Increasing Wheat Protein and Yield through Sulfur Fertilization and Its Relationship with Nitrogen

Gustavo A. Roa 1,2,*, Eber Addí Quintana-Obregón 3, Mariela González-Rentería 4, and Dorivar A. Ruiz Diaz 1

1 Department of Agronomy, Kansas State University, Manhattan, KS 66506, USA; ruizdiaz@ksu.edu
2 Maestría en Seguridad Alimentaria, Universidad Abierta y Distancia de Mexico (UnADM), Mexico City 03330, Mexico
3 CONACyT-Centro de Investigación en Alimentación y Desarrollo, A.C. Coordinación de Tecnología de Alimentos de Origen Vegetal, Hermosillo 83304, Mexico; eber.quintana@ciad.mx
4 División Académica de Ciencias Agropecuarias, Universidad Juárez Autónoma de Tabasco, Villahermosa 86298, Mexico; mgrpvis00007@docente.ujat.mx

* Correspondence: gustavo-roa@live.com or groa@ksu.edu; Tel.: +1-7857706195

Abstract: Sulfur fertilization plays a crucial role in wheat (Triticum aestivum L.) production, influencing both protein concentration and grain yield. Wheat, being one of the most important food crops globally, requires efficient management of essential nutrients, including sulfur and nitrogen, to achieve optimal production. This study aimed to quantify the effect of sulfur fertilization on wheat protein concentration and grain yield and the relationship with nitrogen through two complementary methods: a comprehensive meta-analysis and a controlled greenhouse experiment. The meta-analysis, encompassing 55 studies from 20 countries with 545 comparisons, quantified the overall response of wheat to sulfur fertilization in diverse field environments, examining the effects based on soil texture and organic matter content. The greenhouse study investigated the effects of varying sulfur application rates and sources on protein concentration and grain yield and analyzed the relationship between sulfur and nitrogen concentrations in the grain. The meta-analysis showed overall positive effects of sulfur application on both protein concentration (2.1%) and grain yield (4.2%), with the magnitude of these effects varying based on soil texture and organic matter content. Sandy soils and soils with low organic matter content exhibited the most pronounced responses to sulfur fertilization. The greenhouse experiment revealed responses of both protein concentration and grain yield to increasing sulfur application rates, indicating an optimal rate beyond which additional sulfur may not provide further benefits. A strong positive correlation between sulfur and nitrogen concentrations in the grain highlighted their interdependence in wheat nutrition. These findings emphasize the importance of considering soil properties and the sulfur–nitrogen interaction when developing site-specific sulfur fertilization strategies for wheat. The results provide valuable insights for optimizing grain yield and protein concentration, contributing to more sustainable and efficient wheat production systems.

Keywords: sulfur fertilization; wheat; protein content; grain yield; soil fertility

1. Introduction

Wheat (Triticum aestivum L.) is a staple food crop that plays a crucial role in global food security, providing approximately 20% of all human dietary protein and calories consumed worldwide [1,2]. To meet the growing demand for wheat and ensure high-quality grain production, optimal nutrient management practices are essential [3]. Among the essential nutrients required for wheat growth and development, sulfur (S) has often been overlooked, despite its critical role in various physiological processes and grain quality aspects [4,5].

In recent years, the importance of sulfur fertilization in wheat production has gained increasing attention due to the growing incidence of sulfur deficiency in agricultural soils in many regions worldwide. Currently, the amount of plant-available S in the soil decreased...
by 34–86% between 2000 and 2020 [6,7]. This deficiency can be attributed to several factors, including reduced atmospheric sulfur deposition, the use of sulfur-free fertilizers, and the adoption of intensive cropping systems with high-yielding varieties that have increased sulfur demand [8]. Sulfur deficiency not only limits wheat growth and yield but also adversely affects grain quality, particularly protein content and composition [5,9].

Sulfur is an essential constituent of amino acids such as cysteine and methionine, which are crucial for the synthesis of proteins in wheat grains [10]. Adequate sulfur nutrition has been shown to improve nitrogen use efficiency, as both nutrients are closely linked in the process of protein synthesis [11]. Moreover, the interaction between sulfur and nitrogen plays a vital role in various metabolic processes, including synthesizing proteins, glutathione, chlorophyll, chloroplast membrane lipids, enzymes, coenzyme A, vitamins (biotin and thiamine), nucleic acids, adenosine triphosphate (ATP), and other important plant components that confer resistance to biotic and abiotic stresses [7,12–15].

The interaction between sulfur and nitrogen is of particular importance in wheat production, as both nutrients are essential for optimal growth, yield, and grain quality. Sulfur and nitrogen are involved in the synthesis of amino acids and proteins, and their availability can significantly influence the nutritional value and technological properties of wheat grains [11]. An adequate supply of both sulfur and nitrogen is necessary to achieve high grain yield and protein content in wheat [16]. The synergistic effect of sulfur and nitrogen fertilization has been reported to improve nitrogen uptake, utilization, and remobilization in wheat plants, leading to enhanced grain filling and protein accumulation [17,18].

In addition to its impact on grain protein content, sulfur fertilization has been reported to improve the bread-making quality of wheat flour. Sulfur deficiency can lead to the formation of weaker gluten proteins, resulting in reduced dough elasticity and loaf volume [19,20]. Adequate sulfur nutrition promotes the synthesis of sulfur-containing amino acids, which contribute to the formation of disulfide bonds in gluten proteins, thereby enhancing dough strength and bread-making quality [17].

