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_2, 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_2, temperature and nitrogen on grain protein and grain yield were analysed. Therefore, this study focuses on estimating the effects of CO_2, 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 τ^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_2, 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_2, 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 meta-analysis 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). Meta-analysis 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 between-studies 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_2 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_2 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_2, 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_2, 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_2, 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_2, temperature and nitrogen on grain protein and grain yield. This study focuses on measuring the effects of CO_2, 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_2 (FACE) technology. The researchers conducted the studies under different conditions and various levels of CO_2, 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_2 (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_2, 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 http://cran-project.org. 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)

$$T_i = \mu + u_i. \tag{1}$$

where T_i is an observed effect in the study of i, μ 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 $\bar{T}.$ can be computed as

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

The variance of the combined effect is defined as*:*

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

The standard error of the combined effect is

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

The 95% confidence interval for the combined effect is computed *as*

Lower Limit $= \bar{T}. - 1.96 * SE(\bar{T}.),$ (6)

Upper Limit $= \bar{T}. + 1.96 * SE(\bar{T}.).$ (7)

The *Z*-value can be computed using

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

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

$$p = 1 - \varphi(|Z|), \tag{9}$$

For a two-tailed test by

$$p = 2[1 - (\varphi\ (|Z|))], \tag{10}$$

where φ is the standard normal cumulative distribution function
The random effects model can be written as (Borenstein et al., 2009)

$$T_i = \theta_i + e_i = \mu + \varepsilon_i + e_i. \tag{11}$$

where T_i is the observed effect in study i, θ_i is the true effect, ε_i is the within-study error, μ is the mean of all the true effects, e_i is the between study error.
The weight assigned to each study is

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

where v_i^* is the within-study variance for study i plus the between-studies variance.
The weighted mean $\bar{T}_{.}^{*}$ is then computed as

$$\bar{T}_{.}^{*} = \frac{\sum_{i=1}^{k} w_i^* T_i}{\sum_{i=1}^{k} w_i^*}, \tag{13}$$

The variance of the combined effect is defined as

$$V_{.}^{*} = \frac{1}{\sum_{i=1}^{k} w_i^*}, \tag{14}$$

The standard error of the combined effect is

$$SE = (\bar{T}_{.}^{*}) = \sqrt{V_{.}^{*}}. \tag{15}$$

The 95% confidence interval for the combined effect can be computed as
Lower Limit* $=\bar{T}_{.}^{*} - 1.96^{*}SE(\bar{T}_{.}^{*})$, (16)
Upper Limit* $=\bar{T}_{.}^{*} + 1.96^{*}SE(\bar{T}_{.}^{*})$. (17)
The *Z*-value could be computed using

