The Effects of Inorganic Nitrogen form and \(\text{CO}_2\) Concentration on Wheat Yield and Nutrient Accumulation and Distribution

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The effects of inorganic nitrogen form and CO$_2$ 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 (NH$_4^+$) and nitrate (NO$_3^-$), and management of these forms is vital to agriculture. Wheat can utilize either form alone (Wang and Below, 1992), but mixed N nutrition (e.g., NH$_4$NO$_3$) typically produces the best grain yields and quality in hydroponically grown (Gentry et al., 1989; Heberer and Below, 1989; Wang and Below, 1995) and field-grown plants (Bock, 1987; Camberato and Bock, 1990).

Ammonium and nitrate affect crops differently when either is supplied as the sole N source (Bloom, 1997). Ammonium requires less energy to assimilate into organic compounds (Bloom, 1997), but can prove toxic if it accumulates to high concentrations within plant tissues (Cox and Reisenauer, 1973; Goyal and Huffaker, 1984). Nitrate is generally the predominant form available in aerated, temperate agricultural soils (Haynes, 1986; Bloom, 1997), and may accumulate within plant tissues to high concentrations without toxicity (Goyal and Huffaker, 1984). In wheat, the N form supplied has been found to influence many physiological parameters profoundly including biomass (Wang and Below, 1995, 1996, 1998; Bloom et al., 2002), leaf area (Bloom et al., 2002), tillering (Chen et al., 1998), seed mass (Wang and Below, 1995), protein content (Wang and Below, 1995), and mineral nutrient acquisition and distribution (Gashaw and Mugwira, 1981; Wang and Below, 1998), although such differences can vary among cultivars (Gashaw and Mugwira, 1981; Wang and Below, 1995).

The presence of NH$_4^+$, 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 NH$_4^+$ nutrition (e.g., Gashaw and Mugwira, 1981; Wang and Below, 1998), although results varied among cultivars (Gashaw and Mugwira, 1981). For example, NH$_4^+$ nutrition decreased whole plant and shoot accumulations of K, Ca, Mg, Fe, Mn, and Zn (Wang and Below, 1998). Nutrient allocation to plant tissues also varied between N forms. NH$_4^+$-fed plants distributed a smaller percentage of total P, K, Cu, and B to roots relative to NO$_3^-$-fed plants (Wang and Below, 1995, 1998). Also, a greater percentage of reduced N was allocated to the shoots in NH$_4^+$-fed plants (Wang and Below, 1995).

Elevated atmospheric concentrations of CO$_2$ alter growth and N dynamics of wheat and other C$_3$ plants. Under elevated CO$_2$, 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., 1993; 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 CO$_2$.
and in conjunction with decreased concentrations of micronutrients, bioavailable Zn and Fe are expected to decrease even further under elevated CO₂ (Loladze, 2002; Manoj-Kumar, 2011), as these micronutrients form indigestible complexes with phytate. By contrast, wheat yields (Fangmeier et al., 1996; Amthor, 2001; Hög and Fangmeier, 2008), harvest index (HI; Wu et al., 2004), whole plant biomass (Fangmeier et al., 1996; Hög and Fangmeier, 2008), shoot biomass (Fangmeier et al., 1996; Hög and Fangmeier, 2009), and root biomass (Chaudhuri et al., 1990; Wechsung et al., 1995) typically increase under CO₂ enrichment. In addition, elevated CO₂ concentration can increase tillering (Weigel et al., 1994), nitrogen use efficiency (NUE, Fangmeier et al., 1997), and micro/macro nutrient use efficiencies (Manderscheid et al., 1995). The influence of elevated CO₂ on many of these characteristics may vary among cultivars and research protocols (e.g., FACE vs. controlled environment chamber, greenhouse vs. field; Amthor, 2001; Hög and Fangmeier, 2008).