Despite the recognized importance of sulfur in wheat production, there is a lack of comprehensive understanding of the effect of sulfur fertilization. The most common forms of sulfur fertilizers used in wheat production include ammonium sulfate, ammonium thiosulfate, calcium sulfate, and elemental sulfur [21,22]. Ammonium sulfate is preferred because of its readily available sulfate form. The response of wheat to sulfur fertilization can vary depending on factors such as soil properties, organic matter (OM) content, soil microorganisms, and the interaction with other essential nutrients such as nitrogen [23,24].

While sulfur fertilization is essential for optimizing wheat yield and quality, excessive application of sulfur can lead to negative environmental consequences [25]. Therefore, it is crucial to develop sustainable sulfur fertilization strategies and their relationship with nitrogen that balance crop requirements with environmental concerns.

The study aims to (I) conduct a comprehensive meta-analysis to quantify the effect of sulfur fertilization on wheat protein concentration and grain yield in diverse field environments, examining the effects based on soil texture and OM content; (II) investigate the effect of varying sulfur application rates and sources on the wheat grain protein concentration and grain yield in controlled greenhouse conditions and analyze the relationship between sulfur and nitrogen concentrations in the grain.

2. Materials and Methods
2.1. Meta-Analysis

Data Collection and Selection Criteria

Systematic review and meta-analysis were performed to compile the database according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [26], using electronic databases to search for peer-reviewed articles and technical reports of experiments. The searches were performed in April 2022 across three distinct search engines: Web of Science [27], CAB Direct [28], and Google Scholar [29]. These search
Nitrogen 2024, 5

engines were selected for their comprehensive information and unique, relevant content on agriculture [30].

The search was organized using Boolean logic as follows: “azufre” AND “trigo” OR “Triticum aestivum” AND “proteina” OR “calidad” AND “rendimiento” OR “productividad” in Spanish and “sulfur” OR “sulphur” AND “wheat” OR “Triticum aestivum” AND “protein” OR “quality” AND “yield” OR “productivity” in English. The Rayyan platform [31] was used for article screening.

The articles included in the database for this meta-analysis were screened based on the following criteria:

(I) Studies that included comparisons between a treatment without sulfur and another with sulfur, with the same amounts of other nutrients such as N, P, K, etc. All data were adopted if studies were conducted over multiple years, at multiple sites, or combinations thereof.

(II) Studies that reported at least the grain yield and grain protein concentration. In cases where the grain N content was reported, the protein concentration was calculated by multiplying the N content by 5.49 [32].

(III) Studies that contained the mean (M), the number of samples or replications (NS), and the standard deviation (SD), standard error (SE), or the coefficient of variation (CV) for the control and treatment groups. If a study did not report SD, SE, or CV values, the average CV was calculated within each dataset and then the SD was recalculated.

(IV) Studies conducted in greenhouses or controlled conditions were excluded. If data from the same experiment were reported more than once, the article with the most complete information was used. Some data were extracted using Getdata Graph Digitizer version 2.26 [33] and Extract Table [34].

Based on these criteria, 55 articles were included in our meta-analysis, with 545 comparisons for protein concentration and grain yield. The PRISMA diagram (Figure 1) illustrates the flow of information through the systematic review and meta-analysis phases, including the identification, screening, eligibility assessment, and inclusion of studies. The diagram shows that a total of 1243 records were identified through database searching (Web of Science: 643, CAB Direct: 300, Google Scholar: 300), 964 records were screened after 279 duplicates were removed, 111 full-text articles were assessed for eligibility, and 55 articles were ultimately included in the qualitative synthesis and meta-analysis. The data collected from the reviewed studies included location and soil characteristics before planting, such as texture and OM. When soil organic carbon was reported, it was converted to OM using a conversion factor of 1.72 [35]. Based on the information collected from the studies during the meta-analysis, we identified the study sites and other characteristics (Table 1) and georeferenced them using the R package ‘maps’ [36].

