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CO_2-Induced Changes in Wheat Grain Composition: Meta-Analysis and Response Functions

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Academic Editor: Hans-Joachim Weigel

Received: 30 January 2017; Accepted: 20 April 2017; Published: 25 April 2017

Abstract: Elevated carbon dioxide (eCO_2) stimulates wheat grain yield, but simultaneously reduces protein/nitrogen (N) concentration. Also, other essential nutrients are subject to change. This study is a synthesis of wheat experiments with eCO_2, estimating the effects on N, minerals (B, Ca, Cd, Fe, K, Mg, Mn, Na, P, S, Zn), and starch. The analysis was performed by (i) deriving response functions to assess the gradual change in element concentration with increasing CO_2 concentration, (ii) meta-analysis to test the average magnitude and significance of observed effects, and (iii) relating CO_2 effects on minerals to effects on N and grain yield. Responses ranged from zero to strong negative effects of eCO_2 on mineral concentration, with the largest reductions for the nutritionally important elements of 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 CO_2-induced responses cannot be explained only 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 eCO_2 has a significant effect on wheat grain stoichiometry, with implications for human nutrition in a world of rising CO_2.

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

1. Introduction

The atmospheric concentration of carbon dioxide (CO_2) has steadily increased since the 19th century, from the pre-industrial level of 280 ppm to the current level of 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.

The effects of elevated CO_2 (eCO_2) 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 eCO_2 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-CO_2-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 eCO_2 are according to this argument consequently a result of improved water use efficiency. Comparing the eCO_2 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 eCO_2 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 eCO_2 [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], grain N content could also be affected under eCO_2 by this mechanism.

Changes in crop quality, like nutritional aspects, are of great importance for assessments of climate change and eCO_2 effects on future food production [15]. Loladze [16] pointed out that eCO_2 is likely to induce a shift in the stoichiometry, i.e., the elemental balance of plants, promoting higher concentrations of C and lower concentrations of e.g., N, Fe, and Zn, with important implications for human nutrition. The average effect on protein (hereafter referred to as N) content, estimated in a meta-analysis by Taub et al. [17], 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 eCO_2, such as a reduction in transpiration driven mass flow [18] and impaired N acquisition [19], processes that both can result in a reduced N uptake under eCO_2 even without yield stimulation. According to the mechanism put forward by Bloom [19], the decrease in photorespiration under eCO_2 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 (NO_3^-) to nitrite (NO_2^-), which is the first step of plant NO_3^- assimilation. In line with this, Pleijel and Uddling [20] found that the dilution hypothesis is likely to exist, but cannot fully explain the reduction in N concentration in wheat under eCO_2, 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 CO_2 where the effect of eCO_2 on grain N concentration saturates, analogous to the saturation seen in the response of photosynthesis under eCO_2 of C3 plants [21].

The effects on N content in wheat grains have been observed in a rather large number of studies with wheat grown under eCO_2, while observations of effects on other elements are limited. As suggested by Loladze [16], the decrease in concentrations of some essential mineral nutrients (Fe and Zn) have been documented [22,23], while it is still uncertain to what extent other elements are affected by eCO_2 and the mechanism behind the 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. [24] estimated that the CO_2-induced reduction in Zn concentration in staple crops could substantially increase the number of people at risk of Zn deficiency by 138 million by 2050. Cereals, including wheat, are also an important source of dietary Cadmium (Cd) exposure [25], which could cause injury to kidney and bones [26], hence the CO_2 effect on Cd content is also of importance.

N is often considered to be one of the most limiting elements for crop growth, and thus have the potential to be primarily affected by eCO_2. If there are common mechanisms behind eCO_2 effects on N and other elements it should be possible to detect the correlation of effects. Assuming that dilution is the main process that acts to reduce mineral concentration, the eCO_2 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 eCO_2, where alteration in quality may affect the market value and quality of products (e.g., review by Högy et al. [27]). 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 eCO_2 following the pattern of grain N concentration. Negative effects on various baking properties have been observed in individual experiments [28–32], but to our knowledge no meta-analysis has been made on this aspect.

This study intends to provide an up-to–date review of observed effects of eCO_2 on wheat grain quality, based on all available ecologically realistic experiments. Meta-analysis is used to test the overall magnitude and statistical significance of the effects. There is, however, also a need to understand the gradual change in the dietary value of wheat crops as CO_2 concentration increases. To meet this need, a novel aspect of our study is that we provide response functions for the effects of eCO_2 on the concentration of N and other minerals in wheat and test to what extent the data suggest responses to be linear or non-linear. We also assess the eCO_2 impact on the total production of starch, N, and other nutritionally important elements, by estimating the eCO_2 effect on the content (mass per unit area) of those constituents. As a further novel contribution, we relate the effect of eCO_2 on the concentration of a range of minerals to the eCO_2 effect on N concentration and grain yield. This is done 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 grain yield stimulation. By these three approaches our study aims to examine the following research questions:

1. Are the negative effects of eCO_2 on N concentration and N content independent of the experimental setup, such as exposure system, rooting environment, and concentration level of CO_2 treatment?
2. Is the negative effect of eCO_2 on N concentration saturating at high CO_2?
3. To what extent are the nutritional and baking quality of wheat grain negatively affected by eCO_2?
4. Can starch dilution explain the reduction in concentration of N and minerals under eCO_2?
5. Are effects of eCO_2 on mineral concentration 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 eCO_2 with an overall effect of −8.4% (confidence interval (CI) −9.8 −7.4; Figure 1a). The magnitude of effect was shown to be dependent on the experimental setup where significant differences were observed between exposure systems (FACE < OTC) and the rooting environment (pots > field soil). There was, however, no significant difference between OTC and FACE when excluding eCO_2 treatments >600 ppm (only OTC experiments). A comparison of concentration levels (above or below 600 ppm) in OTC experiments did not show any significant difference, but indicated a larger effect with higher CO_2. Even though N concentration was reduced by eCO_2 there was a significant increase in N content, 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 CO_2 effect on N content; 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 content when comparing OTC with FACE or different CO_2 concentrations.