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

The one-tailed p-value is given by

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

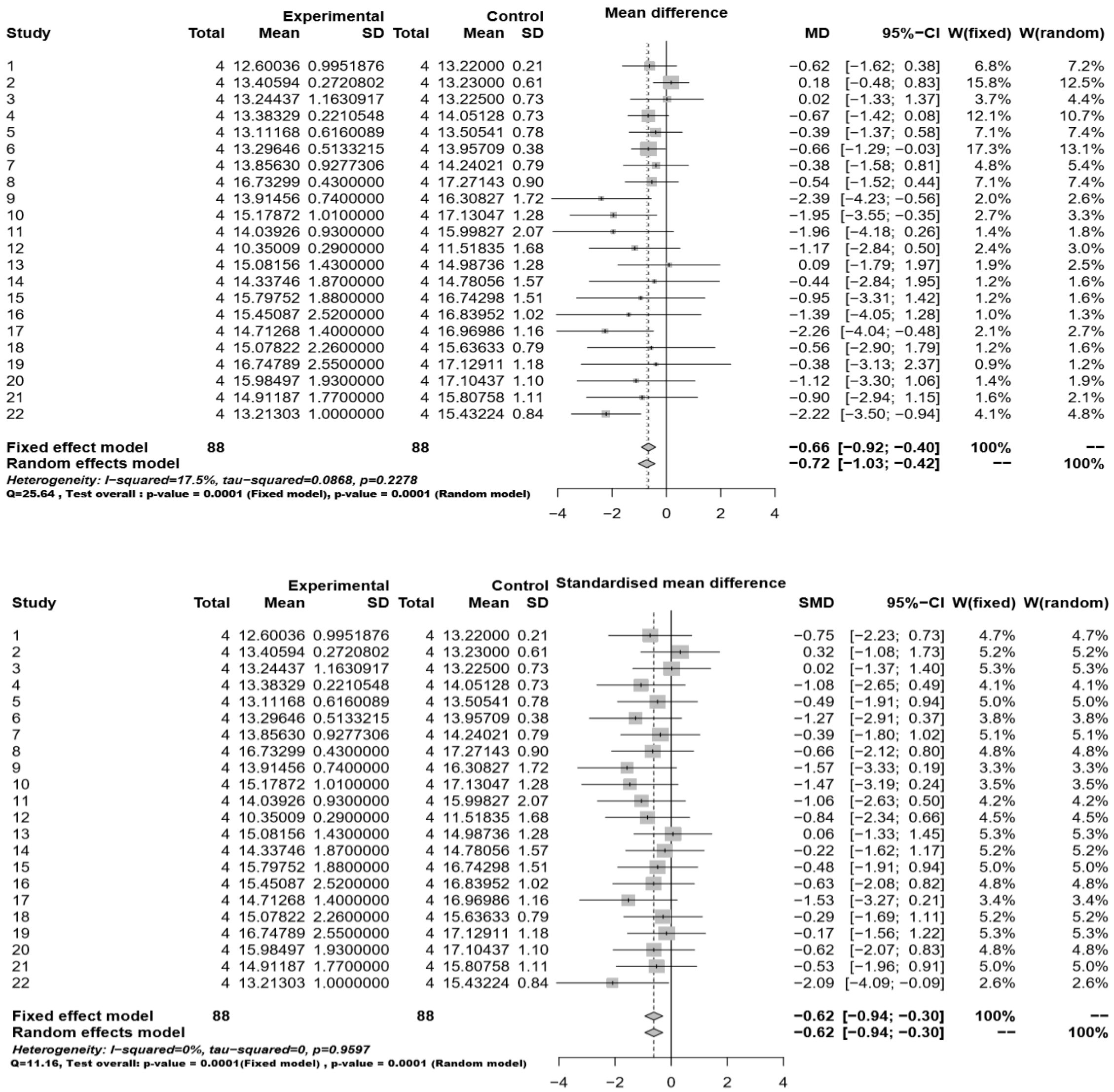
The two-tailed *p*-value by

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

where φ: 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).



	Experimental			Control						
Study	**Total**	**Mean**	**SD**	**Total**	**Mean**	**SD**	**MD**	**95%−CI**	**W(fixed)**	**W(random)**
1	4	12.60036	0.9951876	4	13.22000	0.21	−0.62	[−1.62; 0.38]	6.8%	7.2%
2	4	13.40594	0.2720802	4	13.23000	0.61	0.18	[−0.48; 0.83]	15.8%	12.5%
3	4	13.24437	1.1630917	4	13.22500	0.73	0.02	[−1.33; 1.37]	3.7%	4.4%
4	4	13.38329	0.2210548	4	14.05128	0.73	−0.67	[−1.42; 0.08]	12.1%	10.7%
5	4	13.11168	0.6160089	4	13.50541	0.78	−0.39	[−1.37; 0.58]	7.1%	7.4%
6	4	13.29646	0.5133215	4	13.95709	0.38	−0.66	[−1.29; −0.03]	17.3%	13.1%
7	4	13.85630	0.9277306	4	14.24021	0.79	−0.38	[−1.58; 0.81]	4.8%	5.4%
8	4	16.73299	0.4300000	4	17.27143	0.90	−0.54	[−1.52; 0.44]	7.1%	7.4%
9	4	13.91456	0.7400000	4	16.30827	1.72	−2.39	[−4.23; −0.56]	2.0%	2.6%
10	4	15.17872	1.0100000	4	17.13047	1.28	−1.95	[−3.55; −0.35]	2.7%	3.3%
11	4	14.03926	0.9300000	4	15.99827	2.07	−1.96	[−4.18; 0.26]	1.4%	1.8%
12	4	10.35009	0.2900000	4	11.51835	1.68	−1.17	[−2.84; 0.50]	2.4%	3.0%
13	4	15.08156	1.4300000	4	14.98736	1.28	0.09	[−1.79; 1.97]	1.9%	2.5%
14	4	14.33746	1.8700000	4	14.78056	1.57	−0.44	[−2.84; 1.95]	1.2%	1.6%
15	4	15.79752	1.8800000	4	16.74298	1.51	−0.95	[−3.31; 1.42]	1.2%	1.6%
16	4	15.45087	2.5200000	4	16.83952	1.02	−1.39	[−4.05; 1.28]	1.0%	1.3%
17	4	14.71268	1.4000000	4	16.96986	1.16	−2.26	[−4.04; −0.48]	2.1%	2.7%
18	4	15.07822	2.2600000	4	15.63633	0.79	−0.56	[−2.90; 1.79]	1.2%	1.6%
19	4	16.74789	2.5500000	4	17.12911	1.18	−0.38	[−3.13; 2.37]	0.9%	1.2%
20	4	15.98497	1.9300000	4	17.10437	1.10	−1.12	[−3.30; 1.06]	1.4%	1.9%
21	4	14.91187	1.7700000	4	15.80758	1.11	−0.90	[−2.94; 1.15]	1.6%	2.1%
22	4	13.21303	1.0000000	4	15.43224	0.84	−2.22	[−3.50; −0.94]	4.1%	4.8%
Fixed effect model	**88**			**88**			**−0.66**	**[−0.92; −0.40]**	**100%**	**--**
Random effects model							**−0.72**	**[−1.03; −0.42]**	**--**	**100%**