Table 1. Characteristics of studies included in the meta-analysis: country, number of comparisons, concurrent factors, experimental design, and replications.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study ID</th>
<th>Country</th>
<th>Number of Comparisons</th>
<th>Concurrent Factors †</th>
<th>Experimental Design §</th>
<th>Number of Replications</th>
</tr>
</thead>
<tbody>
<tr>
<td>[37]</td>
<td>1</td>
<td>Australia</td>
<td>2</td>
<td>SY, SS, SV</td>
<td>RCB</td>
<td>4</td>
</tr>
<tr>
<td>[38]</td>
<td>2</td>
<td>India</td>
<td>5</td>
<td>SY, SS, SV</td>
<td>RCB</td>
<td>3</td>
</tr>
<tr>
<td>[39]</td>
<td>3</td>
<td>USA</td>
<td>18</td>
<td>SY, SS, SV</td>
<td>RCB</td>
<td>4</td>
</tr>
<tr>
<td>[40]</td>
<td>4</td>
<td>India</td>
<td>2</td>
<td>SY, SS, SV</td>
<td>SP</td>
<td>4</td>
</tr>
<tr>
<td>[41]</td>
<td>5</td>
<td>UK</td>
<td>139</td>
<td>SY, SS, SV</td>
<td>RCB</td>
<td>3</td>
</tr>
<tr>
<td>[42]</td>
<td>6</td>
<td>India</td>
<td>9</td>
<td>SY, SS, SV</td>
<td>NR</td>
<td>6</td>
</tr>
<tr>
<td>[43]</td>
<td>7</td>
<td>Pakistan</td>
<td>15</td>
<td>SY, SS, SV</td>
<td>RCB</td>
<td>3</td>
</tr>
<tr>
<td>[44]</td>
<td>8</td>
<td>Egypt</td>
<td>4</td>
<td>MY, MS, SV</td>
<td>NR</td>
<td>4</td>
</tr>
<tr>
<td>[45]</td>
<td>9</td>
<td>Spain</td>
<td>2</td>
<td>MY, SS, SV</td>
<td>RCB</td>
<td>4</td>
</tr>
<tr>
<td>[46]</td>
<td>10</td>
<td>Chile</td>
<td>4</td>
<td>SY, SS, SV</td>
<td>RCB</td>
<td>4</td>
</tr>
</tbody>
</table>
Table 1. Cont.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study ID</th>
<th>Country</th>
<th>Number of Comparisons</th>
<th>Concurrent Factors †</th>
<th>Experimental Design §</th>
<th>Number of Replications</th>
</tr>
</thead>
<tbody>
<tr>
<td>[47]</td>
<td>11</td>
<td>Argentina</td>
<td>3</td>
<td>SY, SS, SV</td>
<td>RCB</td>
<td>3</td>
</tr>
<tr>
<td>[48]</td>
<td>12</td>
<td>India</td>
<td>7</td>
<td>MY, SS, SV</td>
<td>RCB</td>
<td>3</td>
</tr>
<tr>
<td>[49]</td>
<td>13</td>
<td>India</td>
<td>10</td>
<td>SY, SS, SV</td>
<td>RCB</td>
<td>3</td>
</tr>
<tr>
<td>[50]</td>
<td>14</td>
<td>India</td>
<td>3</td>
<td>SY, SS, SV</td>
<td>RCB</td>
<td>3</td>
</tr>
<tr>
<td>[51]</td>
<td>15</td>
<td>France</td>
<td>1</td>
<td>SY, SS, SV</td>
<td>RCB</td>
<td>3</td>
</tr>
<tr>
<td>[52]</td>
<td>16</td>
<td>USA</td>
<td>4</td>
<td>SY, SS, MV</td>
<td>SP</td>
<td>4</td>
</tr>
<tr>
<td>[53]</td>
<td>17</td>
<td>Argentina</td>
<td>10</td>
<td>SY, SS, SV</td>
<td>RCB</td>
<td>4</td>
</tr>
<tr>
<td>[54]</td>
<td>18</td>
<td>Estonia</td>
<td>9</td>
<td>SY, SS, SV</td>
<td>NR</td>
<td>4</td>
</tr>
<tr>
<td>[55]</td>
<td>19</td>
<td>Egypt</td>
<td>18</td>
<td>SY, SS, SV</td>
<td>SP</td>
<td>4</td>
</tr>
<tr>
<td>[56]</td>
<td>20</td>
<td>Germany</td>
<td>2</td>
<td>SY, SS, SV</td>
<td>RCB</td>
<td>3</td>
</tr>
<tr>
<td>[57]</td>
<td>21</td>
<td>Canada</td>
<td>16</td>
<td>SY, SS, SV</td>
<td>RCB</td>
<td>4</td>
</tr>
<tr>
<td>[58]</td>
<td>22</td>
<td>UK</td>
<td>1</td>
<td>MY, SS, SV</td>
<td>NR</td>
<td>3</td>
</tr>
<tr>
<td>[59]</td>
<td>23</td>
<td>Norway</td>
<td>12</td>
<td>MY, MS, MV</td>
<td>RCB</td>
<td>3</td>
</tr>
<tr>
<td>[60]</td>
<td>24</td>
<td>Egypt</td>
<td>10</td>
<td>MY, SS, SV</td>
<td>SP</td>
<td>4</td>
</tr>
<tr>
<td>[61]</td>
<td>25</td>
<td>India</td>
<td>24</td>