The response function for the relationship between N concentration and CO_2 (Figure 1b) showed a strong non-linear relationship (r^2 = 0.57), with an initial reduction in N concentration with increasing CO_2, but reaching a minimum at ~600 ppm. N content ($g \cdot m^{-2}$) was positively affected by eCO_2, but showed a rather weak relationship with CO_2 (r^2 = 0.19). Details of the regression models are presented in Table 1.

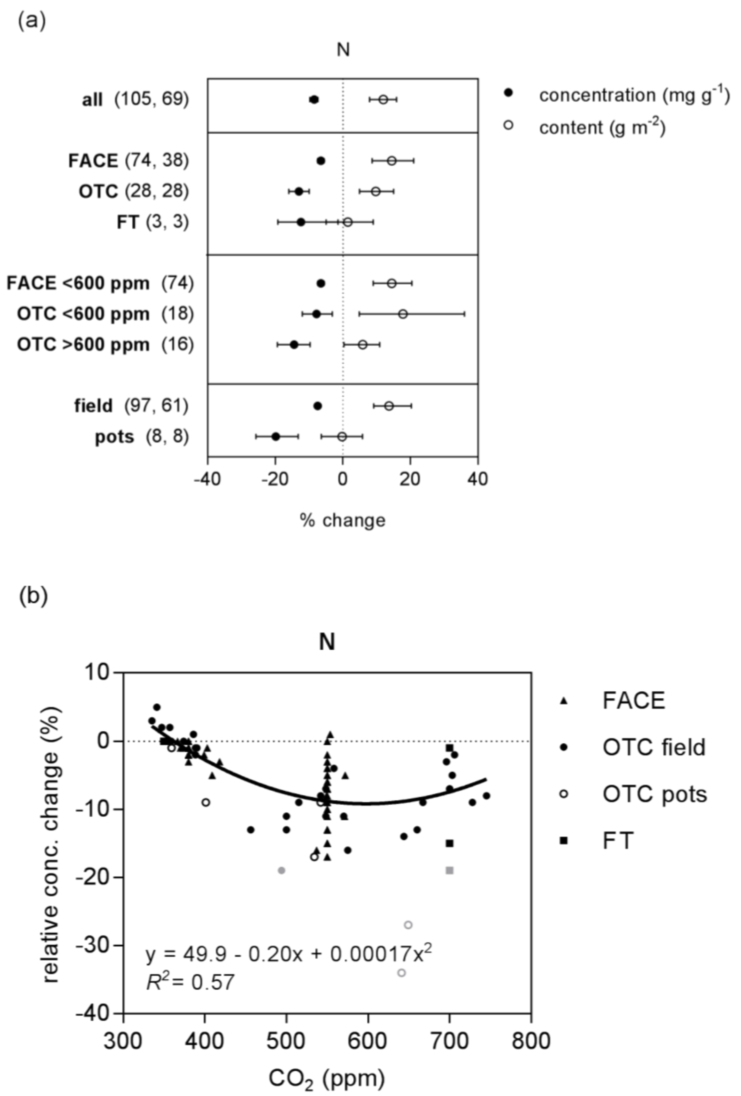

Figure 1. (**a**) Meta-analysis of eCO_2 effects on N concentration and N content using ambient CO_2 as the reference, with subgroup-analysis of exposure systems, rooting environment, and concentration level for eCO_2 treatment. Number of comparisons for concentration and content, respectively, are given within brackets. (**b**) Response function for N concentration (relative to 350 ppm) with CO_2 concentration, grey markers show data points identified as outliers not included in the curve fitting.

Table 1. Response functions for regression of concentration ($mg \cdot g^{-1}$) and content ($g \cdot m^{-2}$) of N, starch, and minerals with CO_2.

Variable		Observations	Regression Model	B0	B1	B2	r^2	Sign.	Preferred Model
N	concentration	132 (4)	linear	9.9	−0.031		0.43	*	
			quadratic	49.9	−0.198	1.66×10^{-4}	0.57		preferred
	content	96 (11)	linear	−5.7	0.025		0.12	*	
			quadratic	−51.9	0.216	-1.85×10^{-4}	0.18		preferred
starch	concentration	30 (3)	linear	0.4	0.001		0.00083	ns	preferred
			quadratic	10.3	−0.039	3.72×10^{-5}	0.028		
	content	30 (2)	linear	−14.3	0.052		0.35	*	preferred
			quadratic	−7.7	0.026	2.46×10^{-5}	0.35		

