INVESTIGATING THE EFFECTS OF INTERACTIONS OF ENVIRONMENTAL FACTORS ON GRAIN QUALITY USING STATISTICAL TECHNIQUES

A Thesis submitted by

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Abstract

The increasing carbon dioxide [CO_2] in the atmosphere increases crop productivity. However, the grain quality of cereals and pulses are substantially decreased and consequently compromise human health. CO_2, temperature, water and nitrogen are considered as the most critical 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.

In this thesis, meta-analysis techniques were employed to investigate the effect of elevated [CO_2] (e[CO_2]) on protein, zinc (Zn) and iron (Fe) concentrations of major food crops including wheat, rice, soybean, field peas and corn considering different levels of temperature, water and nitrogen (N). Each crop, had decreased protein, Zn and Fe concentrations when grown at e[CO_2] concentration compared ambient [CO_2] (a[CO_2]) concentration. However, the responses of protein, Zn, and Fe concentrations to e[CO_2] were modified by water stress and N. There was an increase in Fe concentration in soybean under medium N and wet conditions but nonsignificant. The reductions in protein concentrations for wheat and rice were ~5%–10%, and the reductions in Zn and Fe concentrations were ~3%–12%. For soybean, there was a small and nonsignificant increase of 0.37% in its protein concentration under medium N and dry water, while Zn and Fe concentrations were reduced by ~2%–5%. The protein concentration of field peas decreased by 1.7%, and the reductions in Zn and Fe concentrations were ~4%–10%. The reductions in protein, Zn, and Fe concentrations of corn were ~5%–10%. Bias in the dataset was assessed using a regression test and rank correlation.

Also, randomized trials were carried out based on the conditions of the factorial experiments to show the effect of [e[CO_2]], water, N, and their interactions on protein, Zn and Fe of wheat crop. To determine the effects of interactions of $CO_{2,}$ water and N on protein, Zn and Fe, the designed experiments are implemented in Matlab to investigate all possible possibilities for primary, binary and triple interactions. These results suggested that high [CO_2] concentrations under various levels of environmental conditions affect protein, Zn and Fe concentrations in wheat crop negatively, with protein, Zn and Fe were decreased by 4.5%, 3.5%, 4.1%, respectively, during the three-year experimental period.

The outcomes of this project will inform experts and decision-makers about the effects of CO_2, temperature, water and nitrogen on grain quality, and enable the investigation of suitable solutions.

Certification of Thesis

This Thesis is the work of Ikhlas Ali Hammodi Al-Hadeethi except where otherwise acknowledged, with the majority of the authorship of the papers presented as a Thesis by Publication undertaken by the Student. The work is original and has not previously been submitted for any other award, except where acknowledged.

Principal Supervisor: Prof. Yan Li

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Student and supervisors signatures of endorsement are held at the University.

Statement of Contribution

The following detail is the agreed share of contribution for candidate and co-authors in the presented publications in this thesis:

- **Article I:** Al-Hadeethi, I., Li, Y., Seneweera, S., & Al-Hadeethi, H. (2017, June). Estimating the effects of carbon dioxide, temperature and nitrogen on grain protein and grain yield using meta-analysis. In *Proceedings of the 1st International Conference on Quantitative, Social, Biomedical and Economic Issues 2017 (ICQSBEI2017)* (pp. 107-118). Greek Research Institute for the Study of Quantitative, Social and Biomedical Problems.

  The overall contribution of ***Al-Hadeethi, I.*** was 70% to the concept development, analysis, drafting and revising the final submission; ***Li, Y.*** et al. contributed the other 30% to concept development, analysis, editing and providing important technical inputs.

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Dedication

To
Allah who gave me the power
My beloved parents who prayed for me day and night
My spiritual mother Mama Soaad who have always encouraged me
My soulmate my sister Hanan who consistently supported me
This thesis is dedicated to you

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Table of Contents

List of Figures

List of Tables

Abbreviations

CO_2	Carbon dioxide
$e[CO_2]$	Elevated Carbon dioxide
$a[CO_2]$	Ambient Carbon dioxide
N	Nitrogen
Zn	Zinc
Fe	Iron
SMD	Standardized Mean Differences
WMD	Weighted mean differences
ANOVA	Analysis of Variance
FACE	Free-air CO_2 enrichment technology
MD	Mean difference
Q	Cochran's Statistic
T^2	Tau-squared
I^2	*I*-squared statistic
WHO	World Health Organization
df	A degree of freedom
S.S	A sum of error squares
MS	Mean squares error

CHAPTER 1

Introduction

1.1 Overview and Motivation of the Study

The climate change could result from human activities, including industrial productions, automobile exhausts and logging. These types of activities increase the concentration of carbon dioxide (is a chemical compound of oxygen, and carbon has the chemical formula CO_2) in the atmosphere. If the current trend continues in carbon emissions, temperatures will increase as well. This increase in carbon and temperature will adversely affect the productivity of many crops. As a result, the climate factor is considered the most extensive natural influential member in determining the types of crops that identify areas that can cultivate certain crops. The most crucial climate elements affecting agricultural production are carbon dioxide and temperatures. It was noted that the percentage of carbon dioxide in the earth's atmosphere had risen slightly in recent years. With an elevation of carbon dioxide concentration in the air, the food probably not become nutritionally. The effects of CO_2 on the environment have raised controversy, where it is a material constitute threat to the environment. Also, altitude concentrations of CO_2 threaten global human nutrition by diminution the scales of nutrients of human health (Myers et al., 2014). Many studies shed light on the effects of carbon dioxide on crops (Adams et al., 1997; Asseng et al., 2019; Dietterich et al., 2015; Fitzgerald et al., 2016; Hooper et al., 2015).

Temperature determines the length and type of vegetation growing season. It has importance to assess the production of some yields to maximize economic benefit. Hence, the temperature can influence crop yield significantly if it is cross or decrease the required limit (Liang et al., 2016).

Besides, there is another eloquent factor affecting crops production which is a nitrogen (is a species of fertilizer. Fertilizer is an organic compound or inorganic natural or chemical elements or compounds that are considered individually fertilized soil. The compost material is added to the soil to associate the plant with growing. It contains essential nutrients for plant growth). Farmers utilize significant quantities of fertilizer annually around the world. The increase in production has been reached because of the addition of fertilizer regarding a quarter of the global production of the crop.

However, there is no substantial benefit for nitrogen on crops. Researchers recommended that reducing the nitrogen proportion that gives to the crop since it stimulates magnitude of yield while affects production quality (Njoroge et al., 2014). Furthermore, an ample number of studies have documented the issue of water use efficiency under e[CO_2] levels as well (Chun et al., 2011; Keenan et al., 2013).

CO_2, temperature, water and nitrogen could increase crop quantity but affect yield quality by decreasing proportion of protein in grain subsequently affect human nutrition (Blumenthal et al., 2008; Challinor et al., 2016; Ward, 2007).

Protein is an essential part of the synthesis of a living organism which composes the central part of the enzymes. It is one of the most critical construction materials of plant cells. It is essential for the growth and renewal of tissues, protein also plays a vital role in metabolic processes that occur within the plant, and all the enzymes that help these processes are proteins. Furthermore, it has an active role in the stages of growth and development of plants, especially in crops (Herman & Larkins, 1999). Proteins are composed of building blocks called amino acids. There are twenty different amino, intervention in the synthesis of all proteins, and five of them are carbon, hydrogen, Oxygen, Nitrogen and sulfur (Gamuyao et al., 2012; Rogers et al., 1996). Protein is an essential source for the human body. It's found in muscle, bone, skin, tissue and hair. Protein malnutrition leads to the position known as kwashiorkor, deficiency of protein in human nutrition can cause growth bust (Bernstein et al., 2010). Healthy protein sources exist in the agricultural crop products; many types of research showed that eating quasi one daily serving of beans, lentils, chickpeas and peas can boost fullness by which people can manage their weight (S. S. Li et al., 2014).

Dependents on the latest studies, rising CO_2 begin effect on quality (Pleijel & Uddling, 2011). Where concentrations of the essential elements started in the food (grain), in particular, to fall, causing severe health problems could threaten human life. Since, there are vast numbers of people who rely on the grain for feeding where it is a vital source for iron, zinc. And the iron is responsible for several vital functions in the human body (Myers et al., 2014), and any imbalance in these critical functions lead to morbidity, death and other nutrition elements.

In recent years, many publications were conducted to analyse the impact of CO_2, water and nitrogen on crops using various procedures. Statistical methods are essential instruments to study the influence of climate factors on yields to resolve fundamental issues concerning nutrients. I will use one of the most beneficial statistical tools which is meta-analysis to analyse the effect of CO_2, water and nitrogen on crop protein, Zn, Fe and grain yield then I will expand our techniques to extract the interaction of CO_2, water and N on grain protein, Zn and Fe.

Meta-analysis is a statistical method or a set of statistical methods for combining the results of various studies into a pooled estimate of the effect size. The effect size measures in the meta-analysis depend on the species of the outcome variables. There are two kinds of outcome variables, binary outcome and quantitative. The reported effect size of the binary outcome variables includes odds ratios, risk ratios and risk differences. In contrast, the quantitative outcome variables are the standardized mean difference (SMD), weighted mean difference (WMD) and correlations coefficient (Borenstein et al., 2011). Meta-analysis equips a technique to combine the effect size of all studies to obtain a pooled estimate of the common effect size. There are three models in the meta-analysis (Sutton, 2005), a fixed-effect model (FE) postulates that there is one right effect size for all the studies. That means that all studies are estimating the same effect size, then the combined effect size estimate based on the studies included in the meta-analysis. Random effects model (RE) presumes that the real effect could diverge from study to study. Based on that assumption, each study estimates different effect size and assumes that there is a distribution of proper effect sizes. Under the random-effects model, the mean of this distribution estimates by pooling the effect size of the studies (Goffinet & Gerber, 2000). The IVhet model occupies a position that can procure the fixed effect model-based estimator variance closer to the observed variance by modelling over-dispersion via a quasi-likelihood procedure (Doi et al., 2015). Meta-analysis uses the weighted mean of the effect sizes rather than the simple arithmetic mean. In the fixed-effect model, allocate weights depend on the inverse of the variance. That means the inverse of its variance weights each study, and the variance here is the within-studies variance. The inverse variance approach is used to diminish the variance of the combined effect (Sutton, 2005). In the random-effects model, it also uses inverse variance weights. That means the inverse of its variance weights the effect size of each study. However, the variance here accounts for both the within-studies variation and the between-studies variation. Meta-analysis is widely used in various fields and has abundant applications in medical and social research (Sutton, 2005). Recently, there is a demand for the use of meta-analysis to resolve the controversy about important issues relating to human life because it provides a crucial decision for the decision-makers (Sutton, 2005). Meta-analysis allows for and thematic appraisal of the evidence, which may lead to resolution of suspicion and disagreement (Normand, 1999). There have been many publications investigating the avail and robustness of meta-analysis in biological research such as Shu K Lam et al. (2012); Pan et al. (2016); Haworth et al. (2016).

The experimental design is an accurate balancing of various features including "power", generalizability, different forms of "validity" and practicality. A robust balancing of these features in anticipation will result in an experiment with the best opportunity of providing beneficial evidence to modify the current case of knowledge in a particular scientific area. On the other hand, it is regrettable that many experiments were designed with preventable blemishes. It is only scarcely in these situations that statistical analysis can be the deliverance of the experimenter (Hicks, 1964). The aim is always to actively design an experiment that has the most significant occasion to produce meaningful and justifiable evidence, rather than expecting that proper statistical analysis might be able to correct flaws after the effect. An experimental design is a procedure for planning a study to gather specified goals. Decently planning an experiment is essential to ensure that the right species of data and appropriate sample size and power are obtainable to answer the research questions of interest as obviously and expeditiously as potential (Hinkelmann & Kempthorne, 1994). Factorial experiments include more than one factor concurrently, each at two or more levels. The experimenter is often concerned about the main influences and the interaction effects of various factors (Skillings, 2018). Analysis of variance for a factorial experiment permits investigations into the impact of two or more variables on the mean value of a response variable. Also, diverse combinations of factor 'levels' can be investigated. It is common to repeat a factorial experiment at least two times (Anderson & McLean, 2018). A factorial design is frequently utilized by scientists to comprehend the effects of two or more independent variables upon one dependent variable. Classical research methods mostly study the impact of one variable at a time due to it is statistically easier to manipulate.

To the knowledge of the author, 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. Also, the existing studies have been limited to analysing the impact 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. Besides, a new procedure based on the *dplyr* package in R program will be developed to re-processing data to facilitate meta-analysis.

There are not many published studies on how [CO_2], water and N affect grain protein, zinc and iron concentrations. In addition, most related studies have not been reported. There is a large knowledge gap on how crops response to [CO_2], water and nitrogen. In this project, I hypothesized that grain protein, Zn and Fe concentrations are reduced under e[CO_2], but their responses are modified by factors, such as water stress and nitrogen availability.

There is a minimal understanding on how the interactions of e[CO_2], water and N influenced grain quality traits, such as protein, Fe and Zn within a range of functional groups. In addition, neither of those researches concentrated exclusively on the influences of interactions of e[CO_2] with essential factors, such as water and N fertilization on crop' nutrient composition. Also, there are not many kinds of research on how the interactions of e[CO_2], water and N influence on grain protein, Zn and Fe concentrations. Also, the impacts of the interactions of e[CO_2], N supplies and water on nutrients in crops are still not clear (Al-Hadeethi et al., 2017; Al-Hadeethi et al., 2019).

There are significant knowledge gaps on how crops respond to the interactions of e[CO_2], water and N. When there is more than one factor influencing the production of a particular crop, and each factor has more than one level, there is a need to conduct randomized trials of a specific kind called factorial experiments. Due to it offers the possibility of indicating the significance of each factor as well as the importance of the interactions among them.

In this research, different random trials of the wheat crop were taken. The effects were measured on protein, Zn and Fe by considering several factors of e[CO_2], water and N. This research is also involved in studying the impacts of the interactions of the three factors on nutrient compositions in wheat. The proposed method based on factorial design is implemented to accommodate all potential possibilities for primary, binary and triple interactions. Emphasis was placed on binary and triple interactions. This algorithm produced 49 trials for each experiment conducted for over three years. To the best of my knowledge, there have been no studies that discussed these combinations for each experiment.

1.2 Research Problems

In this research project, I am going to answer the following questions:

1. How to measure the impact of CO_2, temperature, water and nitrogen on grain protein, grain Zn, grain Fe and grain yield?

2. What are the impacts of the interaction of CO_2, water and nitrogen on grain protein, grain Zn, and grain Fe?

The main goal of the proposed research is to develop new techniques for analysing the effects of CO_2, temperature, water and nitrogen on crop quality for understanding how climate changes impact on food nutrient and security. The outcomes of this research can potentially help experts and decision-makers to forecast the corresponding food changes under future climate changes. Two main objectives of this PhD research are to:

1. Analyse the data of several agricultural crops using meta-analysis models to determine the impact of CO_2, water, temperature and nitrogen on grain protein, grain Zn, grain Fe and grain yield.
2. Develop a new model to discover the influence of the interactions among CO_2, water and nitrogen on grain protein, grain Zn and grain Fe.

1.3 Contributions of the Thesis

1. This study developed an effective method for estimating the impacts of CO_2, temperature and nitrogen on grain protein and grain yield. In this work, a new approach based on the *dplyr* package in R was proposed for organizing and categorizing the research data for meta-analysis. The performances of the proposed methods were evaluated using various measurements, such as the Cochran's Q statistic and its *p*-value, I^2 statistic, and tau-squared. Overall, this study aimed to reveal the significance and reliability of a meta-analysis in analysing the effects of carbon dioxide, temperature and nitrogen on the quality of 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 could 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 on the impacts of CO_2, temperature and nitrogen on grain quality, and enable the investigation of suitable solutions.

2. The developed method, meta-analysis, achieved excellent results and accuracy with the effects of CO_2, temperature, water and nitrogen on grain quality, and enable the investigation of suitable solutions. Meta-analysis has been employed to investigate the impacts of elevated [CO_2] (e[CO_2]) on protein, Zn and Fe concentrations of major food crops (542 experimental observations from 135 studies) including wheat, rice, soybean, field peas and corn considering different levels of water and nitrogen (N). Each crop, except soybean, decreased protein, Zn and Fe concentrations when grown at e[CO_2] concentration (≥ 550 μmol mol^{-1}) compared ambient [CO_2] (a[CO_2]) concentration ($\leq$ 380 μmol mol^{-1}). Grain protein, Zn and Fe concentrations were reduced under e[CO_2]. However, the responses of protein, Zn and Fe concentrations to e[CO_2] were modified by water stress and N. There was an increase in Fe concentration in soybean under medium N and wet conditions but non-significant. The reductions in protein concentrations for wheat and rice were ~ 5-10%, and the reductions in Zn and Fe concentrations were ~ 3-12%. For soybean, there was a small and non-significant increase of 0.37% in its protein concentration under medium N and dry water (the water amount include only precipitation or without precipitation and irrigation (Dietterich et al., 2015)) while Zn and Fe concentrations were reduced by ~ 2-5%. The protein concentration of field peas decreased by 1.7%, and the reductions in Zn and Fe concentrations were ~ 4-10%. The reductions in protein, Zn and Fe concentrations of corn were ~ 5-10%. Bias in the datasets was assessed using regression test and rank correlation. The analysis indicated that there were medium levels of bias within published meta-analysis studies of crops responses to Free Air Carbon Dioxide Enrichment (FACE). However,

integration of the influence of reporting bias did not affect the significance or the direction of the [CO_2] effects. These results suggested that increased atmospheric [CO_2] concentrations under different levels of environmental conditions were likely to decrease protein, Zn and Fe concentrations of many food crops.

1. Proposed an efficient method, based on factorial experimental design to study the effect of carbon dioxide [CO_2], water and nitrogen [N] and their binary and triple interactions on the quality of grain.

   Randomized trials were carried out based on the conditions of the factorial experiments to show the effect of elevated carbon dioxide [e[CO_2]], water, N and their interactions on protein, zinc [Zn] and iron [Fe] of the wheat crop. To determine the effects of interactions of CO_2, water and N on protein, Zn and Fe, the designed experiments were implemented in Matlab to investigate all possible possibilities for primary, binary and triple interactions. Emphases were placed on binary and triple interactions. Developed the algorithm based on factorial design to study all possible interactions for three factors (e[CO_2], water and N) on protein, Zn and Fe of the wheat crop. The analysis revealed that all three factors in the three models harmed protein, Zn and Fe values in the wheat crop. These results suggested that high [CO_2] concentrations under various levels of environmental conditions affected protein, Zn and Fe concentrations in the wheat crop negatively, with protein, Zn and Fe were decreased by 4.5%, 3.5%, 4.1%, respectively, during the three-year experimental period.

1.4 Structure of the Thesis

This thesis consists of six chapters, structured as follows:

Chapter 2 provides an overview and background of the effectiveness of climate factors which include CO_2, temperature, water and nitrogen on crops generally and on grain protein, Zn and Fe particularly.

Chapter 3 introduces a useful tool for supporting decision-makers and an efficacious statistical procedure to estimate the influence of CO_2, nitrogen and temperature on grain protein and grain yield utilizing meta-analysis. It was displayed the importance and precision of a meta-analysis in analysing the impacts of carbon dioxide on quality of grain. In this chapter, a new process based on the *dplyr* package in R is suggested for arranging and assorting the study data for meta-analysis. The performance of the presented techniques are assessed using diverse statistics, for instance the Cochran's Q statistic and its p-value, τ-squared and I^2 statistic.

Chapter 4 presents a further enhancement to expand the data of several agriculture crops and the factors to boost the reliability of the procedure of meta-analysis. Meta-analysis models were used to explore the impact of elevated [CO_2] (e[CO_2]) on protein, zinc (Zn) and iron (Fe) concentrations of main food crops (542 experimental observations from 135 studies) including wheat, rice, soybean, field peas and corn taking into account several levels of water and nitrogen (N). The advanced technique, meta-analysis, accomplished outstanding outcomes and precision with the influences of CO_2, water and nitrogen on grain species, and allow the implementing of proper solutions.

Chapter 5 proposes an efficient method, depend on factorial experimental design to research the influence of carbon dioxide [CO_2], water, and nitrogen [N] and their binary and triple interactions on the protein, Zn and Fe grain. In this chapter, randomized trials were implemented out depend on the situations of the factorial experiments to present the impact of elevated carbon dioxide [e[CO_2]], water, N, and their interactions on protein, Zn and Fe of the wheat crop. To define the impacts of interactions of $CO_{2,}$ water and N on protein, Zn and Fe, the designed experiments are carried out in Matlab to explore all potential probabilities for elementary, binary and triple interactions. Assurance was located on binary and triple interactions. The algorithm based on the factorial design was enhanced to examine all probable interactions for three factors (e[CO_2], water and N) on protein, Zn and Fe of the wheat crop.

Chapter 6 provides a summary and the findings of this research presented in this thesis. This chapter also discusses the ideas for future work.

Appendices A provides the simulation code for the proposed approach, which is presented in Chapters 5.

CHAPTER 2

2. Background

There is extensive research work reported about the effectiveness of climate factors which include CO_2, temperature, water and nitrogen on crops generally and on grain protein, Zn and Fe particularly. In this section, we review some of the related research work.

2.1 Carbon dioxide impacts on crops

Carbon dioxide may contribute to increasing the amount of crop and the velocity of its growth rate, but this will be at the expense of quality. Rising levels of CO_2 increase rice yield but change the quality of rice (Ward, 2007). The high proportion of carbon dioxide in the atmosphere affect the installation of grain and thereby alter the type of the crops, at a higher percentage of carbon dioxide, the protein of the crops is influenced clearly (Dietterich et al., 2015). Some crops in response to an increase in carbon dioxide are varying significantly when other elements process them; there is a big difference between the reaction of the wheat crop when exposed to high carbon dioxide and when exposed to O_3 (Pleijel & Uddling, 2011). With the continuous rise of carbon dioxide, the yield will be affected even in the organic functions. It is expected that carbon dioxide increases significantly by 2050. This will affect the physiology and the productivity of the crop, especially wheat crop (Ainsworth & Long, 2005; Fitzgerald et al., 2016).