<td>SY, MS, SV</td>
<td>RCB</td>
<td>3</td>
</tr>
<tr>
<td>[62]</td>
<td>26</td>
<td>Chile</td>
<td>6</td>
<td>SY, SS, SV</td>
<td>RCB</td>
<td>4</td>
</tr>
<tr>
<td>[63]</td>
<td>27</td>
<td>Pakistan</td>
<td>3</td>
<td>SY, SS, SV</td>
<td>SP</td>
<td>4</td>
</tr>
<tr>
<td>[64]</td>
<td>28</td>
<td>Estonia</td>
<td>6</td>
<td>SY, SS, SV</td>
<td>NR</td>
<td>4</td>
</tr>
<tr>
<td>[65]</td>
<td>29</td>
<td>Poland</td>
<td>1</td>
<td>MY, SS, SV</td>
<td>RCB</td>
<td>4</td>
</tr>
<tr>
<td>[66]</td>
<td>30</td>
<td>Czech Republic</td>
<td>10</td>
<td>SY, SS, SV</td>
<td>NR</td>
<td>4</td>
</tr>
<tr>
<td>[67]</td>
<td>31</td>
<td>India</td>
<td>1</td>
<td>MY, SS, SV</td>
<td>RCB</td>
<td>3</td>
</tr>
<tr>
<td>[68]</td>
<td>32</td>
<td>Brazil</td>
<td>1</td>
<td>SY, SS, SV</td>
<td>RCB</td>
<td>4</td>
</tr>
<tr>
<td>[69]</td>
<td>33</td>
<td>Poland</td>
<td>4</td>
<td>MY, SS, SV</td>
<td>SP</td>
<td>4</td>
</tr>
<tr>
<td>[70]</td>
<td>34</td>
<td>India</td>
<td>4</td>
<td>SY, SS, SV</td>
<td>RCB</td>
<td>4</td>
</tr>
<tr>
<td>[71]</td>
<td>35</td>
<td>Egypt</td>
<td>4</td>
<td>SY, SS, SV</td>
<td>SP</td>
<td>3</td>
</tr>
<tr>
<td>[72]</td>
<td>36</td>
<td>Turkey</td>
<td>4</td>
<td>SY, SS, SV</td>
<td>RCB</td>
<td>3</td>
</tr>
<tr>
<td>[73]</td>
<td>37</td>
<td>Germany</td>
<td>2</td>
<td>MY, MS, SV</td>
<td>NR</td>
<td>3</td>
</tr>
<tr>
<td>[74]</td>
<td>38</td>
<td>USA</td>
<td>4</td>
<td>SY, SS, SV</td>
<td>RCB</td>
<td>4</td>
</tr>
<tr>
<td>[75]</td>
<td>39</td>
<td>USA</td>
<td>3</td>
<td>SY, SS, SV</td>
<td>RCB</td>
<td>4</td>
</tr>
<tr>
<td>[76]</td>
<td>40</td>
<td>USA</td>
<td>9</td>
<td>SY, MS, MV</td>
<td>SP</td>
<td>4</td>
</tr>
<tr>
<td>[77]</td>
<td>41</td>
<td>USA</td>
<td>2</td>
<td>MY, MS, MV</td>
<td>SP</td>
<td>4</td>
</tr>
<tr>
<td>[78]</td>
<td>42</td>
<td>Poland</td>
<td>9</td>
<td>SY, SS, SV</td>
<td>NR</td>
<td>4</td>
</tr>
<tr>
<td>[79]</td>
<td>43</td>
<td>Argentina</td>
<td>6</td>
<td>SY, SS, SV</td>
<td>RCB</td>
<td>4</td>
</tr>
<tr>
<td>[80]</td>
<td>44</td>
<td>Italy</td>
<td>9</td>
<td>SY, SS, MV</td>
<td>RCB</td>
<td>4</td>
</tr>
<tr>
<td>[81]</td>
<td>45</td>
<td>Italy</td>
<td>1</td>
<td>SY, SS, MV</td>
<td>SP</td>
<td>3</td>
</tr>
<tr>
<td>[82]</td>
<td>46</td>
<td>USA</td>
<td>18</td>
<td>SY, SS, MV</td>
<td>SP</td>
<td>4</td>
</tr>
<tr>
<td>[83]</td>
<td>47</td>
<td>India</td>
<td>2</td>
<td>SY, SS, SV</td>
<td>RCB</td>
<td>3</td>
</tr>
<tr>
<td>[84]</td>
<td>48</td>
<td>Ethiopia</td>
<td>5</td>
<td>SY, SS, SV</td>
<td>RCB</td>
<td>3</td>
</tr>
<tr>
<td>[85]</td>
<td>49</td>
<td>Argentina</td>
<td>60</td>
<td>SY, SS, SV</td>
<td>SP</td>
<td>3</td>
</tr>
<tr>
<td>[86]</td>
<td>50</td>
<td>Italy</td>
<td>2</td>
<td>SY, SS, MV</td>
<td>SP</td>
<td>3</td>
</tr>
<tr>
<td>[87]</td>
<td>51</td>
<td>Pakistan</td>
<td>18</td>
<td>SY, SS, SV</td>
<td>RCB</td>
<td>3</td>
</tr>
<tr>
<td>[88]</td>
<td>52</td>
<td>USA</td>
<td>9</td>
<td>SY, SS, MV</td>
<td>SP</td>
<td>4</td>
</tr>
<tr>
<td>[89]</td>
<td>53</td>
<td>India</td>
<td>3</td>
<td>MY, SS, SV</td>
<td>RCB</td>
<td>3</td>
</tr>
<tr>
<td>[90]</td>
<td>54</td>
<td>Australia</td>
<td>8</td>
<td>SY, SS, SV</td>
<td>NR</td>
<td>3</td>
</tr>
<tr>
<td>[91]</td>
<td>55</td>
<td>Italy</td>
<td>1</td>
<td>MY, SS, MV</td>
<td>SP</td>
<td>3</td>
</tr>
</tbody>
</table>

