CO2-Induced Changes in Wheat Grain Composition: Meta-Analysis and Response Functions

Malin C. Broberg 1,*, Petra Högy 2 and Håkan Pleijel 1

1 Department of Biological and Environmental Sciences, University of Gothenburg, P.O. Box 461, SE-40530 Göteborg, Sweden; hakan.pleijel@bioenv.gu.se
2 Institute for Landscape and Plant Ecology, University of Hohenheim, Ökologiezentrum 2, August-von-Hartmann Str. 3, D-70599 Stuttgart, Germany; petra.hoegy@uni-hohenheim.de
* Correspondence: malin.broberg@bioenv.gu.se; Tel.: +46-31-786-4805

Abstract: Elevated carbon dioxide (eCO2) stimulates wheat grain yield, but simultaneously reduces protein (N) concentration. Also other essential nutrients are subject to change. This study is a comprehensive synthesis of wheat experiments with eCO2, estimating effects on N, minerals (B, Ca, Cd, Fe, K, Mg, Mn, Na, P, S, Zn), and starch. Analysis was made by i) deriving response functions for the relative effect on element concentration in relation to CO2 concentration, ii) meta-analysis to test the magnitude and significance of observed effects, and iii) relating CO2 effects on minerals to effects on N and grain yield. Responses range from zero to strong negative effects of eCO2 on mineral concentration, with largest reductions for the nutritionally important elements N, Fe, S, Zn and Mg. Together with the positive but small and non-significant effect on starch concentration, the large variation in effects suggests that CO2-induced responses cannot be explained by a simple dilution model. To explain the observed pattern, uptake and transport mechanisms may have to be considered, along with the link of different elements to N uptake. Our study shows that eCO2 has a significant effect on wheat grain stoichiometry, with implications for human nutrition in a world of rising CO2.

Keywords: Triticum aestivum; carbon dioxide; minerals; protein; starch; baking properties; crop quality; food security

1. Introduction

The atmospheric concentration of carbon dioxide (CO2) has steadily increased since the 19th century, from the pre-industrial level of 280 ppm to current 400 ppm [1]. Latest projections by the Intergovernmental Panel on Climate Change [1] suggest that concentrations are likely to reach levels in the range of 420 ppm (RCP2.6) to 1300 ppm (RCP8.5) by the year 2100.

Effects of elevated CO2 (eCO2) on plants are well studied, in particular on food crops due to the strong concern for future food security. Photosynthesis and growth in C3 plants are often enhanced by eCO2 resulting in a higher yield, which has been observed for many crops [2]. The magnitude of yield response has been shown to vary between different crops [3] and crop varieties [4,5], but also to depend on differences in experimental systems [6]. It has been argued that yield stimulation is overestimated due to unrealistic growing conditions in enclosure systems, including open-top chambers (OTCs) [7,8]. In contrast, Ziska and Bunce [9] found that there were no significant differences in yield response for rice, soybean and wheat when comparing experiments using enclosure methodologies with Free-Air-CO2-Enrichment (FACE) technology in a single experiment. According to Körner [10], carbon is rarely the limiting factor for plant growth but soil resources, e.g., nutrients and water, are more likely to determine plant performance and the observed positive effects of eCO2 are according to this argument consequently a result of improved water use efficiency. Comparing the eCO2 effects on plants grown in different experimental systems could possibly reveal if these statements are valid also for effects on wheat crop quality.
Wheat is a major food crop globally, being the second most important energy source for the human population with an annual global production of approximately 700 million tons [11]. The main source of food energy within the wheat grain is starch, accounting for 50-70% of total grain mass. It has been proposed that eCO₂ could enhance concentration of carbohydrates, starch being the major component, and thus reduce the concentrations of other constituents, often referred to as the “dilution hypothesis” [12]. Photosynthetic nitrogen (N) use efficiency can potentially increase under eCO₂ [13], and consequently more carbon can be assimilated with the same amount of N, resulting in a relative decrease in N content in the leaf. Since most of the grain N is translocated from non-reproductive parts of the plant during grain filling [14], also grain N content could be affected under eCO₂ by this mechanism.