Wheat grown under CO₂ enrichment behaves differently under NO₃⁻ and NH₄⁺ nutrition. Exposure to elevated CO₂ inhibits NO₃⁻ photoassimilation in wheat (Bloom et al., 1989, 2002, 2010; Cousins and Bloom, 2004) as well as in all other C₃ and C₄–C₅ intermediate plants tested (Bloom et al., 2012). At elevated CO₂, NH₄⁺-fed plants showed greater increases in leaf area and smaller decreases in shoot protein concentration than NO₃⁻-fed plants (Bloom et al., 2002), which could have consequences for human nutrition. Vegetative plants receiving NH₄⁺ had greater shoot, stem, and root biomass at elevated CO₂ (Bloom et al., 2002). Wheat receiving NO₃⁻ grew slower at elevated CO₂ than at ambient CO₂ (Bloom et al., 2002). Shoot NO₃⁻ concentrations in NH₄⁺-fed plants were undetectable while those in NO₃⁻-fed plants increased by 62% with CO₂ enrichment (Bloom et al., 2002). This increase was associated with an inhibition in NO₃⁻ and NO₂⁻ reductase activities under elevated CO₂ (Bloom et al., 2002).

The interaction between atmospheric CO₂ 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 CO₂ (subambient, ambient, and elevated) and two forms of N nutrition (NH₄⁺ and NO₃⁻). We tested the following hypotheses: (1) plant nutrient concentrations and allocation patterns will respond differently to CO₂ enrichment under the two N forms, and (2) NO₃⁻-fed plants will show a smaller biomass and yield enhancement in response to CO₂ enrichment than NH₄⁺-fed plants as a result of CO₂ inhibition of shoot NO₃⁻ assimilation. Also, we observed both differences in the Zn concentration between plants grown on NH₄⁺ and NO₃⁻ and a strong dependence of Zn absorption on Zn and phytate concentration, indicating that phytate and bioavailable Zn are affected by N form and CO₂. Therefore, we used the well supported Miller equation (Miller et al., 2007) to estimate how N and CO₂ 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 DI water. The seeds were then rolled up in germination paper saturated with 10 mM CaSO₄. The germination paper was placed in a 400 mL beaker with approximately 75 mL of 10 mM CaSO₄ 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 CaSO₄, 1 mM K₂HPO₄, 1 mM KH₂PO₄, 2 mM MgSO₄, 0.2 g L⁻¹ Fe-NaEDTA and micronutrients (20% of a modified Hoagland’s solution with either 0.2 mM KNO₃ or 0.1 mM (NH₄)₂HPO₄ as the N source, Epstein and Bloom, 2005). The nutrient solution was replaced weekly and an additional 0.2 mM of NO₃⁻ or NH₄⁺ – 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 NH₄⁺ and the NO₃⁻ 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⁻² s⁻¹ at plant height. Plants were subjected to one of three CO₂ concentrations: “subambient” (310 ± 30 ppm), “ambient” (410 ± 30 ppm), and “elevated” (720 ± 50 ppm). Subambient CO₂ concentrations were maintained by passing air that entered the growth chamber through wet soda lime, a mixture of KOH, NaOH, and Ca(OH)₂ that was replaced as needed. The elevated CO₂ conditions were maintained in an environmental chamber equipped with non-dispersive infrared analyzers for CO₂ (Horiba model APRA-250E) and valves that added pure CO₂ 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 material was sorted into grain, chaff, shoots, and roots and dried for 48 h at 55°C. Data on kernel number (KN), kernels head⁻¹, and harvest index (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 described by Haug and Lantzsch (1983). The remainder of the grain as well as the shoots and chaff was bulked into five repetitions per treatment and sent to the UC Davis Analytical Laboratory for nutrient analysis. The roots of plants for each CO₂ × 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 CO₂ 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 Wuchler 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).