† SY = Single year, SS = Single site, SV = Single variety, MY = Multiple years, MS = Multiple sites, MV = Multiple varieties. § RCB = Randomized complete block design, SP = Split-plot design, NR = Not reported. Note. USA, United States of America; UK, United Kingdom.
Figure 1. The PRISMA diagram shows the flow of information through the systematic review and meta-analysis phases, including the identification, screening, eligibility assessment, and inclusion of studies.

2.2. Greenhouse Study

2.2.1. Site and Experimental Design

Two experiments (rates and sources) were conducted during the year 2020 at the greenhouse facilities of Kansas State University in Manhattan, Kansas, USA. The soil used for the experiment was classified as sandy loam with low organic matter content (Table 2). Wheat seedlings of the Everest variety were grown in a growth chamber for vernalization for six weeks at a temperature below 9 °C, then transplanted with six plants per pot with incorporated fertilizer. The pots were filled with 1.4 kg of soil each. The experimental design was a completely randomized design with three replications.

The treatments for the rate experiment consisted of the application of ammonium sulfate [(NH₄)₂SO₄] at five different doses (25, 50, 100, 150, and 200 mg of S kg⁻¹ soil) plus control with zero sulfur for a total of six treatments. The treatments for the source experiment consisted of three different sulfur sources: ammonium sulfate [(NH₄)₂SO₄], calcium sulfate [CaSO₄], and elemental sulfur [S₀], applied at a single dose (100 mg of S kg⁻¹ soil). All treatments in both experiments received a balanced dose of 200 mg of N kg⁻¹ soil, using urea [CO(NH₂)₂] as the nitrogen source.
Table 2. Initial soil analysis used for the study (0–15 cm depth).

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (1:1)</td>
<td>5.8</td>
</tr>
<tr>
<td>OM (g kg(^{-1}))</td>
<td>9.0</td>
</tr>
<tr>
<td>P—Mehlich 3 (mg kg(^{-1}))</td>
<td>54</td>
</tr>
<tr>
<td>K (mg kg(^{-1}))</td>
<td>127</td>
</tr>
<tr>
<td>S (mg kg(^{-1}))</td>
<td>1.4</td>
</tr>
<tr>
<td>CEC (cmol(^+) kg(^{-1}))</td>
<td>6.8</td>
</tr>
<tr>
<td>Texture</td>
<td>Sandy loam</td>
</tr>
</tbody>
</table>


2.2.2. Data Collection and Laboratory Analysis

Initial soil samples were analyzed for nutrients and texture (Table 2). Harvesting was performed 13 weeks after transplantation, collecting data on grain yield. Soil samples were dried at 40 °C, and grain samples were dried at 60 °C using a forced air dryer Isotemp 851 F (Fisher Scientific, Waltham, MA, USA), and both were ground to pass a 2 mm sieve. Soil samples were analyzed for pH 1:1 (soil:water) [92], OM using loss on ignition [93], phosphorus content with Mehlich-3 extraction, potassium and cation exchange capacity with ammonium acetate \([\text{NH}_4\text{CH}_3\text{CO}_2]\) extraction [94], and soil sulfur content with dicalcium phosphate \([\text{Ca(HPO}_4)_2]\) extraction. The grain sulfur concentration was determined using nitric-perchloric acid \([\text{HNO}_3-\text{HClO}_4]\) digestion [95], and nutrient concentrations were determined by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) 720-ES (Varian, Palo Alto, CA, USA). The grain nitrogen concentration was determined through a digestion process using sulfuric acid \([\text{H}_2\text{SO}_4]/\text{H}_2\text{O}_2\), following the method described by [96,97]. Nitrogen was analyzed using an indophenol blue colorimetric procedure and a rapid flow analyzer Alpkem RFA-300 (OI Analytical, College Station, TX, USA). The protein concentration for wheat was calculated by multiplying the nitrogen content by 5.49 [32].

2.3. Statistical Analysis

2.3.1. Meta-Analysis

Data analysis for metanalysis was based on the procedures used by [98]. The effect size was calculated as the natural logarithm \((\ln R)\) of the response ratio [99]:

$$\ln R = \ln \left( \frac{M_T}{M_C} \right)$$

where \(M_T\) is the treatment’s mean grain yield or protein content and \(M_C\) is the mean grain yield or protein concentration in the control.

The variance \((V)\) of \(\ln R\) was calculated using the following equation [100,101]:

$$V \ln R = \frac{SD_T^2}{NS_TM_T^2} + \frac{SD_C^2}{NS_CM_C^2}$$

where \(SD_T\) and \(SD_C\) are the standard deviations for the treatment and control groups, respectively. \(NS_T\) and \(NS_C\) are the number of sample sizes or replications for the treatment and control groups, respectively.

The weighting factor for each study was calculated as the reciprocal of the total variance. In cases where studies reported only the SE or CV instead of the SD, the SD was derived using the appropriate formulas as described by [100,101].

The random effects model (RE model) method was employed using functions from the R ‘metafor’ package [102], following the guidelines [103]. The effect size was calculated as the natural logarithm of the response ratio, and the variance of the effect size was estimated using the delta method. The average weighting factor, the reciprocal of the total variance, was used in the analysis.
Influential diagnostics were performed following the procedures described by [104,105], which included examining Cook’s distance, the difference in fit values, and other measures to identify potential outliers and influential cases.

To estimate the effect size of considered moderators, such as soil texture and organic matter content, the bootstrapping method [106] with n = 1000 was employed using parallel processing in R.

