# Estimating the Effects of Carbon Dioxide, Temperature and Nitrogen on Grain Protein and Grain Yield Using Meta-Analysis

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### Abstract

As meta-analysis is an effective tool for assisting decision-makers, there has been a recent increase in demand for its use to solve controversies regarding important human life issues. Meta-analysis allows a thematic appraisal of evidence, which can lead to a resolution of suspicions and disagreements. Carbon dioxide, temperature, and nitrogen are considered as the most important factors influencing crop production. These environmental variables significantly affect grain yield and grain protein concentrations, which are key determinants of grain quality. Consequently, they affect human and animal nutrition. A more detailed understanding of how these environmental factors contribute towards the grain protein content is essential for addressing global nutrient security in the changing climate. To our knowledge, there have been no studies conducted to assess the effect of CO<sub>2</sub>, temperature and nitrogen supply on grain protein and grain yield using meta-analysis. In addition, performance evaluations were mainly conducted in previous studies through traditional statistical measures, and only the combined effect of CO<sub>2</sub>, temperature and nitrogen on grain protein and grain yield were analysed. Therefore, this study focuses on estimating the effects of CO<sub>2</sub>, temperature and nitrogen on grain protein and grain yield using meta-analysis. In this work, a new approach based on the *dplyr* package in R is proposed for organizing and categorizing the research data for meta-analysis. The performances of the proposed methods are evaluated using various measurements, such as the Cochran's Q statistic and its p-value,  $I^2$  statistic, and  $\tau^2$  tau-squared. Overall, the aim of this study was to reveal the significance and reliability of a meta-analysis in analysing the effects of carbon dioxide, temperature and nitrogen on the quality of agricultural crops. The results indicated that the protein concentration was decreased by 0.62% and grain yield was increased by 0.52% under elevated carbon dioxide, ambient temperature and low nitrogen. In contrast, protein concentration was reduced by 0.65% and grain yield was increased by 0.78% under the elevated carbon dioxide, ambient temperature and medium nitrogen. We concluded that meta-analysis can be used to study the effects of CO<sub>2</sub>, temperature and nitrogen on grain protein concentration and grain yield. The outcomes of this project will inform experts and decision-makers about the effects of CO<sub>2</sub>, temperature and nitrogen on grain quality, and enable the investigation of suitable solutions.

Keywords: Meta-analysis, *dplyr* package, grain protein, grain yield.

# **1. Introduction**

Meta-analysis is widely used to assist decision-makers in establishing crucial decisions in various application fields, such as in medical and social research (Jones et al., 2000). As a result, there has been a recent increase in

demand for the use of meta-analysis to solve controversy regarding important human life issues. Meta-analysis allows for thematic appraisal of evidence, which may lead to the resolution of suspicion and disagreement (Normand, 1999). There have been considerable publications investigating the avail and robustness of metaanalysis in biological research (Haworth et al., 2016, Humbert et al., 2016, Niu and Yu, 2016, Zhou et al., 2016, Baig et al., 2015, Doi et al., 2015 and Sutton et al., 2005). Meta-analysis is a statistical method or a set of statistical methods for combining results from various studies into a pooled estimate of the effect size (Schmidt and Hunter, 2014). In meta-analysis, the effect size is measured depending on the species of outcome variables. There are two kinds of outcome variables, binary outcomes and quantitative results. The binary outcome variables include odds ratios, risk ratios and risk differences, while the quantitative outcome variables are standardized mean differences (SMD), weighted mean differences (WMD) and correlations coefficients (Borenstein et al., 2009). A fixed effect model postulates that there is one true effect size for all the studies (Borenstein et al., 2009). This means that all the studies included in the meta-analysis estimated the same effect size. The combined effect size was then estimated based on these studies. Random effect models presume that the true effect could diverge from study to study. Based on this assumption, a different effect size is estimated in each study, with the assumption that there is a distribution of the true effect sizes. Under the random effects model, the mean of the distribution is estimated by pooling the effect size of the studies (Cumming, 2013). Metaanalysis uses the weighted mean of the effect sizes rather than the simple arithmetic mean. In a fixed effect model, the weights are allocated depending on the inverse of the variance. This means that each study is weighted by the inverse of its variance and the variance here is the within-studies variance. The inverse variance approach is used to diminish the variance of the combined effect (Jones et al., 2000). In a random effects model, the inverse of variance weights is also used. This means that the effect size of each study is also weighted by the inverse of its variances. However, the variances here are both the within-studies variation and the betweenstudies variation. It is well known that the concentration of carbon dioxide  $(CO_2)$  in the Earth's atmosphere has risen over the years (nasa.gov). This increase in atmospheric CO<sub>2</sub> levels has resulted in an increase in crop productivity (Ward, 2007), while substantially decreasing grain quality of cereals and pulses. This has consequently compromised human health (Myers et al., 2014). Many studies shed light on the effects of  $CO_2$  on agricultural crops (Fitzgerald et al., 2016, Dietterich et al., 2015, Buchner et al., 2015) but little attention is paid to key environmental variable such as temperature and soil nitrogen availability. For example, temperature often determines the lengths and types of vegetative growths. Therefore, this could influence crop yield and quality (Liang et al., 2016). Another important factor that determines crop yield and quality production is nitrogen (Njoroge et al., 2014). There is a rather large benefit from nitrogen in most crops. However, over-fertilisation with nitrogen is an issue (Njoroge et al., 2014). There is a strong evidence that elevated CO<sub>2</sub> levels interact with temperature and nitrogen, which affect the quality of crops by decreasing the protein concentration in the grain. This subsequently affects the nutritional value of the grain which directly impacts human nutrition (Challinor et al., 2016). In recent years, many publications were reported to analyse the effects of CO<sub>2</sub>, temperature and nitrogen on crops using various methods. Of those, statistical methods were found to be an important approach to study the influences of environmental factors on crops, and to investigate fundamental issues concerning nutrients (Pan et al., 2016). However, the performance evaluations were mainly conducted through traditional statistical measures, for instance, ANOVA (analysis of Variance), t-test,  $\chi^2$  (chi-square), R2 (the coefficient of determination) (Pleijel and Uddling, 2012, Erbs et al., 2015, Sanchez et al., 2014, Wu et al., 2016, Zhang et al., 2016, Valizadeh et al., 2014, Asseng et al., 2015, Tack et al., 2015, Lv et al., 2013, Lobell et al., 2012, Garcia et al., 2015, Cai et al., 2016, Rodrigues et al., 2016, Liu et al., 2014, Panozzo et al., 2014, Fernando et al., 2014, Fernando et al., 2015). These traditional methods have a limitation in analyzing the data, as they depends on individual studies (experiments). Individual studies are not reliable enough to detect significant differences between two treatments or more. In order to overcome this limitation, many researchers found meta-analysis to be a powerful tool in investigating homogeneities among the studies being conducted (Lam et al., 2013, Jablonski et al., 2002). In this research, we will use meta-analysis and other statistical techniques to determine the effect of CO<sub>2</sub>, temperature and nitrogen on grain protein and grain yield. Generalizing the results from a meta-analysis rather than from single studies makes more sense, as it integrates different sets of populations into the analysis. To the knowledge of the authors, no previous studies were conducted to assess the effect of CO<sub>2</sub>, temperature and nitrogen supply on grain protein and grain yield using meta-analysis. In addition, the existing studies have been limited to analysing the effects of CO<sub>2</sub>, temperature and nitrogen on grain protein and grain yield. This study focuses on measuring the effects of CO<sub>2</sub>, temperature and nitrogen on grain protein and grain yield using meta-analysis. In addition, a new procedure based on *dplyr* package in R program will be developed to re-processing data in order to facilitate meta-analysis.

