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Permalink
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Publication Date
2012

DOI
10.3389/fpls.2012.00195

Peer reviewed
The effects of inorganic nitrogen form and CO₂ concentration on wheat yield and nutrient accumulation and distribution

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INTRODUCTION

Nitrogen (N) is the mineral element that most often limits plant growth and primary productivity in natural and agricultural systems. Plants usually acquire N from the soil in the forms of ammonium (NH₄⁺) and nitrate (NO₃⁻), but mixed N nutrition (e.g., NH₄⁺ and nitrate (NO₃⁻)) is also thought to increase or remain the same under elevated CO₂ concentrations (Gashaw and Mugwira, 1981; Wang and Below, 1995), although such differences can vary among cultivars (Gashaw and Mugwira, 1981; Wang and Below, 1995). The presence of NH₄⁺, as either a sole N source or in mixed N nutrition, increased organic N concentration in shoots, roots, and grain and decreased partitioning of dry matter to the roots in wheat (Wang and Below, 1995). Decreased cation uptake has been found in wheat under NH₄⁺ nutrition (e.g., Gashaw and Mugwira, 1981; Wang and Below, 1998), although results varied among cultivars (Gashaw and Mugwira, 1981). For example, NH₄⁺ nutrition decreased whole plant and shoot accumulations of K, Cu, Ca, Mg, Fe, Mn, and Zn (Wang and Below, 1998). Nutrient allocation to plant tissues also varied between N forms. NH₄⁺-fed plants distributed a smaller percentage of total P, K, Cu, and B to roots relative to NO₃⁻-fed plants (Wang and Below, 1995, 1998). Also, a greater percentage of reduced N was allocated to the shoots in NH₄⁺-fed plants (Wang and Below, 1995).

Elevated atmospheric concentrations of CO₂ alter growth and N dynamics of wheat and other C₃ plants. Under elevated CO₂, wheat has lower protein and N concentrations (e.g., Thompson and Woodward, 1994; Bloom et al., 2002; Wu et al., 2004), and lower macro- and micronutrients concentrations (Manderscheid et al., 1995; Fangmeier et al., 1997, 1999; Wu et al., 2004; Hög and Fangmeier, 2008). Grain phytate concentrations are also thought to increase or remain the same under elevated CO₂.
and in conjunction with decreased concentrations of micronutrients, bioavailable Zn and Fe are expected to decrease even further under elevated CO\textsubscript{2} (Loladze, 2002; Manoj-Kumar, 2011), as these micronutrients form indigestible complexes with phytate. By contrast, wheat yields (Fangmeier et al., 1996; Amthor, 2001; Högy and Fangmeier, 2008), harvest index (Hl; Wu et al., 2004), whole plant biomass (Fangmeier et al., 1996; Högy and Fangmeier, 2008), shoot biomass (Fangmeier et al., 1996; Högy et al., 2009), and root biomass (Chaudhuri et al., 1990; Wechsung et al., 1995) typically increase under CO\textsubscript{2} enrichment. In addition, elevated CO\textsubscript{2} concentration can increase tillering (Weigel et al., 1994), nitrogen use efficiency (NUE, Fangmeier et al., 1997), and micro/macronutrient use efficiencies (Manderscheid et al., 1995). The influence of elevated CO\textsubscript{2} on many of these characteristics may vary among cultivars and research protocols (e.g., FACE vs. controlled environment chamber, greenhouse vs. field; Amthor, 2001; Högy and Fangmeier, 2008).

Wheat grown under CO\textsubscript{2} enrichment behaves differently under NO\textsubscript{3}\textsuperscript{−} and NH\textsubscript{4}\textsuperscript{+} nutrition. Exposure to elevated CO\textsubscript{2} inhibits NO\textsubscript{3}\textsuperscript{−} photoassimilation in wheat (Bloom et al., 1989, 2002, 2010; Cousins and Bloom, 2004) as well as in all other C\textsubscript{3} and C\textsubscript{4} intermediate plants tested (Bloom et al., 2012). At elevated CO\textsubscript{2}, NH\textsubscript{4}\textsuperscript{+}-fed plants showed greater increases in leaf area and smaller decreases in shoot protein concentration than NO\textsubscript{3}\textsuperscript{−}-fed plants (Bloom et al., 2002), which could have consequences for human nutrition. Vegetative plants receiving NH\textsubscript{4}\textsuperscript{+} had greater shoot, stem, and root biomass at elevated CO\textsubscript{2} (Bloom et al., 2002). Wheat receiving NO\textsubscript{3}\textsuperscript{−} grew slower at elevated CO\textsubscript{2} than at ambient CO\textsubscript{2} (Bloom et al., 2002). Shoot NO\textsubscript{3}\textsuperscript{−} concentrations in NH\textsubscript{4}\textsuperscript{+}-fed plants were undetectable while those in NO\textsubscript{3}\textsuperscript{−}-fed plants increased by 62% with CO\textsubscript{2} enrichment (Bloom et al., 2002). This increase was associated with an inhibition in NO\textsubscript{3}\textsuperscript{−} and NO\textsubscript{2}\textsuperscript{−} reductase activities under elevated CO\textsubscript{2} (Bloom et al., 2002).