2.3.2. Greenhouse Study

Data analyses for the greenhouse studies were linear; these were performed using the ‘lm’ function from the R [stats] package. The linear plateau model was fit using nonlinear least square ‘nls’ regression implemented using self-starting functions from the [nlraa] R package [107]. Linear plateau models can be expressed as $y = a + b \times x$ if $x \leq c$, and $y = a + b \times c$ if $x > c$, where “a” represents the intercept, “b” represents the slope during the linear phase, and “c” represents the value of x at which the linear model reaches a plateau [108,109]. Additionally, an analysis of variance (ANOVA) was performed using the Kenward–Roger method, with statistical significance set at alpha < 0.05. The analysis utilized a linear mixed-effects model, implemented with the ‘lmer’ function from the R package {lme4} [110], treating replications as a random effect. The linear mixed model was fit by REML (restricted maximum likelihood).

All data analyses from both studies were performed in R version 4.2 [111] and RStudio version 2022.12.0+353 [112].

3. Results and Discussion

3.1. Meta-Analysis: Effects of Sulfur Application on Protein and Yield

The meta-analysis included 55 studies from 20 countries from different continents (Figure 2, Table 1). This study revealed that sulfur application had positive effects on both the wheat protein concentration and grain yield. For the protein concentration, the combined effect was 2.1% (Figure 3a), with moderate heterogeneity ($I^2 = 44.5\%$) among the included studies. The 95% confidence interval (1.1 to 3.1) suggested a consistent positive effect of sulfur application on the protein concentration. Regarding yield, the combined effect was 4.2% (Figure 3b), with high heterogeneity ($I^2 = 74.1\%$) among the studies. The 95% confidence interval (2.3 to 6.2) indicated that while sulfur application generally had a positive effect on yield, the magnitude of the effect varied considerably across studies. These positive effects on both protein and yield are consistent with numerous studies highlighting sulfur’s importance for wheat production, summarized in another meta-analysis [113]. However, the moderate to high heterogeneity among studies suggests that the magnitude of sulfur effects can vary considerably depending on specific conditions, such as climate, genotype, soil texture, organic matter content, other soil properties, and microorganisms.

The meta-analysis also examined the effects of sulfur application on protein concentration and grain yield across different soil textures (Figure 4). Sandy and clay soils showed significant positive effects on protein concentration, while loam soil exhibited a negative effect. For yield, sandy soil had a significant positive effect (2.8%), whereas loam soil showed a slight, non-significant negative effect. Clay and silty soils had small, non-significant positive effects on yield. The analysis of sulfur application effects based on soil OM content (Figure 5) revealed that soils with OM below 20 g kg$^{-1}$ (2%) had significant positive responses in both protein concentration and grain yield. The effect on yield in soils with OM content between 20 and 40 g kg$^{-1}$ showed considerable variation and was positive, but the results were not statistically significant. When OM was greater than 40 g kg$^{-1}$, sulfur application had no significant effect on either the protein concentration or grain yield.
These positive effects on both protein and yield are consistent with numerous studies highlighting sulfur’s importance for wheat production, summarized in another meta-analysis [113]. However, the moderate to high heterogeneity among studies suggests that the magnitude of sulfur effects can vary considerably depending on specific conditions, such as climate, genotype, soil texture, organic matter content, other soil properties, and microorganisms.

**Figure 2.** Geographic distribution of field studies included in the meta-analysis on the effects of sulfur application on wheat protein and yield. Purple circle represents studies sites.

**Figure 3.** Effect diagram or forest plot for the response ratio of protein concentration (a) and grain yield (b) in wheat with the application of sulfur. The sizes of the circles indicate the weight of each study. The red diamond indicates the overall mean estimate.
The meta-analysis also examined the effects of sulfur application on protein concentration (a) and grain yield (b) in different soil types. The asterisk (*) indicates a significant difference ($p < 0.01$). Numbers in parentheses indicate the number of points of comparison.

**Figure 4.** Effects of sulfur application on protein concentration (a) and grain yield (b) in different soil types. The asterisk (*) indicates a significant difference ($p < 0.01$). Numbers in parentheses indicate the number of points of comparison.

This may be because soils with higher organic matter content are rich in available nutrients, supported by their soil texture, which provides greater structural stability, water retention, and gas exchange. Additionally, these soils harbor microorganisms such as fungi, bacteria, viruses, and protozoa that carry out numerous biogeochemical cycles and chemical reactions, including oxidation and fixation, which ensure the availability and sufficiency of essential nutrients [7,14,114].

Sulfur deficiencies are generally more commonly observed in sandy soils with high leaching levels [115]. The risk of sulfur deficiency is higher in sandy soils without organic matter [7,116]. This observation may explain the significant effect of sulfur application in sandy soils, evidenced by grain yield and protein concentration. Several studies also show that a significant portion of the total sulfur stored in the soil is present in organic form, and the decomposition of organic matter results in the mineralization of organic sulfur into sulfate, making it available to plants [117–119]. Sulfur can also be found in some minerals like pyrite and organic molecules like amino acids but in much smaller amounts [120].

The positive effects observed in sandy and clay soils and the negative effects in loam soil suggest that soil texture plays a significant role in sulfur availability and plant uptake [6]. Similarly, the more pronounced effects of sulfur in soils with low organic matter content highlight the importance of soil organic matter as a source of sulfur for plant growth [117]. These results demonstrate that agricultural practices and sulfur fertilization are influenced by the amount of organic matter and that positive effects on grain yield and protein content in soils are linked to the maximum amount of nutrients that can be

**Figure 5.** Effects of sulfur application on protein concentration (a) and grain yield (b) in different organic matter (OM) ranges. The asterisk (*) indicates a significant difference ($p < 0.01$). Numbers in parentheses indicate the number of points of comparison.