Moreover, the CO_2 concentration in the atmosphere is causing many problems in the soil, such as water shortages and severe drought and the loss of some essential elements in the soil including calcium, iron, copper, potassium, manganese, and magnesium which significantly affect the nutrition for both humans and animals (Duval et al., 2012; Erbs et al., 2015; Franzaring et al., 2010). However, various anterior studies notified that elevated CO_2 reinforced the growth of maize beneath thoroughly watered and fertilized situation and precipitation in the process of senescence in wheat. There was slight evidence of boosted biomass accumulation (Hooper et al., 2015; KIM et al., 2006).

2.2 Influence of temperature on yield

The temperature must be at a particular degree for each crop during the growing season. Each crop has a favourite temperature for the growth, and the degree of micro temperature does not grow underneath, and the degree of maximum temperature does not grow above. Liang et al. (2016) showed that in the winter when the temperature is zero for five consecutive days, roots stop growing and when the temperature transcend zero for successive days, the origins starts growing again. Also, each type of crops has a different response to high temperature.

Furthermore, Thornton et al. (2014) and Sánchez et al. (2014) mentioned that rice, wheat and maize are the most sensitive crop to temperature. Each crop needs a specific temperature for growth if the temperature increased or decreased a particular threshold; the crops could be dead. Estrella et al. (2007) demonstrated that eternal crops displayed a sufficiently higher temperature response to an average spring temperature than the annual crops. Rising temperatures cause crops stress. That can adversely affect grain formation and other aspects of crops growth. Wu et al. (2016) illustrated that the temperature affects some phenolic compounds in the sorghum, which is considered one of the important sources of dietary antioxidants. Challinor et al. (2016) indicated that high and low temperatures adversely affect the maize durations and its breeding. Asseng et al. (2015) explained that wheat crop production gradually decreases with temperature increasing.

Furthermore, Valizadeh et al. (2014) showed that the wheat crops growing decreased as a result of rising in temperature also in the future wheat production would be influenced by climate change. García et al. (2015) elucidated that the high temperature affects nitric oxide emission and there is a positive correlation between them which is connected with rice-wheat. Tack et al. (2015) said that the total effect of warming temperature on wheat yields is passive despite the benefits of rising temperature in terms of increased crop growth (Lv et al., 2013). Lobell et al. (2012) expounded that extreme heat hurts wheat crops through accelerating the caducity of grain.

High temperature is a severe threat to the productivity of the crop spread through rodents and diseases. Brzostek et al. (2012) detected that there is a relationship between temperature and pests rendering and how that led to crops damage. Lobell et al. (2012) revealed that a high-temperature increases spread potato beetle in north Europe. García et al. (2015) established that high night temperature has a negative influence on wheat and barley yield during shortening grain yield duration. Zhang et al. (2016) described that the elevation of temperature changes photosynthetic product in wheat seeds and developed bacteria, fungi and actinomycetes.

2.3 The effect of temperature on quality of crops

For bread making, the quality of flour produced from grain developed at high temperatures is poorer. High CO_2 may also have an effect through a reduction in the protein content of wheat grain. For rice, the amylose content of the grain, a major determinant of cooking quality is increased under elevated CO_2 (Conroy et al., 1994). Highest oil content in rape and flax was found at the lowest temperature and a continual decrease was observed with increases in temperature. Fatty acid composition of the oil from safflower and castor bean was not affected by a change in temperature. In the other three species the amount of the more highly unsaturated fatty acids decreased as the temperature was increased (Canvin, 1965). The heating rate and temperature are the most critical parameters in controlling the performance of biomass pyrolysis, particularly with reference to the yield distribution of solid, liquid, and gaseous products (Laird et al., 2009). Charcoal is an important product of biomass-based polygeneration, which can significantly advance the economical profitability of the polygeneration system for the operator. The charcoal content was reduced greatly as the temperature increased (Chen et al., 2012).

2.4 Effect of CO_2 and temperature on crops

Climate change and agriculture operations are interconnected operations that happen on a global scale. The continued increase in the proportion of carbon dioxide in the atmosphere will lead to a rise in temperature. Temperature and CO_2 have significant impacts on conditions that affect agriculture, especially crops yield of grain down with the simultaneous rise CO_2 and temperature (Ingvordsen, 2014). When beans grain exposed to high carbon dioxide under high temperature, the temperature affects the seeds of beans negatively, while carbon dioxide has no possesses any grain effect, overall, there is no advantageous interaction of temperature and CO_2 (Vara Prasad et al., 2003).

Moreover, there is no noticeable impact of the interaction of carbon dioxide and temperature on respiration in crops (Rogers et al., 1996). High temperature and carbon dioxide affect the decrease in grain crops more than rising carbon dioxide alone (Moya et al., 1998). However, increased CO_2 and high temperature raise rice crop productivity and root biomass and thus catalyse methane emission (Cheng et al., 2008; Moya et al., 1998). Although, simultaneous under ambient CO_2 and temperature and high CO_2 and temperature, methane emission decline when using adjusted rice straw biochar (Schrope et al., 1999). Interaction of CO_2 and N_2O has a substantial impact on greenhouse gas emissions under elevation temperature and therefore affect crops production (Rogers et al., 1996). The enhance in temperature in some crops such as wheat, rice and maize leads to a decline in grain yield. At the same time, rise in carbon dioxide causes an increase of grain yield whereas the combination of high temperature and CO_2 resulted in a reduction of grain crops (Bassu et al., 2014; Cai et al., 2016).

Furthermore, it expected by the end of the 21st century that the climate change due to increase of carbon dioxide and temperatures accelerated the loss of several crops such as wheat, rice and peanuts (Conroy et al., 1994; Ruane et al., 2014). Temperature, humidity and light cause deficiency in stomatal conductance in wheat and barley at high CO_2 (Ruane et al., 2014). However, elevated CO_2 can be beneficial on a particular crop where rising CO_2 moderates the effect of temperature on the coffee crop (Rodrigues et al., 2016).

2.5 Impact of nitrogen on yield

Increased nitrogen deposition has ultimately influence on forest and lake food webs, thus affect alimentary levels (Liu et al., 2014). Also, nitrogen affects mountain grasslands, its decrease in plant species but increase plant biomass furthermore, there are abiotic factors that interact with nitrogen lead to plants alterations (Humbert et al., 2016). Whereas, nitrogen has a positive effect on the growth and yield of plants (Cheng et al., 2008). Adding nitrogen is salutary for crops includes wheat, rice, maize, peas and lettuce, it increases the growth of yield and bulk of grain (Ali et al., 2011; Cheng et al., 2008; Manzoor et al., 2006; Millner & Hardacre; Stevovic et al., 2006).

2.6 The effect of nitrogen on quality of crops

The abnormal increase in nitrogen impeded the process of balancing the protein content and carbohydrate content which negatively affected the production by delaying the entry of the plant's maturation stages. Also, increasing the nitrogen of the distant boundaries of the necessary needs led grain crops to produce a crop without grain. The increase in nitrogen obtained in legume crops would increase protein levels. This is due to the fact that nitrogen is the main constituent of amino acids and protein acids that are the basis of proteins in the plant (Al-Hadeethi et al., 2019). Nitrogen often affects amino acid composition of protein and in turn its nutritional quality. In cereals, abundant supply of nitrogen decreases the relative proportion of lysine and threonine, thus, reducing the biological value of the protein. Increasing nitrogen supply generally improves kernel integrity and strength, resulting in better milling properties of the grain. In oil seed crops, protein levels are increased upon nitrogen fertilization, whereas oil concentration is decreased (Blumenthal et al., 2008).

2.7 Protein response to CO_2

Limited publications indicated that CO_2 affects grain composition and has a significant impact on protein grain. Elevated CO_2 causes raise carbohydrate in the grain that leads to a decline in the nitrogen simultaneously high CO_2 boost grain size, however, influence grain quality through decrease protein concentration (Panozzo et al., 2014). Besides, increase CO_2 affect dramatically gluten protein in the wheat crop which is considered one of the most important elements associated with the quality of bread (Fernando et al., 2015; P Högy et al., 2009). Moreover, the interaction between CO_2 and other elements can affect functional processes in crops. Ibrahim et al. (2011) mentioned that interaction between nitrogen and high CO_2 involved in increasing phenylalanine which is an amino acid widely distributed in crop proteins this remarkably influences crop subaltern metabolite production. Overall, rising CO_2 hurts protein in most crops subsequently affect human nutrition (Taub et al., 2008).
Some research has been indicating that carbon dioxide affects grain protein. When the crop is under increasing of CO_2, protein decreases and its composition will change that has an impact on the content of the grain thus yield quality (Conroy et al., 1994; Erbs et al., 2010; Fernando et al., 2012; Fitzgerald et al., 2016; Högy & Fangmeier, 2008; Petra Högy et al., 2009; Nuttall et al., 2017; Saxe et al., 2014).

2.8 Protein response to temperature

In the literature, individual studies found that high temperature has an impact on some of the protein components which affect crops composition. One of the crucial reasons for the diminution of amino acids in the wheat crop is an increased temperature that leads to a decline in photosynthetic produce content (Corbellini, Canevar, et al., 1997; Corbellini, Mazza, et al., 1997; Dupont et al., 2006).

2.9 Protein response to nitrogen

An exuberance of nitrogen affects negatively on cereals protein through reduction of lysine and threonine which is necessary components in amino acid thus, diminishing the vital value of the protein (Blumenthal et al., 2008). However, grain protein concentration raised under nitrogen fertilizer that enhanced yield immutability (Clayton et al., 2004).

2.10 Protein response to carbon dioxide and temperature

Recently there are some debates over the effects of the interaction of temperature and carbon dioxide on the crop protein. Available research indicate that there is no considerable variations in the impact of high CO_2 on wheat seed protein between crops grown at elevated vs the reduced temperature. However, the direction has been for high CO_2 that have the most significant effect on the protein at elevated temperature than at the reduced temperature (Johnson et al., 2010; Taub et al., 2008).

2.11 CO_2 impacts on Zn and Fe

The most important study about the high level of CO_2, and how effects the quality of wheat, by assembling the experimental data available in the published studies (Broberg, 2015). As for the attention of dietary minerals such as (iron, phosphorus, magnesium, calcium, manganese and zinc), it was decreased significantly. With a high carbon dioxide concentration in the atmosphere, where the crops absorb more carbon. That will increase the returns in terms of carbohydrates, but softens the minerals contained in the crop (Saxe et al., 2014). This study evaluated implications that would change the composition of the chemical. It is known that the high temperatures during the grain-filling period are influencing the quality of rice, and there was a negative impact on grain quality through increased carbon dioxide (Ward, 2007). High CO_2 could reduce nutrient concentrations, by reducing carbohydrate ratio. Where it was on the effects of CO_2, calcium, copper, iron, potassium, magnesium, manganese, P, S, and zinc among the four groups of plants and two levels of nitrogen fertilization, note the drop in copper, iron, and magnesium.

As a result, the increase of carbon dioxide rises linked, in an apparent reduction, carbohydrates, leading to reduce the response, nutrients (Duval et al., 2012). Climate change is the most important reasons that may affect human health, as it represents a key to changing the nutritional content of the food role. However, there have been previous attempts to study the effects of increased CO_2 in the atmosphere. Deficiency of iron and zinc in nutrition is considered a global health problem, with international reports indicate that about 2 billion people suffer from this lack, and this is leading to the deaths of 63 million people around the world because these people depend mainly on grain nutrition. This research has concluded the concentrations of zinc and iron are affected under a high level of CO_2 concentration (Myers et al., 2014). Reports indicate that the increase in CO_2 and greenhouse gases (GHGs) in the atmosphere. It is expected that these emissions affect the rice harvest, and this is important because rice is the second largest production of staple crops. With time, many problems began to emerge and become pose a threat to crops, since the wheat of the most important food crops as they represent an essential source of nutrition. The effect of uranium CO_2 on crop and quality of grain as observed adverse effects, biomass and characteristics of quality, as well as influenced by the concentrations of amino acids, also metals decline such as potassium, molybdenum, lead increased. At the same time, manganese, iron, cadmium and silicon dropped, plus they're found high-fat ratio (Petra Högy et al., 2009).

2.12 Meta-analysis

For the above sections of literature, studies have been used traditional statistical instruments, for instance, t-test, χ^2 and R^2 to indicate their results. This section reviews some of the research in which meta-analysis was used.

Providing an excessive quantity of CO_2 affect legumes and rice by increasing a gemmation process as well as decreasing seed nitrogen (Jablonski et al., 2002). Land utilize alterations affect soil C stocks where one of them reduce soil C stocks, whereas others increased it (Guo & Gifford, 2002; Shu Kee Lam et al., 2013). However, rising in a temperature minimizes responses of plants to the impact of high CO_2 (Zvereva & Kozlov, 2006). Raised CO_2 enhances soil gases emissions such as N_2O and CH_4, also affects concentrations of nutrient in the plant which tends to reduce the concentration of certain nutrients while boosts concentration of other mineral elements (Duval et al., 2012; Van Groenigen et al., 2011). Temperature and lowering in precipitation had a significant impact on the organic horizon via increase proteolytic vitality whereas did not influence the mineral soil that affects the fundamental element of the nitrogen cycling (Brzostek et al., 2012). Nitrogen addition has a considerable impact on a plant where elevated nitrogen tends to increase soil respiration as well as biomass production (Y. Li et al., 2016). Besides, belowground nitrogen and carbon affected negatively by grazing (Zhou et al., 2017). It is not potential to identify that there is an effect of interaction high CO_2 and temperature on woody plants (Baig et al., 2015).

2.13 Factorial design

The factorial experimental design was utilized to investigate the impacts of storage time, storage temperature and packaging kind on the colour steadiness of sun-dried tomatoes (Akdeniz et al., 2012). The major effect was located to be the time having the highest coefficient (-3.3 for L and -0.12 for a/b) in storage temperature. The design models were detected to be the least influential factor (Akdeniz et al., 2012). This design provided the best information for the influences of independent variables and their interactions on an experimental model (Dehghan et al., 2010); (de Camargo Forte et al., 2003). Experimentation including discolouration of Orange II has been carried out utilising factorial design procedure for the simulation of the three sensible variables impacting the dye discolouration: concentration of Orange II, pH and concentration of TiO_2. By employing this model, a minimum of well-chosen experiments was implemented to optimise Orange II photo-discolouration. The correlation factor between the experimental and predicted values by the polynomial expression for the discolouration of Orange II was better than 95%. In a typical photo-reactor run at pH_3, the concentration of TiO_2 could be reduced to values <0.5 g/l without affecting the discolouration kinetics (Fernandez et al., 2002). A couple of researchers investigated critically the utilisation of factorial and response surface methodology in modern experimental design and optimization. A survey of significant screening and optimization methods in the literature since 2000 was introduced. Current studies in food technology, biological, environmental and pharmaceutical analysis and industrial-related processes are investigated. Experimental design and optimization methodology are significant in modern research and development efforts. In combination, these two designs can assist in optimizing experimental techniques in a decreased number of studies as well as supplying important information for suitable decisions of the future. This technique is adverse to the traditional univariate approach. Univariate techniques are time-consuming in that the response is examined for each factor while all other factors are held at a fixed level. This method is relatively plain and appropriate for factors that are independent. However, univariate approaches do not take interactive impacts between factors into account. If the impacts are collective in nature, then experimental designs are the optimum choice and require fewer measurements. To address the above concerns, and meet the demands of modern research, suitable experimental design methods considering all factors and their potential interactions were performed (Hanrahan & Lu, 2006). A two-level factorial experimental design approach was utilised to examine the impact of the operating parameters on the production of tomato powder from tomato paste during the spray drying operation. A factorial model was built and used to study all interactions among the considered parameters. Generality interactions between the studied parameters were insignificant (Al-Asheh et al., 2003). Factorial designs are beneficial to psychologists and field scientists as a preliminary study, allowing them to judge whether there is a link between variables while reducing the possibility of experimental errors and confounding variables (Shuttleworth, 2009). A factorial design was carried out to determine the optimal procedure parameters for increased phenol uptake by activated carbon ZSL-C which was made from brown seaweed biomass. The study revealed that the factorial designing was the best instrument for studying the impact of main process parameters on the response factor by significantly decreasing the number of days and henceforth saving time, money and energy (Rathinam et al., 2011). The produce of fatty acid methyl esters, to be utilised as a diesel substitute (biodiesel), was also researched. The procedure of biodiesel production was optimized by the implementation of the factorial design and response surface methodology (Vicente et al., 1998). The factorial design is a robust instrument to analyse the significance of the modifications of the major operation independent variables of a stack. This method was applied to obtain the impacts of the major stack operation independent variables on the cogenerative performance of a proton exchange membrane fuel cell (PEMFC) stack (Torchio et al., 2005). A factorial design was used as a statistical approach to facilitate the process of making the activated carbon (AC) from rice husk by chemical activation. The study elucidated the importance of the multivariable test, decreasing the number of experiments to assess all parameters, making outcome analysis easier, and facilitating the observation of interaction among variables (Isoda et al., 2014). It is substantial to notice that the factorial design was a powerful method to gain the best conditions of extraction for the herbicide atrazine (AT), de-isopropyl atrazine (DIA) and deethylatrazine (DEA) from an oxisol soil sample, particularly the definition of the lower contact time of 30 min (Amadori et al., 2013).

Agricultural science, with a need for field-testing, often uses factorial designs to test the effect of variables on crops. In such large-scale studies, it is difficult and impractical to isolate and test each variable individually.

The factorial design was carried out to obtain the impact of symbiotic N_2 fixation on plants under high CO_2. The outcomes demonstrate that infertile soil and under temperate climatic conditions, symbiotic N_2 fixation *per se* is accountable for the frequently greater magnitude of above-ground biomass and the higher N yield under elevated atmospheric CO_2 (Lüscher et al., 2000). The effect of e[CO_2], temperature (T) and water availability on N_2 fixing alfalfa plants were examined by a factorial design with three factorial treatments (CO_2, temperature and water availability). The results showed that there were no impacts on plant N concentration (Aranjuelo et al., 2005). A factorial design was performed for the independent variables cultivar, CO_2 levels, and irrigation regime; as well as for their interactions on two tomato cultivars. The outcomes displayed that plant water condition was negatively influenced by diminished irrigation regimes but positively affected by high [CO_2] (Pazzagli et al., 2016). A factorial experiment was conducted to determine the effects of e[CO_2], soil temperature and soil N on root development, biomass and nutrient uptake of winter wheat. It was discovered, as predicted, significant soil temperature influences on most of the variables measured (Gavito et al., 2001). The results illustrated that increasing [CO_2] to levels expected for the end of this century leads to a boost in the overall growth of rice crop. However, the results also displayed that the beneficial influence of high [CO_2] on total biomass of rice crop altered incredibly with growth stage, with an overall decrease in response with crop evolution (Myers et al., 2014). The influences of high CO_2 on nutrients on grain quality in spring wheat crop were investigated using the experimental design. This technique revealed that grain quality was diminished by CO_2 enrichment (Fangmeier et al., 1999). Several groups of researchers performed a factorial design to examine the effect of e[CO_2] on crop nutrients (Myers et al., 2014; Fangmeier et al., 1999; Erbs et al., 2010; Pleijel & Danielsson, 2009; Borrill et al., 2014; Petra Högy et al., 2009). They discovered that some nutrient compositions reduced in crop under high [CO_2].

CHAPTER 3

ESTIMATING THE EFFECTS OF CARBON DIOXIDE, TEMPERATURE AND NITROGEN ON GRAIN PROTEIN AND GRAIN YIELD USING META-ANALYSIS

3.1 Introduction

Al-Hadeethi, I., Li, Y., Seneweera, S., & Al-Hadeethi, H. (2017, June). Estimating the effects of carbon dioxide, temperature and nitrogen on grain protein and grain yield using meta-analysis. In *Proceedings of the 1st International Conference on Quantitative, Social, Biomedical and Economic Issues 2017 (ICQSBEI2017)* (pp. 107-118). Greek Research Institute for the Study of Quantitative, Social and Biomedical Problems. (Published)

A meta-analysis is a useful 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 critical 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 my 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 impact 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 tau-squared. Overall, this study aimed to reveal the significance and reliability of a meta-analysis in analysing the effects of carbon dioxide, temperature and nitrogen on the quality of 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 could 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 on the impacts of CO_2, temperature and nitrogen on grain quality, and enable the investigation of suitable solutions.

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)

$$T_i = \mu + u_i. \qquad (1)$$

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:

$$w_i = \frac{1}{v_i}, \qquad (2)$$

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

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

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

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

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

The standard error of the combined effect is

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

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

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

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

$$p = 1 - \varphi(|Z|), \qquad (9)$$

For a two-tailed test by

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

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

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

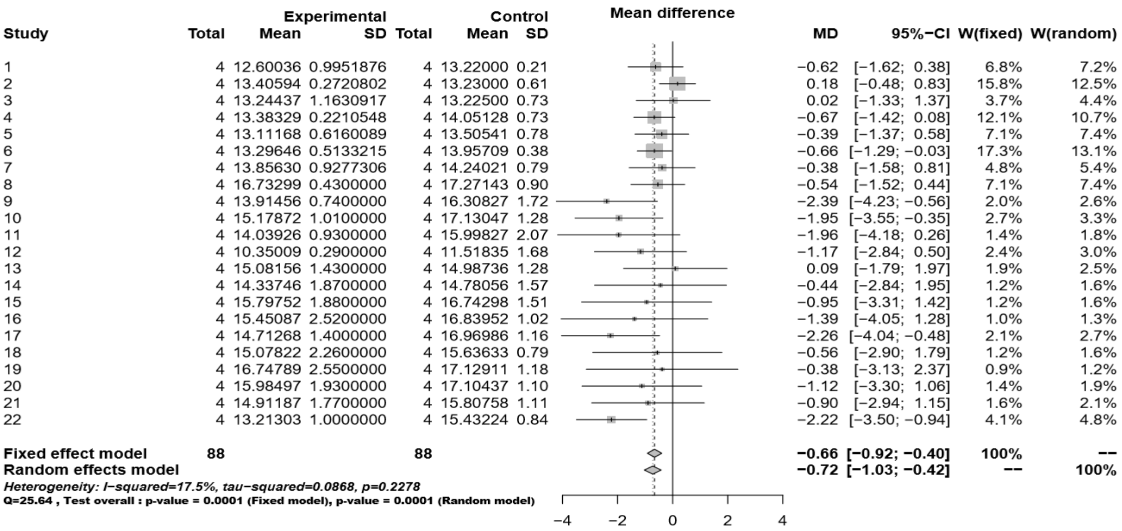
The two-tailed *p*-value by

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

where φ: the standard normal cumulative distribution function.