The interaction between atmospheric CO\textsubscript{2} concentration and inorganic N form and how it influences plant growth and nutrient concentrations has not been examined in wheat or any other crop species grown to senescence. Here, we grew wheat hydroponically in controlled environment chambers and measured mineral nutrition, biomass, and nutrient allocation in response to three concentrations of atmospheric CO\textsubscript{2} (subambient, ambient, and elevated) and two forms of N nutrition (NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{−}). We tested the following hypotheses: (1) plant nutrient concentrations and allocation patterns will respond differently to CO\textsubscript{2} enrichment under the two N forms, and (2) NO\textsubscript{3}\textsuperscript{−}-fed plants will show a smaller biomass and yield enhancement in response to CO\textsubscript{2} enrichment than NH\textsubscript{4}\textsuperscript{+}-fed plants as a result of CO\textsubscript{2} inhibition of shoot NO\textsubscript{3}\textsuperscript{−} assimilation. Also, we observed both differences in the Zn concentration between plants grown on NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{−} and a strong dependence of Zn absorption on Zn and phytate concentration, indicating that phytate and bioavailable Zn are affected by N form and CO\textsubscript{2}. Therefore, we used the well supported Miller equation (Miller et al., 2007) to estimate how N and CO\textsubscript{2} might impact a hypothetical human population. Iron, another important micronutrient that forms complexes with phytate, was not analyzed because we observed no significant differences in iron concentrations between the N forms and because how best to estimate Fe absorption in humans is still uncertain (Welch and Graham, 2004).

**MATERIALS AND METHODS**

**EXPERIMENTAL**

**Wheat seeds** (*Triticum aestivum* cv. Veery 10) were surface sterilized for one minute in 2.6% sodium hypochlorite solution and thoroughly rinsed with DD\textsubscript{2}O water. The seeds were then rolled up in germination paper saturated with 10 mM CaSO\textsubscript{4}. The germination paper was placed in a 400 mL beaker with approximately 75 mL of 10 mM CaSO\textsubscript{4} solution, covered with a plastic bag and placed in an incubator (23°C) for four days. Seedlings were transplanted into 20 L tubs filled with an aerated nutrient solution that contained 1 mM CaSO\textsubscript{4}, 1 mM K\textsubscript{2}HPO\textsubscript{4}, 1 mM KH\textsubscript{2}PO\textsubscript{4}, 2 mM MgSO\textsubscript{4}, and 0.2 g L\textsuperscript{−}1 Fe–NaEDTA and micronutrients (20% of a modified Hoagland’s solution with either 0.2 mM KNO\textsubscript{3} or 0.1 mM (NH\textsubscript{4})\textsubscript{2}HPO\textsubscript{4} as the N source, Epstein and Bloom, 2005). The nutrient solution was replaced weekly and an additional 0.2 mM of NO\textsubscript{3}\textsuperscript{−} or NH\textsubscript{4}\textsuperscript{−} − N was added midweek until harvest. The solution volume was maintained by daily addition of deionized water. Solution pH varied between 6.8 and 7.0 for both of the N forms, and the NH\textsubscript{4}\textsuperscript{−} and the NO\textsubscript{3}\textsuperscript{−} solutions did not differ by more than 0.1 pH units.

The plants were grown in controlled environment chambers (Conviron, Winnipeg, Canada) set at 23/20°C day/night at 60–70% relative humidity with a photoperiod of 15 h. The photosynthetic flux density was 375 μmol m\textsuperscript{−2} s\textsuperscript{−1} at plant height. Plants were subjected to one of three CO\textsubscript{2} concentrations: “subambient” (310 ± 30 ppm), “ambient” (410 ± 30 ppm), and “elevated” (720 ± 50 ppm). Subambient CO\textsubscript{2} concentrations were maintained by passing air that entered the growth chamber through wet soda lime, a mixture of KOH, NaOH, and Ca(OH)\textsubscript{2} that was replaced as needed. The elevated CO\textsubscript{2} conditions were maintained in an environmental chamber equipped with non-dispersive infrared analyzers for CO\textsubscript{2} (Horiba model APBA-250E) and valves that added pure CO\textsubscript{2} to the incoming air stream to hold the chamber concentration at 720 ppm.

The wheat was grown until all aboveground parts turned completely yellow. Plant matter was sorted into grain, chaff, shoots, and roots and dried for 48 h at 55°C. Data on kernel number (KN), kernel mass, number of heads, kernels head\textsuperscript{−1}, and HI were collected prior to sample preparation for nutrient analysis. A portion of the grain was analyzed for phytate using a modification of the method as described by Haug and Lantzsch (1983). The remainder of the grain as well as the shoots and chaff was bulked into five replications per treatment and sent to the UC Davis Analytical Laboratory for nutrient analysis. The roots of plants for each CO\textsubscript{2} × N treatment became entangled within the same tub; therefore, we were unable to separate the roots of the individual plants for analysis. Root data are thus presented as means for each treatment with no standard errors or confidence intervals.

Data were analyzed using PROC MIXED (SAS 9.0 Cary, NC, USA). Nitrogen form and CO\textsubscript{2} factors were treated as fixed independent variables. We used the Tukey–Kramer Honestly Significant Difference test for mean separation. Probabilities less than 0.05 were considered significant. Because some of the transformed variables did not meet the assumption of homogeneity.
of variances, but one-way ANOVAs met the ANOVA assumptions, we analyzed the results via one-way ANOVAs to gain some information on the interactions between CO₂ and N form.

**MODELING THE INFLUENCE OF N FORM ON Zn NUTRITION IN THE HUMAN DIET**

We used a database derived from the United Nation’s Food and Agriculture Organization (FAO)’s national food balance sheets (FBS) to estimate the average daily per capita dietary intake of zinc and phytate from 95 different food commodities in each of 176 countries. This database combines FAO data on per capita intake of food commodities with USDA data on the nutrient or phytate content of each of these commodities. More detailed discussion of the creation of this database for the International Zinc Collaborative Group may be found in Wuehler et al. (2005). Using this database, we produced two datasheets: one containing per capita daily dietary intake of zinc from each food commodity for each country and another containing per capita phytate intake from each food commodity for each country. To calculate total dietary zinc (TDZ) and total dietary phytate (TDP) per country, we summed across the rows of all food commodities for each respective country.