Changes in crop quality, like nutritional aspects, have often been neglected in research synthesis and assessments of future food production. The average effect on protein (hereafter referred to as N) content, estimated in a meta-analysis by Taub et al. [15], showed a significant decrease for several crops, including wheat, barley, rice and soybean. Along with the “dilution hypothesis” a few more hypotheses have been proposed to explain the observed pattern of decreasing N concentration in plants exposed to eCO₂, such as a reduction in transpiration driven mass flow [16] and impaired N acquisition [17], processes that both can result in a reduced N uptake under eCO₂ even without yield stimulation. According to the mechanism put forward by Bloom [17], the decrease in photosynthesis under eCO₂ leads to a reduced malate export from the chloroplasts, and the nicotinamide adenine dinucleotide hydride (NADH) generated from this malate in the cytoplasm powers the reduction of nitrate (NO₃⁻) to nitrite (NO₂⁻), which is the first step of plant NO₃⁻ assimilation. In line with this, Pleijel and Uddling [18] found that the dilution hypothesis is likely to exist, but cannot fully explain the reduction in N concentration and yield in wheat under eCO₂, since N concentration is reduced also where grain yield is unaffected. This suggests a role for the mechanism proposed by Bloom [17]. Another important and related question is if there is a level of CO₂ where the effect of eCO₂ on grain N concentration saturates, analogous to the saturation seen in the response of photosynthesis under eCO₂ of C₃ plants [19].

The effects on N content in wheat grains have been observed in a rather large number of studies with wheat grown under eCO₂, while observations of effects on other elements are limited. Decrease in concentrations of some essential mineral nutrients (Fe and Zn) have been documented [20,21], while it is still uncertain to what extent other elements are affected by eCO₂ and the mechanism behind observed changes. Reduction in concentrations of N and nutrient elements are of great concern for future food security and the issue of so called ‘hidden hunger’, where the amount of calories might be sufficient but with undernourishment with respect to essential nutrients. A modelling study by Myers et al. [22] estimated that the CO₂-induced reduction in Zn concentration in staple crops could substantially increase the number of people at risk of Zn deficiency by 138 million until 2050. Cereals, including wheat, are also an important source of dietary Cadmium (Cd) exposure [23], which could cause injury to kidney and bones [24], hence the CO₂ effect on Cd content is also of importance.

Due to the fact that N is often considered to be one of the most limiting elements for crop growth, the uptake of other nutrients could be expected to match the available N, assuming that excess uptake of other minerals does not occur. With these assumptions the effect on plant nutrients would follow the same pattern as N when wheat is grown under eCO₂, which could be tested by relating the eCO₂ effects on minerals to the effect on N. If dilution is the main process that acts to reduce mineral concentration the eCO₂ effect on grain yield would be closely related to effects on minerals, where a negative effect on mineral concentration will only occur in association with yield stimulation.

Since wheat is used for baking to a large extent, it is also relevant to study how different baking properties are affected by eCO₂, where alteration in quality may affect market value and quality of products (e.g. review by Högy et al. [25]). Many measures of baking properties are related to the content and quality of protein, such as gluten concentration and composition, dough elasticity/resistance, and bread loaf volume, and consequently these variables are likely to be impaired by eCO₂ following the pattern of grain N concentration. Negative effects on various baking
properties have been observed in individual experiments [26-30], but to our knowledge no meta-analysis has been made on this aspect.

This study intends to provide a comprehensive overview of observed effects of eCO$_2$ on wheat grain quality based on all available ecologically realistic experiments, presented as meta-analysis (to test the overall magnitude and statistical significance of the effects) and as response regressions (to assess effect size in relation to CO$_2$ concentration [CO$_2$]). Further, the effect of eCO$_2$ on the concentration of a range of minerals is related to the effect on N concentration and grain yield in order to understand to what extent eCO$_2$ effects are consistent among different minerals and the degree to which they are related to the effects on N concentration and yield stimulation. By these three approaches our study aims to examine the following hypotheses:

1. The negative effects of eCO$_2$ on N concentration and yield are independent of experimental setup, such as exposure system, rooting environment, and concentration level of CO$_2$ treatment.
2. The negative effect of eCO$_2$ on N concentration is saturating at high CO$_2$.
3. Nutritional and baking quality of wheat grain is negatively affected by eCO$_2$.
4. Concentrations of N and minerals are reduced due to starch dilution under eCO$_2$.
5. Effects of eCO$_2$ on minerals concentration are linked to the effect on N concentration and grain yield stimulation.
2. Results