Heterogeneity: I−squared=17.5%, tau−squared=0.0868, p=0.2278

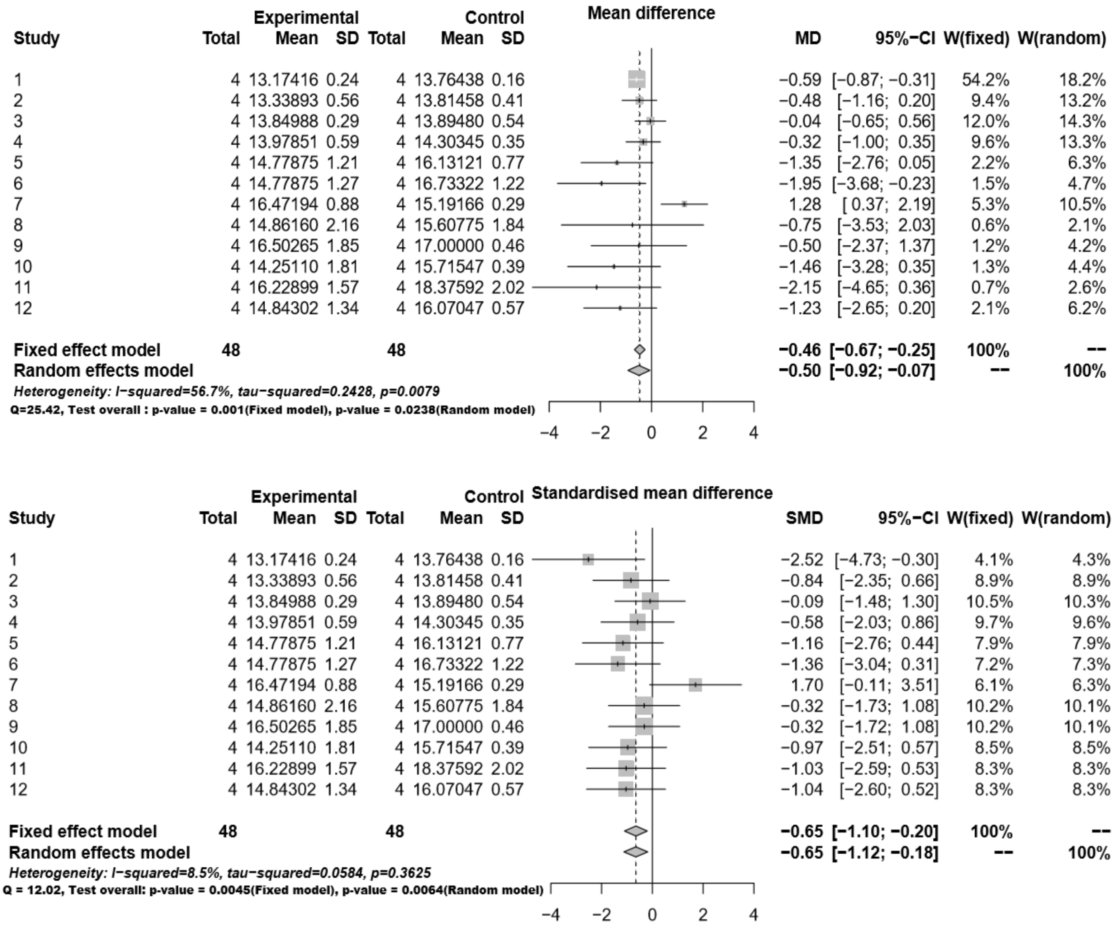
Q=25.64 , Test overall : p-value = 0.0001 (Fixed model), p-value = 0.0001 (Random model)