To determine the proportion of a population at risk for zinc deficiency from a hypothetical least developed country (LDC), we first calculated TDP and TDZ values for a set of 44 countries defined by the United Nations as being least developed. We took the mean TDP and TDZ values for these countries to represent a hypothetical “less developed country.” To calculate the bioavailable zinc portion (TAZ; usually a small fraction of TDZ) we used the Miller equation (Equation 1; Miller et al., 2007).

\[
\text{TAZ} = 0.5 \cdot \left( A_{\text{max}} + \text{TDZ} + K_R \cdot \left( 1 + \frac{\text{TDP}}{K_P} \right) \right) - \sqrt{ \left( A_{\text{max}} + \text{TDZ} + K_R \cdot \left( 1 + \frac{\text{TDP}}{K_P} \right) \right)^2 - 4 \cdot A_{\text{max}} \cdot \text{TDZ} } 
\]

Equation 1 : Miller equation

Mean TDZ and TDP values were converted to mg mmol⁻¹ and put into the Miller equation to compute the average per capita TAZ in our hypothetical LDC. The variables TDZ, TDP, and TAZ are described above, and \( A_{\text{max}} \), \( K_P \), and \( K_R \) are constants as described in Miller et al. (2007).

We made an assumption that our hypothetical LDC receives half of its phytate and half of its zinc from wheat, which is roughly consistent with many of the LDCs in the FAO database. We analyzed the effect of elevated carbon dioxide levels on TDP, TDZ, and TAZ concentrations in a hypothetical LDC population for both NH₄⁺ and NO₃⁻-supplied wheat. To calculate a new TAZ for wheat grown under elevated CO₂ conditions, we first calculated the percent change in TAZ from ambient to elevated levels for wheat receiving NH₄⁺ or NO₃⁻. This computed percent change was then applied to half of the hypothetical TDZ and TDP; meanwhile, the other half of the hypothetical TDZ and TDP remained unmodified. Thus, the total new TDZ and TDZ is the sum of the unmodified and modified portions. These new TDZ and TDZ values for both NH₄⁺ and NO₃⁻-supplied wheat are then put into the Miller equation to compute new hypothetical TAZ values for an LDC. Differences and corresponding percent changes between the new TAZ values and the original TAZ value for a LDC were computed to determine the overall affect of elevated CO₂ on TAZ in NH₄⁺ and NO₃⁻-supplied wheat for an average developing world population. TAZ, TDP, and TDZ concentrations can only be compared within a single N form across the CO₂ concentrations due to methodological constraints of the model.

**RESULTS**

We divide the results here into three categories: first, biomass and yield data for the shoots, grain, and roots; second, tissue concentrations and whole plant micro- and macronutrient contents; and third, nutrient distribution among the different tissues. Values of the statistical significance of the results were place into a table (Table 1) in order to improve the readability of the text.

**BIOMASS AND YIELD**

Plants supplied NH₄⁺ vs. NO₃⁻ nutrition reacted differently to CO₂ enrichment (Figure 1; Table 1). Plants supplied NH₄⁺ differed across CO₂ treatments for most of the yield and biomass measurements. The greatest values typically were found at ambient CO₂ concentrations. Shoot, chaff, grain yield, number of heads, and KN were greatest at ambient CO₂ levels. Individual kernel mass was greatest under both ambient and elevated CO₂ treatments. HI and kernels head⁻¹ showed no change across CO₂ treatments. In contrast, biomass and yield measures of NO₃⁻-supplied plants did not differ among the three CO₂ concentrations.

At subambient CO₂ differences between the NH₄⁺ and NO₃⁻ treatments occurred in shoot biomass and three of the yield components: kernel mass, head number, and kernels head⁻¹. Ammonium-supplied plants had a larger number of heads while NO₃⁻-supplied plants had greater shoot biomass, kernel mass, and kernels head⁻¹. At ambient CO₂, NH₄⁺-supplied plants had a greater number of heads and greater chaff biomass. Plants supplied NO₃⁻ had a larger number of kernels head⁻¹. At elevated CO₂, biomass and yield measures did not differ with N treatment.

**ROOT**

Roots had a smaller mean biomass when supplied NH₄⁺ than when supplied NO₃⁻ at all CO₂ concentrations (Figure 1). Both N treatments had the greatest biomass at ambient CO₂, and the smallest at subambient CO₂. The highest root:shoot ratios for both NH₄⁺ and NO₃⁻-supplied plants were observed at ambient and elevated CO₂. Ammonium-supplied plants always had lower root:shoot ratios and biomass than NO₃⁻-supplied plants at the same CO₂ concentration.

**NUTRIENTS**

**Total plant nutrients**

Total plant nutrients generally followed the same trend within N form, although NH₄⁺-supplied plants exhibited a greater diversity of responses to increasing CO₂ concentrations (Table 2). Total plant P, K, B, Ca, Mg, and Zn decreased with increasing CO₂ under NH₄⁺, while S and Mn were highest under ambient CO₂.
Table 1 | Results of a series of one-way ANOVAs run on the data.

| Among CO₂ cnc. within an N form | Grain | Shoot |
|---------------------------------|-------|-------|
|                                  | NH₄⁺ | NO₃⁻ | NH₄⁺ | NO₃⁻ |
| Total N                         | **    | NS    | ***   | NS    |
| P                               | *     | NS    | **    | NS    |
| K                               | ***   | NS    | NS    | ***   |
| S                               | NS    | NS    | NS    | ***   |
| Ca                              | ***   | NS    | *     | NS    |
| Mg                              | NS    | NS    | NS    | ***   |
| Zn                              | NS    | NS    | ***   | ***   |
| B                               | **    | *     | *     | *     |
| Mn                              | NS    | NS    | ***   | NS    |
| Fe                              | NS    | NS    | NS    | ***   |
| Cu                              | ***   | NS    | NS    | ***   |
| NO₃⁻ − N                       | *     | NS    | ***   | ***   |
| Phytate                         | NS    | NS    | N/A   | N/A   |