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

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 \(NH_{4}^{+}\) and \(NO_{3}^{-}\)-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 \(NH_{4}^{+}\) or \(NO_{3}^{-}\). 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 TDP and TDZ is the sum of the unmodified and modified portions. These new TDP and TDZ values for both \(NH_{4}^{+}\) and \(NO_{3}^{-}\)-supplied wheat were 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 effect of elevated CO2 on TAZ in \(NH_{4}^{+}\) and \(NO_{3}^{-}\}-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 \(NH_{4}^{+}\) vs. \(NO_{3}^{-}\) nutrition reacted differently to CO2 enrichment (Figure 1; Table 1). Plants supplied \(NH_{4}^{+}\) 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 \(NO_{3}^{-}\}-supplied plants did not differ among the three CO2 concentrations.

At subambient CO2 differences between the \(NH_{4}^{+}\) and \(NO_{3}^{-}\) 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 \(NO_{3}^{-}\)-supplied plants had greater shoot biomass, kernel mass, and kernels head\(^{-1}\). At ambient CO2, \(NH_{4}^{+}\)-supplied plants had a greater number of heads and greater chaff biomass. Plants supplied \(NO_{3}^{-}\) 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 \(NH_{4}^{+}\) than when supplied \(NO_{3}^{-}\) 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 \(NH_{4}^{+}\) and \(NO_{3}^{-}\)-supplied plants were observed at ambient and elevated CO2. Ammonium-supplied plants always had lower root:shoot ratios and biomasses than \(NO_{3}^{-}\)-supplied plants at the same CO2 concentration.

**NUTRIENTS**

**Total plant nutrients**

Total plant nutrients generally followed the same trend within N form, although \(NH_{4}^{+}\)-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 \(NH_{4}^{+}\), while S and Mn were highest under ambient CO2.
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 had the greatest concentrations at subambient CO₂. The highest concentrations of B, Fe, and Zn occurred at subambient and elevated 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, F, 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 plants 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 NO₃⁻ – N, P, K, Ca, B, Cu, Fe, 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 highest 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 forms significantly affected grain nutrient concentrations (Table 1; Figure 3). At subambient CO₂, NH₄⁺-supplied plants had higher concentrations of total N, P, 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

### Table 1 | Results of a series of one-way ANOVAs run on the data.

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<thead>
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<th>Among CO₂ cnc. within a N form</th>
<th>Grain</th>
<th>Shoot</th>
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<tr>
<td></td>
<td>NH₄⁺</td>
<td>NO₃⁻</td>
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<tr>
<td>Total N</td>
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</tr>
<tr>
<td>P</td>
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<td>NS</td>
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<tr>
<td>K</td>
<td>***</td>
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</tr>
<tr>
<td>S</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Ca</td>
<td>***</td>
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<td>B</td>
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<td>NS</td>
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<tr>
<td>Cu</td>
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<tr>
<td>NO₃⁻ – N</td>
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<td>Phytate</td>
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<td>Heads</td>
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</tr>
<tr>
<td>Harvest index</td>
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Differences among CO₂ concentrations within an N form and between N forms at each CO₂ concentration for shoot and grain nutrient concentrations. Differences among CO₂ concentrations within an N form or between N forms at each CO₂ concentration for biomass and yield components. The symbols indicate statistical significance (*0.05, **0.01, ***0.001, NS, not significant).
Nitrogen, CO₂ effects on wheat

Yield (g plant⁻¹)

4
5
6
7
8

Sub Amb Elev Sub Amb Elev

Roots (g plant⁻¹)

1.0
1.3
1.6
1.9

Col 1 vs Col 5

Col 1 vs Col 17

Col 1 vs Col 28

Col 1 vs Col 28

Root:Shoot

NH₄⁺ + NO₃⁻

Root:Shoot

Chaff (g plant⁻¹)

1.8
2.1
2.4
2.7
3.0

# Heads (plant⁻¹)

4.28
4.98
5.68
6.38
7.08

Total Kernels (plant⁻¹)

100
125
150
175
200

Harvest Index (g grain/g shoots⁻¹)

0.48
0.50
0.52
0.54

Harvest Index

FIGURE 1 | The effect of N form and CO₂ concentration on biomass and yield components of wheat hydroponically grown to senescence. Closed (NH₄⁺) and open (NO₃⁻) symbols and error bars represent the back-transformed means and 95% confidence intervals (n = 10). Means within N form across CO₂ treatment are significantly different if labeled with different letters. Root-related data do not have error bars.