2.1. Nitrogen and starch

Grain N concentration was significantly reduced by eCO2 with an overall effect of -8.4% (CI -9.8 -7.4; Figure 1a). The magnitude of effect was shown to be dependent on experimental setup where significant differences were observed between exposure systems (FACE < OTC) and rooting environment (pots > field soil). There was, however, no significant difference between OTC and FACE when excluding eCO2 treatments >600 ppm (only OTC experiments). Comparing concentration levels (above or below 600 ppm) in OTC experiments did not show any significant difference, but indicated a larger effect with higher [CO2]. Even though N concentration was reduced by eCO2 there was a significant increase in N yield, with an overall effect of 12% (CI 7.93 15.90; Figure 1a), associated with a strong grain yield stimulation. Subgroup analysis revealed that experiments performed in field tunnels (FT) and pots did not show a significant CO2 effect on N yield; however, it should be noted that those groups have few observations and thus larger CIs. There were no significant differences with regard to the effect on N yield when comparing OTC with FACE or different CO2 concentrations.

The response function for the relationship between N concentration and [CO2] (Figure 1b) showed a strong non-linear relationship ($r^2=0.57$), with an initial reduction in N concentration with increasing [CO2], but reaching a minimum at ~600 ppm. N yield was positively affected by eCO2, but showed a rather weak relationship with [CO2] ($r^2=0.19$). Details of the regression models are presented in Table 1.
Figure 1. a) Meta-analysis of eCO2 effects on N concentration and N yield using ambient [CO2] as the reference, with subgroup-analysis of exposure systems, rooting environment, and concentration level for eCO2 treatment. Number of comparisons for concentration and yield, respectively, are given within brackets. b) Response function for N concentration with [CO2], grey markers show data points identified as outliers not included in the curve fitting.

Figure 2 shows the eCO2 effect on various baking properties, where a significant negative effect is observed for Hagberg falling number (-5.8%, CI -9.9 -1.7), Zeleny value (-21.2%, CI -25.5 -16.9), dry gluten content (-16.5%, CI -22.0 -11.2), wet gluten content (-17.0%, CI -23.0 -11.5), peak resistance (-11.4%, CI -17.3 -3.0), and bread loaf volume (-11.9%, CI -21.3 -2.3). Mixing time significantly increased (11.2%, CI 0.6 21.5) under eCO2, while resistance breakdown remained unaffected (-2.6%, CI -12.5 9.0).

Figure 2. Meta-analysis showing the effect of eCO2 on various baking properties using ambient [CO2] as the reference. Number of comparisons for concentration and yield, respectively, are given within brackets.
Meta-analysis for eCO₂ effect on grain starch concentration (Figure 3a) showed a non-significant positive effect of 2.2% (CI -0.6 6.2). In line with this result the response function for starch concentration with [CO₂] did not reveal any relationship (Figure 3b). Starch yield was significantly positively affected by 20.8% (CI 12.4 30.9). Due to limited amount of data (19 observations) subgroup analysis was not performed for starch concentration and starch yield.