Table 1. *Cont.*

Variable		Observations	Regression Model	B0	B1	B2	r^2	Sign.	Preferred Model
B	concentration	68 (2)	linear	0.9	−0.002		0.00046	ns	preferred
			quadratic						n.a.
	content	32 (4)	linear	−66.4	0.196		0.40	*	preferred
			quadratic						n.a.
Ca	concentration	83 (4)	linear	12.9	−0.037		0.32	*	preferred
			quadratic						n.a.
	content	47 (7)	linear	−16.1	0.056		0.16	*	preferred
			quadratic						n.a.
Cd	concentration	13	linear	12.4	−0.039		0.31	*	preferred
			quadratic	64.0	−0.253	2.07×10^{-4}	0.39		
	content	13	linear	−1.4	0.003		0.0025	ns	preferred
			quadratic	10.3	−0.045	4.71×10^{-5}	0.0068		
Cu	concentration	80 (2)	linear	7.3	−0.020		0.14	*	preferred
			quadratic						n.a.
	content	44 (5)	linear	−33.3	0.104		0.27	*	preferred
			quadratic						n.a.
Fe	concentration	86 (7)	linear	13.7	−0.039		0.51	*	preferred
			quadratic						n.a.
	content	50 (4)	linear	−12.3	0.047		0.07	ns	
			quadratic	−211.7	0.911	-9.00×10^{-4}	0.17		preferred
K	concentration	83 (7)	linear	3.2	−0.008		0.07	*	preferred
			quadratic						n.a.
	content	47 (7)	linear	−37.6	0.116		0.51	*	preferred
			quadratic						n.a.
Mg	concentration	83 (8)	linear	11.7	−0.033		0.61	*	preferred
			quadratic						n.a.
	content	47 (3)	linear	8.2	−0.024		0.39	*	preferred
			quadratic						n.a.
Mn	concentration	84 (3)	linear	6.4	−0.019		0.13	*	
			quadratic	58.9	−0.247	2.39×10^{-4}	0.20		preferred
	content	48 (8)	linear	−20.1	0.067		0.36	*	preferred
			quadratic	−52.3	0.205	-1.41×10^{-4}	0.36		
P	concentration	83 (4)	linear	7.3	−0.022		0.20	*	preferred
			quadratic						n.a.
	content	47 (7)	linear	−27.9	0.018		0.38	*	preferred
			quadratic						n.a.
S	concentration	83 (3)	linear	9.9	−0.028		0.32	*	preferred
			quadratic						n.a.
	content	47 (7)	linear	−19.9	0.065		0.20	*	preferred
			quadratic						n.a.
Zn	concentration	90 (5)	linear	11.3	−0.033		0.18	*	preferred
			quadratic	51.1	−0.205	1.78×10^{-4}	0.21		
	content	54 (6)	linear	−18.0	0.062		0.28	*	
			quadratic	−143.7	0.596	-5.42×10^{-4}	0.43		preferred

Model parameters are presented for both linear (y = B1x + B0) and quadratic (y = B2x^2 + B1x + B0) curve fits, x being the CO_2 concentration and y the response variable. Values within brackets are the number of data points identified as outliers that were excluded from regressions. Sign: * denotes that the slope of linear model (B1) is significantly ($p \leq 0.05$) different from zero. Model fit is compared for each variable and the simpler (linear) model is preferred unless the p-value of the quadratic term is less than 0.05. ns denotes non-significant; n.a. denotes not applicable.

Figure 2 shows the eCO_2 effect on various baking properties, where a significant negative effect is observed for the 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 eCO_2, while resistance breakdown remained unaffected (−2.6%, CI −12.5 9.0).

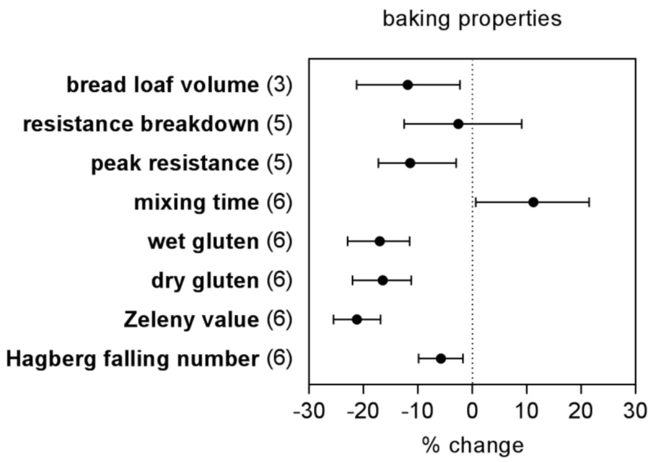

Figure 2. Meta-analysis showing the effect of eCO_2 on various baking properties using ambient CO_2 as the reference. Number of comparisons are given within brackets.

Meta-analysis for the eCO_2 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 CO_2 did not reveal any relationship (Figure 3b). Starch content 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 content.