at subambient CO₂ for NH₄⁺-supplied plants (Figure 4). Subambient CO₂ also produced the lowest phytate concentrations in NO₃⁻-supplied plants. NH₄⁺-supplied plants had greater phytate concentrations than NO₃⁻-supplied plants at subambient CO₂, but not at the other CO₂ concentrations. Grain from plants grown under NH₄⁺ nutrition had roughly 7, 18, and 8% higher bioavailable Zn than NO₃⁻-supplied plants at subambient, ambient, and elevated CO₂, respectively (Figure 4).

Based on this phytate and bioavailable Zn data, we modeled how a human population from a LDC would be affected by
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 plants. Plants at subambient CO$_2$ generally showed a trend toward the shoots at the expense of the roots 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 shoots 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 heterogeneity 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 grain. Phyta, which hinders human absorption of Zn and Fe (Raboy, 2009), showed little variation at ambient and elevated CO$_2$ between NH$_4^+$ and 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,

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Total plant nutrients (mg plant$^{-1}$) as affected by N form and CO$_2$ concentration.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub</td>
<td>Amb</td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>215.66</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>159.39</td>
</tr>
<tr>
<td>B</td>
<td>0.28</td>
</tr>
<tr>
<td>Ca</td>
<td>0.29</td>
</tr>
<tr>
<td>Mg</td>
<td>2.66</td>
</tr>
<tr>
<td>Mn</td>
<td>2.16</td>
</tr>
<tr>
<td>Fe</td>
<td>Cu</td>
</tr>
</tbody>
</table>
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 (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).
Phytic Acid P (mg g⁻¹)

- 3.3
- 3.5
- 3.7
- 3.9
- 4.1

NH₄⁺
Amb

CO₂ concentration

Bioavailable Zn (mg kg⁻¹)

- 3.3
- 3.5
- 3.7
- 3.9
- 4.1

AmbSub Elev

A

B

FIGURE 4 | The effect of N form and CO₂ concentration on (A) grain phytate (phytic acid) and (B) bioavailable Zn concentrations of wheat grown hydroponically to senescence. Closed (NH₄⁺) and open (NO₃⁻) symbols represent the back-transformed means and 95% confidence intervals (n = 4). The model used to calculate the bioavailable Zn did not allow the inclusion of confidence intervals.

Table 3 | Total dietary Zn (TDZ), total dietary phytate (TDP), and total bioavailable Zn (TAZ) of a human population from a hypothetical less developed nation reliant on wheat for 50% of their dietary phytate and Zn as modeled using the Miller equation.

<table>
<thead>
<tr>
<th>Sub → Amb (g/kg⁻¹)</th>
<th>Amb → Elev (g/kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDZ</td>
<td></td>
</tr>
<tr>
<td>NH₄⁺ TDP</td>
<td>2214.92</td>
</tr>
<tr>
<td>TAZ</td>
<td>1.76</td>
</tr>
<tr>
<td>TDZ</td>
<td>8.68</td>
</tr>
<tr>
<td>NO₃⁻ TDP</td>
<td>2346.00</td>
</tr>
<tr>
<td>TAZ</td>
<td>1.64</td>
</tr>
</tbody>
</table>

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 (Wuehler et al., 2009). Baseline values for TDZ, TDP, and TAZ were 8.90, 2278.00, and 1.70 g/kg⁻¹, respectively.

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. Very 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 inceptent 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 CO₂ concentration on root tissue nutrient concentrations of wheat grown hydroponically to senescence. Closed (NH₄⁺) and open (NO₃⁻) 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.
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., 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., Loladze, 2002; Hög and Fangmeier, 2008; Natali et al., 2009). Nitrate-supplied plants accumulated some portion of the greater response of NH₄⁺ 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 (Mn²⁺) 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 (Smirnoff 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.

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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.

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