3. Results

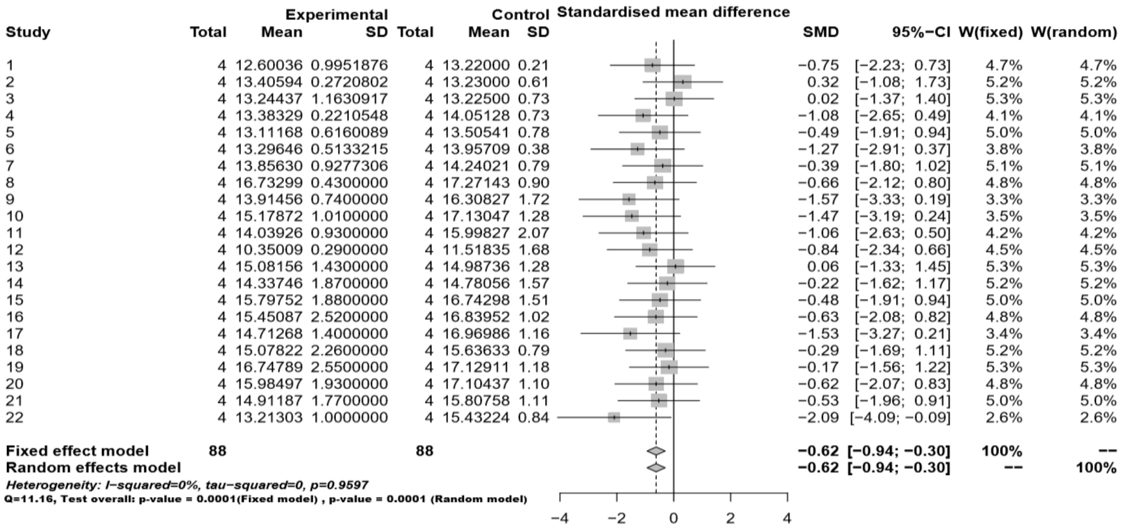
The effect size in the fixed effect model, and the random effects model (p-value) illustrates that there is a significant difference between the two groups. The SMD and MD values indicate that the experimental group has a higher influence on protein concentration than the control group as it reduces the protein concentration in wheat by 0.62%. There was no significant heterogeneity found by the Cochran's Q, *I-sq*uared 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).



Study	Experimental Total	Experimental Mean	Experimental SD	Control Total	Control Mean	Control 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)

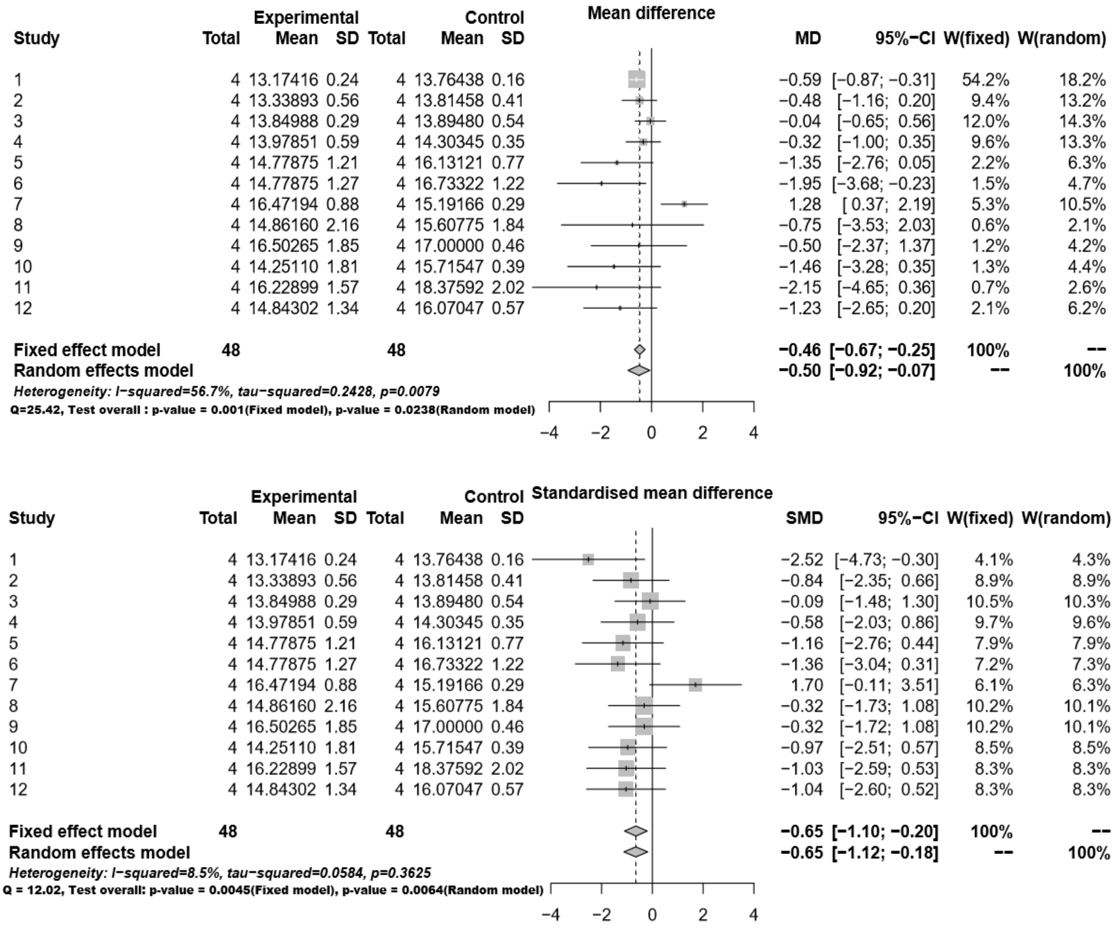


Study	Experimental Total	Experimental Mean	Experimental SD	Control Total	Control Mean	Control SD	SMD	95%−CI	W(fixed)	W(random)
1	4	12.60036	0.9951876	4	13.22000	0.21	−0.75	[−2.23; 0.73]	4.7%	4.7%
2	4	13.40594	0.2720802	4	13.23000	0.61	0.32	[−1.08; 1.73]	5.2%	5.2%
3	4	13.24437	1.1630917	4	13.22500	0.73	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	Mean	SD	Control Total	Mean	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

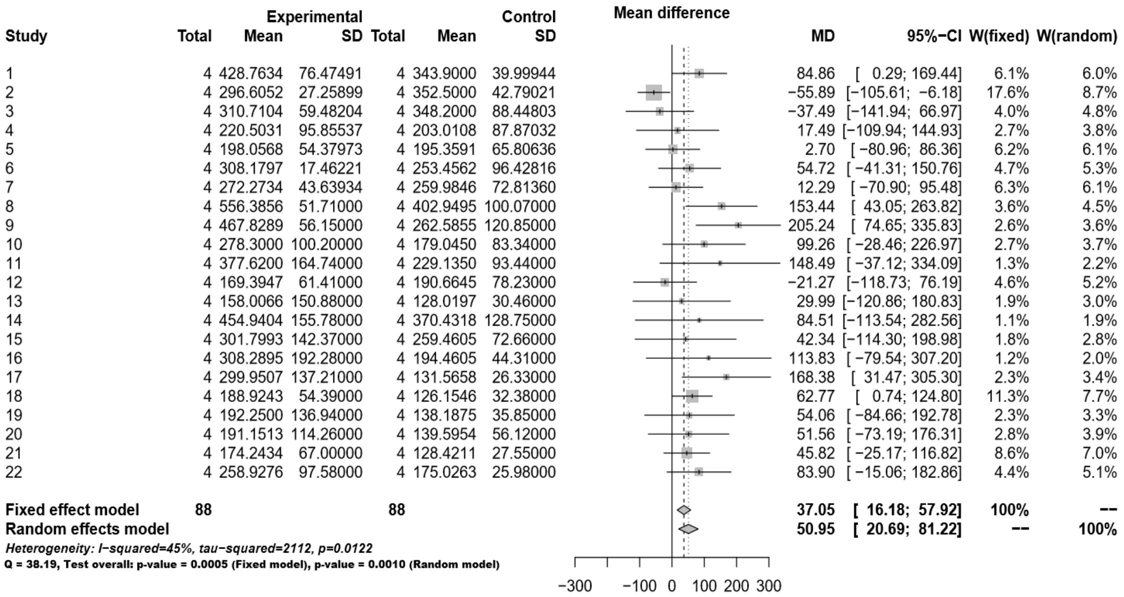
Q=25.42, Test overall : p-value = 0.001(Fixed model), p-value = 0.0238(Random model)

Study	Experimental Total	Mean	SD	Control Total	Mean	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)

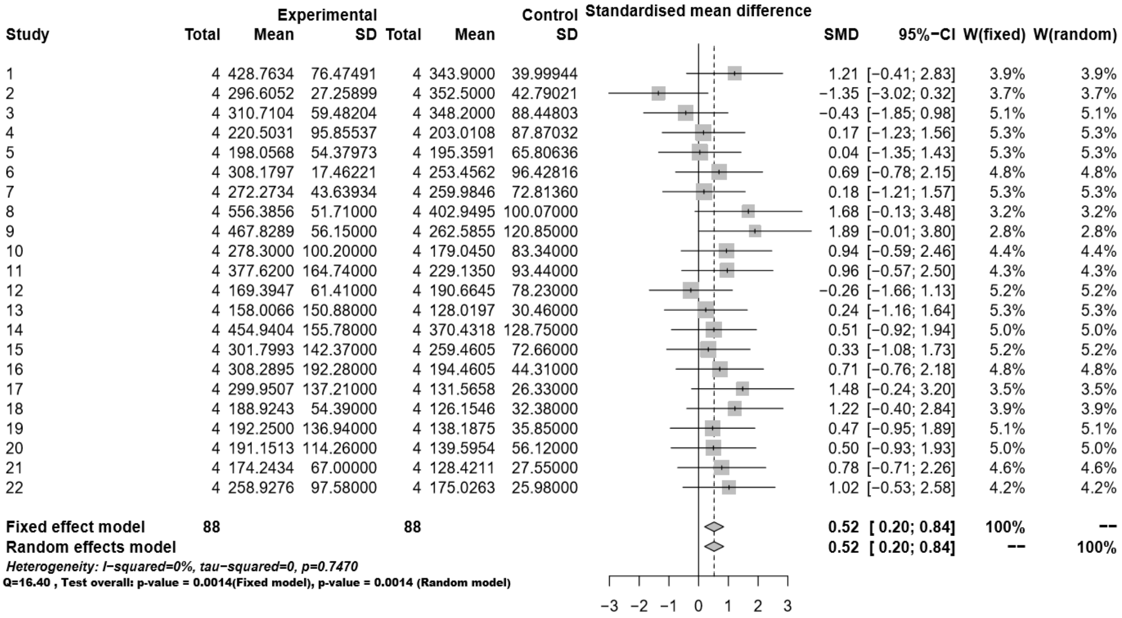
Figure 2. Forest plots for grain proteins under two levels of CO_2, eCO_2 in the experimental group and aCO_2 in the control group. The level of temperature is ambient, and the level of nitrogen is medium.



Study	Experimental Total	Mean	SD	Control Total	Mean	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)

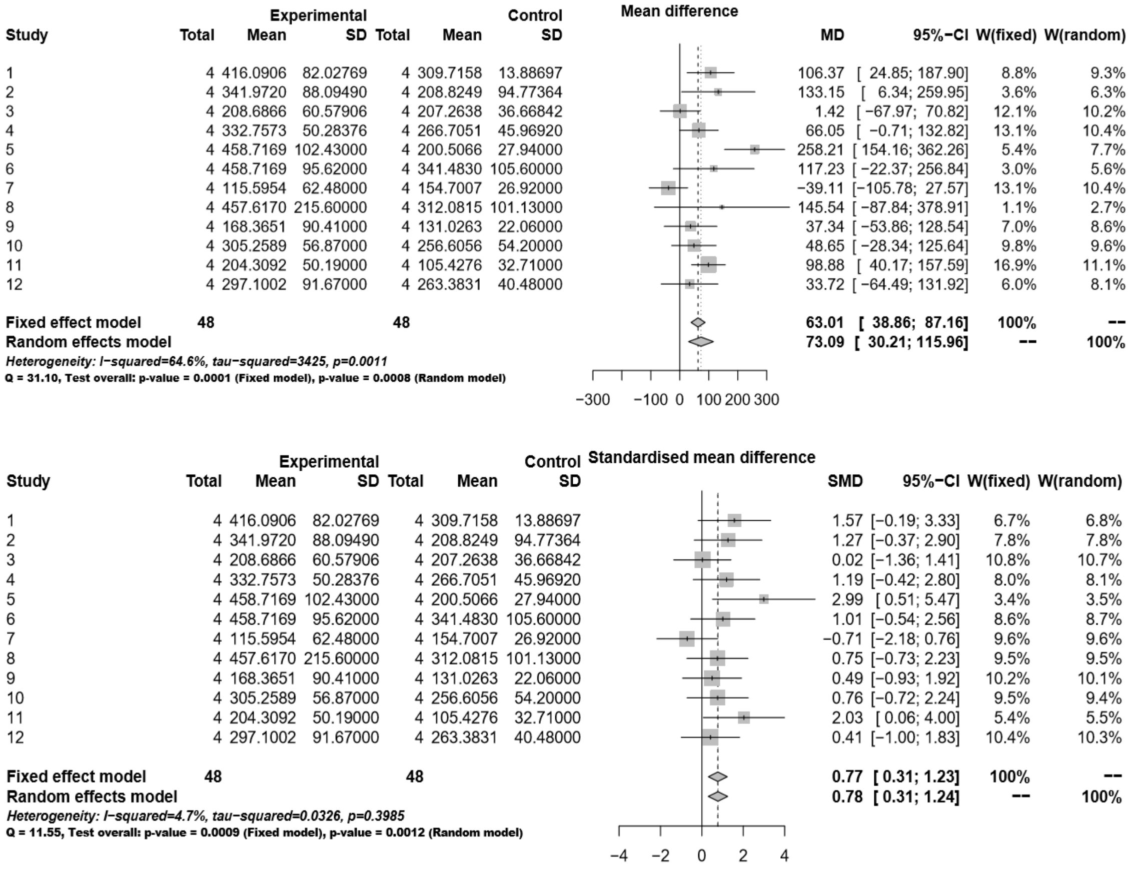


Study	Experimental Total	Experimental Mean	Experimental SD	Control Total	Control Mean	Control SD	SMD	95%-CI	W(fixed)	W(random)
1	4	428.7634	76.47491	4	343.9000	39.99944	1.21	[−0.41; 2.83]	3.9%	3.9%
2	4	296.6052	27.25899	4	352.5000	42.79021	−1.35	[−3.02; 0.32]	3.7%	3.7%
3	4	310.7104	59.48204	4	348.2000	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.



Study	Experimental Total	Experimental Mean	Experimental SD	Control Total	Control Mean	Control 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)

Study	Experimental Total	Experimental Mean	Experimental SD	Control Total	Control Mean	Control 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.

3.3 Discussion

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, I-squared and tau-squared tests.

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, I-squared and tau-squared tests did not show a significant heterogeneity. 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, I-squared and tau-squared tests. 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, I-squared and tau-squared tests.

CHAPTER 4

ASSESSMENT OF GRAIN QUALITY IN TERMS OF FUNCTIONAL GROUP RESPONSE TO ELEVATED [CO_2], WATER AND NITROGEN USING A META-ANALYSIS: GRAIN PROTEIN, ZINC AND IRON UNDER FUTURE CLIMATE

4.1 Introduction

Al-Hadeethi, I., Li, Y., Odhafa, A. K. H., Al-Hadeethi, H., Seneweera, S., & Lam, S. K. (2019). Assessment of grain quality in terms of functional group response to elevated [CO_2], water, and nitrogen using a meta-analysis: Grain protein, zinc, and iron under future climate. *Ecology and evolution*, *9*(13), 7425-7437. (Q1). (Published)

In chapter 3, an efficient statistical method was introduced for estimating the effects of CO_2, temperature and nitrogen on grain protein and grain yield. It was showed the significance and reliability of a meta-analysis in analysing the impacts of carbon dioxide on grain quality.

This chapter makes a further enhancement to expand the data and the factors in Chapter 3 to increase the accuracy of the method in chapter 3.

Meta-analysis techniques were employed to investigate the effect of elevated [CO_2] (e[CO_2]) on protein, zinc (Zn) and iron (Fe) concentrations of major food crops (542 experimental observations from 135 studies) including wheat, rice, soybean, field peas and corn considering different levels of water and nitrogen (N). Each crop, except soybean, had decreased protein, Zn and Fe concentrations when grown at e[CO_2] concentration (≥ 550 μmol mol^{-1}) compared ambient [CO_2] (a[CO_2]) concentration (≤ 380 μmol mol^{-1}). Grain protein, Zn and Fe concentrations were reduced under e[CO_2]. However, the responses of protein, Zn and Fe concentrations to e[CO_2] were modified by water stress and N. There was an increase in Fe concentration in soybean under medium N and wet conditions but non-significant. The reductions in protein concentrations for wheat and rice were ~ 5-10%, and the reductions in Zn and Fe concentrations were ~ 3-12%. For soybean, there was a small and non-significant increase of 0.37% in its protein concentration under medium N and dry water, while Zn and Fe concentrations were reduced by ~ 2-5%. The protein concentration of field peas decreased by 1.7%, and the reductions in Zn and Fe concentrations were ~ 4-10%. The reductions in protein, Zn and Fe concentrations of corn were ~ 5-10%. Bias in the dataset was assessed using regression test and rank correlation. The analysis indicated that there are medium levels of bias within published meta-analysis studies of crops responses to Free Air Carbon dioxide Enrichment FACE. However, integration of the influence of reporting bias did not affect the significance or the direction of the [CO_2] effects. These results suggest that increased atmospheric [CO_2] concentrations under different levels of environmental conditions are likely to decrease protein, Zn and Fe concentrations of many food crops.

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ORIGINAL RESEARCH

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Assessment of grain quality in terms of functional group response to elevated [CO_2], water, and nitrogen using a meta-analysis: Grain protein, zinc, and iron under future climate

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Abstract

The increasing [CO_2] in the atmosphere increases crop productivity. However, grain quality of cereals and pulses are substantially decreased and consequently compromise human health. Meta-analysis techniques were employed to investigate the effect of elevated [CO_2] (e[CO_2]) on protein, zinc (Zn), and iron (Fe) concentrations of major food crops (542 experimental observations from 135 studies) including wheat, rice, soybean, field peas, and corn considering different levels of water and nitrogen (N). Each crop, except soybean, had decreased protein, Zn, and Fe concentrations when grown at e[CO_2] concentration (≥550 µmol/mol) compared to ambient [CO_2] (a[CO_2]) concentration (≤380 µmol/mol). Grain protein, Zn, and Fe concentrations were reduced under e[CO_2]; however, the responses of protein, Zn, and Fe concentrations to e[CO_2] were modified by water stress and N. There was an increase in Fe concentration in soybean under medium N and wet conditions but nonsignificant. The reductions in protein concentrations for wheat and rice were ~5%–10%, and the reductions in Zn and Fe concentrations were ~3%–12%. For soybean, there was a small and nonsignificant increase of 0.37% in its protein concentration under medium N and dry water, while Zn and Fe concentrations were reduced by ~2%–5%. The protein concentration of field peas decreased by 1.7%, and the reductions in Zn and Fe concentrations were ~4%–10%. The reductions in protein, Zn, and Fe concentrations of corn were ~5%–10%. Bias in the dataset was assessed using a regression test and rank correlation. The analysis indicated that there are medium levels of bias within published meta-analysis studies of crops responses to free-air [CO_2] enrichment (FACE). However, the integration of the influence of reporting bias did not affect the significance or the direction of the [CO_2] effects.

KEYWORDS
elevated CO_2 (e[CO_2]), iron, meta-analysis, nitrogen, protein, water, zinc

1 | INTRODUCTION

Climate change factors, including high temperature and atmospheric CO_2 concentration ($[CO_2]$), are among the most pervasive environmental changes (Mueller et al., 2016). Since the industrial revolution, the increase in $[CO_2]$ has been documented and is predicted to increase more in the middle of the century (IPCC, 2014). Changes in these environmental variables directly or indirectly affect plant growth, development, grain yield, and quality (Fernando et al., 2012; Panozzo et al., 2014; Thilakarathne et al., 2013). Stimulation of photosynthesis together with plant nutrient metabolism alters the grain nutrient quality of many cereals and pulses. Quantitative reviews of different studies demonstrated that elevated $[CO_2]$ ($e[CO_2]$) stimulated the grain yields of many crops. For example, the yields of C_3 legumes and C_4 plants were increased by 11%–31% and 14%–54%, respectively, under $e[CO_2]$ (Kimball, 1983; Tubiello et al., 2007), but $e[CO_2]$ reduced the grain N or protein concentrations of C_3 nonlegumes (10%–15%) and had little effect on protein concentrations of legumes (−1.4%) (Jablonski, Wang, & Curtis, 2002; Taub, Miller, & Allen, 2008). Such changes in grain N, Zn, and Fe concentrations affected nutrient requirements of all cropping systems. Furthermore, the demand for these nutrients can be modified by genetic and environmental factor cropping systems. Thus, understanding grain quality trait responses to $e[CO_2]$ under a range of climate stressors is required to develop adaptation strategies to inevitable climate change.