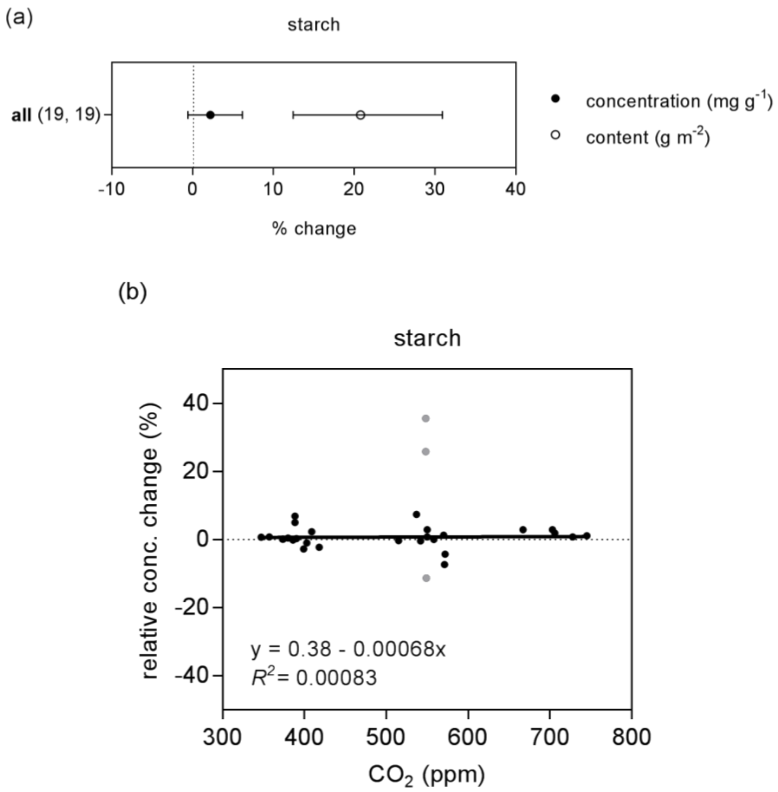

Figure 3. (**a**) Meta-analysis of eCO_2 effects on starch concentration and content using ambient CO_2 as a reference. Number of comparisons for concentration and content, respectively, are given within brackets. (**b**) Response function for starch concentration (relative to 350 ppm) with CO_2. Grey markers show data points identified as outliers and that were not included in the curve fitting.

2.2. Minerals

Meta-analysis (Figure 4) showed that eCO_2 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 content 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 content) among the different elements.

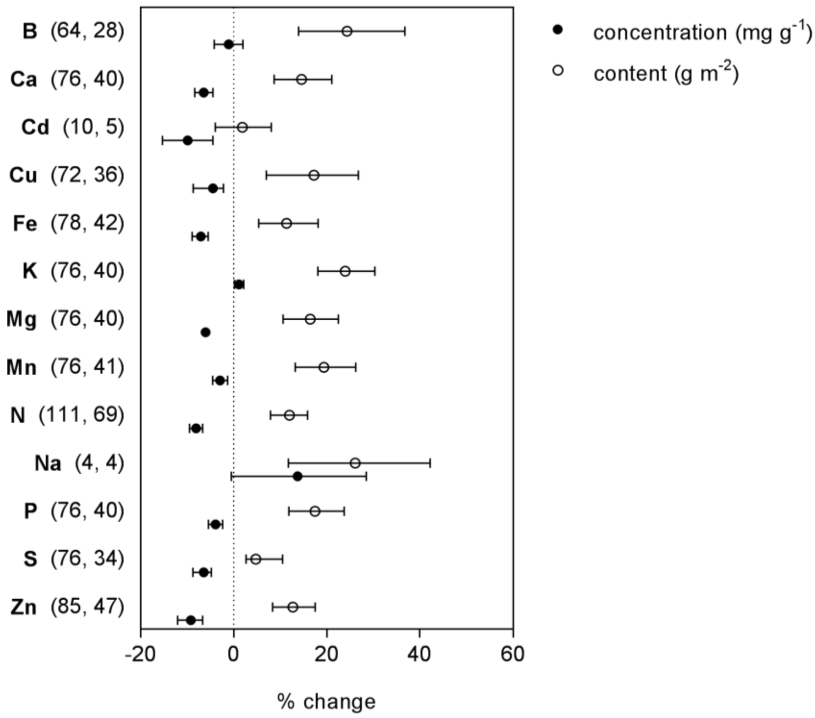

Figure 4. Meta-analysis output for mineral concentration and content using ambient CO_2 as the reference. Numbers within brackets gives the number of comparisons for the concentration and content, respectively.

Response functions in Figure 5 show that concentrations of several mineral nutrients had a strong linear relationship with increasing CO_2, with a significant negative slope for all elements (Fe, Mg, P, S, and Zn) except K. Regression models for the remaining elements are presented in Table 1. Concentrations of Ca, Cd, and Cu also showed a significant linear decrease with higher CO_2, however, a quadratic model had a better fit for Mn, while B did not show any relationship with CO_2. 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 content showed a positive relationship with CO_2 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 r^2 between 0.40 and 0.68.