	Experimental			Control						
Study	**Total**	**Mean**	**SD**	**Total**	**Mean**	**SD**	**SMD**	**95%−CI**	**W(fixed)**	**W(random)**
1	4	12.60036	0.9951876	4	13.22000	0.21	−0.75	[−2.23; 0.73]	4.7%	4.7%
2	4	13.40594	0.2720802	4	13.23000	0.61	0.32	[−1.08; 1.73]	5.2%	5.2%
3	4	13.24437	1.1630917	4	13.22500	0.73	0.02	[−1.37; 1.40]	5.3%	5.3%
4	4	13.38329	0.2210548	4	14.05128	0.73	−1.08	[−2.65; 0.49]	4.1%	4.1%
5	4	13.11168	0.6160089	4	13.50541	0.78	−0.49	[−1.91; 0.94]	5.0%	5.0%
6	4	13.29646	0.5133215	4	13.95709	0.38	−1.27	[−2.91; 0.37]	3.8%	3.8%
7	4	13.85630	0.9277306	4	14.24021	0.79	−0.39	[−1.80; 1.02]	5.1%	5.1%
8	4	16.73299	0.4300000	4	17.27143	0.90	−0.66	[−2.12; 0.80]	4.8%	4.8%
9	4	13.91456	0.7400000	4	16.30827	1.72	−1.57	[−3.33; 0.19]	3.3%	3.3%
10	4	15.17872	1.0100000	4	17.13047	1.28	−1.47	[−3.19; 0.24]	3.5%	3.5%
11	4	14.03926	0.9300000	4	15.99827	2.07	−1.06	[−2.63; 0.50]	4.2%	4.2%
12	4	10.35009	0.2900000	4	11.51835	1.68	−0.84	[−2.34; 0.66]	4.5%	4.5%
13	4	15.08156	1.4300000	4	14.98736	1.28	0.06	[−1.33; 1.45]	5.3%	5.3%
14	4	14.33746	1.8700000	4	14.78056	1.57	−0.22	[−1.62; 1.17]	5.2%	5.2%
15	4	15.79752	1.8800000	4	16.74298	1.51	−0.48	[−1.91; 0.94]	5.0%	5.0%
16	4	15.45087	2.5200000	4	16.83952	1.02	−0.63	[−2.08; 0.82]	4.8%	4.8%
17	4	14.71268	1.4000000	4	16.96986	1.16	−1.53	[−3.27; 0.21]	3.4%	3.4%
18	4	15.07822	2.2600000	4	15.63633	0.79	−0.29	[−1.69; 1.11]	5.2%	5.2%
19	4	16.74789	2.5500000	4	17.12911	1.18	−0.17	[−1.56; 1.22]	5.3%	5.3%
20	4	15.98497	1.9300000	4	17.10437	1.10	−0.62	[−2.07; 0.83]	4.8%	4.8%
21	4	14.91187	1.7700000	4	15.80758	1.11	−0.53	[−1.96; 0.91]	5.0%	5.0%
22	4	13.21303	1.0000000	4	15.43224	0.84	−2.09	[−4.09; −0.09]	2.6%	2.6%
Fixed effect model	**88**			**88**			**−0.62**	**[−0.94; −0.30]**	**100%**	**--**
Random effects model							**−0.62**	**[−0.94; −0.30]**	**--**	**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 (Random model)

Figure 1. Forest plots for grain proteins under two levels of CO_2, elevated CO_2 (eCO_2) in the experimental group and ambient CO_2 (aCO_2) 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.