## 2. Materials and methods

#### Database

The dataset was obtained from the studies published in the publicly available *nature* website (Dietterich et al., 2015). It can be accessed on the URL of: http://www.nature.com/articles/sdata201536#data-records. In the dataset, researchers from several countries conducted a large-scale study on several agricultural crops. Data were collected from three countries: the USA, Australia and Japan, for six crops (wheat, soybean, sorghum, corn, rice and field peas) grown using free-air CO<sub>2</sub> (FACE) technology. The researchers conducted the studies under different conditions and various levels of CO<sub>2</sub>, nitrogen, water and temperature. They investigated their effects on nutritional elements, such as iron, zinc and protein of the crops. In this proposal, we focus on investigating grain protein and grain yield for wheat crops in Victoria, Australia under two levels of CO<sub>2</sub> (ambient and elevated), two different nitrogen levels (low and medium), and one temperature level (ambient). We used a procedure based on the dplyr package in R program (Wickham, 2011) to re-arrange the data from each individual study separately under certain conditions to make them suitable for the meta-analysis format. Conducting a meta-analysis requires a set of clear and consistent information about the individual studies, such as the study name, years, level of each factor and outcomes for each study. Therefore, we created a template that contained all the relevant information for this purpose. The aforementioned procedure was applied to the data to make them suitable for meta-analysis. We have built a dataset template containing the name of study, level of CO<sub>2</sub> level of temperature, level of nitrogen, name of crop, year, city, state, country, cultivar, sowing time and replicate.

#### Meta-analysis

Meta-analysis was carried out using the standardized mean difference (SMD) and the mean difference (MD) for the continuous outcome measures (mean and standard deviation). We applied a random effects model and a fixed effect model using the inverse variance weighted approach to combine the data (Memon et al., 2011). Cochran's Q Statistic, tau-squared and *I*-squared statistic were used to assess the heterogeneity among the studies (Memon et al., 2011). Forest plots were used to interpret the statistics. All the estimates were calculated using a computer software written in R, version 3.2.5 (2016), and all the plots were calculated using the "metafor", "meta", "nmeta" packages, URL <u>http://cran-project.org</u>. To test the hypothesis of the equality of effect sizes, the paper reports the values of the testing statistics and associated p-values for the various study variables.

#### Meta-analysis models

The fixed effect model is given by (Borenstein et al., 2009)

$$T_i = \mu + u_i.$$
 (1)  
where  $T_i$  is an observed effect in the study of  $i$ ,  $\mu$  is the common effect,  $u_i$  is the within-study error.

The weight assigned to each study is defined as:

$$w_i = \frac{1}{v_i},\tag{2}$$

where  $v_i$  is the within study variance for study *i*.

Then the weighted mean  $\overline{T}$ . can be computed as

$$\bar{T}. = \frac{\sum_{i=1}^{k} w_i}{\sum_{i=1}^{k} w_i},$$
(3)

The variance of the combined effect is defined as:

$$V. = \frac{1}{\sum_{i=1}^{k} w_i},$$
(4)

The standard error of the combined effect is

$$SE(\overline{T}.) = \sqrt{V.}$$
 (5)

The 95% confidence interval for the combined effect is computed as Lower Limit  $=\overline{T}.-1.96 * SE(\overline{T}.),$  (6) Upper Limit  $=\overline{T}.+1.96 * SE(\overline{T}.).$  (7)

The Z-value can be computed using

$$Z = \frac{\overline{T}}{SE(\overline{T}_{...})} \,. \tag{8}$$

For a one-tailed test, the *p*-value is given by

$$p = 1 - \varphi(|Z|),$$
For a two-tailed test by
$$p = 2[1 - (\varphi(|Z|))],$$
(10)

where  $\boldsymbol{\varphi}$  is the standard normal cumulative distribution function

The random effects model can be written as (Borenstein et al., 2009)

$$T_i = \theta_i + e_i = \mu + \varepsilon_i + e_i.$$
(11)  
where  $T_i$  is the observed effect in study  $i, \theta_i$  is the true effect,  $\varepsilon_i$  is the within-study error,  $\mu$  is the mean of

where  $T_i$  is the observed effect in study t,  $\sigma_i$  is the true effect,  $\varepsilon_i$  is the within-study error,  $\mu$  is the mean of all the true effects,  $e_i$  is the between study error.

The weight assigned to each study is

$$w_i^* = \frac{1}{v_i^*}$$
, (12)

where  $v_i^*$  is the within-study variance for study *i* plus the between-studies variance.