Figure 3. a) Meta-analysis of eCO₂ effects on starch concentration using ambient [CO₂] as reference. Number of comparisons for concentration and yield, respectively, are given within brackets. b) Response function for starch concentration with [CO₂]. Grey markers show data points identified as outliers and not included in the curve fitting.
Table 1. Response functions for regression of concentration and yield of N, starch, and minerals with 
[CO₂]. Model parameters are presented for both linear (y=B1x+B0) and quadratic (y=B2x²+B1x+B0) 
curve fit. The AICc comparison gives the probability of model (linear vs. quadratic) being correct. 
Data points identified as outliers were excluded from regressions.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Observations (outliers)</th>
<th>B0</th>
<th>B1</th>
<th>B2</th>
<th>R²</th>
<th>Probability¹ (%)</th>
<th>Sign.</th>
</tr>
</thead>
<tbody>
<tr>
<td>N concentration</td>
<td>132 (4)</td>
<td>1.10</td>
<td>3.10E-04</td>
<td>1.66E-06</td>
<td>0.43</td>
<td>&lt;0.01 *</td>
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</tr>
<tr>
<td>yield</td>
<td>96 (11)</td>
<td>0.94</td>
<td>2.49E-04</td>
<td>-1.85E-06</td>
<td>0.12</td>
<td>11.8 *</td>
<td></td>
</tr>
<tr>
<td>Starch</td>
<td>30 (3)</td>
<td>1.00</td>
<td>6.79E-06</td>
<td>3.71E-07</td>
<td>0.00</td>
<td>73.4 ns</td>
<td></td>
</tr>
<tr>
<td>yield</td>
<td>30 (2)</td>
<td>0.86</td>
<td>5.18E-04</td>
<td>2.47E-07</td>
<td>0.35</td>
<td>79.4 *</td>
<td></td>
</tr>
<tr>
<td>B concentration</td>
<td>68 (2)</td>
<td>1.01</td>
<td>2.46E-05</td>
<td></td>
<td>0.00</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>yield</td>
<td>32 (4)</td>
<td>0.34</td>
<td>1.96E-03</td>
<td></td>
<td>0.40</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Ca concentration</td>
<td>83 (4)</td>
<td>1.13</td>
<td>3.69E-04</td>
<td></td>
<td>0.32</td>
<td>*</td>
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<tr>
<td>yield</td>
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<td>0.84</td>
<td>5.64E-04</td>
<td></td>
<td>0.16</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Cd concentration</td>
<td>13</td>
<td>1.12</td>
<td>3.95E-04</td>
<td>2.08E-06</td>
<td>0.31</td>
<td>81.2 *</td>
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</tr>
<tr>
<td>yield</td>
<td>13</td>
<td>0.99</td>
<td>3.19E-05</td>
<td>4.70E-07</td>
<td>0.00</td>
<td>89.5 ns</td>
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</tr>
<tr>
<td>Cu concentration</td>
<td>80 (2)</td>
<td>1.07</td>
<td>2.03E-04</td>
<td></td>
<td>0.14</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>yield</td>
<td>44 (5)</td>
<td>0.67</td>
<td>1.04E-03</td>
<td></td>
<td>0.27</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Fe concentration</td>
<td>86 (7)</td>
<td>1.14</td>
<td>3.87E-04</td>
<td></td>
<td>0.51</td>
<td>*</td>
<td></td>
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<tr>
<td>yield</td>
<td>50 (4)</td>
<td>0.88</td>
<td>4.71E-04</td>
<td>9.11E-03</td>
<td>0.07</td>
<td>19.4 ns</td>
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<tr>
<td>K concentration</td>
<td>87 (4)</td>
<td>1.02</td>
<td>4.44E-05</td>
<td></td>
<td>0.01</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>yield</td>
<td>51 (8)</td>
<td>0.58</td>
<td>1.27E-03</td>
<td>9.00E-06</td>
<td>0.17</td>
<td>80.6</td>
<td></td>
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<tr>
<td>Mg concentration</td>
<td>83 (8)</td>
<td>1.12</td>
<td>3.33E-04</td>
<td></td>
<td>0.61</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>yield</td>
<td>47 (7)</td>
<td>0.75</td>
<td>7.85E-04</td>
<td></td>
<td>0.42</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Mn concentration</td>
<td>84 (3)</td>
<td>1.06</td>
<td>1.91E-04</td>
<td>2.39E-06</td>
<td>0.13</td>
<td>8.1 *</td>
<td></td>
</tr>
</tbody>
</table>

¹ AICc comparison gives the probability of model (linear vs. quadratic) being correct.
2.2. Minerals

Meta-analysis (Figure 4) showed that eCO₂ significantly reduced the concentration of various minerals (Ca, Cd, Cu, Fe, Mg, Mn, P, S, and Zn) in wheat grains, while others were unaffected (B and Na) or significantly increased by a small amount (K). A significant increase in yield was observed for all minerals except for Cd. It should be noted that there was a considerable variation in the magnitude of response (concentration and yield) among the different elements.
Figure 4. Meta-analysis output for mineral concentration and yield using ambient [CO2] as the reference. Numbers within brackets gives the number of comparisons for concentration and yield, respectively.

Response functions in Figure 5 show that concentrations of several mineral nutrients had a strong linear relationship with increasing [CO2], with a significant negative slope for all elements (Fe, Mg, P, S, and Zn) except K. Regression models for remaining elements are presented in Table 1. Ca, Cd, and Cu also showed a significant linear decrease with higher [CO2], however, a quadratic model had a better fit for Mn, while B did not show any relationship with [CO2]. Na was excluded from this analysis due to the small number of observations. The slope of the linear regression line suggests a reduction in mineral concentration of about 2-4% per 100 ppm for all minerals except for B and K, which had a non-significant slope close to zero. Mineral yield showed a positive relationship with [CO2] and a significant slope for all elements except for Cd and Fe (Table 1). The strongest relationships were found for B, K, Mg, and P with an r² between 0.40 and 0.68.