The effect of $e[CO_2]$ on different plant physiological processes, such as photosynthesis and stomatal conductance, is well researched (Leakey et al., 2009; Thilakarathne et al., 2013). It has been well established that elevated $[CO_2]$ increases photosynthetic rates (Drake, Gonzàlez-Meler, & Long, 1997; Ehleringer & Cerling, 2002; Rosenthal & Tomeo, 2013; Yamori, Hikosaka, & Way, 2014), while stomatal conductance decreases across a range of plant species (Ainsworth & Long, 2005; Ainsworth & Rogers, 2007; Farquhar & Sharkey, 1982; Medlyn et al., 2001). Correspondingly, a number of researchers have considered the concept of food security in regard to $e[CO_2]$ (Ziska et al., 2012). Furthermore, an ample number of studies have documented the issue of water use efficiency under $e[CO_2]$ levels as well (Chun, Wang, Timlin, Fleisher, & Reddy, 2011; Keenan et al., 2013). However, the effect of $e[CO_2]$ on plant quality, including nutrition, has yet to be fully investigated. Through photosynthesis, plants convert CO_2 into sugar and other carbohydrates to take up minerals and other nutrients from the soil (Loladze, 2014). Each nutrient response to $e[CO_2]$ largely varies between functional groups and even within the same species (Ainsworth et al., 2008). Therefore, understanding the response of each functional group to $e[CO_2]$ under different environmental stresses is essential to addressing global food security. Recently, Loladze (2014) demonstrated that $e[CO_2]$ reduced wheat grain protein and nitrogen concentrations. Similarly, studies by Taub et al. (2008), De Graaff, Van Groenigen, Six, Hungate, and van Kessel (2006), Conroy (1992), and Giri, Armstrong, and Rajashekar (2016) investigated the response of grain protein to $e[CO_2]$ under different N regimes. Several experiments were carried out to investigate the responses of biomass and productivity to $e[CO_2]$ among different functional groups (Hooper & Vitousek, 1998; Reich et al., 2004). Research shows that the effects of $[CO_2]$ are not just presented in cereals (Wohlfahrt, Smith, Tittmann, Honermeier, & Stoll, 2018). Wohlfahrt et al. reported an increased yield of grapevines under FACE. However, there is very limited understanding on how $e[CO_2]$ influences grain quality traits, such as protein, Fe, and Zn under water and nitrogen stress within a range of functional groups.

Large differences in the responses of grain yields and quality to $e[CO_2]$ have been reported across a number of functional groups (Kimball, Kobayashi, & Bindi, 2002). Micronutrients requirements, particularly Fe and Zn, in grain and the consequences of not having these micronutrients at the required amount are well explained by the World Health Organization. Studies have shown different impacts including child mortality, mental impairment, and anemia due to the lack of Fe and Zn in different species of food crops (Cakmak, Pfeiffer, & McClafferty, 2010). Hence, assessing the status of macronutrients in different food crops is crucial as they are documented as changing with $e[CO_2]$. A number of studies have been conducted to explain lower micronutrient concentrations in cereal crops under $e[CO_2]$ (Erbs et al., 2010; Kimball et al., 2001; Seneweera, Blakeney, & Milham, 1996). However, there is very limited understanding of how grain protein, Zn, and Fe respond to $e[CO_2]$ under a range of stress conditions, particularly water and nitrogen limitations.

There have been a number of meta-analysis studies to discuss the impact of climate change on crop quality (Baig, Medlyn, Mercado, & Zaehle, 2015; Haworth, Hoshika, & Killi, 2016; Humbert, Dwyer, Andrey, & Arlettaz, 2016; Niu et al., 2016; Sutton, 2005; Zhou et al., 2017). A number of studies have shed light on the effects of carbon dioxide $[CO_2]$ on agricultural crops (Buchner et al., 2015; Dietterich et al., 2015; Fitzgerald et al.., 2016). Some meta-analyses utilized a very limited number of studies for grain quality studies (Al-Hadeethi, Li, Seneweera, & Al-Hadeethi, 2017). Jablonski et al. (2002) conducted a meta-analysis to combine the data on eight reproductive traits from 159 CO_2 enrichment studies that reported the information on 79 species. They found that crops were responsive to high $[CO_2]$ more than wild species. In addition, grain N was not affected by the elevated $[CO_2]$ concentrations in legumes but reduced significantly in most nonlegumes. Other groups of researchers performed a comprehensive meta-analysis to explore the influence of $e[CO_2]$ on crop nutrients compositions (Broberg, Högy, & Pleijel, 2017; Duval, Blankinship, Dijkstra, & Hungate, 2012; Ingvordsen et al., 2016; Lam, Chen, Mosier, & Roush, 2013; Lam, Chen, Norton, Armstrong, & Mosier, 2012; Li, Niu, & Yu, 2016; Myers, Wessells, Kloog, Zanobetti, & Schwartz, 2015; Taub et al., 2008). They reported that many nutrient compositions decreased in crops under elevated $[CO_2]$. Neither of those studies were concentrated exclusively on the effects of high $[CO_2]$ on crops nutrient composition taking into consideration of the influence of water and nitrogen fertilization. And little attention was given to the impacts of key environmental factors such as water and soil nitrogen availability on crops. The abnormal increase in nitrogen impeded the process of balancing the protein content and carbohydrate content which negatively affected the production by delaying the entry of the plant's maturation stages. Also, increasing the nitrogen of the distant boundaries of the necessary needs led grain crops to produce a crop without grain. In addition, low wetness level inhibited cell growth and led to the closure of stomatal and reduced photosynthesis, and each plant process was directly or indirectly affected by water availability. To address these issues, a meta-analysis has been carried out to analyze the effect of e[CO_2] on protein, zinc, and iron for five different crops under different functional groups considering different levels of water and N. The study includes five different crops: wheat, rice, maize as a cereal crops and soybean and field peas as legumes. These crops define different functional groups including cereal and legumes, along with C_3 and C_4 photosynthetic groups. The functional group cereals and legumes best define the issues relating to protein and micronutrients. Cereals are grown for their grains which are high in protein and carbohydrates and legumes are among the most versatile and nutritious foods available. In a recent meta-analysis, Al-Hadeethi et al. (2017) found that the protein concentrations in wheat diminished slightly under e[CO_2]; however, grain yields increased. In this previous study, we examined protein concentration and grain yield in a wheat crop under three environmental factors in Australia. The analysis showed that there were decreases in the Zn concentrations of some major food crops, including staple foods, such as rice, wheat, and corn. The WHO (2017) estimated the risk of an inadequate Zn uptake for approximately 17.3% of the population worldwide, including an annual death of 433,000 children under the age of five due to Zn deficiency. Therefore, deficiencies in micronutrients are not only limited to production or biomass but also more pronounced in terms of the diets and well-being of humans.

There are not many published studies on how [CO_2], water, and N affect grain protein, zinc, and iron concentrations. In addition, most related studies have not been reported. There is a large knowledge gap on how crops response to [CO_2], water, and nitrogen. In this paper, we hypothesized that grain protein, Zn, and Fe concentrations are reduced under e[CO_2], but their responses are modified by factors, such as water stress and nitrogen availability.

2 | MATERIALS AND METHODS

2.1 | Data selection

In 2017, a database of the effect of [CO_2], temperature, and nitrogen on grain protein and grain yield was created (Al-Hadeethi et al., 2017). This database was obtained from the website of the journal scientific data (http://www.nature.com/articles/sdata201536#data-records; Dietterich et al., 2015). The investigation was focused on grain proteins and grain yields of wheat crops in Victoria, Australia, under two different [CO_2] levels (ambient and elevated), two levels of nitrogen (low and medium), and one level of temperature (ambient). A procedure based on the *dplyr* package in R program (Wickham, 2011) was utilized to re-arrange data from individual studies, separately, under the conditions considered in this study to make them suitable for meta-analysis. 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 was created. Limitations faced in previous studies included (a) data compiled from one place and for one crop, (b) crops being cultivated under the same field conditions, and (c) crops grown at e[CO_2] in studies using the single [CO_2] enrichment technology free-air [CO_2] enrichment (FACE). In this study, those limitations were overcome by considering several crops including wheat, rice, soybean, corn, and field peas grown in different countries such as Australia, Japan, United States, and Germany. Furthermore, the effect of diverse environmental variables (nitrogen supply and water supply) on the magnitude of the [CO_2] effect was investigated. In addition, the effect of [CO_2] with the aforementioned environmental factors on the concentration of the basic types of micronutrient such as protein, Zn, and Fe was examined.

The data obtained from the website of the journal scientific data were expanded. In addition, a compilation of additional data from literature using a comprehensive keyword search in various databases (Web of Science, Scopus, and Natural Resources Index) and an examination of lists of references were conducted (although there was paucity of studies that contained the effect of [CO_2] on protein, Zn, and Fe considering different levels of nitrogen and water) with the search terms are listed in Appendix S2. This study focused on investigating grain protein, Zn, and Fe for wheat, rice, soybean, corn, and field peas in Australia, Japan, United States, and Germany under two different levels of [CO_2] (ambient and elevated), three levels of nitrogen (low, medium, and high), and two levels of water (wet and dry). The areas were chosen because we had the full access of the relevant information data, and we were able to employ meta-analysis to investigate those published studies. An extensive reprocessing of data to the data compatible for meta-analysis was carried out. Conducting a meta-analysis demands a set of clear and proportionate information about the individual studies. The following criteria were important to selecting appropriate studies to be included in this analysis. First, sample size, mean, and standard deviation or standard error had to be reported for the treatments of e[CO_2] and a[CO_2]. Second, crop species and experimental design were identified. Finally, for studies that did not report grain protein concentration, protein values were calculated based on a measurement of nitrogen and a conversion to protein using Equation (1), where k = 5.36 (Myers et al., 2014).

$$\text{protein (weight \%)} = k \times \text{nitrogen (weight \%)} \tag{1}$$

The different levels of [CO_2] treatments were classified as “elevated” (CO_2 concentration ≥ 550 μmol/mol) and as “ambient” (CO_2 concentration ≤ 380μmol/mol). The water status was classified as “wet” (water amount include precipitation + irrigation) or as “dry” (water amount include only precipitation or without precipitation + irrigation). Nitrogen concentrations (the amount of nitrogen) were classified as "low" (nitrogen concentration equivalent to zero kg N per ha), "medium" (50 kg N/ha ≤ nitrogen concentration < 120 kg N/ha), and "high" (nitrogen concentration ≥ 120 kg N/ha). The database contained 542 observations from 135 studies, including 280 observations for wheat, 118 for rice, 40 for field peas, 88 for soybean, and 16 for corn. The database of the meta-analysis is presented in Table S1, and it will made available online.

2.2 | Meta-analysis

The meta-analysis was carried out as described by Curtis and Wang (1998) and Ainsworth et al., (2002). The response ratio representing the ratio of several measures of outcomes in the treatment group to that of the control group were estimated (Rosenberg, Adams, & Gurevitch, 2000). This analysis has the merit of estimating the effect as a proportionate alteration resulting from experimental manipulation. For summarizing the influences of [CO_2] on ecosystems, the natural log of the response ratio has been widely used (Ainsworth et al., 2002; Curtis & Wang, 1998; Hedges, Gurevitch, & Curtis, 1999). Therefore, the natural log of the response ratio (*r* = response to e[CO_2]/ response to a[CO_2]) was used as a metric for the analysis. The results were reported as the percentage change under e[CO_2] ((*r* − 1) × 100). Negative values indicated a decrease in the variable compared with the ambient status, and positive percentage changes indicate an increase in the account of e[CO_2] conditions. In previous meta-analyses on [CO_2] effects, effect sizes were weighted using the inverse of pooled variance (Ainsworth & Long, 2005; Duval et al., 2012), replication (Adams, Gurevitch, & Rosenberg, 1997; Blankinship, Niklaus, & Hungate, 2011), or unweighted effect sizes (Wang, 2007). In the database of this study, the collected studies did not constantly include published variance. Furthermore, the variance-based weighting function might result in excessive weights for some studies while weighting using replication could produce less excessive weights (Van Groenigen, Osenberg, & Hungate, 2011). Thus, the studies were weighted by replication using a function of sample size given by Equation (2).

$$\text{weight} = (n_a \times n_e)/(n_a + n_e), \tag{2}$$

where n_a and n_e represent the number of replicates of the ambient and elevated [CO_2], respectively (Adams et al., 1997; Van Groenigen et al., 2011; Hedges & Olkin, 1985). To calculate mean effect sizes and 95% confidence intervals, bootstrapping techniques were used. For the bootstrapping using statistical software MetaWin 2.1 (Rosenberg et al., 2000), 4,999 iterations were used. Technically, a mixed-effects model or a fixed-effects model is not viable for non-parametric meta-analytic methods based on weighting by replication. However, a fixed-effects model had to be adopted to implement a valid bootstrapping using MetaWin. The fixed-effect model is given by Equation (3) (Borenstein, Hedges, Higgins, & Rothstein, 2009).

$$T_i = \mu + u_i \tag{3}$$

where T_i is an observed effect in the study of *i*, μ is the common effect, and u_i is $u_i$is the within-study error.

The weight assigned to each study is defined as:

$$w_i = \frac{1}{v_i} \tag{4}$$

where v_i is within-study variance for study *i*.

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

$$\bar{T}. = \frac{\sum_{i=1}^{k} w_i}{\sum_{i=1}^{k} w_i}. \tag{5}$$

The variance of the combined effect is defined as:

$$V. = \frac{1}{\sum_{i=1}^{k} w_i} \tag{6}$$

The standard error of the combined effect is

$$SE(\overline{T}.) = \sqrt{V.} \tag{7}$$

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

$$\text{Lower limit} = \overline{T}. - 1.96 * SE(\overline{T}.), \tag{8}$$

$$\text{Upper limit} = \overline{T}. + 1.96 * SE(\overline{T}.). \tag{9}$$

The *Z*-value can be computed using

$$Z = \frac{\overline{T}.}{SE(\overline{T}..)}. \tag{10}$$

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

$$p = 1 - \varphi(|Z|). \tag{11}$$

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

$$p = 2[1 - (\varphi(|Z|))] \tag{12}$$

where φ is the standard normal cumulative distribution function.

The e[CO_2] effects on a response variable were considered significant if the confidence interval did not overlap with zero. The means of various categorical variables were considered significantly different if their 95% confidence intervals did not overlap.

2.3 | Techniques to assess publication bias

Although meta-analysis provides an accurate technique to combine the effect size from all the studies to obtain a pooled estimate of the common effect size, however, if the studies are biased of all relevant studies, then the effect size will reflect this bias (Borenstein et al., 2009). Various researches indicate that studies that report comparatively high effect sizes are more probable to be published than studies that report lower effect sizes. Also, published studies have considerable opportunity to find their path into a meta-analysis, and it is possible the bias in the literature could be reflected in the meta-analysis also. This case is commonly called publication bias.

The issue of publication bias affects the researchers who compose a narrative review. Though, meta-analyses and systematic reviews be given more attention, perhaps due to these advanced techniques are more accurate than other methods to synthesizing research. An approach to examining whether a review is liable to publication bias is to utilize funnel plots.

The funnel plot is a technique for presenting the connection between effect size and study size. The funnel plot was plotted with treatment effects on the X-axis and the measure of every study's size such as inverse of variance on the Y-axis (Light & Pillemer, 1984). To test for and assess the possible impacts of bias, we performed a random effects meta-analysis using the metafor package (Viechtbauer, 2010) in R statistical software. Bias in the dataset was assessed using regression (Egger, Smith, Schneider, & Minder, 1997) and rank correlation (Begg & Mazumdar, 1994).

3 | RESULTS

3.1 | Response of protein to e[CO_2] under different N and water

Elevated [CO_2] significantly decreased the protein concentration in wheat (Figure 1). The average reduction in the protein concentration was 6.5% across a range of environmental conditions (Figure 1). Under low N supplies, the reduction in the grain protein concentration was 6.9% greater than the suboptimal N levels. Overall, e[CO_2] significantly decreased the protein concentration in rice by 5.32%. Elevated [CO_2] resulted in a small and nonsignificant reduction in protein concentration (2.69%) under medium N level, but a greater and significant reduction in protein concentration (9.36%) under high N. Overall, a small

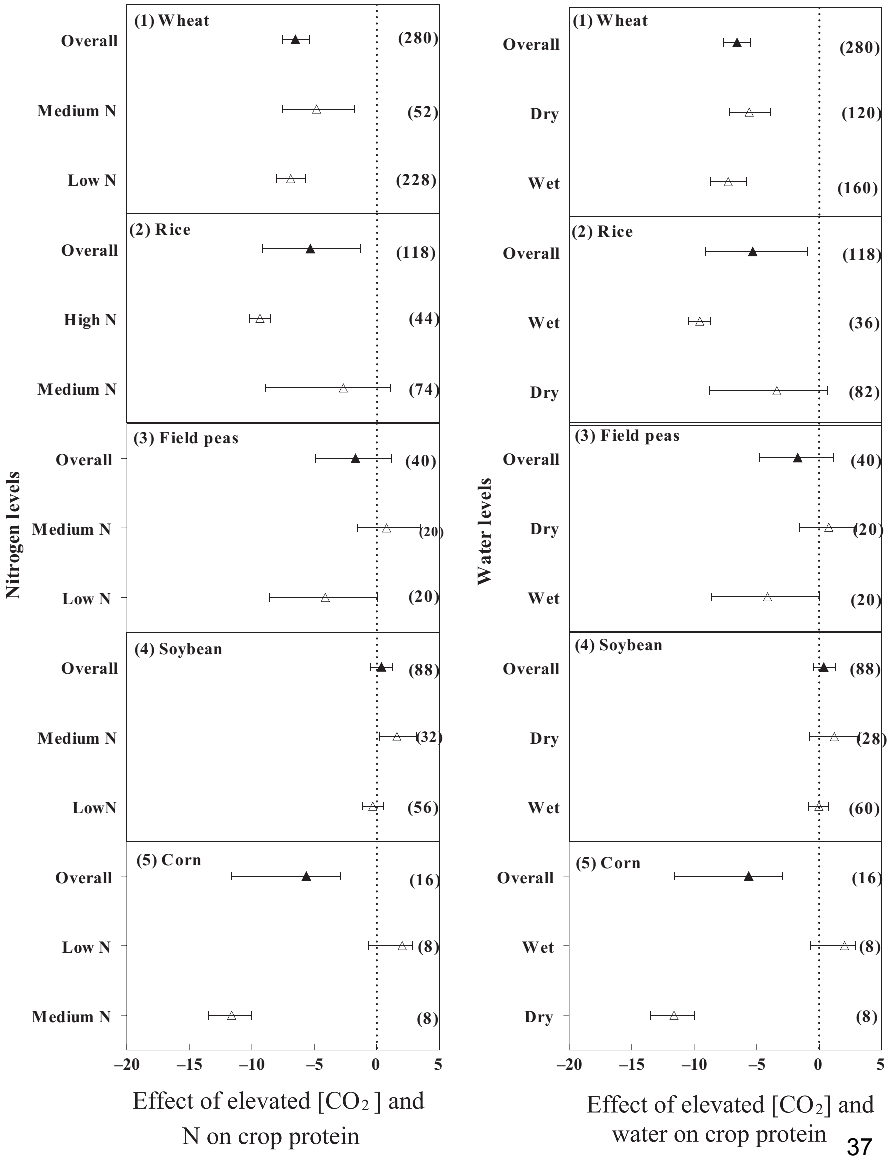


FIGURE 1 Effects of e[CO_2] on protein for wheat, rice, field peas, soybean, and corn. Means and 95% confidence intervals are depicted. The numbers of experimental observations are in parentheses. Low N, medium N, and high N refer to nitrogen concentration equivalent to zero kg N per ha, 50 kg N/ha ≤ nitrogen concentration < 120 kg N/ha, and nitrogen concentration ≥120 kg N/ha, respectively. Wet and dry refer to the water amount including precipitation + irrigation and the water amount including only precipitation or without precipitation + irrigation, respectively

and nonsignificant reduction in the protein concentration in field peas was observed under e[CO_2] (1.75%). The protein concentration showed a nonsignificant decrease under low N (4.12%), and there was no significant increase under medium N (0.79%). Overall, a small and nonsignificant increase in the protein concentration in soybean was observed under e[CO_2] (0.37%). The reduction in protein concentration was nonsignificant under low N (0.33%). The increase in protein concentration was not significant under medium N (1.6%). Overall, e[CO_2] significantly decreased the protein concentration in corn by 5.63%. The protein concentration decreased significantly under medium N (11.61%) but there was no significant reduction under low N (2.9%).

The reduction in wheat protein concentration significantly varied between the different water levels, 7.3% and 5.6% under well-watered conditions and less well-watered conditions, respectively. Elevated [CO_2] resulted in a respectable reduction in protein concentration in rice by (5.31%). A nonsignificant reduction in protein concentration under dry conditions (3.38%) and a significant reduction in protein concentration under wet conditions (9.55%) were observed. Elevated [CO_2] caused a nonsignificant decrease in the protein concentration in field peas (1.71%). The protein concentration showed a nonsignificant decrease of 4.12% under wet conditions and a nonsignificant increase under dry condition (0.79%). There was a nonsignificant increase in the protein concentration in soybean under e[CO_2] (0.37%). The protein concentration showed a nonsignificant decrease under wet conditions (0.02%) and a nonsignificant increase under dry conditions (1.22%). Elevated [CO_2] significantly decreased the protein concentration in corn by 5.63%. The protein concentration decreased substantially under dry condition (11.615), while a nonsignificant reduction in the protein concentration was recorded under wet conditions (2.9%).