The weighted mean  $\overline{T}^*$  is then computed as

$$\bar{T}_{\cdot}^{*} = \frac{\sum_{i=1}^{k} w_{i}^{*} T_{i}}{\sum_{i=1}^{k} w_{i}^{*}},$$
(13)

The variance of the combined effect is defined as

$$V_{\cdot}^{*} = \frac{1}{\sum_{i=1}^{k} w_{i}^{*}},$$
(14)  
The standard error of the combined effect is

$$SE = (\overline{T}^*) = \sqrt{V^*}.$$
(15)

The 95% confidence interval for the combined effect can be computed as

Lower Limit<sup>\*</sup> =  $\overline{T}^* - 1.96^* SE(\overline{T}^*)$ , (16) Upper Limit<sup>\*</sup> =  $\overline{T}^* + 1.96^* SE(\overline{T}^*)$ . (17)

The Z-value could be computed using

$$Z^* = \frac{T^*_{.}}{SE(\bar{T}^*)} \,. \tag{18}$$

The one-tailed p-value is given by  

$$p^* = 1 - \varphi(|Z^*|),$$
(19)

The two-tailed *p*-value by  

$$p^* = 2[1 - \varphi(|Z^*|)],$$
(20)

where  $\varphi$ : the standard normal cumulative distribution function.

## 3. Results

The effect size in the fixed effect model, and the random effects model (p-value) illustrates that there is a significant difference between the two groups. The SMD and MD values indicate that the experimental group has a higher influence on protein concentration than the control group as it reduces the protein concentration in wheat by 0.62%. There was no significant heterogeneity found by the Cochran's Q, *I*-squared and tau-squared tests (Figure 1). The effect size (p-value) shows that there is a significant difference between the two groups. The (SMD, MD) values indicated that the protein concentration was negatively affected in the experimental group and was decreased by 0.65%. The Cochran's Q, *I*-squared and tau-squared tests did not show a significant heterogeneity (Figure 2). The effect size (p-value) of the fixed effect model and the random effects model indicated a significant difference between the two groups. The (SMD and MD) values showed that grain yield was increased by 0.52% for the experimental group. There was no significant heterogeneity found by the Cochran's Q, *I*-squared and tau-squared tests (Figure 3). The effect size (p-value) of fixed effect model and random effects model showed a significant difference was found between the two groups. SMD and MD values demonstrated that the grain yield was increased by 0.78% under the experimental group. No significant heterogeneity was found from the Cochran's Q, *I*-squared and tau-squared tests (Figure 4).

	Experim	ental	Control	Mean difference				
Study	Total Mean		an SD		MD	95%-CI	W(fixed)	W(random)
,				3			. ,	, ,
1	4 12.60036 0.995	51876 4 13.220	00 0.21	-+	-0.62	[-1.62; 0.38]	6.8%	7.2%
2	4 13.40594 0.272	20802 4 13.230	00 0.61	ŝ- <b> -</b> -	0.18	[-0.48; 0.83]	15.8%	12.5%
3	4 13.24437 1.163	80917 4 13.225	00 0.73		0.02	[-1.33; 1.37]	3.7%	4.4%
4	4 13.38329 0.221	0548 4 14.051	28 0.73		-0.67	[-1.42; 0.08]	12.1%	10.7%
5	4 13.11168 0.616	60089 4 13.505	41 0.78		-0.39	[-1.37; 0.58]	7.1%	7.4%
6	4 13.29646 0.513	33215 4 13.957	09 0.38		-0.66	[-1.29; -0.03]	17.3%	13.1%
7	4 13.85630 0.927	7306 4 14.240	21 0.79		-0.38	[-1.58; 0.81]	4.8%	5.4%
8	4 16.73299 0.430	00000 4 17.271	43 0.90	- <u>je</u> -	-0.54	[-1.52; 0.44]	7.1%	7.4%
9	4 13.91456 0.740	00000 4 16.308	27 1.72		-2.39	[-4.23; -0.56]	2.0%	2.6%
10	4 15.17872 1.010	00000 4 17.130	47 1.28		-1.95	[-3.55; -0.35]	2.7%	3.3%
11	4 14.03926 0.930	00000 4 15.998	27 2.07		-1.96	[-4.18; 0.26]	1.4%	1.8%
12	4 10.35009 0.290	00000 4 11.518	35 1.68		-1.17	[-2.84; 0.50]	2.4%	3.0%
13	4 15.08156 1.430	00000 4 14.987	36 1.28		0.09	[-1.79; 1.97]	1.9%	2.5%
14	4 14.33746 1.870	00000 4 14.780	56 1.57		-0.44	[-2.84; 1.95]	1.2%	1.6%
15	4 15.79752 1.880	00000 4 16.742	98 1.51		-0.95	[-3.31; 1.42]	1.2%	1.6%
16	4 15.45087 2.520	00000 4 16.839	52 1.02		-1.39	[-4.05; 1.28]	1.0%	1.3%
17	4 14.71268 1.400	00000 4 16.969	86 1.16		-2.26	[-4.04; -0.48]	2.1%	2.7%
18	4 15.07822 2.260	00000 4 15.636	33 0.79		-0.56	[-2.90; 1.79]	1.2%	1.6%
19	4 16.74789 2.550	00000 4 17.129	11 1.18		-0.38	[-3.13; 2.37]	0.9%	1.2%
20	4 15.98497 1.930	00000 4 17.104	37 1.10		-1.12	[-3.30; 1.06]	1.4%	1.9%
21	4 14.91187 1.770	00000 4 15.807	58 1.11		-0.90	[-2.94; 1.15]	1.6%	2.1%
22	4 13.21303 1.000	00000 4 15.432	24 0.84		-2.22	[-3.50; -0.94]	4.1%	4.8%
				3				
Fixed effect model	88	88		\$	-0.66	[-0.92; -0.40]	100%	
Random effects mode				<b></b>	-0.72	[-1.03; -0.42]		100%
Heterogeneity: I-squared=					_			
Q=25.64 , Test overall : p-val	ue = 0.0001 (Fixed model), p-	value = 0.0001 (Kandom						
				-4 -2 0 2	4			
				Standardised mean differe				
e	Experin			Standardised mean differe		0.5%		
Study	Total Mean	SD Total Me	an SD	1.1	SMD	95%-C	W(fixed)	W(random)
1	4 12.60036 0.99	51876 4 13.220	00 0 21		-0.75	[-2.23; 0.73]	4.7%	4.7%
2	4 13.40594 0.27				0.32	[-2.23, 0.73] [-1.08; 1.73]		5.2%
3	4 13.24437 1.16				0.02	[-1.37; 1.40]		5.3%
4	4 13.38329 0.22				-1.08	[-2.65; 0.49]	4.1%	4.1%
5	4 13.11168 0.61				-0.49	[-1.91; 0.94]	5.0%	5.0%
6	4 13.29646 0.51				-1.27	[-2.91; 0.37]	3.8%	3.8%
7	4 13.85630 0.92				-0.39	[-1.80; 1.02]		5.1%
8	4 16.73299 0.43				-0.66	[-2.12; 0.80]		4.8%
9	4 13.91456 0.74				-1.57	[-3.33; 0.19]		3.3%
10	4 15.17872 1.01				-1.47			3.5%
11	- 10.17072 1.01							
	4 14 03926 0 93	00000 4 15 998	327 2 07					
	4 14.03926 0.93 4 10.35009 0.29				-1.06	[-2.63; 0.50]		4.2% 4.5%
12	4 10.35009 0.29	00000 4 11.518	335 1.68		-0.84	[-2.34; 0.66]	4.5%	4.5%
		00000 4 11.518 00000 4 14.987	335 1.68 36 1.28					