| Between N forms within a CO₂ cnc. | Grain | Shoot |
|------------------------------------|-------|-------|
|                                    | Sub   | Amb   | Elev | Sub   | Amb   | Elev |
| Total N                           | **    | NS    | NS   | NS    | NS    | **   |
| P                                 | NS    | NS    | NS   | NS    | NS    | NS   |
| K                                 | **    | NS    | NS   | NS    | NS    | NS   |
| S                                 | NS    | NS    | NS   | NS    | NS    | NS   |
| Ca                                | ***   | NS    | NS   | NS    | NS    | NS   |
| Mg                                | NS    | NS    | NS   | NS    | NS    | NS   |
| Zn                                | NS    | NS    | NS   | NS    | NS    | NS   |
| B                                 | NS    | NS    | NS   | NS    | NS    | NS   |
| Mn                                | NS    | NS    | NS   | NS    | NS    | NS   |
| Fe                                | NS    | NS    | NS   | NS    | NS    | NS   |
| Cu                                | NS    | NS    | NS   | NS    | NS    | NS   |
| NO₃⁻ − N                         | *     | NS    | NS   | NS    | NS    | NS   |
| Phytate                           | NS    | NS    | N/A  | N/A   | N/A   | N/A  |

| Among CO₂ cnc. or between N forms | Sub   | Amb   | Elev | NH₄⁺ | NO₃⁻ |
|------------------------------------|-------|-------|------|------|------|
| Yield                              | NS    | *     | NS   | **   | NS   |
| Shoot                              | **    | NS    | NS   | **   | NS   |
| Chaff                              | NS    | NS    | NS   | NS   | NS   |
| Grain number                       | NS    | NS    | NS   | *    | NS   |
| Grain mass                         | ***   | NS    | NS   | NS   | NS   |
| Grains head−1                      | ***   | NS    | NS   | NS   | NS   |
| Heads                              | **    | NS    | NS   | NS   | NS   |
| Harvest index                      | NS    | NS    | NS   | NS   | NS   |

Ammonium-supplied plants had the greatest amounts of Fe and total N at subambient CO₂. Nitrate-supplied plants accumulated the greatest amounts of total N, P, K, S, B, Ca, Zn, Mn, and Mg at ambient CO₂. Only three nutrients – K, S, and Fe – had the lowest contents at elevated CO₂.

Shoot
Under NH₄⁺ supply, plants varied with CO₂ concentration for total N, P, S, Ca, Cu, B, Mn, Zn, and NO₃⁻ − N (Table 1; Figure 2). Calcium and Cu were highest under subambient CO₂. Total N and S were greatest at subambient and elevated CO₂. Nitrate-N was greatest at ambient CO₂. Phosphorus was highest at elevated CO₂ concentrations. Boron, Zn, and Mn increased with CO₂ concentration.

Plants supplied NO₃⁻ showed significant variation across CO₂ treatments for K, Ca, Mg, B, Fe, Cu, Zn, and NO₃⁻ − N (Table 1; Figure 2). Calcium and Cu had the greatest concentrations at subambient CO₂. The highest concentrations of B, Fe, and Zn occurred at subambient and elevated CO₂. Potassium concentrations were highest at elevated CO₂. Nitrate-N increased with CO₂. Magnesium showed the opposite trend, decreasing with CO₂ concentration.

Differences between N forms were also evident. At subambient CO₂, NH₄⁺-supplied plants had increased concentrations of P, S, and Zn, while NO₃⁻-supplied plants had greater concentrations of B, Mg, Mn, and NO₃⁻ − N (Table 1; Figure 2). Concentrations of K, Zn, and Cu were higher in plant supplied NH₄⁺ at ambient CO₂, while Mg, Mn, and NO₃⁻ − N were greater in plants supplied NO₃⁻. At elevated CO₂, concentrations of N, P, S, and Zn were higher in plants supplied NH₄⁺, while concentrations of B, Mg, Mn, and NO₃⁻ − N were greater in plants supplied NO₃⁻.

Grain
Grain nutrient concentrations. Plants supplied NH₄⁺ showed significant variation across the CO₂ treatments in the concentrations of total N, P, K, Ca, Cu, B, Mn, and NO₃⁻ − N (Table 1; Figure 3). The greatest concentrations of total N, P, K, Ca, and Cu were found at subambient CO₂. Iron concentrations were high at both subambient and ambient CO₂. Boron was equally high at subambient and elevated CO₂. Manganese was greatest at elevated CO₂. Nitrate-N decreased with increasing CO₂.

Significant differences among the NO₃⁻-supplied plants across CO₂ treatments were only observed in S and B. The greatest concentrations of B were found at subambient CO₂. Sulfur was highest at ambient CO₂.

Nitrogen form significantly affected grain nutrient concentrations (Table 1; Figure 3). At subambient CO₂, NH₄⁺-supplied plants had higher concentrations of total N, K, S, Ca, Zn, and Cu than NO₃⁻ plants. At ambient CO₂, Ca, Zn, and Cu were greatest under NH₄⁺. Ammonium-supplied plants also had the highest concentrations of K, S, Ca, Zn, and Cu at elevated CO₂. At no CO₂ concentration did plants supplied NH₄⁺ have significantly lower concentrations of any micro- or macronutrient than those supplied NO₃⁻.

Phytate and bioavailable Zn. Phytate was relatively insensitive to CO₂ concentration. Phytate concentrations were highest
at subambient CO$_2$ for NH$_4^+$-supplied plants (Figure 4). Subambient CO$_2$ also produced the lowest phytate concentrations in NO$_3^-$-supplied plants. NH$_4^+$-supplied plants had greater phytate concentrations than NO$_3^-$-supplied plants at subambient CO$_2$, but not at the other CO$_2$ concentrations. Grain from plants grown under NH$_4^+$ nutrition had roughly 7, 18, and 8% higher bioavailable Zn than NO$_3^-$-supplied plants at subambient, ambient, and elevated CO$_2$, respectively (Figure 4).