The positive effects observed in sandy and clay soils and the negative effects in loam soil suggest that soil texture plays a significant role in sulfur availability and plant uptake [6]. Similarly, the more pronounced effects of sulfur in soils with low organic matter content highlight the importance of soil organic matter as a source of sulfur for plant growth [117]. These results demonstrate that agricultural practices and sulfur fertilization are influenced by the amount of organic matter and that positive effects on grain yield and protein content in soils are linked to the maximum amount of nutrients that can be
processed and assimilated by the plant [15]. Studies reveal that the amount of sulfur required in soils with high organic matter content is lower [121].

3.2. Meta-Analysis: Relationship between Protein and Yield

The relationship between grain yield and protein concentration showed a negative trend (Figure 6a), with protein concentration tending to decrease as yield increased. However, the relationship was weak ($R^2 = 0.14$). This negative relationship, often referred to as the “yield-protein trade-off” [122], is attributed to the competing demands for nitrogen between vegetative growth and grain protein synthesis [123]. Essentially, this reduction is due to the dilution of protein concentration due to increased grain yield [124]. It is important to note that the relationship between yield and protein concentration does not follow a linear pattern and may be subject to the influence of environmental and genetic factors. For example, previous research has shown that nitrogen availability and other nutrients or management practices [125,126] can affect both yield and protein quality.

![Figure 6](image_url)

**Figure 6.** Relationship between (a) protein concentration and grain yield, and (b) protein yield and grain yield. Black circles represent data with sulfur fertilizer application, and gray triangles represent data without sulfur fertilizer application.

Wheat grain protein concentrations typically range from 10 to 18% [127,128]. In the present meta-analysis, an average protein concentration of 12% was observed across the included studies. The negative trend between protein concentration and grain yield has been documented in the literature. A three-year study evaluating protein concentrations in relation to wheat grain yield and found consistent negative correlations [114]. This inverse trend has also been reported in other cereal crops, such as rice [129], highlighting the need for a paradigm shift in crop production strategies. These findings emphasize the importance of adopting a holistic approach to crop production that prioritizes nutritional quality alongside yield improvements.

In contrast, the relationship between grain yield and protein yield exhibited a strong positive trend (Figure 6b), with an $R^2$ of 0.87, which is expected. This positive relationship suggests that increasing grain yield through appropriate management practices, including sulfur fertilization, can produce a higher overall protein amount.
These results highlight the importance of considering soil properties, such as texture and organic matter content, when assessing the potential benefits of sulfur fertilization on wheat grain protein concentration and yield. The findings also underscore the complex relationship between grain yield and protein concentration, while emphasizing the potential for increasing overall protein production through yield improvements.

3.3. Greenhouse: Study with Different Sulfur Rates and Sources

This greenhouse study investigated the effects of different sulfur rates and sources on wheat grain protein concentration and yield. The relationship between sulfur rate and protein concentration was analyzed using a linear plateau model (Figure 7a). Protein concentration increased up to a sulfur rate of approximately 113 mg kg$^{-1}$, beyond which it did not change. The model explained 91% of the variability in protein concentrations, indicating a strong fit to the observed data. This finding highlights that sulfur application positively affected grain quality.

Similarly, grain yield response to sulfur application rates was evaluated using a linear plateau model (Figure 7b). Grain yield peaked at a sulfur rate of around 57 mg kg$^{-1}$ and then remained constant at higher rates. The model accounted for 94% of the variation in grain yield, suggesting that sulfur application had an impact on wheat productivity. The response observed for both protein concentration and grain yield indicates an optimal sulfur rate, beyond which additional sulfur may not provide further benefits. This optimal sulfur rate will vary for each specific site and condition.

The choice of the linear plateau model for this analysis was based on its ability to capture complex, non-linear relationships between variables, which are common in agricultural research. The response of crop yield and quality parameters to varying levels of nutrient application often follows a non-linear pattern, characterized by a plateau or even diminishing returns at higher application rates [130,131].

The strong positive linear relationship ($R^2 = 0.80$) between sulfur and nitrogen concentrations in the grain (Figure 8) highlights the interdependence of these nutrients in wheat. This finding is consistent with previous studies that have reported the importance of balanced sulfur and nitrogen nutrition for optimizing grain yield and quality [132]. The linear equation indicates that the nitrogen concentration in the grain increases in proportion to the sulfur concentration. This provides a simple yet effective tool for understanding the stoichiometric relationship between these two essential nutrients in wheat grain. The
The strong positive linear relationship \( R^2 = 0.80 \) between sulfur and protein concentrations in wheat grain suggests that sulfur fertilization can significantly increase protein concentration. This relationship highlights the importance of characterizing growing areas and determining the effect of sulfur fertilization on protein concentration and grain yield. Future studies could explore the potential differences among sulfur sources. Field studies may be necessary to fully evaluate the potential differences in sulfur responsiveness in wheat.

Limitations of this study include the high heterogeneity among the studies included in the meta-analysis and the short duration of the greenhouse experiment. Future research should focus on conducting long-term experiments to assess the effects of sulfur on wheat performance. The lack of consistent results among the studies may be due to the relatively short duration of the experiments and plant development. Field studies may be necessary to fully evaluate the potential differences among sulfur sources.