4 15.79752 1.8800000 4 15.45087 2.520000 4 14.71268 1.400000 4 15.07822 2.260000 4 16.74789 2.5500000 4 15.98497 1.930000 4 14.91187 1.7700000 16.74298 1.51 16.83952 1.02 16.96986 1.16 15.63633 0.79 17.12911 1.18 17.10437 1.10 [-1.91; 0.94] [-2.08; 0.82] [-3.27; 0.21] [-1.69; 1.11] [-1.56; 1.22] [-2.07; 0.83] [-1.96; 0.91] [-4.09; -0.091] -0.48 -0.63 -1.53 -0.29 -0.17 -0.62 5.0% 4.8% 3.4% 5.2% 5.3% 4.8% 15 16 17 18 19 20 21 22 4444444 5.0% 4.8% 3.4% 5.2% 5.3% 4.8% 5.0% 2.6% 5.0% 2.6% 4 15.80758 1.11 -0.53 13.21303 1.0000000 15.43224 0.84 -2.09 4.09; -0.09] Fixed effect model Random effects model -0.62 [-0.94; -0.30] -0.62 [-0.94; -0.30] 88 88 100% ... \_\_\_ 100% Heterogeneity: *I-squared=0%, tau-squared=0, p=0.9597* Q=11.16, Test overall: p-value = 0.0001(Fixed model) , p-value = 0.0001 -4 -2 0 2 4

**Figure 1.** Forest plots for grain proteins under two levels of  $CO_2$ , elevated  $CO_2$  (eCO<sub>2</sub>) in the experimental group and ambient  $CO_2$  (aCO<sub>2</sub>) in the control group. The level of temperature is ambient and the level of nitrogen is low. In Figure 1, the text and values on the right are the study identification, standardized mean difference (SMD), mean difference (MD), lower and upper limits of 95% confidence interval (CI) and weights of studies (W). On the left are the mean and standard deviations (SD). In the graph, the squares elucidate the point estimates of the treatment effect (SD and mean for experimental and control group) and the size of squares represents the weights assigned to each study. The pooled estimates of the SMD and MD were determined by combing all the mean differences using the inverse variance weighted approach and it is represented by a diamond.

		Experim	ental		Co	ntrol	Mean difference				
Study	Total	Mean			Mean	SD		MD	95%-CI	W(fixed)	W(random)
							2				
1		13.17416			13.76438				[-0.87; -0.31]	54.2%	18.2%
2		13.33893			13.81458				[-1.16; 0.20]	9.4%	13.2%
3		13.84988			13.89480				[-0.65; 0.56]	12.0%	14.3%
4	4	13.97851	0.59	4	14.30345	0.35		-0.32	[-1.00; 0.35]	9.6%	13.3%
5	4	14.77875	1.21	4	16.13121	0.77		-1.35	[-2.76; 0.05]	2.2%	6.3%
6	4	14.77875	1.27	4	16.73322	1.22		-1.95	[-3.68; -0.23]	1.5%	4.7%
7	4	16.47194	0.88	4	15.19166	0.29	3   <del></del>	1.28	[0.37; 2.19]	5.3%	10.5%
8	4	14.86160	2.16	4	15.60775	1.84		-0.75	[-3.53; 2.03]	0.6%	2.1%
9	4	16.50265	1.85	4	17.00000	0.46		-0.50	[-2.37; 1.37]	1.2%	4.2%
10	4	14.25110	1.81	4	15.71547	0.39		-1.46	[-3.28; 0.35]	1.3%	4.4%
11	4	16.22899	1.57	4	18.37592	2.02		-2.15	[-4.65; 0.36]	0.7%	2.6%
12	4	14.84302	1.34	4	16.07047	0.57		-1.23	[-2.65; 0.20]	2.1%	6.2%
							3				
Fixed effect model	48			48			-	-0.46	[-0.67; -0.25]	100%	
Random effects mode	el 👘						<u>ه</u> -	-0.50	[-0.92; -0.07]		100%
Heterogeneity: I-squared=	=56.7%, t	au-squared	=0.242	28, p=0.	0079		3		- / -		
Q=25.42, Test overall : p-valu	e = 0.001	(Fixed mode	I), p-va	lue = 0.0	0238(Random	n mode	ı)				
							-4 -2 0 2 4				
		Experime					Standardised mean difference				
Study	Total	Mean	SD	Total	Mean	SD		SMD	95%-CI	W(fixed)	W(random)
					10 70 10 -	0.46					1.05
1		13.17416			13.76438				[-4.73; -0.30]	4.1%	4.3%
2		13.33893			13.81458				[-2.35; 0.66]	8.9%	8.9%
3		13.84988			13.89480		1	-0.09	[-1.48; 1.30]	10.5%	10.3%
4	4	13.97851	0.59	4	14.30345	0.35		-0.58	[-2.03; 0.86]	9.7%	9.6%