Based on this phytate and bioavailable Zn data, we modeled how a human population from a LDC would be affected by
Table 2 | Total plant nutrients (mg plant\(^{-1}\)) as affected by N form and CO\(_2\) concentration.

|          | Sub | Amb | Elev | Sub | Amb | Elev | Sub | Amb | Elev | Sub | Amb | Elev |
|----------|-----|-----|------|-----|-----|------|-----|-----|------|-----|-----|------|
| Total N  | NH\(_4^+\) | 215.66 | 191.62 | 208.56 | 80.84 | 73.96 | 68.69 | 228.91 | 202.92 | 196.33 | 49.98 | 50.72 | 46.82 |
|          | NO\(_3^-\) | 159.39 | 210.26 | 164.88 | 63.21 | 85.02 | 67.75 | 208.32 | 259.07 | 198.79 | 42.21 | 50.84 | 38.25 |
| P        | NH\(_4^+\) | 0.28 | 0.25 | 0.18 | 23.18 | 19.55 | 19.24 | 42.41 | 38.62 | 35.34 | 0.62 | 0.54 | 0.45 |
|          | NO\(_3^-\) | 0.29 | 0.41 | 0.31 | 21.10 | 25.54 | 22.48 | 45.26 | 52.45 | 52.45 | 0.27 | 0.48 | 0.36 |
| Ca       | NH\(_4^+\) | 6.26 | 2.93 | 2.24 | 1.93 | 1.26 | 1.47 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 |
|          | NO\(_3^-\) | 2.16 | 3.54 | 2.52 | 2.16 | 2.71 | 1.75 | 0.05 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 |
| Mg       | NH\(_4^+\) | 2.66 | 2.93 | 2.24 | 1.93 | 1.26 | 1.47 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 |
|          | NO\(_3^-\) | 2.16 | 3.54 | 2.52 | 2.16 | 2.71 | 1.75 | 0.05 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 |
| Zn       | NH\(_4^+\) | 2.66 | 2.93 | 2.24 | 1.93 | 1.26 | 1.47 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 |
|          | NO\(_3^-\) | 2.16 | 3.54 | 2.52 | 2.16 | 2.71 | 1.75 | 0.05 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 |

Changes in atmospheric CO\(_2\) concentrations (Table 3). The calculations were based on differences among CO\(_2\) concentrations; therefore, modeled TDZ, TDP, and TAZ values cannot be compared between NH\(_4^+\) and NO\(_3^-\)-supplied grain. Grain from plants supplied the different N forms behaved differently as CO\(_2\) concentration increased. We found that under NH\(_4^+\) supply, TAZ would increase 3.6% with the rise in CO\(_2\) from subambient to ambient, and decrease 1.6% with the rise from ambient to elevated CO\(_2\) (Figure 4). Humans provided NO\(_3^-\)-supplied wheat would experience a decrease in TAZ of 3.5% going from subambient to ambient, and an increase 5.6% from ambient to elevated CO\(_2\) (Figure 4).

**Roots**

Ammonium-supplied plants generally showed a trend toward decreasing nutrient concentrations with increasing CO\(_2\) concentration while NO\(_3^-\)-supplied plants varied widely across CO\(_2\) treatments (Figure 5). The decrease in nutrient concentrations under NH\(_4^+\) supply corresponded to an increase in root mass. Nitrate-supplied plants tended to have their highest nutrient concentrations in the ambient and elevated CO\(_2\) treatments. Ammonium-supplied plants had higher concentrations of Zn and Mn across all of the CO\(_2\) treatments, as well as higher total N and Fe at subambient CO\(_2\). Nitrate-supplied plants typically had higher concentrations of the other nutrients at all CO\(_2\) concentrations.

**Distribution of nutrients**

The distribution of nutrients and micronutrients among plant parts followed similar patterns in both the NH\(_4^+\) and NO\(_3^-\)-supplied plants, although the NH\(_4^+\)-supplied plant distributions were slightly more variable (Table 4). Allocations to root and grain usually were greatest at ambient CO\(_2\), and those to chaff and shoots at either subambient or elevated CO\(_2\). Grain typically contained the largest proportion of total N, P, Zn, and Cu, although the organ with the largest percentage of Cu varied with CO\(_2\) treatment among NO\(_3^-\)-supplied plants. Plants at subambient and elevated CO\(_2\) allocated more Cu to the grain, while those at ambient CO\(_2\) allocated more to the roots. In general shoots received the majority of K, S, B, Ca, and Mg for all N and CO\(_2\) treatments. Ammonium-supplied plants allocated slightly more Mn to the roots at subambient CO\(_2\), but allocated increasing amounts to the shoots at the expense of the roots as CO\(_2\) concentration increased. In contrast, NO\(_3^-\)-supplied plants allocated most of the Mn to the shoots. Ammonium-supplied plants typically allocated more resources to the chaff while NO\(_3^-\)-supplied plants allocated a greater percentage of elements to the roots.

**DISCUSSION**

No other study to our knowledge has examined the influence of N form (NH\(_4^+\) vs. NO\(_3^-\)) on plant nutrient relations at three different atmospheric CO\(_2\) concentrations. Overall, N form affected growth, total plant nutrient contents, and nutrient distribution in senescing wheat shoots, grain, and roots. The influence of NH\(_4^+\) and NO\(_3^-\) on growth and nutrient status were so distinct that they should be treated as separate nutrients and not bundled into a general category of N nutrition. Wheat size and nutrition at senescence responded to CO\(_2\) concentration in a non-linear manner. As was previously shown (Bloom et al., 2012), we found that plants supplied with NH\(_4^+\) were more responsive to CO\(_2\) concentration than those supplied with NO\(_3^-\).