2.3. Effects on minerals in relation to effects on N concentration and grain yield

Figure 6 shows the relationship between eCO2 effects on concentration of various minerals and eCO2 effect on N concentration. The correlation coefficient provides an estimate of the association of effects, and a strong association (r>0.75) is found for S and Fe (r=0.87 and r=0.79, respectively). Ca, Cd, Mg, P, and Zn show a moderately association (0.5<r<0.75), while it was rather weak for remaining elements (B, Cu, K, and Mn).

Regression analysis of the eCO2 effect on mineral concentrations with the CO2 effect on N concentration (Table 2) showed a strong relationship for S (R²=0.75) and Fe (R²=0.63), while the relationships were rather weak for B, Cu, K, and Mn (R²<0.25). Remaining elements were found in the intermediate range (0.25<R²<0.50). A deviation of the fitted curve from the 1:1 line indicates that the element:N ratio was affected by eCO2, hence that the grain stoichiometry was altered. The majority of minerals (Ca, Cu, K, Mg, Mn, P, and S) were less affected by eCO2 compared to N (slope of line <1), whereas the regression lines for Zn and Fe were close to 1, and for B and Cd the slope was >1. Relating the eCO2 effect on minerals to the effect on grain yield showed a weak and non-significant relationship for most elements (Table 2), except for concentrations of K, P, and Zn that had a significantly negative relationships with the effects on grain yield (Figure 7).
Figure 5. Response-functions for mineral concentrations of P, Mg, Fe, K, Zn, and S with [CO₂]. Grey markers show data points identified as outliers and not included in the curve fitting.
Figure 6. Relative effect of eCO2 on mineral concentration (B, Ca, Cd, Cu, Fe, K, Mg, Mn, P, S, and Zn) related to the relative effect on N concentration. Correlation coefficient (r) and its significance is presented in each plot. Black solid lines represent the linear regression model, for which parameters and model performance are presented in Table 2. Grey markers show data points identified as outliers not included in the curve fitting. Gray dashed lines represent the hypothetical situation where the effect of eCO2 on mineral concentration is equal to the effect on N concentration.
Figure 7. Relative effect of eCO\textsubscript{2} on mineral concentration of B, Ca, Cd, Cu, Fe, K, Mg, Mn, N, P, S, and Zn vs. the relative effect on grain yield. Black solid lines represent the linear regression model, for which parameters and model performance are presented in Table 2.
Table 2. Response functions for regression between the relative eCO₂ effect on the concentration of various minerals with eCO₂ effect on N concentration and grain yield. Data points identified as outliers were excluded from the regression analysis.

<table>
<thead>
<tr>
<th>Mineral</th>
<th>N/grain yield</th>
<th>Observations (outliers)</th>
<th>R²</th>
<th>Intercept</th>
<th>Slope</th>
<th>Significance</th>
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<tbody>
<tr>
<td>B</td>
<td>N</td>
<td>64 0.20</td>
<td>0.076</td>
<td>1.28</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>grain yield</td>
<td>28 0.00</td>
<td>-0.019</td>
<td>0.03</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td>N</td>
<td>69 0.46</td>
<td>0.018</td>
<td>0.80</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>grain yield</td>
<td>40 0.04</td>
<td>-0.051</td>
<td>-0.07</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>N</td>
<td>6 0.49</td>
<td>-0.042</td>
<td>1.29</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td></td>
<td>grain yield</td>
<td>10 0.11</td>
<td>-0.039</td>
<td>-0.42</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>N</td>
<td>65 (1) 0.17</td>
<td>-0.007</td>
<td>0.48</td>
<td>*</td>
<td></td>
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<tr>
<td></td>
<td>grain yield</td>
<td>36 (1) 0.00</td>
<td>-0.012</td>
<td>-0.24</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Fe</td>
<td>N</td>
<td>70 0.63</td>
<td>0.008</td>
<td>0.99</td>
<td>*</td>
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<tr>
<td></td>
<td>grain yield</td>
<td>42 0.04</td>
<td>-0.071</td>
<td>-0.07</td>
<td>ns</td>
<td></td>
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<tr>
<td>K</td>
<td>N</td>
<td>69 (1) 0.11</td>
<td>0.026</td>
<td>0.21</td>
<td>*</td>
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<td></td>
<td>grain yield</td>
<td>42 0.59</td>
<td>0.036</td>
<td>-0.14</td>
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<tr>
<td>Mg</td>
<td>N</td>
<td>76 0.32</td>
<td>-0.029</td>
<td>0.42</td>
<td>*</td>
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<td></td>
<td>grain yield</td>
<td>40 0.09</td>
<td>-0.044</td>
<td>-0.51</td>
<td>ns</td>
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<tr>
<td>Mn</td>
<td>N</td>
<td>74 (1) 0.08</td>
<td>-0.008</td>
<td>0.31</td>
<td>*</td>
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<td></td>
<td>grain yield</td>
<td>43 0.01</td>
<td>-0.026</td>
<td>-0.02</td>
<td>ns</td>
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<tr>
<td>P</td>
<td>N</td>
<td>69 (3) 0.46</td>
<td>0.018</td>
<td>0.80</td>
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<tr>
<td></td>
<td>grain yield</td>
<td>42 0.36</td>
<td>-0.014</td>
<td>-0.14</td>
<td>*</td>
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<td>S</td>
<td>N</td>
<td>68 0.75</td>
<td>-0.002</td>
<td>0.74</td>
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<td>-0.07</td>
<td>ns</td>
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<td>Zn</td>
<td>N</td>
<td>83 0.30</td>
<td>-0.001</td>
<td>1.11</td>
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<td></td>
<td>grain yield</td>
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<td>-0.22</td>
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<td>87 (6) 0.18</td>
<td>-0.057</td>
<td>-0.08</td>
<td>*</td>
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3. Discussion