Q = 12.02, Test overall: p-value			-2 0 2	4		
Random effects model Heterogeneity: I-squared=8		n=0 3625	\$	-0.65 [-1.12; -0.18]		100%
Fixed effect model	48	48	\$	-0.65 [-1.10; -0.20]	100%	
12	4 14.84302 1.34	4 16.07047 0.57		-1.04 [-2.60; 0.52]	8.3%	8.3%
11	4 16.22899 1.57	4 18.37592 2.02		-1.03 [-2.59; 0.53]	8.3%	8.3%
10	4 14.25110 1.81	4 15.71547 0.39		-0.97 [-2.51; 0.57]	8.5%	8.5%
9	4 16.50265 1.85	4 17.00000 0.46		-0.32 [-1.72; 1.08]	10.2%	10.1%
8	4 14.86160 2.16	4 15.60775 1.84		-0.32 [-1.73: 1.08]	10.2%	10.1%
7	4 16.47194 0.88	4 15,19166 0.29		- 1.70 [-0.11; 3.51]	6.1%	6.3%
6	4 14.77875 1.27	4 16.73322 1.22		-1.36 [-3.04; 0.31]	7.2%	7.3%
5	4 14.77875 1.21	4 16.13121 0.77		-1.16 [-2.76; 0.44]	7.9%	7.9%
3	4 13.97851 0.59	4 14.30345 0.35		-0.58 [-2.03; 0.86]	9.7%	9.6%
3	4 13.84988 0.29	4 13.89480 0.54		-0.09 [-1.48; 1.30]	10.5%	10.3%

Figure 2. Forest plots for grain proteins under two levels of  $CO_2$ ,  $eCO_2$  in the experimental group and  $aCO_2$  in the control group. The level of temperature is ambient, and the level of nitrogen is medium.

		xperimental		Control	Mean difference				
Study	Total Mea		Total Mean	SD		MD	95%-CI	W(fixed)	W(random)
1	4 428.763		4 343.9000	39.99944		84.86		6.1%	6.0%
2	4 296.605		4 352.5000	42.79021			[-105.61; -6.18]	17.6%	8.7%
3	4 310.710		4 348.2000	88.44803			[-141.94; 66.97]	4.0%	4.8%
4	4 220.503		4 203.0108	87.87032			[-109.94; 144.93]	2.7%	3.8%
5	4 198.056		4 195.3591	65.80636			[-80.96; 86.36]	6.2%	6.1%
6	4 308.179		4 253.4562	96.42816			[ -41.31; 150.76]	4.7%	5.3%
7	4 272.273		4 259.9846	72.81360			[-70.90; 95.48]	6.3%	6.1%
8	4 556.385					153.44		3.6%	4.5%
9	4 467.828		4 262.5855			- 205.24		2.6%	3.6%
10		0 100.20000	4 179.0450	83.34000		99.26	[-28.46; 226.97]	2.7%	3.7%
11		0 164.74000	4 229.1350	93.44000			[-37.12; 334.09]	1.3%	2.2%
12	4 169.394		4 190.6645	78.23000			[-118.73; 76.19]	4.6%	5.2%
13		6 150.88000		30.46000			[-120.86; 180.83]	1.9%	3.0%
14		4 155.78000					[-113.54; 282.56]	1.1%	1.9%
15		3 142.37000	4 259.4605	72.66000			[-114.30; 198.98]	1.8%	2.8%
16		5 192.28000	4 194.4605	44.31000			[-79.54; 307.20]	1.2%	2.0%
17		7 137.21000	4 131.5658	26.33000	1	168.38		2.3%	3.4%
18	4 188.924		4 126.1546	32.38000		62.77		11.3%	7.7%
19	4 192.250	0 136.94000	4 138.1875	35.85000		54.06	[-84.66; 192.78]	2.3%	3.3%
20	4 191.15	3 114.26000	4 139.5954	56.12000		51.56	[-73.19; 176.31]	2.8%	3.9%
21	4 174.243	4 67.00000	4 128.4211	27.55000	+*-	45.82	[-25.17; 116.82]	8.6%	7.0%
22	4 258.927	6 97.58000	4 175.0263	25.98000	+ : =	83.90	[ -15.06; 182.86]	4.4%	5.1%
<b>_</b> <i></i>									
Fixed effect model	88		88		•	37.05		100%	
Random effects model					<b></b>	50.95	[ 20.69; 81.22]		100%
Heterogeneity: I-squared=4									
Q = 38.19, Test overall: p-valu	e = 0.0005 (Fixed	nodel), p-value =	0.0010 (Random mo	del)					