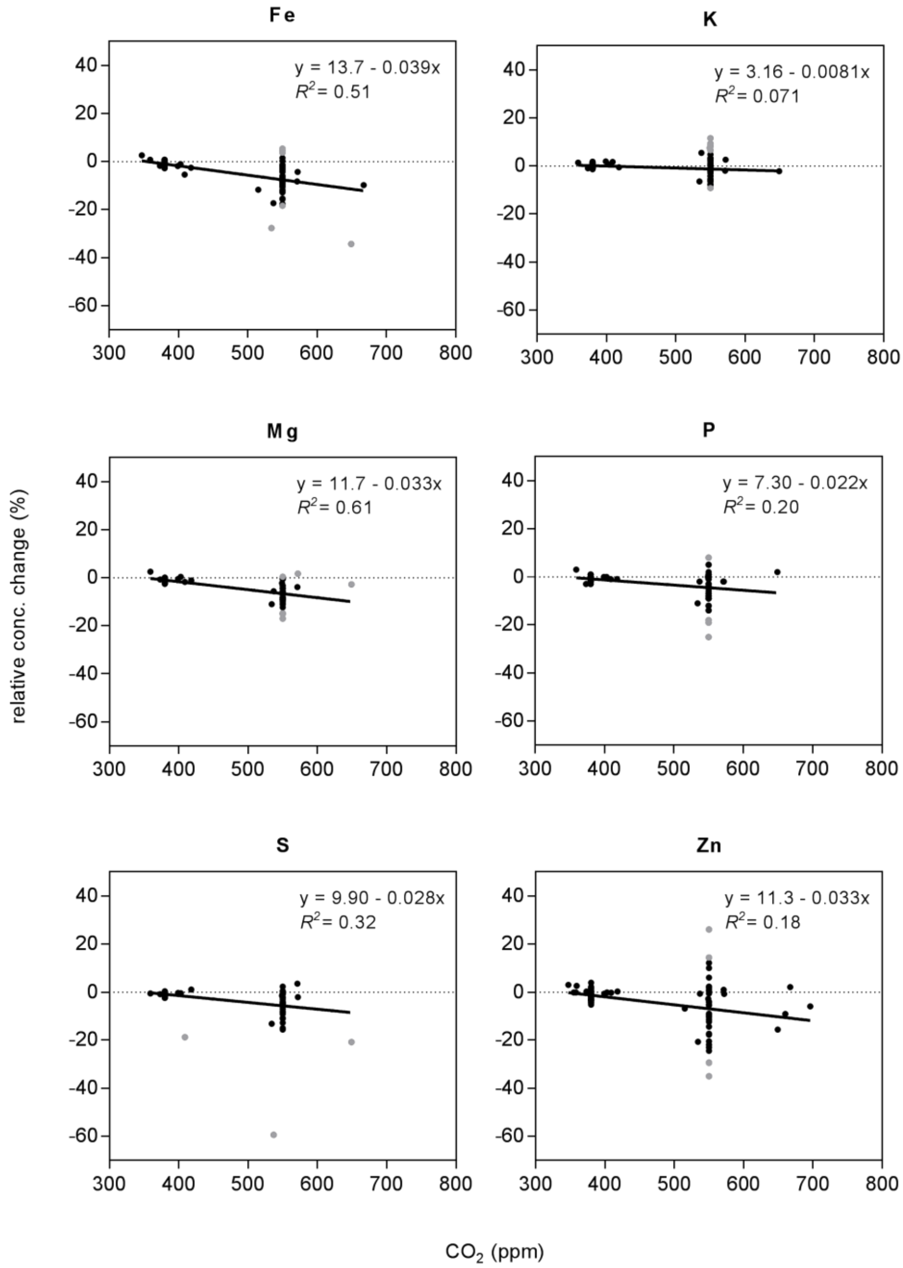

Figure 5. Response-functions for mineral concentrations of P, Mg, Fe, K, Zn, and S (relative to 350 ppm) with CO_2 concentration. Grey markers show data points identified as outliers and not included in the curve fitting.

2.3. Effects on Minerals in Relation to the Effects on N Concentration and Grain Yield

Figure 6 shows the relationship between eCO_2 effects on the concentration of various minerals and the eCO_2 effect on the 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 moderate association ($0.5 < r < 0.75$), while it was rather weak for the remaining elements (B, Cu, K, and Mn).