The overall results from this study suggest that eCO₂ can cause a significant shift in wheat grain stoichiometry, with concentration reductions for N and several nutritionally important minerals together with a decreased baking quality and thus lower commodity value. This is the most comprehensive synthesis of eCO₂ effects on mineral elements in wheat, with meta-analyses including more than 60 pair of observations for most mineral elements and 105 for N.

Our results support the first hypothesis, where a significant negative effect on N concentration was observed regardless of experimental setup, although magnitudes of effects were significantly different as shown in the subgroup analysis comparing experimental methods. The negative effect of eCO₂ on N concentration observed in some recent studies was estimated to be between 6.3% [21] and 9.8% [15], which is in line with the overall results in this study (8.4%). The large amount of data gives robust results (small CIs) and allows for subgroup analysis to unravel sources of variation within the data.

The response function for the relationship between N concentration and [CO₂] indicates that the negative effect on N concentration saturates around 600 ppm, supporting the second hypothesis. The meta-analysis, however, points to a stronger response in experiments using an eCO₂ level above 600 ppm compared to below 600 ppm, although this difference was not statistically significant. The significant difference detected when comparing OTC and FACE for all data was indicated to be a
The consequence of the different levels of eCO$_2$ used, and not the exposure system itself, since the difference was not found when comparing FACE with the subset of OTC experiment data with eCO$_2$ concentrations below 600 ppm (average [CO$_2$] 550 ppm and 528 ppm respectively).

The comparison of rooting environment showed that there was a much stronger negative effect on N concentration in potted plants compared to those grown in field soil. This is in line with the results from Taub et al. [15] where wheat grown in OTCs showed a similar difference in response between rooting environments. Assuming that experiments in field soil are more realistic, this suggests that potted experiments may strongly overestimate the negative effect of eCO$_2$ on N concentration. Potted plants are more likely to suffer from nutrient limitation due to their restricted rooting space, thus nutrient uptake cannot increase with the same rate as photosynthesis under eCO$_2$. It should, however, be noted that only 8 pairs of observations from potted plants were included in this study, compared to 97 observations for field soil, and the large CIs for potted plants indicate that conclusions about them are uncertain.

As a consequence of the decrease in N concentration, eCO$_2$ had a significant negative effect on most baking properties (Figure 2), even though the number of observations is rather small. A reduction in gluten proteins results in lower elasticity and resistance of the dough and smaller bread loaf volume, but also longer mixing time [31]. In addition, the falling number was reduced under eCO$_2$ reflecting an increase in $\alpha$-amylase activity, which is associated with poor baking properties, such as sticky dough and poorly structured loaves [32], but also shortens the storage time of flour and grains [33].

No significant effect of eCO$_2$ on starch concentration could be demonstrated and consequently the negative effect on N could not be explained by starch dilution, thus the third hypothesis was not supported. On the other hand the number of observations is rather small, resulting in large CIs and low statistical power. Since starch is a major component of the wheat grain (50-70 %) even a small change in its concentration could alter the grain stoichiometry considerably. To detect an effect with small magnitude a large sample size is required and the non-significant results found here could be a consequence of power failure and a dilution effect by starch may be of importance for some elements. Further investigations would be needed to confirm this.