-300 -100 0 100 200 300

		Ex	perimental			00111101	Standardised mean difference				
Study	Total	Mean	SD	Total	Mean	SD	1.1	SMD	95%-CI	W(fixed)	W(random)
1	4	428.7634	76.47491	4	343.9000	39.99944		1.21	[-0.41; 2.83]	3.9%	3.9%
2	4	296.6052	27.25899	4	352.5000	42.79021		-1.35	[-3.02; 0.32]	3.7%	3.7%
3	4	310.7104	59.48204	4	348.2000	88.44803		-0.43	[-1.85; 0.98]	5.1%	5.1%
4	4	220.5031	95.85537	4	203.0108	87.87032		0.17	[-1.23; 1.56]	5.3%	5.3%
5	4	198.0568	54.37973	4	195.3591	65.80636	i	0.04	[-1.35; 1.43]	5.3%	5.3%
6	4	308.1797	17.46221	4	253.4562	96.42816		0.69	[-0.78; 2.15]	4.8%	4.8%
7	4	272.2734	43.63934	4	259.9846	72.81360		0.18	[-1.21; 1.57]	5.3%	5.3%
8	4	556.3856	51.71000	4	402.9495	100.07000		1.68	[-0.13; 3.48]	3.2%	3.2%
9	4	467.8289	56.15000	4	262.5855	120.85000		1.89	[-0.01; 3.80]	2.8%	2.8%
10	4	278.3000	100.20000	4	179.0450	83.34000		0.94	[-0.59; 2.46]	4.4%	4.4%
11	4	377.6200	164.74000	4	229.1350	93.44000		0.96	[-0.57; 2.50]	4.3%	4.3%
12	4	169.3947	61.41000	4	190.6645	78.23000		-0.26	[-1.66; 1.13]	5.2%	5.2%
13	4	158.0066	150.88000	4	128.0197	30.46000		0.24	[-1.16; 1.64]	5.3%	5.3%
14	4	454.9404	155.78000	4	370.4318	128.75000		0.51	[-0.92; 1.94]	5.0%	5.0%
15	4	301.7993	142.37000	4	259.4605	72.66000		0.33	[-1.08; 1.73]	5.2%	5.2%
16	4	308.2895	192.28000	4	194.4605	44.31000		0.71	[-0.76; 2.18]	4.8%	4.8%
17	4	299.9507	137.21000	4	131.5658	26.33000	+	1.48	[-0.24; 3.20]	3.5%	3.5%
18	4	188.9243	54.39000	4	126.1546	32.38000		1.22	[-0.40; 2.84]	3.9%	3.9%
19	4	192.2500	136.94000	4	138.1875	35.85000		0.47	[-0.95; 1.89]	5.1%	5.1%
20	4	191.1513	114.26000	4	139.5954	56.12000		0.50	[-0.93; 1.93]	5.0%	5.0%
21	4	174.2434	67.00000	4	128.4211	27.55000		0.78	[-0.71; 2.26]	4.6%	4.6%
22	4	258.9276	97.58000	4	175.0263	25.98000		1.02	[-0.53; 2.58]	4.2%	4.2%
Fixed effect model	88			88			-	0.52	[ 0.20; 0.84]	100%	
Random effects mode	el						$\diamond$	0.52	[ 0.20; 0.84]		100%
Heterogeneity: I-squared									- / -		
Q=16.40 , Test overall: p-val	ue = 0.001	4(Fixed mod	el), p-value = 0	.0014 (F	landom mode	el)					
							-3 -2 -1 0 1 2 3				

**Figure 3.** Forest plots for grain yield under two levels of  $CO_2$ ,  $eCO_2$  in the experimental group and  $aCO_2$  in the control group. The level of temperature is ambient and the level of nitrogen is low.

		Experimental		Control	Mean difference				
Study	Total Me	an SD	Total Mean	SD		MD	95%-CI	W(fixed)	W(random)
1	4 416.09	06 82.02769	4 309.7158	13.88697		106.37	[ 24.85; 187.90]	8.8%	9.3%
2	4 341.97	20 88.09490	4 208.8249	94.77364		133.15	[ 6.34; 259.95]	3.6%	6.3%
3	4 208.68	66 60.57906	4 207.2638	36.66842		1.42	[-67.97; 70.82]	12.1%	10.2%
4	4 332.75	73 50.28376	4 266.7051	45.96920		66.05	[ -0.71; 132.82]	13.1%	10.4%
5	4 458.71	69 102.43000	4 200.5066	27.94000		258.21	[154.16; 362.26]	5.4%	7.7%
6	4 458.71	69 95.62000	4 341.4830	105.60000		117.23	[-22.37; 256.84]	3.0%	5.6%
7	4 115.59	54 62.48000	4 154.7007	26.92000		-39.11	[-105.78; 27.57]	13.1%	10.4%
8	4 457.61	70 215.60000	4 312.0815	101.13000		- 145.54	[-87.84; 378.91]	1.1%	2.7%
9	4 168.36	51 90.41000	4 131.0263	22.06000		37.34	[-53.86; 128.54]	7.0%	8.6%
10	4 305.25	89 56.87000	4 256.6056	54.20000		48.65	[-28.34; 125.64]	9.8%	9.6%
11	4 204.30	92 50.19000	4 105.4276	32.71000		98.88	[ 40.17; 157.59]	16.9%	11.1%
12	4 297.10	02 91.67000	4 263.3831	40.48000		33.72	[-64.49; 131.92]	6.0%	8.1%
Fixed effect model	48		48			63.01	[ 38.86; 87.16]	100%	
Random effects mode	el					73.09	[ 30.21; 115.96]		100%
Heterogeneity: I-squared Q = 31.10, Test overall: p-va				del)			-		

-300	-100	0	100 200 300

		Ex	perimental			Control	Standardised mean difference				
Study	Total	Mean	. SD	Total	Mean	SD		SMD	95%-CI	W(fixed)	W(random)
1	4	416.0906	82.02769	4	309.7158	13.88697		1.57	[-0.19; 3.33]	6.7%	6.8%
2	4	341.9720	88.09490	4	208.8249	94.77364		1.27	[-0.37; 2.90]	7.8%	7.8%
3	4	208.6866	60.57906	4	207.2638	36.66842		0.02	[-1.36; 1.41]	10.8%	10.7%
4	4	332.7573	50.28376	4	266.7051	45.96920		1.19	[-0.42; 2.80]	8.0%	8.1%
5	4	458.7169	102.43000	4	200.5066	27.94000	+	2.99	[ 0.51; 5.47]	3.4%	3.5%
6	4	458.7169	95.62000	4	341.4830	105.60000		1.01	[-0.54; 2.56]	8.6%	8.7%
7	4	115.5954	62.48000	4	154.7007	26.92000		-0.71	[-2.18; 0.76]	9.6%	9.6%
8	4	457.6170	215.60000	4	312.0815	101.13000	-+	0.75	[-0.73; 2.23]	9.5%	9.5%
9	4	168.3651	90.41000	4	131.0263	22.06000		0.49	[-0.93; 1.92]	10.2%	10.1%
10	4	305.2589	56.87000	4	256.6056	54.20000		0.76	[-0.72; 2.24]	9.5%	9.4%
11	4	204.3092	50.19000	4	105.4276	32.71000		2.03	[0.06; 4.00]	5.4%	5.5%
12	4	297.1002	91.67000	4	263.3831	40.48000		0.41	[-1.00; 1.83]	10.4%	10.3%
Fixed effect model	48			48			<b>\</b>	0.77	[ 0.31; 1.23]	100%	
Random effects model							\$	0.78	[ 0.31; 1.24]		100%
Heterogeneity: I-squared=4											
Q = 11.55, Test overall: p-valu	ie = 0.00	09 (Fixed mo	del), p-value =	0.0012	(Random mo	del)					
							-4 -2 0 2 4				