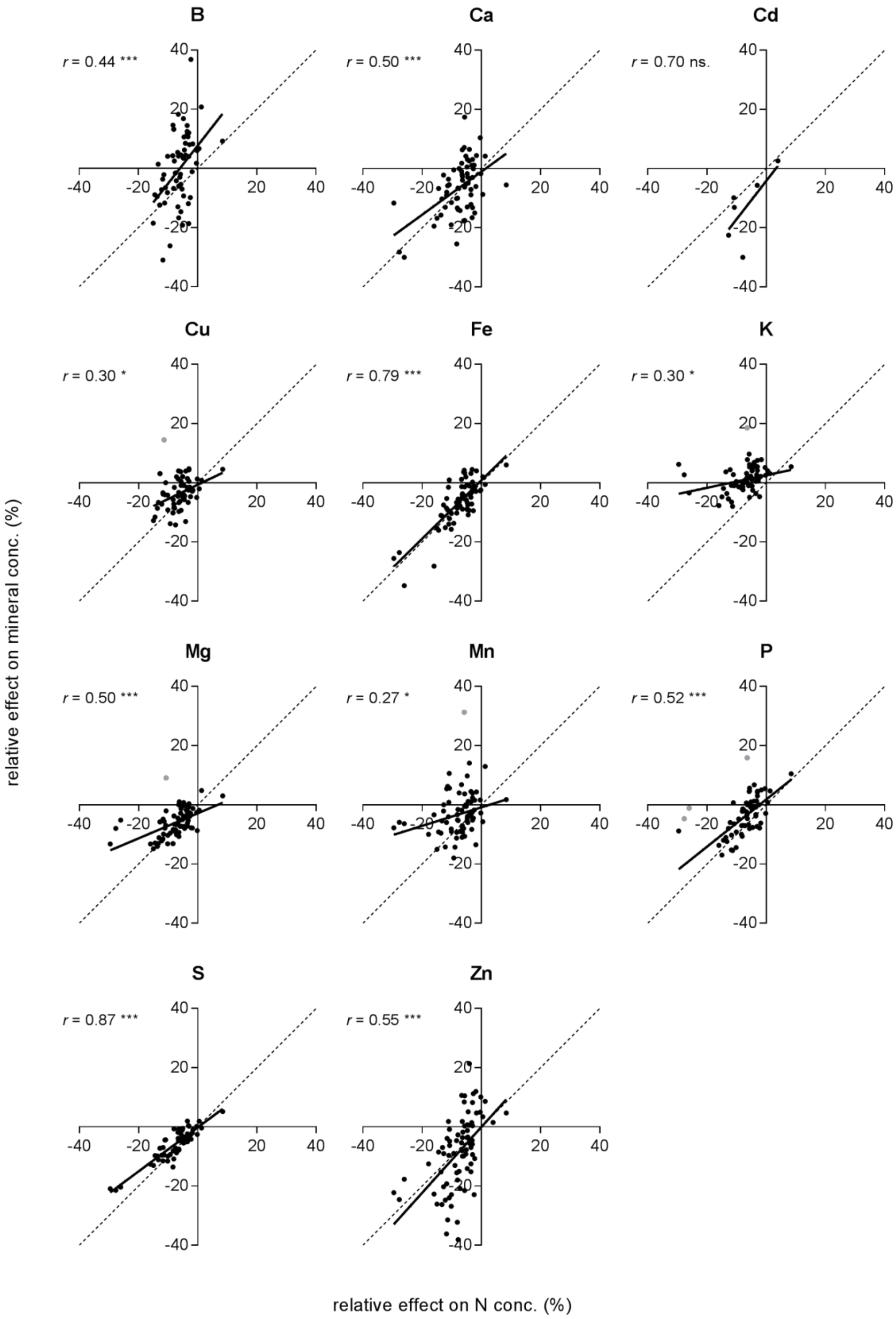

Figure 6. Relative effect of eCO_2 on mineral concentration (B, Ca, Cd, Cu, Fe, K, Mg, Mn, P, S, and Zn) related to the relative effect on N concentration. Correlation coefficient (r) and its significance is presented in each plot. Black solid lines represent the linear regression model, for which parameters and model performance are presented in Table 2. Grey markers show data points identified as outliers not included in the curve fitting. Dashed lines represent the hypothetical situation where the effect of eCO_2 on mineral concentration is equal to the effect on N concentration.

Regression analysis of the eCO_2 effect on mineral concentrations with the CO_2 effect on the N concentration (Table 2) showed a strong relationship for S (R^2 = 0.75) and Fe (R^2 = 0.63), while the relationships were rather weak for B, Cu, K, and Mn (R^2 < 0.25). Remaining elements were found in the intermediate range (0.25 < R^2 < 0.50). A deviation of the fitted line from the 1:1 line indicates that the element:N ratio was affected by eCO_2, hence the grain stoichiometry was altered. Cu, K, Mg, Mn, and S had a slope smaller and significantly different from 1 (Table 2), while the remaining elements did not having regression slopes significantly different from the 1:1 line. Relating the eCO_2 effect on minerals to the effect on grain yield showed a weak and non-significant relationship for most elements (Table 2), except for the concentrations of K, N, P, and Zn that had significantly negative relationships with the effects on grain yield (Figure 7).