Although not explicitly addressed here because of the heterogeneousity of variances, interactions between CO\(_2\) and N treatments likely existed for a number of the biomass and nutrient measures. Most nutrient concentrations were generally higher in NH\(_4^+\)-supplied plants, with the exceptions of NO\(_3^-\)-supplied plants, which, in conjunction with the observed greater bioavailability of Zn in NH\(_4^+\)-supplied plants, may have consequences for human nutrition. Distribution of nutrients to the shoots, roots, chaff, and grain in response to CO\(_2\) concentration and N form was also non-linear and varied by nutrient.

**BIOMASS AND YIELD**

The data support our hypothesis that NO\(_3^-\)-supplied plants would show a more limited biomass and yield enhancement with CO\(_2\) enrichment than NH\(_4^+\)-supplied plants. Nevertheless, mean biomass and yield decreased from ambient to elevated CO\(_2\) in both NO\(_3^-\)- and NH\(_4^+\)-supplied plants in contrast to biomass increases in prior work on wheat seedlings (Bloom et al., 2002). NO\(_3^-\)-supplied plants allocated more biomass to roots and had larger root:shoot ratios than NH\(_4^+\)-supplied plants regardless of CO\(_2\) concentrations as has been reported previously (Wang and Below, 2009).
FIGURE 2 | The effect of N form and CO$_2$ concentration on shoot nutrient concentrations of wheat grown hydroponically to senescence. Closed (NH$_4^+$) and open (NO$_3^-$) symbols represent back-transformed means and 95% confidence intervals (n = 5). Macro- and micronutrients are listed in the upper left of each frame. Differences are significant within N form if letters are different. Differences between N forms at each CO$_2$ concentration are generally significantly different if error bars do not overlap (see Table 1 for statistical significance).
FIGURE 3 | The effect of N form and CO$_2$ concentration on grain nutrient concentrations of wheat grown hydroponically to senescence. Closed ($\text{NH}_4^+$) and open (NO$_3^-$) symbols represent back-transformed means and 95% confidence intervals ($n=5$). Macro- and micronutrients are listed in the upper left of each frame. Differences are significant within N form if letters are different. Differences between N forms at each CO$_2$ concentration are generally significantly different if error bars do not overlap (see Table 1 for statistical significance).
Table 3 | Total dietary Zn (TDZ), total dietary phytate (TDP), and total bioavailable Zn (TAZ) of a human population from a hypothetic less developed nation reliant on wheat for 50% of their dietary phytate and Zn as modeled using the Miller equation.

|                | Sub → Amb (g/kg⁻¹) | Amb → Elev (g/kg⁻¹) |
|----------------|---------------------|----------------------|
| TDZ            | 9.21                | 8.69                 |
| TDP            | 2241.92             | 2264.70              |
| TAZ            | 1.76                | 1.67                 |
| NH₄⁺-TDP       | 2346.00             | 2275.33              |
| NH₄⁺-TAZ       | 1.64                | 1.79                 |
| NO₃⁻-TDP       | 2346.00             | 2275.33              |
| NO₃⁻-TAZ       | 1.64                | 1.79                 |

The data columns represent the change in TDZ, TDP, and TAZ concentration going from subambient to ambient and ambient to elevated CO₂ concentrations, respectively. The values are calculated as deviations from the mean TDZ, TDP and TAZ concentrations as produced from FAO and USDA data (Mueller et al., 2009). Baseline values for TDZ, TDP, and TAZ were 8.90, 2278.00, and 1.70 g kg⁻¹, respectively.

1995; Bloom et al., 2002), but increased root mass at elevated CO₂ concentration for NO₃⁻-supplied plants reported previously (Bloom et al., 2002) were not observed here. The shoot biomass data suggest that growth differences measured early in the lifespan of wheat supplied with NH₄⁺ or NO₃⁻ or NH₄⁺ (i.e., greater shoot biomass in plants supplied NH₄⁺ relative to those supplied NO₃⁻ at elevated CO₂ concentrations; Bloom et al., 2002) do not necessarily carry through to senescence. This may be due in part to a shift in NO₃⁻ assimilation to the root (Kruse et al., 2003), allowing NO₃⁻-supplied plants to compensate for the decrease in shoot NO₃⁻ assimilation that occurs at elevated atmospheric CO₂ concentrations (Bloom et al., 2002, 2010, 2012).

The decrease in yield and biomass measures at elevated CO₂ concentrations does not agree with field observations where wheat yields as well as overall biomass increased with elevated CO₂ (Högy and Fangmeier, 2008; Taub et al., 2008). Similarly, our results that the greatest values for other yield measures (e.g., heads, kernel mass, KN) occurred at ambient CO₂ concentrations vary from the literature. High CO₂ has been found to increase flowering tillers (Havelka et al., 1984; Fangmeier et al., 1996), KN (McKee et al., 1997), and kernel mass (i.e., thousand grain weight; McKee et al., 1997). Conflicting results, however, have also been reported (e.g., Havelka et al., 1984). Many of the field and open top chamber studies were grown under natural light and thus received substantially greater photosynthetic flux density than our chamber-grown plants. These higher light conditions would be more favorable to biomass accumulation. Also, these studies typically applied high amounts of mixed N fertilizer (e.g., NH₄NO₃), and yields and biomass have been found to be greater under mixed N nutrition than under either NH₄⁺ or NO₃⁻ alone (Cox and Reisenauer, 1973; Gentry et al., 1989; Heberer and Below, 1989; Wang and Below, 1995). Finally, the wheat cultivar we used (T. aestivum cv. Verry 10) is a short-statured variety that has rarely been used in other studies and may have accounted for some of the differences between our study and other published data.