3.2 | Response of Zn to e[CO_2] under different N and water

Overall, the Zn concentration in wheat decreased by 9.1% under e[CO_2] as shown in Figure 2. The reduction in the grain Zn

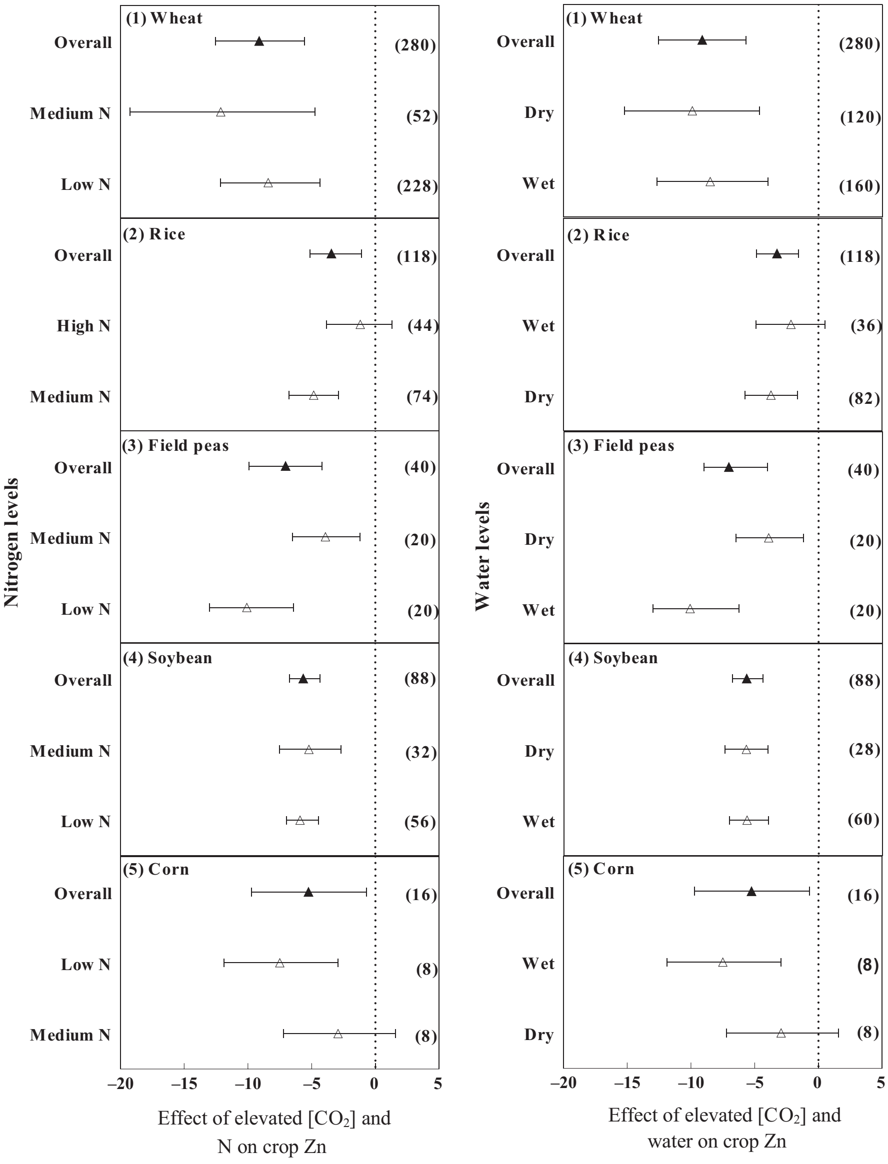


FIGURE 2 Effects of e[CO_2] on zinc for wheat, rice, field peas, soybean, and corn. Means and 95% confidence intervals are depicted. The numbers of experimental observations are in parentheses. Low N, medium N, and high N refer to nitrogen concentration equivalent to zero kg N per ha, 50 kg N/ha ≤ nitrogen concentration < 120 kg N/ha, and nitrogen concentration ≥ 120 kg N/ha, respectively. Wet and dry refer to the water amount including precipitation + irrigation and the water amount including only precipitation or without precipitation + irrigation, respectively

concentration was significant at 8.4% and 12.12% for low and medium N levels, respectively. The Zn concentration in rice decreased under e[CO_2] (3.44%). The reduction in the Zn concentration was considerable under medium N (4.82%) but nonsignificant under high N (1.18%). Elevated [CO_2] decreased the Zn concentration in field peas (7.04%). The reduction in the Zn concentration was large under low N (10.08%) and under medium N (3.91%). Elevated [CO_2] decreased the Zn concentration in soybean by 5.64%. The Zn concentration decreased significantly under low and medium N by 5.89% and 5.2%, respectively. Elevated [CO_2] significantly decreased the Zn concentration in corn by 5.24%. A small and nonsignificant reduction of 2.92% in the Zn concentration under medium N was observed, but the reduction was significant under low N (7.5%).

The reduction in the wheat Zn concentration was higher under a low water level compared to high water availability. There was also a significant reduction in the Zn concentration in rice under e[CO_2] (3.24%). Under dry conditions, the Zn concentration decreased significantly by 3.71% but was nonsignificant under wet conditions (2.15%). Elevated [CO_2] decreased the Zn concentration in field peas significantly by 7.04%. The Zn concentration decreased significantly both under wet and dry conditions by 10.08% and 3.91%, respectively. Elevated [CO_2] decreased the Zn concentration in soybean significantly by 5.64%. There were significant reductions in the Zn concentration under wet (5.62%) and dry conditions (5.68%). Elevated [CO_2] significantly decreased the Zn concentration in corn by (5.24%). The Zn concentration decreased under both dry and wet conditions by 2.925% and 7.5%, respectively.

3.3 | Response of Fe to e[CO_2] under different N and water

The Fe concentration in wheat decreased under e[CO_2] by 4.6% (Figure 3). The reduction in grain Fe concentration was significant under low N (5.6%), but this response was not observed in medium N levels. Elevated [CO_2] decreased the Fe concentration in rice significantly by 5.39%. Under medium and high N levels, the Fe concentration decreased significantly by 5.29% and 5.54%, respectively. Elevated [CO_2] decreased the Fe concentration in field peas (4.44%).

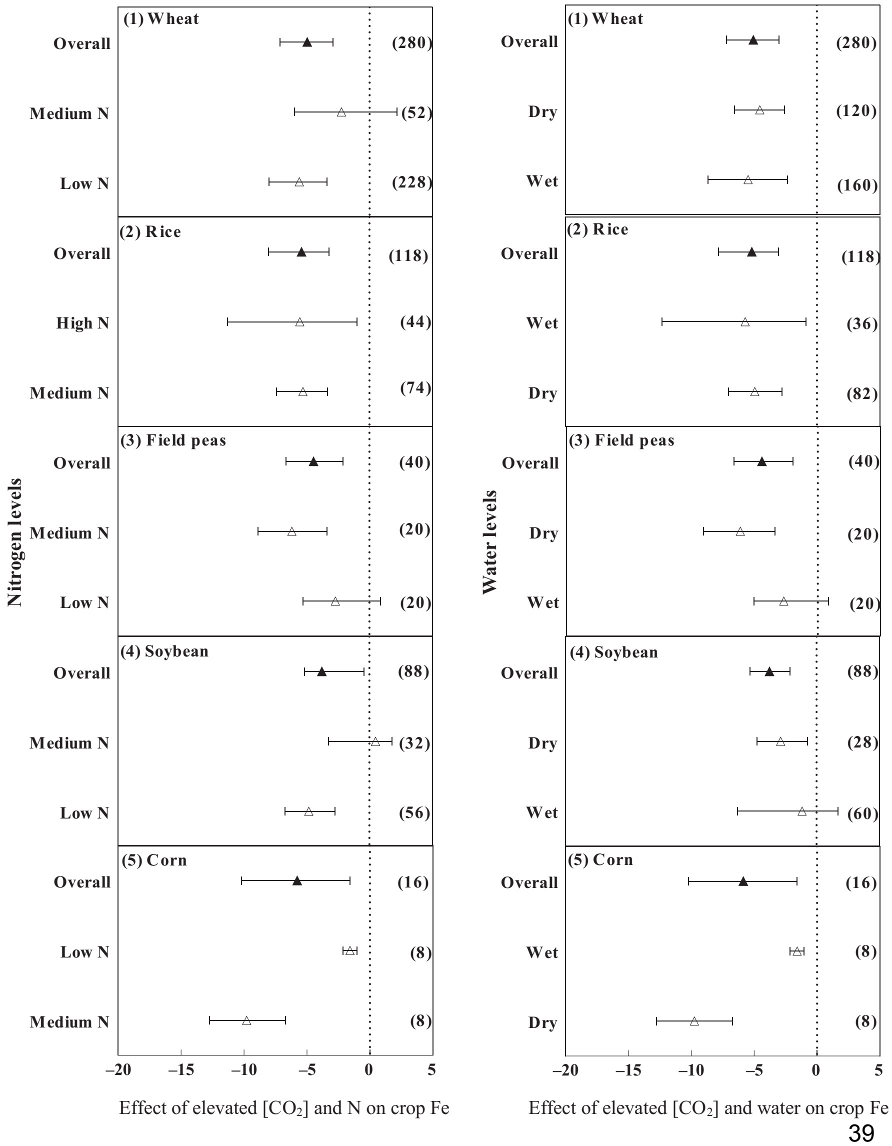


FIGURE 3 Effects of e[CO_2] on iron for wheat, rice, field peas, soybean, and corn. Means and 95% confidence intervals are depicted. The numbers of experimental observations are in parentheses. Low N, medium N, and high N refer to nitrogen concentration equivalent to zero kg N/ha, 50 kg N/ha ≤ nitrogen concentration < 120 kg N/ha, and nitrogen concentration ≥ 120 kg N/ha, respectively. Wet and dry refer to the water amount including precipitation + irrigation and the water amount including only precipitation or without precipitation + irrigation, respectively

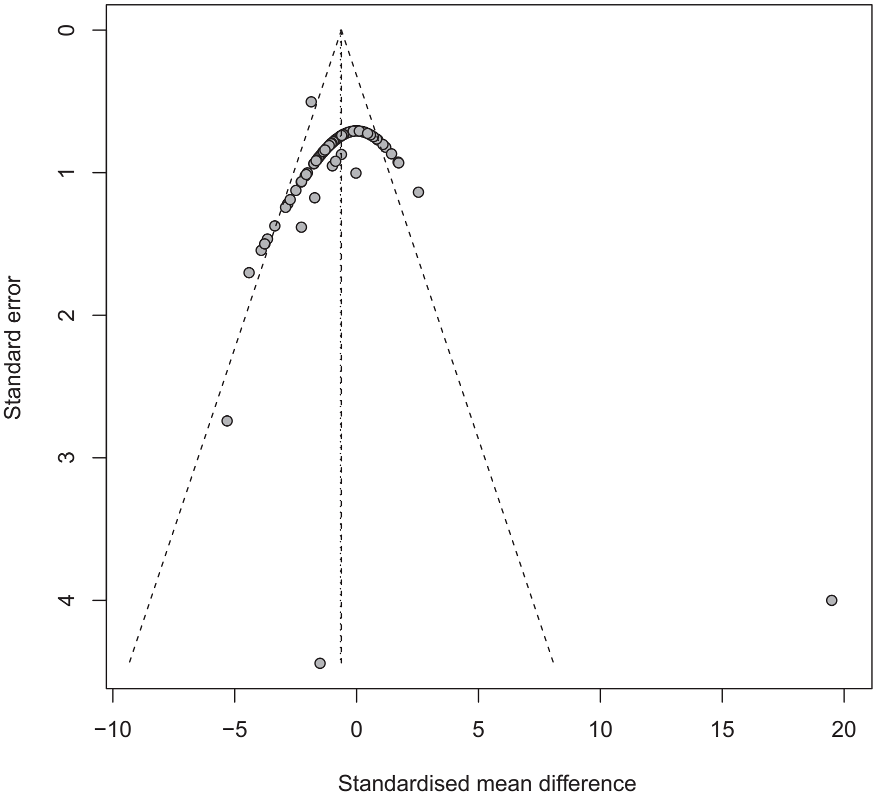


FIGURE 4 Funnel plots of crop protein (*n* = 137) show the distribution of data. Data from the studies used in the meta-analysis are represented by solid black circles. The dashed vertical line indicates the mean effect size computed by the meta-analysis. The funnel plot shows the Begg–Mazumdar (Begg & Mazumdar, 1994) rank correlation coefficient using Kendall's τ and Egger's regression test (Egger et al., 1997). Rank correlation test of asymmetry: τ = 0.552; = 0.0004; Regression test for asymmetry: z = -7.76; = 0.0001

A small and nonsignificant reduction in the Fe concentration was observed under low N (2.7%) while a greater and significant reduction was observed under medium N (6.16%). Under e[CO_2], the Fe concentration in soybean decreased significantly (3.77%). Additionally, the Fe concentration decreased under low N (4.81%), but there was a nonsignificant increase in the Fe concentration under medium N (1.8%). The Fe concentration in corn decreased significantly under e[CO_2] (5.77%). Under medium and low N, the Fe concentration decreased significantly by 9.785% and 1.585%, respectively.

The Fe concentration in wheat decreases more under wet conditions (5.5%) than dry conditions (4.5%). Under e[CO_2], the Fe concentration in rice decreased significantly by 5.17%. Reductions in the Fe concentrations under dry and wet conditions were 4.94% and 5.7%, respectively. The concentration of Fe in field peas showed a nonsignificant decrease under e[CO_2] (4.44%). It also showed a nonsignificant decrease under wet conditions (2.7%) but a large decrease under dry conditions (6.16). The reduction in the Fe concentration in soybean under elevated [CO_2] (2.1%) was statistically significant. The Fe concentration decreased significantly under dry conditions (3.09%), but a nonsignificant increase in the Fe concentration under wet conditions (1.1%). The reduction in the Fe concentration in corn was significant under elevated [CO_2] (5.77%). The Fe concentration decreased substantially under dry and wet conditions by 9.78% and 1.58%, respectively.

3.4 | Hypothetical bias

A hypothetical publication bias induced reductions in [CO_2] effect size of 28.02% in crop protein (Figure 4), 30.9% in crop Zn (Figure 5), and 11.23% in crop Fe (Figure 6). Our analysis is indicative of medium levels of bias within published meta-analysis studies of crops responses to FACE. Although the integration of the influence of reporting bias did not affect the significance or the direction of the [CO_2] effects, the outcomes of these studies should be treated with a degree of caution (Haworth et al., 2016).

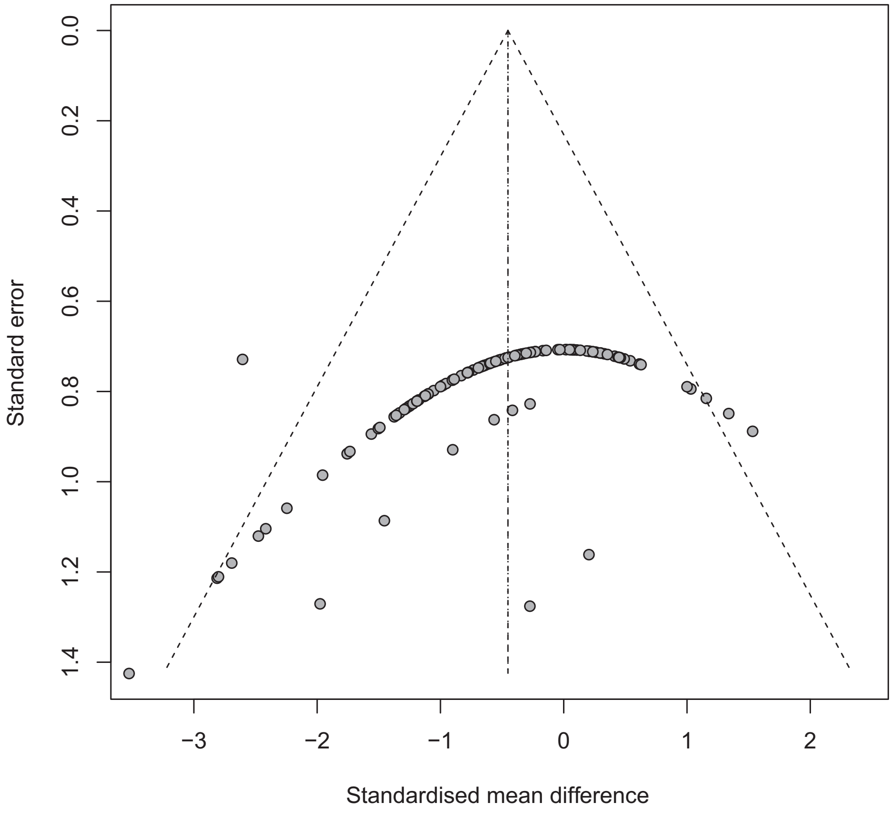
4 | DISCUSSIONS

4.1 | Effect of CO_2, N, and water on grain protein

The overall results were in line with our hypothesis that e[CO_2] would reduce the protein concentration in most of the selected crops. Several studies such as Jablonski et al. (2002) and Loladze (2002) had a similar results related to a decrease in protein concentration under e[CO_2]. The overall decreases in the protein concentrations of the selected crops were found to be more influenced by N and water content. The variations in protein concentration under low, medium, and high N levels including dry and wet water conditions showed a different response in different crops.

In most of the nonlegume C_3 and C_4 crops including corn, wheat, and rice, the protein concentrations decreased under medium N and dry conditions. The decreased protein concentrations in the nonlegume crops under e[CO_2] are a consequence of decreasing protein concentrations in their photosynthetic tissues (Fangmeier, Chrost, Högy, & Krupinska, 2000; Fangmeier et al., 1999). Studies have demonstrated that a decrease in protein results from a decreased rubisco concentration (Ainsworth & Long,

FIGURE 5 Funnel plots of crop Zn (*n* = 136) show the distribution of data. Data from the studies used in the meta-analysis are represented by solid black circles. The dashed vertical line indicates the mean effect size computed by the meta-analysis. The funnel plot shows the Begg–Mazumdar (Begg & Mazumdar, 1994) rank correlation coefficient using Kendall's τ and Egger's regression test (Egger et al., 1997). Rank correlation test of asymmetry: τ = 0; = 0.653; Regression test for asymmetry: z = -6.80; = 0.0001



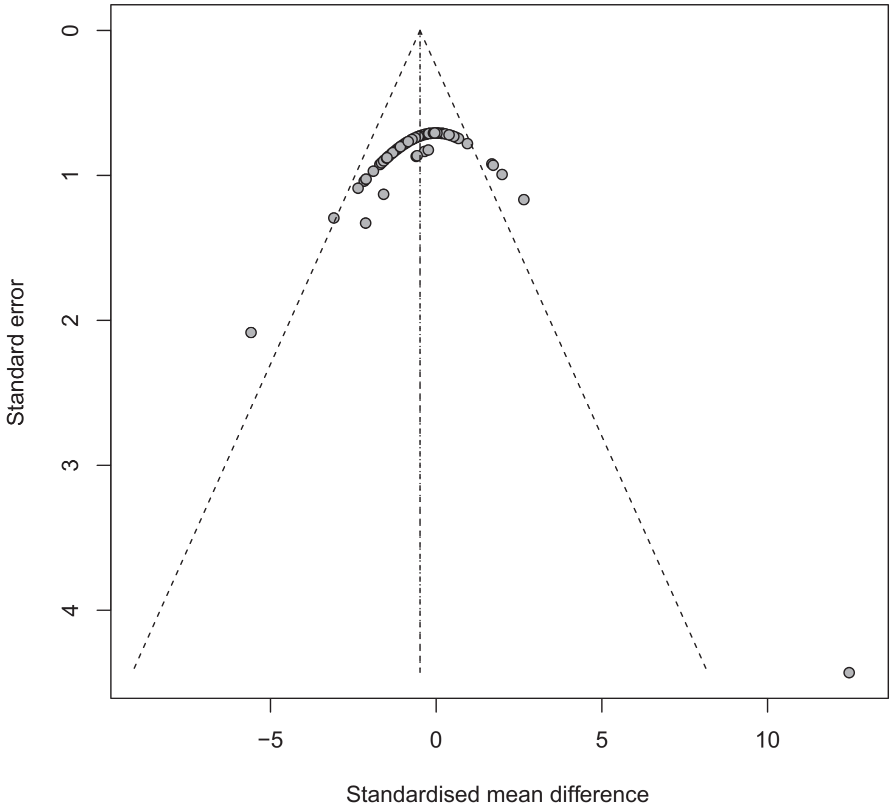


FIGURE 6 Funnel plots of crop Fe (*n* = 136) show the distribution of data. Data from the studies used in the meta-analysis are represented by solid black circles. The dashed vertical line indicates the mean effect size computed by the meta-analysis. The funnel plot shows the Begg–Mazumdar (Begg & Mazumdar, 1994) rank correlation coefficient using Kendall's τ and Egger's regression test (Egger et al., 1997). Rank correlation test of asymmetry: τ = 0; = 0.635; Regression test for asymmetry: z = -7.20; = 0.0001

2005) and a carbohydrate-dependent decrease in the expression of photosynthetic genes (Moore, Cheng, Sims, & Seemann, 1999). In contrast to the nonlegume C_3 and C_4 crops, the selected legumes including field peas and soybean showed a slight increase in protein concentration under medium N and dry water conditions. The increase in nitrogen obtained in legume crops would increase protein levels. This is due to the fact that nitrogen is the main constituent of amino acids and protein acids that are the basis of proteins in the plant. In addition, water is an essential component of all these reactions and the formation of acids. Therefore, drought conditions or water shortages are the causes of a specific increase in protein concentrations. Legumes are able to use the increased carbon gained under e[CO_2] to increase N_2-fixation (Allen & Boote, 2000), thus increasing grain components (Jablonski et al., 2002). Studies have shown that N_2-fixing legumes are typically more responsive to CO_2 than other nonleguminous plants (Poorter, 1993; Wand, Midgley, Jones, & Curtis, 1999). Although the concentration of grain protein tends to increase slightly under low N in legumes, on average, the overall concentration of grain protein decreased. The reason for the slight increase and decrease could be that the different features of the functional group of the crops contributed to the different responses to e[CO_2] under different N and water levels.

4.2 | Effect of CO_2, N, and water on grain Zn

The analysis confirmed our hypothesis related to the reduction in the Zn concentration under e[CO_2]. Different studies have also stated that exposure to e[CO_2] tends to reduce the concentration of mineral elements in all crops at their harvest (Fangmeier, Temmerman, Black, Persson, & Vorne, 2002). Similarly, studies have shown that CO_2 enrichment affects nutrient uptake and distribution in a complex manner (Fangmeier, Grüters, Högy, Vermehren, & Jäger, 1997). The analysis confirms that there was a decrease in Zn concentration under e[CO_2] in different functional group crops including legumes and nonlegume C_3 and C_4 crops. Furthermore, the analysis shows there was a relationship of N availability and water conditions in the reduction of the zinc concentration. The amount of N used affects the Zn concentration as smaller application of nitrogen fertilizer correlates to lower Zn grain concentrations (Cakmak et al., 2010).