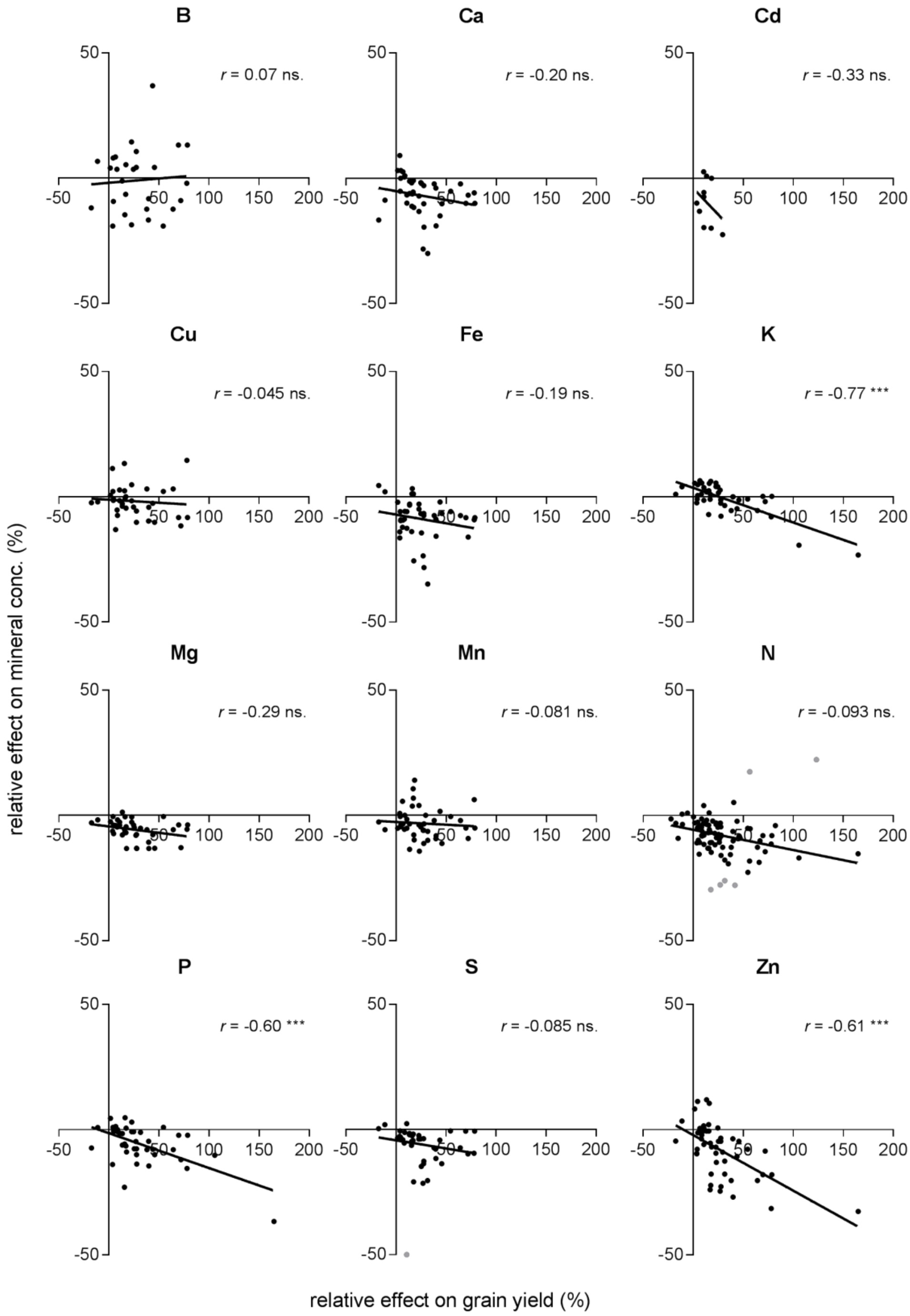

Figure 7. Relative effect of eCO_2 on the mineral concentration of B, Ca, Cd, Cu, Fe, K, Mg, Mn, N, P, S, and Zn vs. the relative effect on grain yield ($g{\cdot}m^{-2}$). Black solid lines represent the linear regression model, for which parameters and model performance are presented in Table 2.

Table 2. Response functions for the linear regression between the relative eCO_2 effect on the concentration of various minerals with the eCO_2 effect on N concentration and grain yield.

x	Element	Observations	r^2	B0	B1	Sign.
N	B	64	0.20	7.65	1.28	ns.
	Ca	69	0.46	1.80	0.80	ns.
	Cd	6	0.49	−4.17	1.29	ns.
	Cu	65 (1)	0.17	−0.66	0.48	*
	Fe	70	0.63	0.80	0.99	ns.
	K	69 (1)	0.11	2.57	0.21	*
	Mg	76	0.32	−2.86	0.42	*
	Mn	74 (1)	0.084	−0.84	0.31	*
	P	69 (3)	0.46	1.80	0.80	ns.
	S	68	0.75	−0.18	0.74	*
	Zn	83	0.30	−0.084	1.11	ns.
Grain yield	B	28	0.0049	−1.87	0.034	ns.
	Ca	40	0.042	−5.10	−0.070	ns.
	Cd	10	0.11	−3.90	−0.42	ns.
	Cu	36 (1)	0.0021	−1.18	−0.24	ns.
	Fe	42	0.038	−7.08	−0.069	ns.
	K	42	0.59	3.61	−0.14	*
	Mg	40	0.087	−4.42	−0.51	ns.
	Mn	43	0.0065	−2.63	−0.022	ns.
	P	42	0.36	−1.38	−0.14	*
	S	40 (1)	0.063	−4.26	−0.065	ns.
	Zn	50	0.37	−21.1	−0.22	*
	N	87 (6)	0.18	−5.70	−0.081	*

Values within brackets are the number of data points identified as outliers that have been excluded from the regressions. B0 gives the intercept and B1 gives the slope of regression line. Sign.: * denotes that slope (B1) is significantly ($p \leq 0.05$) different from 1, when x = effect on N, and significantly different from zero when x = effect on grain yield. ns, denotes non-significant.

3. Discussion

The overall results from this study suggest that eCO_2 can cause an overall significant shift in wheat grain stoichiometry, with concentration reductions for N and several nutritionally important minerals, in line with the conclusion of Loladze [12,16], together with a decreased baking quality and thus lower commodity value. This is the most comprehensive synthesis of eCO_2 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 showed a significant negative effect of eCO_2 on N concentration regardless of experimental setup. The negative effect of eCO_2 on N concentration observed in some recent studies was estimated to be between 6.3% [23] and 9.8% [17], 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 the sources of variation within the data.

The response function for the relationship between N concentration and CO_2 indicates that there is a gradual reduction in N concentration that saturates around 600 ppm. This has not been highlighted before and is of importance e.g., for scenarios of how the nutritional value in crops will gradually change over the present century in response to rising CO_2. The meta-analysis, however, points to a stronger response in experiments using an eCO_2 level above 600 ppm compared to that 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 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 eCO_2 550 ppm and 528 ppm, respectively).

The comparison of the rooting environments 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. [17] 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 eight 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 [33]. In addition, the falling number was reduced under eCO_2 reflecting an increase in α-amylase activity, which is associated with poor baking properties, such as sticky dough and poorly structured loaves [34], but also shortens the storage time of flour and grains [35].

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 dilution 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. Even with a small effect size, a dilution effect by starch is likely to be of importance for all elements although other factors, such as transpiration-driven mass flow, N acquisition, and variation in plant demand-to-availability, can modify and even overshadow the dilution effect [12]. Further investigations would be needed to outline the relative importance of different mechanisms for different elements and growing conditions.

