figs 3, 4). The partitioning of $\text{NO}_3^-$ assimilation between root and shoot was not determined here, but lamina NRA increased substantially with an increased $\text{NO}_3^-$ supply of 0.5–6 mol m$^{-3}$ $\text{NO}_3^-$ at ambient and elevated CO$_2$ in Experiment 4 (Fig. 5). This result indicates that lamina $\text{NO}_3^-$ assimilation increased substantially with increased $\text{NO}_3^-$ supply at both ambient and elevated CO$_2$ in Experiment 4. At 4 mol m$^{-3}$ applied $\text{NO}_3^-$ in Experiment 3 and across the range of $\text{NO}_3^-$ supply in Experiment 4, lamina NRA was not affected by CO$_2$ supply indicating that elevated CO$_2$ did not inhibit lamina $\text{NO}_3^-$ assimilation. Also, the in vivo NRA assay used here relies on endogenous NADH to reduce $\text{NO}_3^-$ to $\text{NO}_2^-$ (NADH is not included in the assay buffer), and thus similar values for lamina NRA at ambient and elevated CO$_2$ indicate that NADH is not limiting $\text{NO}_3^-$ reduction under elevated relative to ambient CO$_2$.

For wheat supplied NH$_4^+$ in Experiment 4, shoot DW increased with increased NH$_4^+$ supply to 3 mol m$^{-3}$, then changed little with increased supply to 6 mol m$^{-3}$ at both ambient and elevated CO$_2$ (Fig. 4A). As with the $\text{NO}_3^-$ treatments, shoot DW and total N were greater and shoot N content per unit DW lower at elevated CO$_2$ (Fig. 4B, C). At both ambient and elevated CO$_2$, shoot DW and total N were greater but shoot N content lower with $\text{NO}_3^-$ than with NH$_4^+$ at application rates above 0.5 mol m$^{-3}$. Wheat did not respond more positively to elevated CO$_2$ under NH$_4^+$ nutrition than under $\text{NO}_3^-$ nutrition. In the studies of Bloom and co-workers, the effects of elevated CO$_2$ on growth and N assimilation of wheat supplied NH$_4^+$ or $\text{NO}_3^-$ as N source were not consistent across experiments. The data obtained for $\text{NO}_3^-$-fed wheat plants across Experiments 2–4 here are similar to those reported for wheat by Bloom et al. (2002), the study in which elevated CO$_2$ gave a substantially greater response in NH$_4^+$- than $\text{NO}_3^-$-fed plants. However, lower growth with NH$_4^+$ than with $\text{NO}_3^-$ under ambient and elevated CO$_2$ in Experiment 4 contrasts with the findings of Bloom et al. (2002), but is in agreement with the reports of Bloom et al. (2012) and Rubio-Asensio et al. (2015), and this is an important point of inconsistency across these studies that needs to be resolved.

In summary, across Experiments 1–4, elevated CO$_2$ substantially increased growth of common bean and wheat under $\text{NO}_3^-$ nutrition. Also, for both species, at limiting and optimal

### Table 2

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<thead>
<tr>
<th>CO$_2$</th>
<th>Total plant DW (g)</th>
<th>Shoot N content (mg N g$^{-1}$ DW)</th>
<th>Total plant N (mg)</th>
<th>NRA (μmol NO$_2^-$ g$^{-1}$ FW h$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient</td>
<td>1.02 ± 0.08</td>
<td>20.5 ± 1.9</td>
<td>17.2 ± 0.11</td>
<td>2.24 ± 0.54</td>
</tr>
<tr>
<td>Elevated</td>
<td>1.41 ± 0.10</td>
<td>17.0 ± 0.5</td>
<td>20.04 ± 1.00</td>
<td>1.91 ± 0.14</td>
</tr>
</tbody>
</table>

Variability quoted is standard deviation; $n = 6$.
NO$_3^-$ supply, total plant reduced N was greater at elevated than at ambient CO$_2$ indicating that greater NO$_3^-$ assimilation had occurred at elevated CO$_2$. Nevertheless, the proportional increase in total plant N content was not as great as that for DW, and thus tissue N content per unit DW was consequently lower with elevated CO$_2$. This increase in total plant DW relative to total plant N and the resultant decrease in tissue N per unit DW has been termed N ‘dilution’ and has been linked to increased accumulation of non-structural carbohydrates and plant secondary compounds (Taub and Wang, 2008). The effects of elevated CO$_2$ on growth and N assimilation of common bean and wheat were similar regardless of N form supplied. In particular, neither species responded more positively to an elevated atmospheric [CO$_2$] under NH$_4^+$ nutrition than under NO$_3^-$ nutrition.

Overall, we consider that the weight of evidence in the literature indicates that elevated atmospheric CO$_2$ concentration does not inhibit NO$_3^-$ assimilation of C$_3$ plants and the new data reported here strongly support that conclusion. Additionally, the results obtained for common bean and wheat in this study extend that conclusion by suggesting the effects of elevated atmospheric CO$_2$ concentration on N assimilation and growth of C$_3$ plants will be similar regardless of the form of N assimilated.

References


Seabra AR, Pereira PA, Becker JD, Carvalho HG. 2012. Inhibition of glutamine synthetase by phosphinositbin leads to transcription reprogramming in root nodules of Medicago truncatula. Molecular Plant-Microbe Interactions 25, 976–992.