4.3 | Effect of CO_2, N, and water on grain Fe

This study used a meta-analysis to show the decrease in Fe concentrations for different functional groups of crops under e[CO_2]. For Zn, the amount of N used was also found to affect the Fe concentration as a lower application of nitrogen fertilizer correlates to lower Fe grain concentrations as well (Cakmak et al., 2010).

An imbalance of different micronutrients, including Fe, is expected from e[CO_2] as e[CO_2] alters the leaf demand for nitrogen in different plant species (Fangmeier et al., 1997). Nitrogen fertilization makes the response of Fe in crops greater because of the presence of CO_2. This may be due to the presence of N as a nutrient that makes the plant grow as its best. Nutrients increase the rate of the vegetative growth and increase plant activity such as photosynthesis, subsequently increasing the ability of plant to benefit from other nutrients, including Fe. This is linked to the increase in CO_2, which is the basis of the process of photosynthesis that improves the growth and activity of the plant.

4.4 | Assessing the publication bias

Figures 4 and 5 show that the choice of the axis representation can influence the appearance of a funnel plot. For example, the plot of crop protein and crop Fe has a clear funnel shape because there is a medium variation for the sample size. Crop Fe has a funnel shape with a little variation for the sample size as shown in Figure 6. Funnel plots should be seen as a generic means of examining whether small studies in a meta-analysis would show larger intervention effects that may be suggestive of publication bias (Higgins and Green, 2006). However, even if small studies are associated with larger intervention effects, this may be due to other reasons rather than publication bias (Higgins and Green, 2006; Sterne et al., 2011).

5 | CONCLUSIONS

Raising atmospheric [CO_2] is likely to decrease protein, Zn, and Fe concentrations in many crops such as wheat, rice, and corn. However, protein and Fe concentrations increase in soybean under e[CO_2]. Nevertheless, reduction in protein, Zn, and Fe concentrations was found to be consistent over diverse species across a wide range of experimental techniques and environmental conditions. Increased use of nitrogen fertilizers and water may lessen the effects of elevated [CO_2] on protein, Zn, and Fe concentrations in rice. However, this approach might be only a partial solution. In other crops such as corn, high nitrogen could result in high reductions in protein, Zn, and Fe concentrations. The analysis indicated that there are medium levels of bias within published meta-analysis studies of crop responses to FACE. However, the integration of the influence of reporting bias did not affect the significance or the direction of the [CO_2] effects The effects of atmospheric [CO_2] on protein, Zn, and Fe in crops are, therefore, likely to be of substantial importance to human nutrition in and beyond the 21st century. These results suggest that increased [CO_2] under different levels of environmental conditions is likely to decrease protein, Zn, and Fe concentrations of many food crops.

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CONFLICT OF INTEREST

None declared.

DATA ACCESSIBILITY

The data have been deposited in Dryad. All the relevant information has been included in the data. Provisional https://doi.org/10.5061/dryad.1h1f63h.

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CHAPTER 5

ESTIMATING THE EFFECT OF INTERACTIONS OF ENVIRONMENTAL FACTORS ON GRAIN QUALITY BASED ON FACTORIAL EXPERIMENTAL DESIGN

5.1 Introduction

Al-Hadeethi, I., Li, Y. 2020. Estimating the effect of interactions of environmental factors on grain quality based on factorial experimental design. Journal of Ecology and Evolution. Q1, (Under review).

In chapter 4, the developed method meta-analysis achieved excellent results and accuracy with the effects of CO_2, temperature, water and nitrogen on grain quality, and enable the investigation of suitable solutions.

This chapter proposes an efficient method based on factorial experimental design to study the effect of carbon dioxide [CO_2], water, and nitrogen [N] and their binary and triple interactions on the quality of grain.

In this chapter, randomized trials were carried out based on the conditions of the factorial experiments to show the effect of elevated carbon dioxide [e[CO_2]], water, N, and their interactions on protein, zinc [Zn] and iron [Fe] of the wheat crop. To determine the effects of interactions of $CO_{2,}$ water and N on protein, Zn and Fe, the designed experiments are implemented in Matlab to investigate all possible possibilities for primary, binary and triple interactions. Emphasis was placed on binary and triple interactions. I developed the algorithm based on factorial design to study all possible interactions for three factors (e[CO_2], water and N) on protein, Zn and Fe of the wheat crop. The analysis revealed that all three factors in the three models harmed protein, Zn and Fe values in the wheat crop. These results suggested that high [CO_2] concentrations under various levels of environmental conditions affect protein, Zn and Fe concentrations in the wheat crop negatively, with protein, Zn and Fe were decreased by 4.5%, 3.5%, 4.1%, respectively, during the three-year experimental period.

Appendix A provides a Matlab code for the proposed method.

Title:

Estimating the effect of interactions of environmental factors on grain quality based on factorial experimental design

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Keywords:

Elevated CO_2 (e[CO_2]), Factorial Experimental Design, Protein, Zinc, Iron, Nitrogen, Water

Paper type:

Primary Research

Abstract

To study the effect of Carbon dioxide [CO_2], water, and nitrogen [N] and their interactions, a factorial experimental design has been proposed in this paper.

In this research, randomized trials were carried out based on the conditions of the factorial experiments to show the effect of elevated carbon dioxide [e[CO_2]], water, N, and their interactions on protein, zinc [Zn] and iron [Fe] of wheat crop. To determine the effects of interactions of CO_2, water and N on protein, Zn and Fe, the designed experiments are implemented in Matlab to investigate all possible possibilities for primary, binary and triple interactions. Emphasis was placed on binary and triple interactions. We developed the algorithm based on factorial design to study all possible interactions for three factors (e[CO_2], water and N) on protein, Zn and Fe of the wheat crop. The analysis revealed that all three factors in the three models harmed protein, Zn and Fe values in the wheat crop. These results suggested that high [CO_2] concentrations under various levels of environmental conditions affect protein, Zn and Fe concentrations in wheat crop negatively, with protein, Zn and Fe were decreased by 4.5%, 3.5%, 4.1%, respectively, during the three-year experimental period.

Introduction:

In the recent decade, the concentration of CO_2 in the atmosphere of the earth has climbed over the years, according to the world health organisation (WHO, https://www.who.int/bulletin/volumes/94/10/15-167031/en/). This raised CO_2 in the atmospheric has fulfilled a boost in crop productivity (Ward, 2007) while diminishing grain quality of cereals and legumes. This raising has consequently harmed human health (Myers et al., 2014). Much research has shed light on the impacts of CO_2 on crops (Buchner et al., 2015; L. H. Dietterich et al., 2015; Fitzgerald et al., 2016). However, little attention is driven to critical environmental factors, such as the level of both N and W in soil. For example, an abundance of studies have recorded the affair of W use competence, under e[CO_2] levels. Another substantial factor that defines the quality production by N (Njoroge et al., 2014). There is significant usefulness from N in most crops. However, over-fertilisation with N is also a problem (Njoroge et al., 2014). There is strong evidence that elevated CO_2 level can interact with N and water, which influence the quality of crops by detracting protein, Zn and Fe concentrations in the cereal. Thus, modelling the impact of the interaction of high CO_2, water and N on protein, Zn and Fe of crops will provide vital information on how these crops will be influenced in future climate conditions to take convenient measures to cope with this challenge. One of the best methods to offer reasonable solutions for biologists to this phenomenon is the experimental design.

The experimental design is an accurate balancing of various features including "power", generalizability, different forms of "validity" and practicality. A robust balancing of these features in anticipation will result in an experiment with the best opportunity of providing beneficial evidence to modify the current case of knowledge in a particular scientific area. On the other hand, it is regrettable that many experiments were designed with preventable blemishes. It is only scarcely in these situations that statistical analysis can be the deliverance of the experimenter (Hicks, 1964). The aim is always to actively design an experiment that has the most significant occasion to produce meaningful and justifiable evidence, rather than expecting that proper statistical analysis might be able to correct flaws after the effect. Decently planning an experiment is essential to ensure that the right species of data and appropriate sample size and power are obtainable to answer the research questions of interest as obviously and expeditiously as potential (Hinkelmann & Kempthorne, 1994). The experimenter is often concerned about the main influences and the interaction effects of various factors (Skillings, 2018). A factorial design is frequently utilized by scientists to comprehend the effects of two or more independent variables upon one dependent variable.

The factorial experimental design provided the best information for the influences of independent variables and their interactions on an experimental model (Dehghan et al., 2010); (de Camargo Forte et al., 2003). The most important utilities of this method are that the effects of single parameters as well as their relative significance are determined and that the interactions of two or more factors could be confirmed (Hunt et al., 2013; Mtaallah et al., 2017); (Salerno et al., 2018); (Carmona et al., 2005); (Fernandez et al., 2002). Current studies in food technology, biological, environmental, medical, psychological and pharmaceutical analysis and industrial-related processes are investigated using experimental design (Hanrahan & Lu, 2006); (Fangueiro et al., 2012); (Al-Asheh et al., 2003); (Abdel-Ghani et al., 2009); (Can & Yildiz, 2006); (Lee et al., 2006); (Shuttleworth, 2009); (Feldman et al., 1997);

(Sonebi, 2004); (Cestari et al., 2008); (Meshkini et al., 2010); (Wang & Wan, 2009); (Abdulra'uf & Tan, 2013) (Abdulra'uf & Tan, 2013) (Rathinam et al., 2011); (Vicente et al., 1998); (Amadori et al., 2013).

Agricultural science, with a need for field-testing, often uses factorial designs to test the effect of variables on crops. In such large-scale studies, it is difficult and impractical to isolate and test each variable individually (Lüscher et al., 2000); (Aranjuelo et al., 2005) (Gavito et al., 2001; Pazzagli et al., 2016); (KIM et al., 2003) (Fangmeier et al., 1999). Several groups of researchers performed a factorial design to examine the effect of e[CO_2] on crop nutrients ((Myers et al., 2014); (Fangmeier et al., 1999); (Erbs et al., 2010); (Pleijel & Danielsson, 2009); (Borrill et al., 2014); (D.-X. Wu et al., 2004); (Högy et al., 2009). They discovered that some nutrient compositions reduced in crop under high [CO_2]. Recently, many studies were reported to analyse the impacts of CO_2, water and N on crops employing diverse methods. Of those, statistical techniques were found to be a significant approach to study the effects of environmental factors on crops, and to examine essential issues in connection with nutrients (Asseng et al., 2015; Cai et al., 2016; Erbs et al., 2015; Fernando et al., 2015; Fernando et al., 2014; García et al., 2015; Liu et al., 2014; Lobell et al., 2012; Lv et al., 2013; Panozzo et al., 2014; Rodrigues et al., 2016; Sánchez et al., 2014; Tack et al., 2015; Valizadeh et al., 2014; G. Wu et al., 2016; Zhang et al., 2016). However, there is a very limited understanding on how the interactions of e[CO_2], water and N influenced grain quality traits, such as protein, Fe and Zn within a range of functional groups. In addition, neither of those researches concentrated exclusively on the influences of interactions of e[CO_2] with essential factors, such as water and N fertilization on crop' nutrient composition. Also, there are not many kinds of research on how the interactions of e[CO_2], water and N influence on grain protein, Zn and Fe concentrations. Also, the impacts of the interactions of e[CO_2], N supplies and water on nutrients in crops are still not clear (Al-Hadeethi et al., 2017), (Al-Hadeethi et al., 2019).

There are significant knowledge gaps on how crops respond to the interactions of e[CO_2], water and N. When there is more than one factor influencing the production of a particular crop, and each factor has more than one level, there is a need to conduct randomized trials of a particular kind called factorial experiments. Due to it offers the possibility of indicating the significance of each factor as well as the importance of the interactions among them.

In this research, different random trials of the wheat crop were taken. The effects were measured on protein, Zn and Fe by considering several factors of e[CO_2], water and N. This research is also involved in studying the impacts of the interactions of the three factors on nutrient compositions in wheat. The proposed method based on factorial design is implemented to accommodate all potential possibilities for primary, binary and triple interactions. Emphasis was placed on binary and triple interactions. This algorithm produced 49 trials for each experiment conducted for over three years. To the best of our knowledge, there have been no studies that discussed these combinations for each experiment.

Materials and Methods

This project proposes an efficient method for analysing the effects of the interactions of CO_2, water and N on grain protein, Zn and Fe concentrations. It consists of five phases. The first phase is the information about the datasets we used. The second phase deals with the classification and organization of the data to efficiently representing the data sets, while the third phase includes building a statistical model to describe the influence of the interactions of CO_2, water and N on grain protein, Zn and Fe of wheat crop. The methodology was implemented in Matlab to investigate all possible interactions for the three factors of e[CO_2], water, and N on protein, Zn and Fe in wheat crop. The fourth phase presents the results through tables, graphs and discussions. The final stage summarises the levels of interactions of the three factors and discussions of the results. Figure 1 illustrates the methodology of this study.

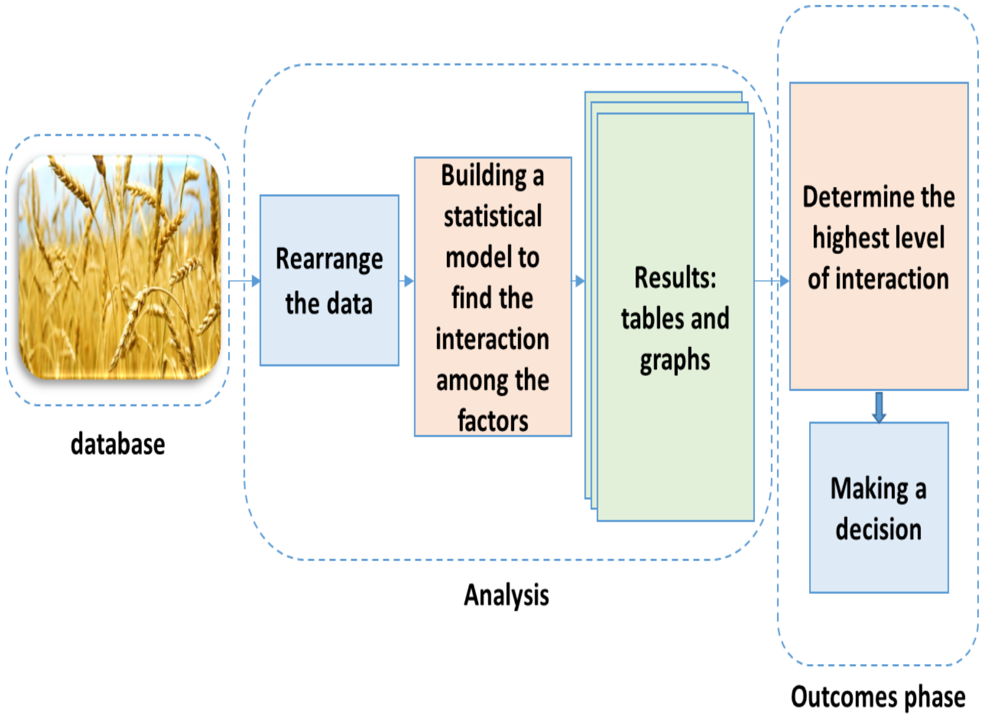

Fig. 1 Illustration of the methodology.

Data selection

The data used in this study was obtained from the website of *Nature (T. G. Dietterich & Horvitz, 2015)* through its URL[1]. This database contains two parts. One of them is the study results that have been used in our previous research (Al-Hadeethi et al., 2019). The second part is the primary data, the raw data that were used in this research. An extensive reprocessing to the data to make it appropriate to the method implemented in Matlab was performed. The data were sorted by the levels of the studies that we need to analyze. I rearranged the data based on individual experiments. Each experiment datasets contains *replication, year, crop, city, level of* CO_2*, level of W and level of N*. This study focused on examining the interactions of two different levels of [CO_2] (ambient and elevated), two levels of N (low, medium) and two levels of W (wet and dry) on grain protein, Zn and Fe for wheat in Australia for 2007, 2008 and 2009.

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[1] http://www.nature.com/articles/sdata201536#data-records

Method

Factorial experimental design

In a factorial model, the influences of all experimental variables, factors, and interaction effects on the response or responses are investigated. If the combinations of k factors are investigated at two levels, a factorial design will consist of 2^k experiments (Lundstedt et al., 1998). The choice of sampling or experimental design is fundamental to any statistical study. Factors and levels must be carefully selected by an individual or team who understands both the mathematical models and the issues that the study will address (Coy et al., 2001). Analysis of variance ANOVA was used to analyse the data to obtain the amount of effects of the interactions among the factors.

To investigate the effects of the interactions of e[CO_2], water and N on protein, Zn and Fe in crops, the factorial experimental design was carried out for choosing three factors' practical responses. These three factors of CO_2, water, N were represented by *a*, *b* and *c*, respectively, in the experiments. *ab, bc* and *ac* represent binary interactions of the three factors, and *abc* indicates their triple interaction. Table 1, 2 and 3 explain all the possible combinations of the three factors.

Table 1. Individual factors

Trial Number	**Factor (individual factor)**
1	***a***
2	***b***
3	***c***

Table 2. Binary interaction

Trial Number	Factor (Binary interaction)
4	***ab1***
5	***ab2***
6	***ab3***
7	***ab4***
8	***ab5***
9	***ab6***
10	***ac1***
11	***ac2***
12	***ac3***
13	***ac4***
14	***ac5***
15	***ac6***
16	***bc1***
17	***bc2***
18	***bc3***
19	***bc4***
20	***bc5***
21	***bc6***

Table 3. Triple interaction

Trial Number	Factor (Triple interaction)
22	***abc1***
23	***abc2***
24	***abc3***
25	***abc4***
26	***abc5***
27	***abc6***
28	***abc7***
29	***abc8***
30	***abc9***
31	***abc10***
32	***abc11***
33	***abc12***
34	***abc13***
35	***abc14***
36	***abc15***
37	***abc16***
38	***abc17***
39	***abc18***
40	***abc19***
41	***abc20***
42	***abc21***
43	***abc22***
44	***abc23***
45	***abc24***
46	***abc25***
47	***abc26***
48	***abc27***
49	***abc28***

where

Factors: a = CO_2, b = water, c = N (nitrogen); *ab*, *bc* and *ac* represent binary interactions among the three factors, and *abc* indicates their triple interaction.

Binary interaction: interaction between two factors

Triple interaction: interaction among three factors

Each of the factors of CO_2, water and N have two different levels of conditions coded as (-1 and +1) as shown in Table 4.

Table 4. Factor and their levels

Factor	**Level**	
CO_2	Ambient	Elevate
Water	Wet	Dry
N	Low	Medium

A 2^3 full factorial design was carried out to set the mathematical relationships and to represent how protein, Zn and Fe depend on CO_2, water and N. The running order for each run was randomized to minimize possible systematic errors. All the factors and their interaction terms were taken into account. A model can be presented as follows:

$$Y = \beta_o + \beta_{1a} + \beta_{2b} + \beta_{3c} + \beta_{12ab} + \beta_{13ac} + \beta_{23bc} + \beta_{123abc} \tag{1}$$

where Y is either protein, Zn or Fe. β_o is the constant; β_1, β_2 and β_3 were coefficients for the coded variables *a*, *b* and *c*, respectively; and β_{12}, β_{13}, β_{23} and β_{123} were the interaction effects among variables. All the design and analyses of the experiments were implemented in Matlab (version R17). The proposed method was implemented in Matlab to investigate all the possible interactions for the three factors (CO_2, water, and N) on protein, Zn and Fe of the wheat crop. The main effects and interactions of the factors on crop' nutrients were determined. As well as the standard error of the estimates, the sum of squares of the errors, *F* statistics, *p*-value and *t*-test.

From the *p*-values at the smallest level of significance to the rejection of the null hypothesis, it shows that the significant effect of each factor and the interaction impacts are statistically significant when *p*-values are less than 0.05. Since a 95% confidence level and 56 factorial tests, $F_{0,05,1,56,}$ is equal to 4.40, all the effect with *F*-values higher than 4.49 are significant. Student's *t*-test was also carried out to define whether the calculated main interaction impacts were significantly different from zero.

With a 95% confidence level (0.05, 63) degrees of freedom, the *t*-value was equal to 2.571.

The hypotheses that we will test are as follows:

1. H_0: Interaction of CO_2, water and N have no main effect on protein (2)
   H_1: Interaction of CO_2, water and N have a main effect on protein
2. H_0: Interaction of CO_2, water and N have no main effect on Zn (3)
   H_1: Interaction of CO_2, water and N have a main effect on Zn
3. H_0: Interaction of CO_2, water and N have no main effect on Fe (4)
   H_1: Interaction of CO_2, water and N have a main effect on Fe

Results

An experimental factorial design is a cost-effective method with a minimum number of trials (Yann et al., 2005). By using an experimental factorial design, a mathematical model was set to analyse the effects of interactions of e[CO_2], water and N on protein, Zn and Fe in the wheat crop. The factors of CO_2, water and N were represented as *a, b* and *c* in the model. The results of ANOVA for protein, Zn and Fe for the three-year period were given in Tables 3-11. ANOVA results indicated that three models were significant with the *F*-values. The results showed that all of the three factors in the three models were found to be statistically significant at $p<0.05$ in a 95% confidence interval. This result indicated that the model conditions of *a*, *b*, *c*, *ab*, *ac*, *bc* and *abc* in the three models were all statistically significant.

The results in Tables 5-13 showed that all the three factors CO_2, water and N in the three models had a significant effect on protein, Zn and Fe values in wheat crop. The highest impact of the three factors on the protein at an average value of 31 is 4.5% (Fig. 2.), under conditions of elevated CO_2, well- water and low N.

For Zn, the high effect of e[CO_2], water, and N was at an average value of 32 is 3.5% (Fig 3.), under conditions of elevated CO_2, low water and medium N.

While the strong influence of three factors on Fe was at an average value of 25 is 4.1% under elevated CO_2 conditions, wet and low N. (Fig 4.)