Study	Experimental Total	Experimental Mean	Experimental SD	Control Total	Control Mean	Control SD	MD	95%−CI	W(fixed)	W(random)
1	4	13.17416	0.24	4	13.76438	0.16	−0.59	[−0.87; −0.31]	54.2%	18.2%
2	4	13.33893	0.56	4	13.81458	0.41	−0.48	[−1.16; 0.20]	9.4%	13.2%
3	4	13.84988	0.29	4	13.89480	0.54	−0.04	[−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	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%
Fixed effect model	**48**			**48**			**−0.46**	**[−0.67; −0.25]**	**100%**	**--**
Random effects model							**−0.50**	**[−0.92; −0.07]**	**--**	**100%**

Heterogeneity: I−squared=56.7%, tau−squared=0.2428, p=0.0079

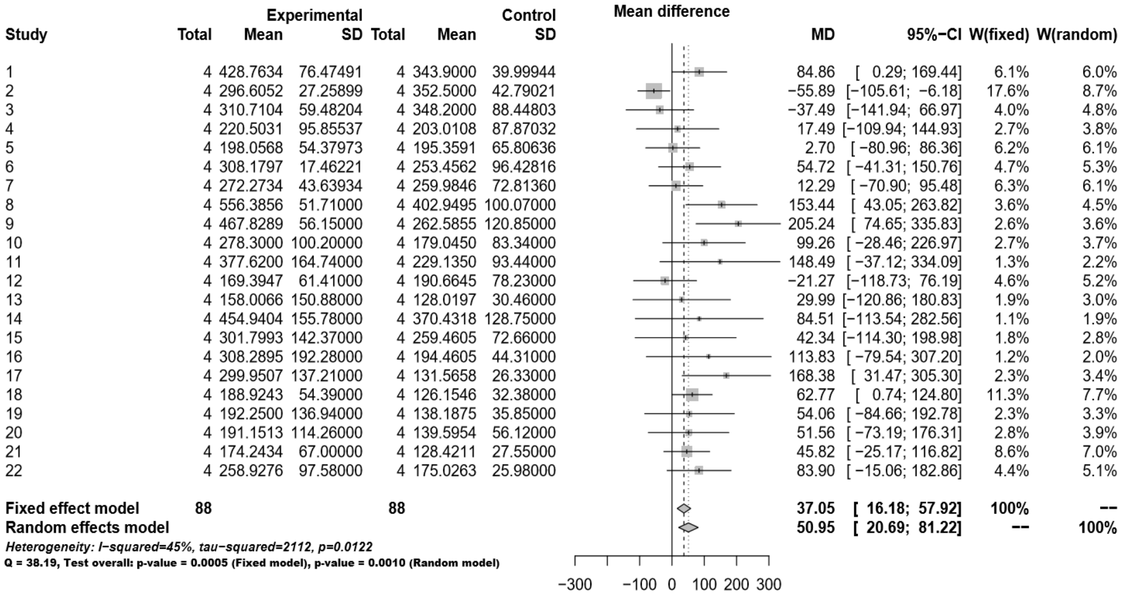
Q=25.42, Test overall : p-value = 0.001(Fixed model), p-value = 0.0238(Random model)

Study	Experimental Total	Experimental Mean	Experimental SD	Control Total	Control Mean	Control SD	SMD	95%−CI	W(fixed)	W(random)
1	4	13.17416	0.24	4	13.76438	0.16	−2.52	[−4.73; −0.30]	4.1%	4.3%
2	4	13.33893	0.56	4	13.81458	0.41	−0.84	[−2.35; 0.66]	8.9%	8.9%
3	4	13.84988	0.29	4	13.89480	0.54	−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%
5	4	14.77875	1.21	4	16.13121	0.77	−1.16	[−2.76; 0.44]	7.9%	7.9%
6	4	14.77875	1.27	4	16.73322	1.22	−1.36	[−3.04; 0.31]	7.2%	7.3%
7	4	16.47194	0.88	4	15.19166	0.29	1.70	[−0.11; 3.51]	6.1%	6.3%
8	4	14.86160	2.16	4	15.60775	1.84	−0.32	[−1.73; 1.08]	10.2%	10.1%
9	4	16.50265	1.85	4	17.00000	0.46	−0.32	[−1.72; 1.08]	10.2%	10.1%
10	4	14.25110	1.81	4	15.71547	0.39	−0.97	[−2.51; 0.57]	8.5%	8.5%
11	4	16.22899	1.57	4	18.37592	2.02	−1.03	[−2.59; 0.53]	8.3%	8.3%
12	4	14.84302	1.34	4	16.07047	0.57	−1.04	[−2.60; 0.52]	8.3%	8.3%
Fixed effect model	**48**			**48**			**−0.65**	**[−1.10; −0.20]**	**100%**	**--**
Random effects model							**−0.65**	**[−1.12; −0.18]**	**--**	**100%**