Meta-analysis for eCO$_2$ effect on mineral concentrations (Figure 4) showed that there was a large variation in magnitude of effects, ranging from effects close to zero to reductions of about 10%. Together with the non-significant effect on starch concentration, the large variation in effects on mineral elements indicates that CO$_2$-induced responses cannot be explained by a simple growth dilution model. In addition, almost all elements (except K) showed a weak relationship when comparing eCO$_2$ effects on mineral concentration with grain yield stimulation. If dilution was the only mechanism operating, the reduction in mineral concentration would closely follow the increase in biomass and would be the same for all elements.

The eCO$_2$ effects on Fe and S were closely related to the effects on N (Figure 5) and those elements were also among the ones most strongly negatively affected by eCO$_2$ in the meta-analysis (Figure 4). In contrast, the effect on minerals (B, Cu, K, and Mn) that showed a weak relationship with effects on N, were observed to be little (B, Cu, Mn) or not significantly (K) affected by eCO$_2$. This suggests that eCO$_2$ effects on N may play a role also for other minerals such as Fe and S. The regression of effects between B and N gives a slope >1, however, this should not be interpreted as a stronger effect on B than N since it is mainly a result of large response range in B (with both positive and negative effects) compared to N. As shown in the meta-analysis (Figure 4) the large variation of eCO$_2$ effects on B cancel each other out, resulting in a net zero effect.

The different response patterns of mineral elements could possibly be attributed to their different functions in the plant. In a study by Ågren and Weih [34] stoichiometric clusters of mineral elements were identified in leaves of six *Salix* genotypes grown under altered water and nutrient supply. Changes in concentration for one group of elements (N, P, S, and Mn) were associated with growth, the second group (K, Ca, and Mg) followed changes in biomass, while the third group (Fe, B, Zn, and Al) were believed to be limited by soil availability. It was also suggested that these groups could be associated with different biochemical functions, where elements of the first group are linked...
to nucleic acids/proteins, the second group is related to structure/photosynthesis, and the third group
is associated with enzymes. The significant relationship between K and grain yield stimulation
(Figure 7) confirms that K concentration is associated with changes in biomass, while the
respective relationships was rather weak (non-significant) for Ca and Mg. With the current data
it is not possible to test if the elements most strongly affected by eCO2 in our study, Fe and Zn, are
reduced due to soil limitation or if they are functionally linked to N. It is also important to note that
these effects on element concentration in leaves do not necessarily translate to the same response in seeds.

The mineral concentration in wheat grains is generally a result of total plant uptake, biomass
accumulation, and the rate of translocation from vegetative tissues during grain filling. Waters et al.
[35] showed that the translocation of Fe, Zn, and N from vegetative tissues to grain is partly regulated
by the same proteins in wheat plants. eCO2 could possibly affect translocation rates indirectly
through higher leaf temperatures due to lower transpiration rates [36]. Increase in leaf temperature
can lead to heat stress, which is known to promote senescence [37], and thus shorten the grain filling
period [38]. This is, however, likely to increase concentrations of minerals since starch accumulation
is often more strongly reduced than N and minerals [37]. If the rate and efficiency of translocation
were strongly affected by eCO2, Fe, Zn, and N could be expected to follow the same response pattern.

The yield of N (Figure 1) and all minerals, except for Cd (Figure 4), were significantly increased
under eCO2, which indicates that there is an increase in total soil uptake of these elements. As a
potential mitigation strategy more fertilizers could be added to the agricultural system, however,
with the risk of also increasing leaching of nutrients and enhanced emissions of N2O.

Web of Science, Scopus, and Google Scholar were used to survey all peer-reviewed literature
published between 1980 and 2016 (May) related to the response of wheat grain quality to eCO2.
Experimental data were included in the database if at least one of the following variables were
reported: grain protein concentration (or N concentration), grain starch concentration, grain mineral
concentrations (B, Ca, Cd, Cu, Fe, K, Mg, Mn, Na, P, S, and Zn), grain yield (to calculate yield of
N/protein and other minerals), and baking properties (Hagberg falling number, Zeleny value, gluten
content, mixing time, peak resistance, resistance breakdown, bread loaf volume). In order to only
include ecologically realistic data, experiments performed in greenhouse or closed growth chambers
were excluded. For factorial design experiments with elevated ozone only treatments without ozone
fumigation were included, since ozone is known to have significant effects on both yield and grain
quality [39]. Data sources for the included experiments are presented in Supplementary (Table 1,
Table 2).