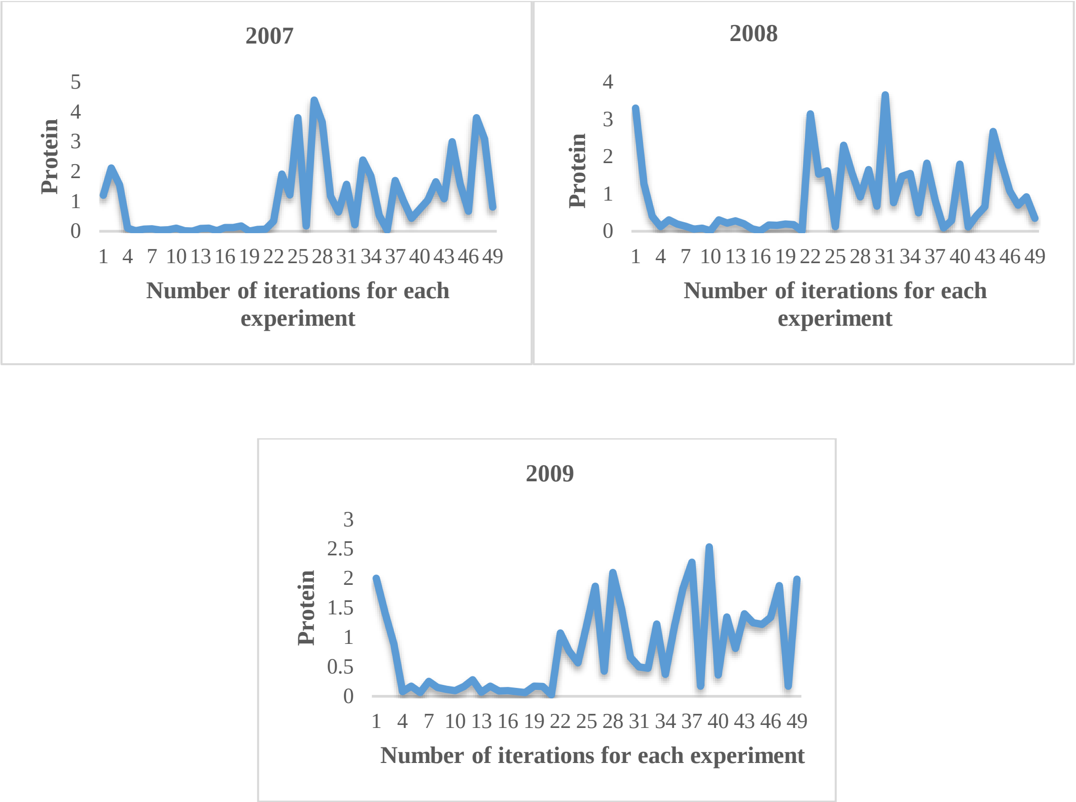

Fig. 2 Effects of elevated [CO_2], water and N on crop protein

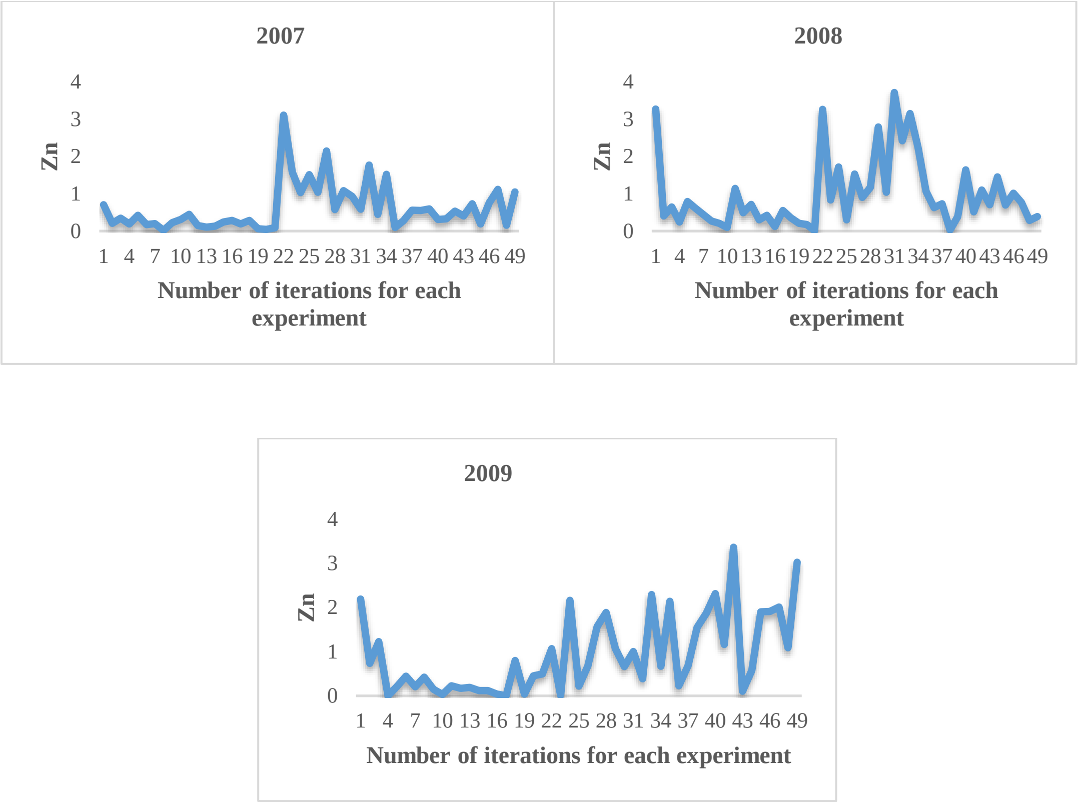

Fig. 3. Effects of elevated [CO_2], water and N on crop Zn

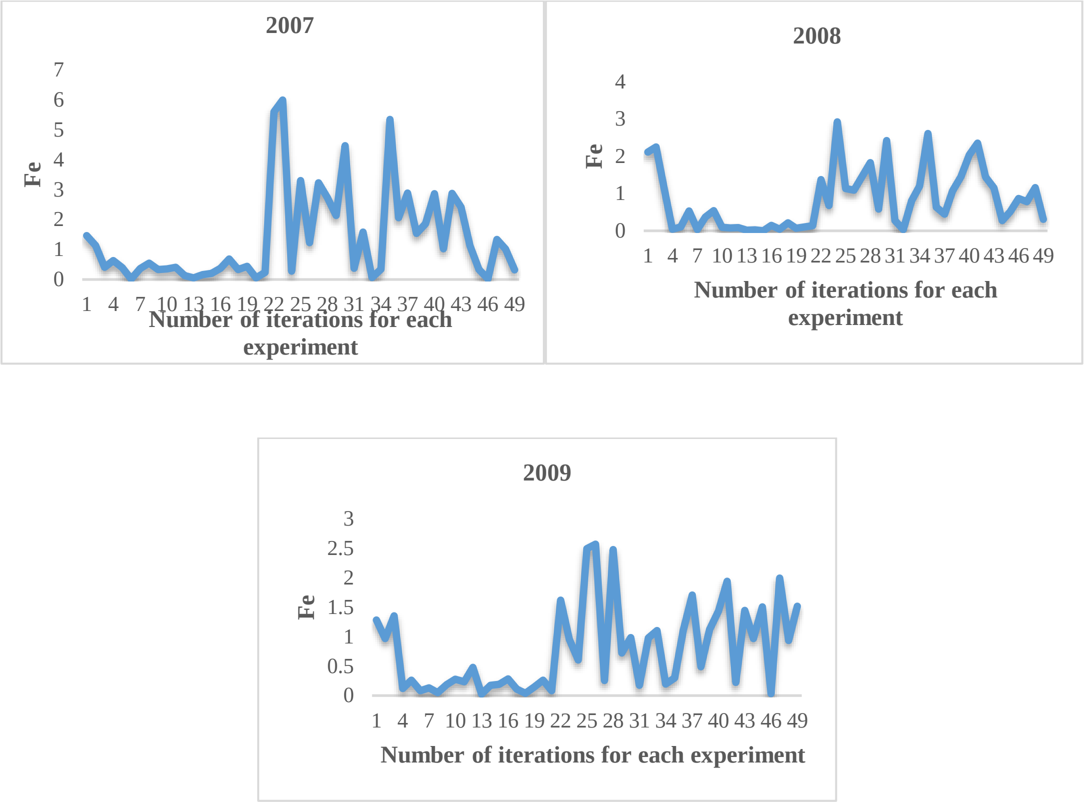

Fig. 4 Effects of elevated [CO_2], water and N on crop Fe

Table 5. Analyses of variance for the factorial model (Protein 2007)

Treatments	*df*	*S.S*	*MS*	*F*	*p-values*
a	1	0.669811	0.669811	20.31639	**
b	1	3.143946	3.143946	95.36063	***
c	1	1.33568	1.33568	40.5132	**
ab	1	3.81575	3.81575	115.7375	***
ac	1	2.386557	2.386557	72.38788	**
bc	1	4.505701	4.505701	136.6647	***
abc	1	5.982861	5.982861	181.4692	***
Error	56	1.846265	0.032969		
Total	63				

* Non-significance

** Medium significance

*** High significance

df is a degree of freedom

S.S is a sum of error squares

MS is mean squares error

The results approved the hypothesis of the interaction of CO_2, water and N having the main effect on the protein. A *p*-value less or equal to 0.05 that was accepted, while those with a value exceeding 0.05 were rejected. The reduction in protein concentration was highly significant at binary interactions of (e[CO_2] with water, and water with N) and triple interactions of (e[CO_2], water and N). At the same time, the decrease in protein was medium significant at e[CO_2], N and binary interactions of (e[CO_2] and N).

Table 6. Analyses of variance for the factorial model (Protein 2008)

Treatments	*df*	*S.S*	*MS*	*F*	*p*-values
a	1	11.5113	11.5113	12.55297	**
b	1	0.53374	0.53374	0.582038	*
c	1	1.087325	1.087325	1.185718	*
ab	1	21.96574	21.96574	23.95343	**
ac	1	13.63625	13.63625	14.8702	**
bc	1	1.622914	1.622914	1.769772	*
abc	1	24.17823	24.17823	26.36614	**
Error	56	51.35304	0.917019		
Total	63				

The hypothesis was true. The protein concentration showed a medium decrease at e[CO_2] and binary interactions of (e[CO_2] with water and e[CO_2] with N) and at triple interactions of (e[CO_2], water and N).

Table 7. Analyses of variance for the factorial model (Protein 2009)

Treatments	*df*	*S.S*	*MS*	*F*	*p-values*
a	1	19.88103	19.88103	18.90737	**
b	1	4.530512	4.530512	4.308634	*
c	1	5.045504	5.045504	4.798404	**
ab	1	24.67493	24.67493	23.4665	**
ac	1	25.69616	25.69616	24.43771	**
bc	1	9.582308	9.582308	9.113021	**
abc	1	31.30538	31.30538	29.77222	**
Error	56	58.88379	1.051496		
Total	63				

The results approved the hypothesis. The lowering in protein concentration had a medium significant, approximately at all individuals, binary interactions and triple interactions of factors.

Table 8. Analyses of variance for the factorial model (Zn 2007)

Treatments	*df*	*S.S*	*MS*	*F*	*p*-values
a	1	0.020306	0.020306	0.000723	*
b	1	0.286225	0.286225	0.010186	*
c	1	0.041006	0.041006	0.001459	*
ab	1	50.71653	50.71653	5.804835	**
ac	1	115.8927	115.8927	4.824242	**
bc	1	0.373456	0.373456	10.01329	**
abc	1	191.7854	191.7854	6.825013	**
Error	56	1573.621	28.10037		
Total	63				

The results approved the hypothesis of the interaction of CO_2, water and N having the main effect on Zn. A *p*-value less or equal to 0.05 that was accepted, while those with a value exceeding 0.05 were rejected.

The reduction in Zn concentration was a medium significant at binary interactions of (e[CO_2] with water, e[CO_2] with N, and water with N) and triple interactions of (e[CO_2], water and N).

Table 9. Analyses of variance for the factorial model (Zn 2008)

Treatments	*df*	*S.S*	*MS*	*F*	*p*-value
a	1	462.6263	462.6263	23.7065	**
b	1	2.398627	2.398627	0.122914	*
c	1	46.56356	46.56356	2.386071	*
ab	1	568.7846	568.7846	29.14641	**
ac	1	599.7013	599.7013	30.73068	**
bc	1	50.60379	50.60379	22.593106	**
abc	1	720.2908	720.2908	36.91008	**
Error	56	1092.826	19.51474		
Total	63				

The hypothesis was approved. The reduction in Zn concentration was a medium significant at binary interactions and triple interactions of factors.

Table 10. Analyses of variance for the factorial model (Zn 2009)

Treatments	*df*	*S.S*	*MS*	*F*	*p*-values
a	1	405.5189	405.5189	55.0772	**
b	1	0.039006	0.039006	0.005298	*
c	1	27.58876	27.58876	3.747079	*
ab	1	521.0129	521.0129	70.76349	***
ac	1	443.8989	443.8989	60.28993	***
bc	1	40.91379	40.91379	5.556872	**
abc	1	618.953	618.953	84.06561	***
Error	56	412.3133	7.362737		
Total	63				

The results confirmed the hypothesis. The reduction in Zn concentration was highly significant at binary interactions of (e[CO_2] with water, and e[CO_2] with N) and triple interactions of (e[CO_2] with water and N) while the lowering in Zn concentration was a medium significant at binary interactions of (water with N).

Table 11. Analyses of variance for the factorial model (Fe 2007)

Treatments	*df*	*S.S*	*MS*	*F*	*p*-values
a	1	37.34738	37.34738	6.570862	**
b	1	43.05	43.05	7.574177	**
c	1	8.143889	8.143889	1.432828	*
ab	1	216.7322	216.7322	38.13166	**
ac	1	45.51974	45.51974	8.008701	**
bc	1	51.40664	51.40664	9.044437	**
abc	1	453.1651	453.1651	79.72944	***
Error	56	318.292	5.683786		
Total	63				

* Non-significance

** Medium significance

*** High significance

The results approved the hypothesis of the interaction of CO_2, water and N having the main effect on Fe. A *p*-value less or equal to 0.05 that were accepted, while those with values exceeding 0.05 were rejected.

The decrease in Fe concentration was a medium significant at e[CO_2] and W and binary interactions of (e[CO_2] with water, e[CO_2] with N, and water with N) while the reduction in Fe concentration was highly significant at triple interactions of (e[CO_2] with W and N).

Table 12. Analyses of variance for the factorial model (Fe 2008)

Treatments	*df*	*S.S*	*MS*	*F*	*p*-values
a	1	87.28231	87.28231	11.9626	**
b	1	137.007	137.007	18.77769	**
c	1	0.154056	0.154056	0.021114	*
ab	1	278.3486	278.3486	38.14945	**
ac	1	159.008	159.008	21.79306	**
bc	1	149.0119	149.0119	20.42303	**
abc	1	381.6387	381.6387	52.30602	**
Error	56	408.5909	7.296267		
Total	63				

The results approved the hypothesis. Approximately the reduction in Fe concentration was a medium significant at individuals, binary and triple interactions of factors.

Table 13. Analyses of variance for the factorial model (Fe 2009)

Treatments	*df*	*S.S*	*MS*	*F*	*p*-values
a	1	90.01266	90.01266	7.77056	**
b	1	50.33903	50.33903	4.345638	*
c	1	85.0084	85.0084	7.338556	**
ab	1	152.6718	152.6718	13.17976	**
ac	1	186.5132	186.5132	16.1012	**
bc	1	165.9007	165.9007	14.32178	**
abc	1	308.0013	308.0013	26.58896	**
Error	56	648.6931	11.58381		
Total	63				

Again, the results demonstrated the hypothesis was true. The concentration of Fe decreased had a medium significant at all levels of interactions of factors (individually, binary and triple).

Discussions

In statistics, the factorial experiments allow a researcher to study how the response variable affected based on each factor, and the effects of interactions among different levels of elements on the response variable. This work suggested that designing factorial experiments to measure the impact of e[CO_2], water and N on the essential nutrients in wheat crop. The experimental models are designed to be able to accommodate many factors with different levels. The experiments were designed to investigate the three essential nutrition elements of protein, Zn and Fe under the influence of three factors e[CO_2], water and N, and each factor has two levels of conditions (for example, wet and dry). This paper focuses on studying binary interactions and triple interactions among factors (CO_2, water or N), to explore the levels of impact on the nutrition elements of protein, Zn and Fe concentrations. For the triple interaction, it was designed to find out the effect of three factors on each individual nutrition element. Fig. 5 shows the diagram of the influences of interactions of CO_2, water and N on protein, Zn and Fe in wheat crop.

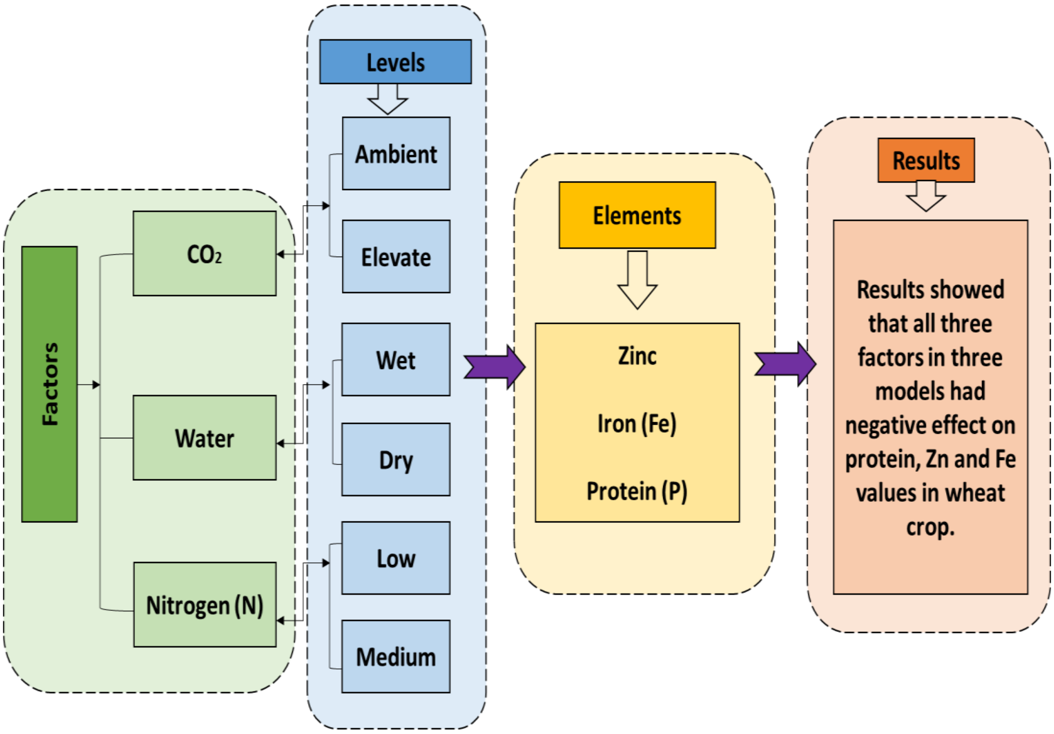

Fig. 5. The diagram of interactions of CO_2, water and N on protein, Zn and Fe in wheat crop.

Conclusions

The 2^3 factorial design was used to investigate the effects of the interactions of e[CO_2], water and N on protein, Zn and Fe in wheat crop. The experimental models indicated that the nutrients in the crop are susceptible to e[CO_2] under water and N conditions. The research results in this study showed that the interactions of e[CO_2], water and N can affect the protein, Zn and Fe concentrations in wheat crop negatively. Where protein, Zn and Fe were reduced by 4.5%, 3.5%, 4.1%, respectively, during the three-year experimental period. The results also indicated that with a careful selection of the water and N conditions under e[CO_2], crop quality can be improved. The proposed method was implemented in Matlab to investigate all possible interactions for three factors (CO_2, water, and N) on protein, Zn and Fe in wheat crop.

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CHAPTER 6

CONCLUSION AND FUTURE WORK

6.1 Conclusion

In chapter three, this study aimed to analyse the effects of e[CO_2], temperature and nitrogen on grain protein and grain yield. The proposed techniques will improve the accuracy of the 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 e[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 different data.

In chapter four, rising atmospheric [CO_2] is likely to decrease protein, Zn and Fe concentrations in many crops such as wheat, rice and corn. However, protein and Fe concentrations increase in soybean under e[CO_2]. Nevertheless, reduction in protein, Zn and Fe concentrations were found to be consistent over diverse species across a wide range of experimental techniques and environmental conditions. Increased use of nitrogen fertilizers and water may lessen the effects of elevated [CO_2] on protein, Zn and Fe concentrations in rice. However, this approach might be only a partial solution. In other crops such as corn, high nitrogen could result in high reductions in protein, Zn and Fe concentrations. The analysis indicated that there are medium levels of bias within published meta-analysis studies of crops responses to FACE. However, integration of the influence of reporting bias did not affect the significance or the direction of the [CO_2] effects. The effects of atmospheric [CO_2] on protein, Zn and Fe in crops are, therefore, likely to be of substantial importance to human nutrition in and beyond the 21st century.

In chapter five, The 2^3 factorial design was used to investigate the effects of the interactions of e[CO_2], water and N on protein, Zn and Fe in wheat crop. The experimental models indicated that the nutrients in the crop are susceptible to e[CO_2] under water and N conditions. The research results in this study showed that the interactions of e[CO_2], water and N can affect the protein, Zn and Fe concentrations in wheat crop negatively. Where protein, Zn and Fe were reduced by 4.5%, 3.5%, 4.1%, respectively, during the three-year experimental period. The results also indicated that with a careful selection of the water and N conditions under e[CO_2], crop quality could be improved. The proposed method was implemented in Matlab to investigate all possible interactions for three factors (CO_2, water, and N) on protein, Zn and Fe in wheat crop.

6.2 Future work

The techniques presented in this thesis provide excellent performances in determining the impacts of environmental factors on crop qualities. The future work can be to explore the potential applications of those methods. A few key areas below are suggested for the further improvement of this work.