Figure 4. Forest plot for grain yield under two levels of  $CO_2$ ,  $eCO_2$  in the experimental group and  $aCO_2$  in the control group. The level of temperature is ambient and the level of nitrogen is medium.

	-	Test for ov	verall	Tests for heterogeneity					
Experiments	MD	SMD	P-value	$\tau^2$	<b>I</b> <sup>2</sup>	Q	P-value		
1	-0.66	-0.62	0.0001(Fixed)						
	-0.72	-0.62	0.0001(Random)	0	0%	11.16	0.9597		
2	-0.46	-0.65	0.0045(Fixed)						
	-0.50	-0.65	0.0064(Random)	0.0584	8.5%	12.02	0.3625		
3	37.05	0.52	0.0014(Fixed)						
	50.95	0.52	0.0014(Random)	0	0%	16.40	0.7470		
4	63.01	0.77	0.0009(Fixed)						
	73.09	0.78	0.0012(Random)	0.0326	4.7%	11.55	0.3985		

Table 1. Summary statistics of the pooled data

### 4. Conclusion

The aim of this study was to analyse the effects of  $CO_2$ , temperature and nitrogen on grain protein and grain yield. The proposed techniques will improve the accuracy of analysis. The results showed that the protein concentration was decreased by 0.62% and grain yield was increased by 0.52% under elevated carbon dioxide, ambient temperature and low nitrogen. In contrast, protein concentration was reduced by 0.65% and grain yield was increased by 0.78% under the elevated carbon dioxide, ambient temperature and medium nitrogen. They can be used to analyse the effect of  $CO_2$  and temperature on grain protein content and grain yield. These methods have the potential to aid experts and decision makers in making better decisions regarding crops production. They can also be applied to other fields of study, such as plants, forest, food webs and biomedical engineering. In addition, the proposed procedure draws the line for other researchers to follow the same strategy to represent other data.

## References

- Asseng, S., Ewert, F., Martre, P., Rötter, R. P., Lobell, D., Cammarano, D., White, J. W. (2015). Rising temperatures reduce global wheat production. *Nature Climate Change*, 5(2), 143-147.
- Baig, S., Medlyn, B. E., Mercado, L. M., & Zaehle, S. (2015). Does the growth response of woody plants to elevated CO2 increase with temperature? A model-oriented meta-analysis. *Global change biology*, 21(12), 4303-4319.
- Borenstein, M., Hedges, L. V., Higgins, J., & Rothstein, H. R. (2009). References: Wiley Online Library.
- Buchner, P., Tausz, M., Ford, R., Leo, A., Fitzgerald, G. J., Hawkesford, M. J., & Tausz-Posch, S. (2015). Expression patterns of C-and N-metabolism related genes in wheat are changed during senescence under elevated CO 2 in dry-land agriculture. *Plant Science*, 236, 239-249.
- Cai, C., Yin, X., He, S., Jiang, W., Si, C., Struik, P. C., Xiong, Y. (2016). Responses of wheat and rice to factorial combinations of ambient and elevated CO2 and temperature in FACE experiments. *Global change biology*, 22(2), 856-874.
- Challinor, A. J., Koehler, A.-K., Ramirez-Villegas, J., Whitfield, S., & Das, B. (2016). Current warming will reduce yields unless maize breeding and seed systems adapt immediately. *Nature Climate Change*, 6(10), 954-958.
- Cumming, G. (2013). Understanding the new statistics: Effect sizes, confidence intervals, and meta-analysis: Routledge.
- Dietterich, L. H., Zanobetti, A., Kloog, I., Huybers, P., Leakey, A. D., Bloom, A. J., Hasegawa, T. (2015). Impacts of elevated atmospheric CO2 on nutrient content of important food crops. *Scientific data*, 2.
- Doi, S. A., Barendregt, J. J., Khan, S., Thalib, L., & Williams, G. M. (2015). Advances in the meta-analysis of heterogeneous clinical trials I: the inverse variance heterogeneity model. *Contemporary Clinical Trials*, 45, 130-138.
- Erbs, M., Manderscheid, R., Hüther, L., Schenderlein, A., Wieser, H., Dänicke, S., & Weigel, H.-J. (2015). Freeair CO2 enrichment modifies maize quality only under drought stress. Agronomy for sustainable development, 35(1), 203-212.