Heterogeneity: I−squared=8.5%, tau−squared=0.0584, p=0.3625

Q = 12.02, Test overall: p-value = 0.0045(Fixed model), p-value = 0.0064(Random model)

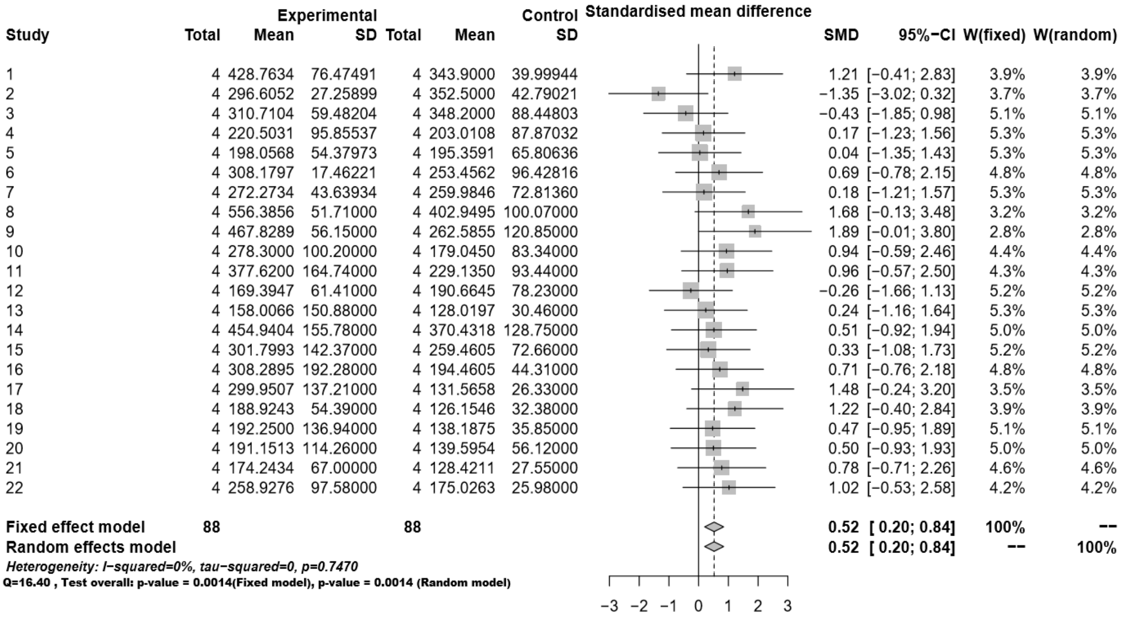
Figure 2. Forest plots for grain proteins under two levels of CO_2, eCO_2 in the experimental group and aCO_2 in the control group. The level of temperature is ambient, and the level of nitrogen is medium.