Our results that NH₄⁺-supplied plants had greater yield and yield components than NO₃⁻-supplied plants at ambient CO₂ have been observed previously (Wang and Below, 1996; Chen et al., 1998). Wang and Below (1995) observed greater numbers of kernels head⁻¹ and KN in plants supplied NO₃⁻ that was not observed here. Their study, however, supplied NH₄⁺ at relatively high levels (~8.9 vs. 0.2 mM NH₄⁺ – N in our study). Several studies (Bennett and Adams, 1970; Cox and Reisenauer, 1973) have found that incipient NH₄⁺ toxicity can start appearing at N levels as low as 0.08–0.2 mM NH₄⁺, although the onset of NH₄⁺ toxicity depends on light level (Magalhaes and Wilcox, 1984; Britto and Kronzucker, 2002) and solution pH (Findenegg, 1987). The poorer performance of the NH₄⁺ treatment in Wang and Below (1995), therefore, might derive from NH₄⁺ toxicity. We have previously determined that the 0.2 mM NH₄⁺-supplied to our plants to be sufficiently high for normal growth, but low enough to avoid toxicity problems under our experimental conditions (Bloom et al., 2002).

PLANT NUTRIENTS

Our second hypothesis, that nutrient concentrations are differentially affected by the inorganic N form supplied to the plants and CO₂ enrichment, was supported by our data. CO₂ concentration...
FIGURE 5 | The effect of N form and CO$_2$ concentration on root tissue nutrient concentrations of wheat grown hydroponically to senescence. Closed (NH$_4^+$) and open (NO$_3^-$) symbols represent the bulked treatment mean (n = 10). Macro- and micronutrients are listed in the upper left of each frame. The lack of error bars reflects that the root mass for each treatment was bulked and analyzed as a unit.
Table 4 | Organ nutrient allocation as percentage of the plant total under the CO₂ and N form treatments.

|        | Root | Cha | Shoots | Grain | Root | Cha | Shoots | Grain | Root | Cha | Shoots | Grain |
|--------|------|-----|--------|-------|------|-----|--------|-------|------|-----|--------|-------|
|        |      |     |        |       |      |     |        |       |      |     |        |       |
| Total N|      |     |        |       |      |     |        |       |      |     |        |       |
| Sub NH₄⁺ | 5.22 | 9.23 | 10.35  | 75.20 | 4.34 | 14.68 | 38.99  | 41.98 | 2.24 | 22.75 | 58.15 | 16.86 |
| Amb    | 5.02 | 6.36 | 9.08   | 79.54 | 4.56 | 12.10 | 36.78  | 46.55 | 2.38 | 16.30 | 62.59 | 18.73 |
| Elev   | 4.32 | 8.36 | 9.79   | 77.52 | 4.13 | 12.96 | 40.89  | 42.02 | 2.48 | 16.33 | 63.12 | 18.06 |
| Sub NO₃⁻ | 6.45 | 4.66 | 11.89  | 77.00 | 6.46 | 10.21 | 41.24  | 42.10 | 10.05 | 10.50 | 64.04 | 15.41 |
| Amb    | 7.96 | 4.95 | 10.48  | 76.61 | 9.03 | 10.91 | 35.97  | 44.09 | 17.87 | 10.54 | 56.04 | 15.56 |
| Elev   | 6.67 | 6.88 | 9.82   | 76.64 | 7.30 | 12.78 | 36.04  | 43.88 | 12.74 | 12.44 | 59.35 | 15.48 |
| B      |      |     |        |       |      |     |        |       |      |     |        |       |
|        |      |     |        |       |      |     |        |       |      |     |        |       |
| Ca     |      |     |        |       |      |     |        |       |      |     |        |       |
|        |      |     |        |       |      |     |        |       |      |     |        |       |
| Mg     |      |     |        |       |      |     |        |       |      |     |        |       |
|        |      |     |        |       |      |     |        |       |      |     |        |       |
| Zn     |      |     |        |       |      |     |        |       |      |     |        |       |
| N      |      |     |        |       |      |     |        |       |      |     |        |       |
| Mn     |      |     |        |       |      |     |        |       |      |     |        |       |
|        |      |     |        |       |      |     |        |       |      |     |        |       |
| Cu     |      |     |        |       |      |     |        |       |      |     |        |       |
|        |      |     |        |       |      |     |        |       |      |     |        |       |

and N form interactions may alter tissue demands for nutrients. For many nutrients, ratios between different elements are typically maintained within a narrow range (Garten, 1976; Bloom et al., 1985; Lodalz, 2002). CO₂ concentration and N form may disturb the balance between different nutrients, leading to a cascade of changes in demand, accumulation, and allocation among the different plant tissues (e.g., Lodalz, 2002; Högy and Fangmeier, 2008; Natali et al., 2009). Nitrate-supplied plants accumulated the greatest amounts of nutrients at ambient CO₂ (Table 2). Some portion of the greater response of NH₄⁺-supplied plants to CO₂ derived from a dilution effect from the greater biomass at ambient CO₂ concentrations (Figures 2 and 3). Total amounts of nutrients tended to decline with CO₂ enrichment for NH₄⁺-supplied plants, which had the greatest amounts of macro/micronutrients at subambient CO₂ (Table 2). These results have not been observed in other published studies (e.g., Fangmeier et al., 1997; Wu, et al., 2004). Growth chamber studies, however, tend to have more exaggerated differences among treatments than field and greenhouse experiments (Högy and Fangmeier, 2008), and N source cannot be well-controlled in field and greenhouse experiments.

The observed increase in NO₃⁻–N concentration with CO₂ concentration in NO₃⁻-supplied plants has been reported previously (Bloom et al., 2002), and adds further support to the hypothesis that elevated CO₂ concentrations and the resulting decrease in photorespiration inhibit shoot NO₃⁻ photoassimilation. Nevertheless, tissue NO₃⁻ – N concentrations observed here were substantially lower than those in the earlier study (Bloom et al., 2002). Again, this may derive from difference in life stages in the two studies. Most of the N available to the plant for grain filling comes from N translocation rather than uptake from the substrate (Simpson et al., 1983). Probably, the plants continued to assimilate plant NO₃⁻ using a non-photorespiratory dependent process such as root assimilation after root N uptake slowed or stopped. Loss of NO₃⁻ through root efflux to the nutrient solution also may have contributed to the lower concentration of NO₃⁻–N.