Data from figures were extracted using software GetData Graphic Digitizer [43]. For
experiments where ambient [CO2] were not reported it was assumed to be equal to the global mean
for the year the study was conducted, with the Mauna Loa record used as reference (retrieved from National Oceanic & Atmospheric Administration, (NOAA), www.noaa.gov).

4.2. Meta-analysis

Meta-analysis was performed using a meta-analytical software package MetaWin [44]. The experimental treatment with ambient CO₂ was used as control, and parameter values were considered independent if they were made on different cultivars, different [CO₂], or different years, in line with previous meta-analysis [41,45]. The effect size used was the natural log of the response ratio (r, the ratio of the means of two groups, experimental and control) reported as percentage change from the control [41,44,46]. All variables were analyzed using an un-weighted approach due to lack of data for computation of sample variance (standard deviation or standard error with degree of replication). In line with previous meta-analysis [39,44] variance of the effect size was calculated using a resampling method with 9999 iterations, and confidence intervals (CI) were calculated using the bootstrap method. If the 95% CI did not overlap zero the average effect size for each variable was considered to be significant, and for subgroup analysis the different groups were considered significantly different if the 95% CI did not overlap [45].

Experiments with additional treatments were included, such as different application levels of N, water supply, temperature, and time of sowing. However, only the effect of eCO₂ was tested in the meta-analysis, and interactions of eCO₂ and additional treatments were not further examined. Subgroup analysis was performed for N concentration and yield, for which a substantial amount of data was available, where data was categorized by 1) exposure system, Free-Air-CO₂-Enrichment (FACE), Open-Top-Chamber (OTC), and Field Tunnel (FT), 2) rooting environment, pots or field soil, and 3) the concentration level of the eCO₂ treatment, above or below 600 ppm (only applicable for OTC experiments).

4.3. Response functions

Response functions were derived through regression between the relative effect of each variable and the corresponding [CO₂] for the treatment. The response was related to the effect estimated at 350 ppm by linear regression for each individual experiment. At 350 ppm the variables were set to take the value of 1 on a relative scale. Both a linear (first order polynomial) and quadratic (second order polynomial) model was fitted to the data, and the model preferred by Akaike information criterion (AICc) comparison method [47] was chosen. All additional treatments, such as low N, drought, and high temperature, were excluded from the response functions since they were observed to cause large scatter not related to the effect of [CO₂]. All response functions were derived using automatic outlier removal [48].

4.4. Comparison of CO₂ effects on different response variables

The eCO₂ effect was related to the control treatment (ambient [CO₂]) when relating the effects on minerals to effects on N or grain yield. The correlation coefficient was calculated to estimate the association of effects, while regression was used to test if effects on minerals are dependent on effects on N or grain yield. Only linear regression was used to explore the relationship with N, since the slope of a linear trend line could be compared to a 1:1 line that represents the theoretical situation where the mineral and N concentrations are equally affected. For regression between effect on minerals and effect on grain yield both linear and quadratic curve fit was tested, and best fit chosen with AICc comparison method.

5. Conclusions

Our study, based on an extensive database, shows that eCO₂ has significant negative effects on the concentration of several minerals and N in wheat grain, and that the effects on N translates into reduced baking quality. Subgroup analysis of experimental systems reveals that N concentration was more strongly affected in potted plants than plants grown in field soil. Also, the significant difference found between FACE and OTC studies could be attributed to the different concentration levels used
and not the enclosure system itself. The pattern of effects by eCO₂ on different minerals was complex, showing that a single mechanism cannot account for the diversity of responses. Although the positive effect on starch concentration was not statistically significant, a dilution effect by starch may be of importance for some elements. However, for most of the minerals the eCO₂ effect was not strongly related to the effect on grain yield, suggesting that dilution was not of large importance. The association with N was strong for eCO₂ effects on S and Fe, elements being important components of proteins, and fairly strong also for P. The response functions and relationships between different elements and N presented in this study can be used in risk assessments of effects on nutrition in a future high CO₂ world.

Supplementary Materials: The following are available online at www.mdpi.com/link, Table S1: Data sources, grain yield, N, starch, and minerals, Table S2: Data sources for baking properties.

Acknowledgments: The work by M.B. and H.P. was supported by the strategic research area Biodiversity and Ecosystem Services in a Changing Climate (BECC, http://www.becc.lu.se/).

Author Contributions: M.B. and H.P. conceived and designed the study; data collection was made by M.B. in close collaboration with P.H.; all authors participated in the analysis of the data; M.B. wrote the paper with substantial input from P.H. and H.P.

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, and in the decision to publish the results.

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