The analysis of the eCO_2 effect on mineral concentrations (Figures 4, 6 and 7) showed that there was a variation in the magnitude of effects, ranging from effects close to zero to reductions of about 10%. Together with the non-significant effect on starch concentration, this indicates that CO_2-induced responses cannot be explained only 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 strongly correlated 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 the 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 [36] stoichiometric clusters of mineral elements were identified in the 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 corresponding relationships were 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 eCO_2 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 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. [37] showed that the translocation of Fe, Zn, and N from vegetative tissues to grain is partly regulated by the same proteins in wheat plants. eCO_2 could possibly affect translocation rates indirectly through higher leaf temperatures due to lower transpiration rates [38]. Increase in leaf temperature can lead to heat stress, which is known to promote senescence [39], and thus shorten the grain filling period [40]. This is, however, likely to increase the concentrations of minerals since starch accumulation is often more strongly reduced than N and minerals [39]. If the rate and efficiency of translocation were strongly affected by eCO_2, Fe, Zn, and N could be expected to follow the same response pattern. Our results (Figure 6) show a strong correlation between eCO_2 effects on Fe and N ($r = 0.79$), while the relationship is moderately strong for Zn and N ($r = 0.55$), suggesting that additional mechanisms are of importance in terms of wheat grain concentrations for Zn.

In line with other minerals, Cd concentration was significantly reduced under eCO_2, which could be considered a positive effect due to the toxicity of Cd. A reduction in Cd concentration was also observed for wheat grown under CO_2 enrichment [20] and ozone exposure [41]. Cd is a non-essential element for the plant and the uptake is known to be dependent on transpiration driven mass-flow [42], therefore lower concentrations could be expected since transpiration rates are often reduced under both eCO_2 and high ozone [43].

The content of N (Figure 1) and all minerals, except for Cd (Figure 4), were significantly increased under eCO_2, 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 the leaching of nutrients and enhanced emissions of nitrous oxide (N_2O), ammonia (NH_3), and nitrogen oxide (NO).

In order to fully understand mechanisms behind the shift in wheat grain composition, further research is needed. The response of nutrient concentration under eCO_2 has to be tested under different levels of fertilizers and water supply to identify possible interaction of these factors, which has been done for N in a few experiments (e.g., Li et al. [44]) but not for other nutrients. It would also be possible to follow translocation rates of elements from straw and leaves, by measuring element composition of all plant parts during growth. Simultaneous measurements of transpiration could test the strength of the link between eCO_2 effects on different minerals in crop yield and transpiration-driven mass flow.

eCO_2-induced reductions in the concentration of N, as a proxy for protein, and essential minerals can have significant impacts on human nutrition. Fe and Zn deficiency is already an urgent issue in many parts of the world. An estimated two billion people suffer from these deficiencies [45], especially in regions where people depend on C3 grains such as wheat as their primary dietary source of Zn and Fe. Consequently, these factors are also important to take into account when assessing the effects of CO_2 and climate change on global food security.

4. Materials and Methods

4.1. Database

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 eCO_2. 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, 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 [41]. Data sources for the included experiments are presented in the Supplementary Information (Tables S1 and S2).

Data from figures were extracted using the software GetData Graphic Digitizer [46]. For experiments where the ambient CO_2 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 the National Oceanic & Atmospheric Administration (NOAA) [47].

4.2. Meta-Analysis

Meta-analysis was performed using a meta-analytical software package MetaWin [48]. The experimental treatment with ambient CO_2 was used as control, and parameter values were considered independent if they were made on different cultivars, different (CO_2), or different years, in line with previous meta-analysis [43,49]. 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 [43,48,50]. All variables were analyzed using an un-weighted approach due to lack of data for the computation of sample variance (standard deviation or standard error with degree of replication). In line with previous meta-analyses [41,48], 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 [49].

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 eCO_2 was tested in the meta-analysis, and interactions of eCO_2 and additional treatments were not further examined. Subgroup analysis was performed for the N concentration and N content, for which a substantial amount of data was available, where data was categorized by (1) exposure system, Free-Air-CO_2-Enrichment (FACE), Open-Top-Chamber (OTC), and Field Tunnel (FT), (2) rooting environment, pots or field soil, and (3) the concentration level of the eCO_2 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 CO_2 concentration 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 0 on a relative scale. Both a linear (first order polynomial) and quadratic (second order polynomial) model was fitted to the data, and the simpler model was preferred if the second parameter (in quadratic model) did not significantly improve the model fit. 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 eCO_2. All response functions were derived using automatic outlier removal [51].

4.4. Comparison of CO_2 Effects on Different Response Variables

The eCO_2 effect was related to the control treatment (ambient CO_2) when relating the effects on minerals to the 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. The deviation from the 1:1 line was tested for each regression model. For regressions between the eCO_2 effect on minerals and the effect on grain yield, it was tested if the slope deviated from zero, where a slope close to zero indicates a poor relationship between the effects on mineral concentrations with grain yield stimulation.

5. Conclusions

Our study, based on an extensive database, shows that eCO_2 has significant negative effects on the concentration of several minerals and N (as a proxy for protein) 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 eCO_2 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 element concentration. However, for most of the minerals the eCO_2 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 eCO_2 effects on S and Fe, elements that are important components of proteins, and fairly strong also for P. The response functions and relationships between different elements and N presented in this study show a gradual change in nutritional quality and can be used in risk assessments of the effects on nutrition in a future high CO_2 world.

Supplementary Materials: The following are available online at www.mdpi.com/2073-4395/7/4/32/s1, 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.C.B. and H.P. conceived and designed the study; data collection was performed by M.C.B. in close collaboration with P.H.; all authors participated in the analysis of the data; M.C.B. wrote the paper with substantial input from P.H. and H.P.

Conflicts of Interest: The authors declare no conflict of interest.

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