- Expand data collections from more regions, for example, in the Middle East to discover the effects of environmental factors on crops in a different atmosphere and weather conditions. Particularly in Iraq, the weather is entirely different from the north to the middle, and to the south.

- Improve meta-analysis models to absorb the binary and triple interactions for more accuracy when involving massive data from different fields and for an extended period. The models will be developed in several stages which include,
  - to derive a new framework fitting with the interaction of the four elements of CO_2, temperature, water and nitrogen.
  - to combine two models to obtain a more accurate method that will be able to accommodate the new data collected in the future.

- Meta-analysis models can be applied to many different application fields, for example:
  - for biomedical data like EEG and ECG signals.

- for medical data - an example involves using meta-analysis to analysing breast cancer data collected from the period of 2010 to 2020.
- for food industry data, such as meta-analysis is used by food processing laboratories to investigate the effect of using preservatives before and after adding them to the food.
- for economic data, for example, in Iraq, to determine the impacts of wars on the economy of Iraq and to define how the economy was before and after the wars.
- meta-analysis models can also be applied to other fields of study, such as plants and forest. Due to the robustness of meta-analysis models, there is no limitation in applying it to different types of areas.`

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APPENDIX A

APPENDIX A

Matlab simulation code for Chapter 5

Estimating the effect of interactions of environmental factors on grain quality based on factorial experimental design

The experiment results were obtained using Matlab programming language (version R17).

```
y = dlmread('f1.txt');
n=8;
a=2;
b=2;
c=2;
k=8;
s1=0;
s2=0;
for i=1:n
   for j=1:k
      s1=s1+y(i,j);
      s2=s2+y(i,j)^2;
   end
end
cf=s1^2/(n*k);
sst=s2-cf;
for j=1:k
   fol=0;
   for i=1:n
      fol=fol+y(i,j);
   end
```

```
    t(j)=fol;
end

% main factor calculations
fa1=t(1)+t(3)+t(5)+t(7);
fa2=t(2)+t(4)+t(6)+t(8);
ssa=(fa1^2+fa2^2)/(n*b*c)-cf;

fb1=t(1)+t(2)+t(5)+t(6);
fb2=t(3)+t(4)+t(7)+t(8);
ssb=(fb1^2+fb2^2)/(n*a*c)-cf;

fc1=t(1)+t(2)+t(3)+t(4);
fc2=t(5)+t(6)+t(7)+t(8);
ssc=(fc1^2+fc2^2)/(n*a*b)-cf;

%  interaction  calculations
fa1b1=t(1)+t(5);
fa1b2=t(3)+t(7);
fa2b1=t(2)+t(6);
fa2b2=t(4)+t(8);
ssab=(fa1b1^2+fa1b2^2+fa2b1^2+fa2b2^2)/(n*c)-cf;

fa1c1=t(1)+t(3);
fa1c2=t(5)+t(7);
fa2c1=t(2)+t(4);
fa2c2=t(6)+t(8);
ssac=(fa1c1^2+fa1c2^2+fa2c1^2+fa2c2^2)/(n*b)-cf;

fb1c1=t(1)+t(2);
```

```
fb1c2=t(5)+t(6);
fb2c1=t(3)+t(4);
fb2c2=t(7)+t(8);
ssbc=(fb1c1^2+fb1c2^2+fb2c1^2+fb2c2^2)/(n*a)-cf;

ssabc=(t(1)^2+t(2)^2+t(3)^2+t(4)^2+t(5)^2+t(6)^2+t(7)^2+t(8)^2)/n-cf;
sse=sst-(ssa+ssb+ssc+ssab+ssac+ssbc+ssabc);

msa=ssa/(a-1);
msb=ssb/(b-1);
msc=ssc/(c-1);
msab=ssab/(a-1)*(b-1);
msac=ssac/(a-1)*(c-1);
msbc=ssbc/(b-1)*(c-1);
msabc=ssabc/(a-1)*(b-1)*(c-1);

dfe=(n*a*b*c)-((a-1)+(b-1)+(c-1)+(a-1)*(b-1)+(a-1)*(c-1)+(b-1)*(c-1)+(a-1)*(b-1)*(c-1))-1;
mse=sse/dfe;

% F test calculations
fa=msa/mse;
fb=msb/mse;
fc=msc/mse;
fab=msab/mse;
fac=msac/mse;
fbc=msbc/mse;
fabc=msabc/mse;
ddf(1)=(a-1);
ddf(2)=(b-1);
ddf(3)=(c-1);
```

```
ddf(4)=(a-1)*(b-1);
ddf(5)=(a-1)*(c-1);
ddf(6)=(b-1)*(c-1);
ddf(7)=(a-1)*(b-1)*(c-1);
ddf(8)=dfe;
ddf(9)=n*a*b*c-1;
dss(1)=ssa;
dss(2)=ssb;
dss(3)=ssc;
dss(4)=ssab;
dss(5)=ssac;
dss(6)=ssbc;
dss(7)=ssabc;
dss(8)=sse;
dms(1)=msa;
dms(2)=msb;
dms(3)=msc;
dms(4)=msab;
dms(5)=msac;
dms(6)=msbc;
dms(7)=msabc;
dms(8)=mse;
dft(1)=fa;
dft(2)=fb;
dft(3)=fc;
dft(4)=fab;
dft(5)=fac;
dft(6)=fbc;
dft(7)=fabc;
```

```
ak1=ddf';
ak2=dss';
ak3=dms';
ak4=dft';



for j=1:k
    fol=0;
    for i=1:n
        fol=fol+y(i,j);
    end
    t(j)=fol;
end
ro=0;
for j=1:k
    ro=ro+1;
    ma1(ro)=y(j,1);
    ma2(ro)=y(j,2);
    mb1(ro)=y(j,1);
    mb2(ro)=y(j,3);
    mc1(ro)=y(j,1);
    mc2(ro)=y(j,5);

end
ro=k;
for j=1:k
    ro=ro+1;
    ma1(ro)=y(j,3);
    ma2(ro)=y(j,4);
    mb1(ro)=y(j,2);
```

```
    mb2(ro)=y(j,4);
    mc1(ro)=y(j,2);
    mc2(ro)=y(j,6);

end
ro=2*k;
for j=1:k
    ro=ro+1;
    ma1(ro)=y(j,5);
    ma2(ro)=y(j,6);
    mb1(ro)=y(j,5);
    mb2(ro)=y(j,7);
    mc1(ro)=y(j,3);
    mc2(ro)=y(j,7);

end
ro=3*k;
for j=1:k
    ro=ro+1;
    ma1(ro)=y(j,7);
    ma2(ro)=y(j,8);
    mb1(ro)=y(j,6);
    mb2(ro)=y(j,8);
    mc1(ro)=y(j,4);
    mc2(ro)=y(j,8);
end

ro=0;
for j=1:k
    ro=ro+1;
```

```
mab11(ro)=y(j,1);
mac11(ro)=y(j,1);
mbc11(ro)=y(j,1);
mab12(ro)=y(j,3);
mac12(ro)=y(j,5);
mbc12(ro)=y(j,5);
mab21(ro)=y(j,2);
mac21(ro)=y(j,2);
mbc21(ro)=y(j,3);
mab22(ro)=y(j,4);
mac22(ro)=y(j,6);
mbc22(ro)=y(j,7);
end
ro=2*k;
for j=1:k
    ro=ro+1;
mab11(ro)=y(j,5);
mac11(ro)=y(j,3);
mbc11(ro)=y(j,2);
mab12(ro)=y(j,7);
mac12(ro)=y(j,7);
mbc12(ro)=y(j,6);
mab21(ro)=y(j,6);
mac21(ro)=y(j,4);
mbc21(ro)=y(j,4);
mab22(ro)=y(j,8);
mac22(ro)=y(j,8);
mbc22(ro)=y(j,8);
end
```

```
xa1=0;
xb1=0;
xc1=0;
xa2=0;
xb2=0;
xc2=0;
for j=1:3*k
xa1=xa1+ma1(j)/(3*k);
xa2=xa2+ma2(j)/(3*k);
xb1=xb1+mb1(j)/(3*k);
xb2=xb2+mb2(j)/(3*k);
xc1=xc1+mc1(j)/(3*k);
xc2=xc2+mc2(j)/(3*k);
end
xab11=0;
xab12=0;
xab21=0;
xab22=0;
xac11=0;
xac12=0;
xac21=0;
xac22=0;
xbc11=0;
xbc12=0;
xbc21=0;
xbc22=0;

for i=1:2*k
    xab11=xab11+mab11(i)/(2*k);
    xab12=xab12+mab12(i)/(2*k);
```

```
    xab21=xab21+mab21(i)/(2*k);
    xab22=xab22+mab22(i)/(2*k);
xac11=xac11+mac11(i)/(2*k);
    xac12=xac12+mac12(i)/(2*k);
    xac21=xac21+mac21(i)/(2*k);
    xac22=xac22+mac22(i)/(2*k);
xbc11=xbc11+mbc11(i)/(2*k);
    xbc12=xbc12+mbc12(i)/(2*k);
    xbc21=xbc21+mbc21(i)/(2*k);
    xbc22=xbc22+mbc22(i)/(2*k);

end


sxa1=0;
sxb1=0;
sxc1=0;
sxa2=0;
sxb2=0;
sxc2=0;
for j=1:3*k
sxa1=sxa1+((ma1(j)-xa1)^2)/(3*k-1);
sxa2=sxa2+((ma2(j)-xa2)^2)/(3*k-1);
sxb1=sxb1+((mb1(j)-xb1)^2)/(3*k-1);
sxb2=sxb2+((mb2(j)-xb2)^2)/(3*k-1);
sxc1=sxc1+((mc1(j)-xc1)^2)/(3*k-1);
sxc2=sxc2+((mc2(j)-xc2)^2)/(3*k-1);
end
sxab11=0;
sxab12=0;
```

```
sxab21=0;
sxab22=0;
sxac11=0;
sxac12=0;
sxac21=0;
sxac22=0;
sxbc11=0;
sxbc12=0;
sxbc21=0;
sxbc22=0;

for i=1:2*k
sxab11=sxab11+((mab11(j)-xab11)^2)/(2*k-1);
sxab12=sxab12+((mab12(j)-xab12)^2)/(2*k-1);
sxab21=sxab21+((mab21(j)-xab21)^2)/(2*k-1);
sxab22=sxab22+((mab22(j)-xab22)^2)/(2*k-1);
sxac11=sxac11+((mac11(j)-xac11)^2)/(2*k-1);
sxac12=sxac12+((mac12(j)-xac12)^2)/(2*k-1);
sxac21=sxac21+((mac21(j)-xac21)^2)/(2*k-1);
sxac22=sxac22+((mac22(j)-xac22)^2)/(2*k-1);
sxbc11=sxbc11+((mbc11(j)-xbc11)^2)/(2*k-1);
sxbc12=sxbc12+((mbc12(j)-xbc12)^2)/(2*k-1);
sxbc21=sxbc21+((mbc21(j)-xbc21)^2)/(2*k-1);
sxbc22=sxbc22+((mbc22(j)-xbc22)^2)/(2*k-1);
end
df1=3*k;
df2=2*k;
sa=(((df1-1)*sxa1+(df1-1)*sxa2)/(df1+df1-2))^0.5;
sb=(((df1-1)*sxb1+(df1-1)*sxb2)/(df1+df1-2))^0.5;
sc=(((df1-1)*sxc1+(df1-1)*sxc2)/(df1+df1-2))^0.5;
```

```
sab1=(((df2-1)*sxab11+(df2-1)*sxab12)/(df2+df2-2))^0.5;
sab2=(((df2-1)*sxab11+(df2-1)*sxab21)/(df2+df2-2))^0.5;
sab3=(((df2-1)*sxab11+(df2-1)*sxab22)/(df2+df2-2))^0.5;
sab4=(((df2-1)*sxab12+(df2-1)*sxab21)/(df2+df2-2))^0.5;
sab5=(((df2-1)*sxab12+(df2-1)*sxab22)/(df2+df2-2))^0.5;
sab6=(((df2-1)*sxab21+(df2-1)*sxab22)/(df2+df2-2))^0.5;

sac1=(((df2-1)*sxac11+(df2-1)*sxac12)/(df2+df2-2))^0.5;
sac2=(((df2-1)*sxac11+(df2-1)*sxac21)/(df2+df2-2))^0.5;
sac3=(((df2-1)*sxac11+(df2-1)*sxac22)/(df2+df2-2))^0.5;
sac4=(((df2-1)*sxac12+(df2-1)*sxac21)/(df2+df2-2))^0.5;
sac5=(((df2-1)*sxac12+(df2-1)*sxac22)/(df2+df2-2))^0.5;
sac6=(((df2-1)*sxac21+(df2-1)*sxac22)/(df2+df2-2))^0.5;

sbc1=(((df2-1)*sxbc11+(df2-1)*sxbc12)/(df2+df2-2))^0.5;
sbc2=(((df2-1)*sxbc11+(df2-1)*sxbc21)/(df2+df2-2))^0.5;
sbc3=(((df2-1)*sxbc11+(df2-1)*sxbc22)/(df2+df2-2))^0.5;
sbc4=(((df2-1)*sxbc12+(df2-1)*sxbc21)/(df2+df2-2))^0.5;
sbc5=(((df2-1)*sxbc12+(df2-1)*sxbc22)/(df2+df2-2))^0.5;
sbc6=(((df2-1)*sxbc21+(df2-1)*sxbc22)/(df2+df2-2))^0.5;

for j=1:k
  xabc(j)=t(j)/k;
end
for j=1:k
    ssss=0;
    for i=1:n
        ssss=ssss+((y(i,j)-xabc(j))^2)/(k-1);
    end
```

```
    sabc(j)=ssss;
end
sabc1=(((k-1)*sabc(1)+(k-1)*sabc(2))/(k+k-2))^0.5;
sabc2=(((k-1)*sabc(1)+(k-1)*sabc(3))/(k+k-2))^0.5;
sabc3=(((k-1)*sabc(1)+(k-1)*sabc(4))/(k+k-2))^0.5;
sabc4=(((k-1)*sabc(1)+(k-1)*sabc(5))/(k+k-2))^0.5;
sabc5=(((k-1)*sabc(1)+(k-1)*sabc(6))/(k+k-2))^0.5;
sabc6=(((k-1)*sabc(1)+(k-1)*sabc(7))/(k+k-2))^0.5;
sabc7=(((k-1)*sabc(1)+(k-1)*sabc(8))/(k+k-2))^0.5;
sabc8=(((k-1)*sabc(2)+(k-1)*sabc(3))/(k+k-2))^0.5;
sabc9=(((k-1)*sabc(2)+(k-1)*sabc(4))/(k+k-2))^0.5;
sabc10=(((k-1)*sabc(2)+(k-1)*sabc(5))/(k+k-2))^0.5;
sabc11=(((k-1)*sabc(2)+(k-1)*sabc(6))/(k+k-2))^0.5;
sabc12=(((k-1)*sabc(2)+(k-1)*sabc(7))/(k+k-2))^0.5;
sabc13=(((k-1)*sabc(2)+(k-1)*sabc(8))/(k+k-2))^0.5;
sabc14=(((k-1)*sabc(3)+(k-1)*sabc(4))/(k+k-2))^0.5;
sabc15=(((k-1)*sabc(3)+(k-1)*sabc(5))/(k+k-2))^0.5;
sabc16=(((k-1)*sabc(3)+(k-1)*sabc(6))/(k+k-2))^0.5;
sabc17=(((k-1)*sabc(3)+(k-1)*sabc(7))/(k+k-2))^0.5;
sabc18=(((k-1)*sabc(3)+(k-1)*sabc(8))/(k+k-2))^0.5;
sabc19=(((k-1)*sabc(4)+(k-1)*sabc(5))/(k+k-2))^0.5;
sabc20=(((k-1)*sabc(4)+(k-1)*sabc(6))/(k+k-2))^0.5;
sabc21=(((k-1)*sabc(4)+(k-1)*sabc(7))/(k+k-2))^0.5;
sabc22=(((k-1)*sabc(4)+(k-1)*sabc(8))/(k+k-2))^0.5;
sabc23=(((k-1)*sabc(5)+(k-1)*sabc(6))/(k+k-2))^0.5;
sabc24=(((k-1)*sabc(5)+(k-1)*sabc(7))/(k+k-2))^0.5;
sabc25=(((k-1)*sabc(5)+(k-1)*sabc(8))/(k+k-2))^0.5;
sabc26=(((k-1)*sabc(6)+(k-1)*sabc(7))/(k+k-2))^0.5;
sabc27=(((k-1)*sabc(6)+(k-1)*sabc(8))/(k+k-2))^0.5;
sabc28=(((k-1)*sabc(7)+(k-1)*sabc(8))/(k+k-2))^0.5;
```

```
dft1=((1/df1)+(1/df1))^0.5;
dft2=((1/df2)+(1/df2))^0.5;
dft3=((1/k)+(1/k))^0.5;

ta=abs(xa1-xa2)/(sa*dft1);
tb=abs(xb1-xb2)/(sa*dft1);
tc=abs(xc1-xc2)/(sa*dft1);



tab1=abs(xab11-xab12)/(sab1*dft2);
tab2=abs(xab11-xab21)/(sab2*dft2);
tab3=abs(xab11-xab22)/(sab3*dft2);
tab4=abs(xab12-xab21)/(sab4*dft2);
tab5=abs(xab12-xab22)/(sab5*dft2);
tab6=abs(xab21-xab22)/(sab6*dft2);

tac1=abs(xac11-xac12)/(sac1*dft2);
tac2=abs(xac11-xac21)/(sac2*dft2);
tac3=abs(xac11-xac22)/(sac3*dft2);
tac4=abs(xac12-xac21)/(sac4*dft2);
tac5=abs(xac12-xac22)/(sac5*dft2);
tac6=abs(xac21-xac22)/(sac6*dft2);

tbc1=abs(xbc11-xbc12)/(sbc1*dft2);
tbc2=abs(xbc11-xbc21)/(sbc2*dft2);
tbc3=abs(xbc11-xbc22)/(sbc3*dft2);
tbc4=abs(xbc12-xbc21)/(sbc4*dft2);
tbc5=abs(xbc12-xbc22)/(sbc5*dft2);
```

```
tbc6=abs(xbc21-xbc22)/(sbc6*dft2);

tabc1=abs(xabc(1)-xabc(2))/(sabc1*dft3);
tabc2=abs(xabc(1)-xabc(3))/(sabc2*dft3);
tabc3=abs(xabc(1)-xabc(4))/(sabc3*dft3);
tabc4=abs(xabc(1)-xabc(5))/(sabc4*dft3);
tabc5=abs(xabc(1)-xabc(6))/(sabc5*dft3);
tabc6=abs(xabc(1)-xabc(7))/(sabc6*dft3);
tabc7=abs(xabc(1)-xabc(8))/(sabc7*dft3);
tabc8=abs(xabc(2)-xabc(3))/(sabc8*dft3);
tabc9=abs(xabc(2)-xabc(4))/(sabc9*dft3);
tabc10=abs(xabc(2)-xabc(5))/(sabc10*dft3);
tabc11=abs(xabc(2)-xabc(6))/(sabc11*dft3);
tabc12=abs(xabc(2)-xabc(7))/(sabc12*dft3);
tabc13=abs(xabc(2)-xabc(8))/(sabc13*dft3);
tabc14=abs(xabc(3)-xabc(4))/(sabc14*dft3);
tabc15=abs(xabc(3)-xabc(5))/(sabc15*dft3);
tabc16=abs(xabc(3)-xabc(6))/(sabc16*dft3);
tabc17=abs(xabc(3)-xabc(7))/(sabc17*dft3);
tabc18=abs(xabc(3)-xabc(8))/(sabc18*dft3);
tabc19=abs(xabc(4)-xabc(5))/(sabc19*dft3);
tabc20=abs(xabc(4)-xabc(6))/(sabc20*dft3);
tabc21=abs(xabc(4)-xabc(7))/(sabc21*dft3);
tabc22=abs(xabc(4)-xabc(8))/(sabc22*dft3);
tabc23=abs(xabc(5)-xabc(6))/(sabc23*dft3);
tabc24=abs(xabc(5)-xabc(7))/(sabc24*dft3);
tabc25=abs(xabc(5)-xabc(8))/(sabc25*dft3);
tabc26=abs(xabc(6)-xabc(7))/(sabc26*dft3);
tabc27=abs(xabc(6)-xabc(8))/(sabc27*dft3);
tabc28=abs(xabc(7)-xabc(8))/(sabc28*dft3);
```

```
ttt(1)=ta;
ttt(2)=tb;
ttt(3)=tc;
ttt(4)=tab1;
ttt(5)=tab2;
ttt(6)=tab3;
ttt(7)=tab4;
ttt(8)=tab5;
ttt(9)=tab6;
ttt(10)=tac1;
ttt(11)=tac2;
ttt(12)=tac3;
ttt(13)=tac4;
ttt(14)=tac5;
ttt(15)=tac6;
ttt(16)=tbc1;
ttt(17)=tbc2;
ttt(18)=tbc3;
ttt(19)=tbc4;
ttt(20)=tbc5;
ttt(21)=tbc6;
ttt(22)=tabc1;
ttt(23)=tabc2;
ttt(24)=tabc3;
ttt(25)=tabc4;
ttt(26)=tabc5;
ttt(27)=tabc6;
ttt(28)=tabc7;
ttt(29)=tabc8;
```

```
ttt(30)=tabc9;
ttt(31)=tabc10;
ttt(32)=tabc11;
ttt(33)=tabc12;
ttt(34)=tabc13;
ttt(35)=tabc14;
ttt(36)=tabc15;
ttt(37)=tabc16;
ttt(38)=tabc17;
ttt(39)=tabc18;
ttt(40)=tabc19;
ttt(41)=tabc20;
ttt(42)=tabc21;
ttt(43)=tabc22;
ttt(44)=tabc23;
ttt(45)=tabc24;
ttt(46)=tabc25;
ttt(47)=tabc26;
ttt(48)=tabc27;
ttt(49)=tabc28;

ak5=ttt';
```