Study	Experimental Total	Experimental Mean	Experimental SD	Control Total	Control Mean	Control SD	MD	95%−CI	W(fixed)	W(random)
1	4	428.7634	76.47491	4	343.9000	39.99944	84.86	[0.29; 169.44]	6.1%	6.0%
2	4	296.6052	27.25899	4	352.5000	42.79021	−55.89	[−105.61; −6.18]	17.6%	8.7%
3	4	310.7104	59.48204	4	348.2000	88.44803	−37.49	[−141.94; 66.97]	4.0%	4.8%
4	4	220.5031	95.85537	4	203.0108	87.87032	17.49	[−109.94; 144.93]	2.7%	3.8%
5	4	198.0568	54.37973	4	195.3591	65.80636	2.70	[−80.96; 86.36]	6.2%	6.1%
6	4	308.1797	17.46221	4	253.4562	96.42816	54.72	[−41.31; 150.76]	4.7%	5.3%
7	4	272.2734	43.63934	4	259.9846	72.81360	12.29	[−70.90; 95.48]	6.3%	6.1%
8	4	556.3856	51.71000	4	402.9495	100.07000	153.44	[43.05; 263.82]	3.6%	4.5%
9	4	467.8289	56.15000	4	262.5855	120.85000	205.24	[74.65; 335.83]	2.6%	3.6%
10	4	278.3000	100.20000	4	179.0450	83.34000	99.26	[−28.46; 226.97]	2.7%	3.7%
11	4	377.6200	164.74000	4	229.1350	93.44000	148.49	[−37.12; 334.09]	1.3%	2.2%
12	4	169.3947	61.41000	4	190.6645	78.23000	−21.27	[−118.73; 76.19]	4.6%	5.2%
13	4	158.0066	150.88000	4	128.0197	30.46000	29.99	[−120.86; 180.83]	1.9%	3.0%
14	4	454.9404	155.78000	4	370.4318	128.75000	84.51	[−113.54; 282.56]	1.1%	1.9%
15	4	301.7993	142.37000	4	259.4605	72.66000	42.34	[−114.30; 198.98]	1.8%	2.8%
16	4	308.2895	192.28000	4	194.4605	44.31000	113.83	[−79.54; 307.20]	1.2%	2.0%
17	4	299.9507	137.21000	4	131.5658	26.33000	168.38	[31.47; 305.30]	2.3%	3.4%
18	4	188.9243	54.39000	4	126.1546	32.38000	62.77	[0.74; 124.80]	11.3%	7.7%
19	4	192.2500	136.94000	4	138.1875	35.85000	54.06	[−84.66; 192.78]	2.3%	3.3%
20	4	191.1513	114.26000	4	139.5954	56.12000	51.56	[−73.19; 176.31]	2.8%	3.9%
21	4	174.2434	67.00000	4	128.4211	27.55000	45.82	[−25.17; 116.82]	8.6%	7.0%
22	4	258.9276	97.58000	4	175.0263	25.98000	83.90	[−15.06; 182.86]	4.4%	5.1%
Fixed effect model	**88**			**88**			**37.05**	**[16.18; 57.92]**	**100%**	**--**
Random effects model							**50.95**	**[20.69; 81.22]**	**--**	**100%**

Heterogeneity: I−squared=45%, tau−squared=2112, p=0.0122

Q = 38.19, Test overall: p-value = 0.0005 (Fixed model), p-value = 0.0010 (Random model)