- Fernando, N., Panozzo, J., Tausz, M., Norton, R., Fitzgerald, G., Khan, A., & Seneweera, S. (2015). Rising CO 2 concentration altered wheat grain proteome and flour rheological characteristics. *Food chemistry*, 170, 448-454.
- Fernando, N., Panozzo, J., Tausz, M., Norton, R. M., Fitzgerald, G. J., Myers, S., Seneweera, S. (2014). Intraspecific variation of wheat grain quality in response to elevated [CO 2] at two sowing times under rainfed and irrigation treatments. *Journal of Cereal Science*, 59(2), 137-144.
- Fitzgerald, G. J., Tausz, M., O'Leary, G., Mollah, M. R., Tausz-Posch, S., Seneweera, S., McNeil, D. (2016). Elevated atmospheric [CO2] can dramatically increase wheat yields in semi-arid environments and buffer against heat waves. *Global change biology*.
- García, G. A., Dreccer, M. F., Miralles, D. J., & Serrago, R. A. (2015). High night temperatures during grain number determination reduce wheat and barley grain yield: a field study. *Global change biology*, 21(11), 4153-4164.
- Haworth, M., Hoshika, Y., & Killi, D. (2016). Has the Impact of Rising CO2 on Plants been exaggerated by Meta-Analysis of Free Air CO2 Enrichment Studies? *Frontiers in Plant Science*, 7.
- Humbert, J. Y., Dwyer, J. M., Andrey, A., & Arlettaz, R. (2016). Impacts of nitrogen addition on plant biodiversity in mountain grasslands depend on dose, application duration and climate: a systematic review. *Global change biology*, 22(1), 110-120.
- Jablonski, L. M., Wang, X., & Curtis, P. S. (2002). Plant reproduction under elevated CO2 conditions: a meta-analysis of reports on 79 crop and wild species. *New Phytologist*, 156(1), 9-26.
- Lam, S. K., Chen, D., Mosier, A. R., & Roush, R. (2013). The potential for carbon sequestration in Australian agricultural soils is technically and economically limited. *Scientific reports*, *3*.
- Li, Y., Niu, S., & Yu, G. (2016). Aggravated phosphorus limitation on biomass production under increasing nitrogen loading: a meta-analysis. *Global change biology*, 22(2), 934-943.
- Liang, H., Hu, K., Batchelor, W. D., Qi, Z., & Li, B. (2016). An integrated soil-crop system model for water and nitrogen management in North China. *Scientific reports*, 6.
- Liu, C.-W., Sung, Y., Chen, B.-C., & Lai, H.-Y. (2014). Effects of nitrogen fertilizers on the growth and nitrate content of lettuce (Lactuca sativa L.). *International journal of environmental research and public health*, 11(4), 4427-4440.
- Lobell, D. B., Sibley, A., & Ortiz-Monasterio, J. I. (2012). Extreme heat effects on wheat senescence in India. *Nature Climate Change*, 2(3), 186-189.
- Lv, Z., Liu, X., Cao, W., & Zhu, Y. (2013). Climate change impacts on regional winter wheat production in main wheat production regions of China. *Agricultural and forest meteorology*, *171*, 234-248.
- Myers, S. S., Zanobetti, A., Kloog, I., Huybers, P., Leakey, A. D., Bloom, A. J., Hasegawa, T. (2014). Increasing CO2 threatens human nutrition. *Nature*, *510*(7503), 139-142.
- Njoroge, P., Mburu, M., Githiri, S., & Shibairo, S. (2014). Effects of Nitrogen Application on Growth and Yield of Snowpeas (Pisum sativum).
- Normand, S.-L. T. (1999). Tutorial in biostatistics meta-analysis: formulating, evaluating, combining, and reporting. *Statistics in medicine*, 18(3), 321-359.
- Panozzo, J., Walker, C., Partington, D., Neumann, N., Tausz, M., Seneweera, S., & Fitzgerald, G. (2014). Elevated carbon dioxide changes grain protein concentration and composition and compromises baking quality. A FACE study. *Journal of Cereal Science*, 60(3), 461-470.
- Pleijel, H., & Uddling, J. (2012). Yield vs. Quality trade-offs for wheat in response to carbon dioxide and ozone. *Global change biology*, 18(2), 596-605.
- Rodrigues, W. P., Martins, M. Q., Fortunato, A. S., Rodrigues, A. P., Semedo, J. N., Simões-Costa, M. C., ... Goulao, L. (2016). Long-term elevated air [CO2] strengthens photosynthetic functioning and mitigates the impact of supra-optimal temperatures in tropical Coffea arabica and C. canephora species. *Global change biology*, 22(1), 415-431.
- Sánchez, B., Rasmussen, A., & Porter, J. R. (2014). Temperatures and the growth and development of maize and rice: a review. *Global change biology*, 20(2), 408-417.
- Schmidt, F. L., & Hunter, J. E. (2014). *Methods of meta-analysis: Correcting error and bias in research findings*: Sage publications.
- Subramanya, M. S., Hossain, M., Khan, S., Memon, B., & Memon, M. A. (2010). *Meta-analysis of D1 versus D2 gastrectomy for gastric adenocarcinoma*. Paper presented at the Proceedings of the Tenth Islamic Countries Conference on Statistical Sciences (ICCS-X).
- Sutton, A. J., Abrams, K. R., Jones, D. R., Jones, D. R., Sheldon, T. A., & Song, F. (2000). Methods for metaanalysis in medical research.
- Sutton, A. J., Rothstein, H., Sutton, A., & Borenstein, M. (2005). Evidence concerning the consequences of publication and related biases. *Publication bias in meta-analysis: Prevention, assessment, and adjustments*, 175-192.

- Tack, J., Barkley, A., & Nalley, L. L. (2015). Effect of warming temperatures on US wheat yields. *Proceedings* of the National Academy of Sciences, 112(22), 6931-6936.
- Valizadeh, J., Ziaei, S., & Mazloumzadeh, S. (2014). Assessing climate change impacts on wheat production (a case study). *Journal of the Saudi Society of Agricultural Sciences*, 13(2), 107-115.
- Ward, R. M. (2007). Potential impact of temperature and carbon dioxide levels on rice quality.
- Wickham, H. (2011). The split-apply-combine strategy for data analysis. *Journal of Statistical Software*, 40(1), 1-29.
- Wu, G., Johnson, S. K., Bornman, J. F., Bennett, S. J., Clarke, M. W., Singh, V., & Fang, Z. (2016). Growth temperature and genotype both play important roles in sorghum grain phenolic composition. *Scientific reports*, 6.
- Zhang, T., Li, T., Yang, X., & Simelton, E. (2016). Model biases in rice phenology under warmer climates. Scientific reports, 6.
- Zhou, G., Zhou, X., He, Y., Shao, J., Hu, Z., Liu, R., Hosseinibai, S. (2017). Grazing intensity significantly affects belowground carbon and nitrogen cycling in grassland ecosystems: a meta-analysis. *Global change biology*, 23(3), 1167-1179.