The partitioning and accumulation of all mineral elements was affected in some manner by the CO₂ treatment and N form supplied to the plants. Observations that cation concentrations decrease under NH₄⁺ supply (e.g., Cox and Reisenauer, 1973; Gashaw and Mugwira, 1981; Wang and Below, 1998) relative to NO₃⁻ supply were not apparent in this study. Again, this could be partly due to the relatively low concentration of NH₄⁺-supplied in our study, the age of the plants at harvest, and differences among wheat cultivars.

Allocation of nutrients within the plant followed similar trends for both N forms, with the exceptions of Mn and Cu (Table 2). Interestingly, in NO₃⁻-supplied plants, shoot Mn concentrations increased slightly with CO₂, and these plants allocated far more Mn to the shoots than NH₄⁺-supplied plants at all CO₂ concentrations. Manganese (Mn⁴⁺) has been found to activate Rubisco in place of Mg²⁺ and the Rubisco-Mn complex has been observed to decrease Rubisco carboxylase activity while minimally affecting or even enhancing oxygenase activity (Jordan and Ogren, 1983). The slight increase in shoot Mn with CO₂ corresponded to a large 23% decrease in Mg concentration. Manganese, which can act as a cofactor for glutamine synthetase (Sinnorff and Stewart, 1987), was also the only nutrient that NH₄⁺-supplied plants allocated a
greater percentage to the roots at the expense of the shoots. NO$_3^-$-supplied plants typically allocated a higher percentage of most nutrients to the roots, as has been reported previously (Wang and Below, 1995, 1998).

Phytate, which forms complexes with divalent cations, has been found to hinder human Zn and Fe absorption during digestion and thus has been labeled an "anti-nutrient." It may serve a number of valuable functions, however, including roles as an anti-oxidant and anti-cancer agent (Raboy, 2009). Phytate is also the major repository of grain P, and variation in P supply to the developing seed is the major determinant of net seed phytate accumulation (Raboy, 1997, 2009; Cakmak et al., 2010). To our knowledge, no published studies have explicitly looked at how phytate is affected by CO$_2$ concentration. Elevated CO$_2$ has been found to have a much larger negative impact on Zn and Fe concentrations than on P in wheat (Loladze, 2002; Cakmak et al., 2010). Several studies (e.g., Fangmeier et al., 1999; Hogy and Fangmeier, 2008) have observed that P increases slightly with CO$_2$ concentration, and because the majority of P is tied up in phytate, this may cause increases in grain phytate concentrations as atmospheric CO$_2$ rises. As a result, bioavailable Zn and Fe–Zn and Fe not bound to phytate – is expected to decrease even further (Loladze, 2002).

Nonetheless, we did not observe such trends in macro- and micronutrient concentrations in this study. The mechanism behind these contrasting results is not clear, although the environmental conditions and nutrient solution in which the plants were grown likely had some role. The modeled data demonstrated only a small negative impact of CO$_2$ concentration on bioavailable Zn concentrations (Table 4), which was unexpected. Indeed, the grain from NO$_3^-$-supplied plants actually showed a slight increase in bioavailable Zn between ambient and elevated CO$_2$. These results combined with the differences in grain bioavailable Zn between NH$_4^+$ and NO$_3^-$-supplied plants demonstrates that N form may differentially affect the nutritional status of this important nutrient, especially in less developed countries that might be more dependent on phytate-rich grains for their Zn nutrition (Table 3). The milling process removes some, if not most, of the phytate and grain mineral content with the bran fraction of the grain (Guttieri et al., 2006). Regardless, with over 50% of the human population suffering from Zn deficiencies, even small increases in bioavailable Zn would be beneficial (Loladze, 2002). This modeling exercise, however, is not a prediction of how increasing CO$_2$ will affect wheat nutrition so much as illustrates that N source may mediate, to some extent, the effects of CO$_2$ on phytate and bioavailable Zn, and that N source will become an even more important agricultural consideration in the future.

In summary, both CO$_2$ concentration and N form strongly affect biomass and yield in hydroponically grown wheat, as well as nutrient concentrations in above- and belowground tissues. Interactions among plant nutrient concentrations, CO$_2$ concentrations, and N form are complex and non-linear. The impact of N form and CO$_2$ concentration on the mechanisms affecting nutrient accumulation and distribution requires further research and extension to more realistic and agriculturally relevant growing conditions found in greenhouse and field studies. Of course, in greenhouse and field studies, control of N source is limited and control of atmospheric CO$_2$ concentration is expensive. The effects of CO$_2$ and N form on agriculture and human nutrition observed here are interesting and suggest a new area of research on mitigating the effects of climate change on agriculture. The supply of fertilizers (e.g., urea, NH$_4$NO$_3$, anhydrous NH$_3$, organic amendments) or addition of nitrification inhibitors that increase the amount of available NH$_4^+$ may have beneficial effects for human nutrition, particularly in regards to micronutrient deficiencies such as Zn and Fe that currently affect billions of people worldwide. In the face of the potentially negative consequences of climate change on agriculture, all avenues of mitigation must be examined, and even small improvements may prove worthwhile.

ACKNOWLEDGMENTS

The authors would like to thank Kenneth Brown and Jan Peerson at the International Zinc Collaborative Group for providing access to their food balance sheet database which was used in calculating total dietary zinc and phytate values. We would also like to thank Hsien Easlon for his advice and critical review of the manuscript. Finally we thank two anonymous reviewers for their critical reviews of the manuscript. This work was supported by NSF IOS-08-18435 and the National Research Initiative Competitive Grant no. 2008-35100-04459 from the USDA National Institute of Food and Agriculture.
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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.