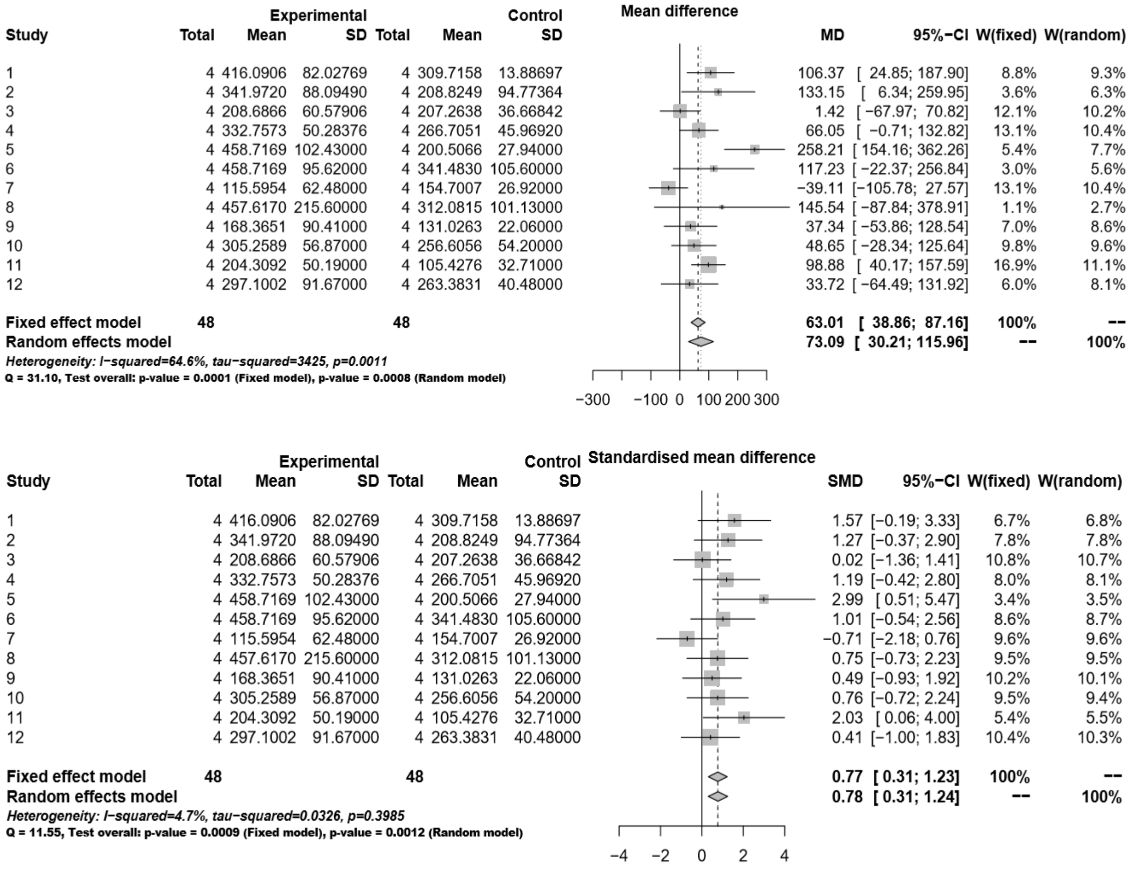


	Experimental			Control			Standardised mean difference			
Study	Total	Mean	SD	Total	Mean	SD	SMD	95%-CI	W(fixed)	W(random)
1	4	428.7634	76.47491	4	343.9000	39.99944	1.21	[−0.41; 2.83]	3.9%	3.9%
2	4	296.6052	27.25899	4	352.5000	42.79021	−1.35	[−3.02; 0.32]	3.7%	3.7%
3	4	310.7104	59.48204	4	348.2000	88.44803	−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	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 model							**0.52**	**[0.20; 0.84]**	**--**	**100%**

Heterogeneity: I−squared=0%, tau−squared=0, p=0.7470

Q=16.40 , Test overall: p-value = 0.0014(Fixed model), p-value = 0.0014 (Random model)

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	Mean	SD	Total	Mean	SD	MD	95%-CI	W(fixed)	W(random)
1	4	416.0906	82.02769	4	309.7158	13.88697	106.37	[24.85; 187.90]	8.8%	9.3%
2	4	341.9720	88.09490	4	208.8249	94.77364	133.15	[6.34; 259.95]	3.6%	6.3%
3	4	208.6866	60.57906	4	207.2638	36.66842	1.42	[−67.97; 70.82]	12.1%	10.2%
4	4	332.7573	50.28376	4	266.7051	45.96920	66.05	[−0.71; 132.82]	13.1%	10.4%
5	4	458.7169	102.43000	4	200.5066	27.94000	258.21	[154.16; 362.26]	5.4%	7.7%
6	4	458.7169	95.62000	4	341.4830	105.60000	117.23	[−22.37; 256.84]	3.0%	5.6%
7	4	115.5954	62.48000	4	154.7007	26.92000	−39.11	[−105.78; 27.57]	13.1%	10.4%
8	4	457.6170	215.60000	4	312.0815	101.13000	145.54	[−87.84; 378.91]	1.1%	2.7%
9	4	168.3651	90.41000	4	131.0263	22.06000	37.34	[−53.86; 128.54]	7.0%	8.6%
10	4	305.2589	56.87000	4	256.6056	54.20000	48.65	[−28.34; 125.64]	9.8%	9.6%
11	4	204.3092	50.19000	4	105.4276	32.71000	98.88	[40.17; 157.59]	16.9%	11.1%
12	4	297.1002	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 model							**73.09**	**[30.21; 115.96]**	**--**	**100%**

Heterogeneity: I−squared=64.6%, tau−squared=3425, p=0.0011

Q = 31.10, Test overall: p-value = 0.0001 (Fixed model), p-value = 0.0008 (Random model)

	Experimental			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**			**0.77**	**[0.31; 1.23]**	**100%**	**--**
Random effects model							**0.78**	**[0.31; 1.24]**	**--**	**100%**

Heterogeneity: I−squared=4.7%, tau−squared=0.0326, p=0.3985

Q = 11.55, Test overall: p-value = 0.0009 (Fixed model), p-value = 0.0012 (Random model)

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.

Table 1. Summary statistics of the pooled data

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

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.

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