Figure 8. Linear relationship between nitrogen concentration and sulfur concentration in wheat grain yield at different rates using ammonium sulfate as the sulfur fertilizer source.

However, the application of various sulfur sources (ammonium sulfate, calcium sulfate, and elemental sulfur) did not result in significant differences in protein concentration, grain yield, or nitrogen and sulfur concentrations in the grain (Figure 9) under the specific conditions of this study. This is in contrast with previous studies that have reported varying effects of sulfur sources on wheat performance [135,136]. The lack of source effects in the current study may be due to the relatively short duration of the experiment and plant development. Field studies may be necessary to fully evaluate the potential differences among sulfur sources.

Figure 9. Effects of different sulfur sources (ammonium sulfate, calcium sulfate, and elemental sulfur) on (a) nitrogen concentration and (b) sulfur concentration in wheat grain. Means with the same letter are not significantly different \( (p > 0.05) \). Error bars represent standard error (SE) from the model.
4. Conclusions

This study contributes to a better understanding of the effects of sulfur on wheat protein concentration and grain yield and its relationship with nitrogen by employing a comprehensive meta-analysis and a complementary greenhouse experiment. The meta-analysis showed overall positive effects of sulfur application on both protein concentration and grain yield, with the magnitude of these effects varying based on soil texture and organic matter content, highlighting the importance of characterizing growing areas. Our findings imply that sulfur fertilization has a greater impact on protein concentration and grain yield in sandy soils and in soils with lower organic matter content, particularly those with less than 20 g kg\(^{-1}\). With respect to the relationship between protein concentration and grain yield, the relationship was weak, in contrast to the relationship between grain yield and protein yield. These findings derived from the meta-analysis suggest efforts should be made to produce wheat of both high quality and quantity. The results indicate that sulfur fertilization strategies should be tailored to specific soil conditions to maximize the benefits of sulfur application. The greenhouse experiment conducted under the described conditions suggests that higher sulfur application rates do not provide further benefits while highlighting the strong relationship \((R^2 = 0.80)\) between sulfur and nitrogen in wheat nutrition. The integration of the meta-analysis and greenhouse experiment results demonstrates the need to characterize and determine the effects of sulfur and nitrogen; this approach provides a more comprehensive understanding of the factors influencing sulfur responsiveness in wheat. The greenhouse experiment showed that there are no statistically significant differences between sulfur sources with respect to nitrogen and sulfur concentration.

Limitations of this study include the high heterogeneity among the studies included in the meta-analysis and the short duration of the greenhouse experiment. Future research should focus on conducting long-term field studies to validate the findings and develop site-specific sulfur fertilization recommendations that optimize both protein concentration and grain yield while considering the strong relationship between sulfur and nitrogen nutrition. Additionally, future studies could explore the influence of other variables besides soil texture and organic matter content, such as climate zones, genotypes, and management practices to further refine sulfur fertilization strategies and provide a more comprehensive understanding of the factors affecting sulfur responsiveness in wheat.

In conclusion, this study presents an advancement in understanding the effects of sulfur fertilization on wheat protein concentration and grain yield, as well as its relationship with nitrogen. The joint meta-analysis and greenhouse experiment highlight the importance of characterizing growing areas and considering the synergistic effects of sulfur and nitrogen when developing fertilization strategies.


**Funding:** This research received no external funding.

**Data Availability Statement:** The original contributions presented in the greenhouse study are included in the article, and further inquiries can be directed to the corresponding author. The original data presented in the meta-analysis study are openly available in [Harvard Dataverse] at [https://doi.org/10.7910/DVN/4RLPP1]. This dataset includes the compiled data from the 55 studies used in the meta-analysis, which were sourced from the published literature. While the original data for each individual study falls under the purview of the respective authors, the curated dataset used for the meta-analysis in this study is made publicly available to ensure transparency and reproducibility. The dataset contains information such as study location, soil characteristics, fertilization rates, sources, wheat grain yield, and protein concentration responses, enabling other
researchers to further investigate the effects of sulfur fertilization on wheat performance across diverse environments.

**Acknowledgments:** The authors acknowledge the Kansas State University library staff for providing access to essential research papers, Livia Olsen for her initial guidance on conducting systematic reviews, and Nicolas Giordano for his recommendations and guidance that contributed to the meta-analysis. We also extend our appreciation to Brenda Santiago Ruiz and Dafne Itzel Orozco Rojas for their input and review of the original work that formed the basis for this manuscript. Their support has been instrumental in the successful completion of this research.

**Conflicts of Interest:** The authors declare no conflicts of interest.

**References**


7. Sharma, R.K.; Cox, M.S.; Oglesby, C.; Dhillon, J.S. Revisiting the Role of Sulfur in Crop Production: A Narrative Review. *J. Agric. Food Res.* 2024, 15, 101013. [CrossRef]


Acknowledgments: The authors acknowledge the Kansas State University library staff for providing access to essential research papers, Livia Olsen for her initial guidance on conducting systematic reviews, and Nicolas Giordano for his recommendations and guidance that contributed to the meta-analysis. We also extend our appreciation to Brenda Santiago Ruiz and Dafne Itzel Orozco Rojas for their input and review of the original work that formed the basis for this manuscript. Their support has been instrumental in the successful completion of this research.

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


74. Chatterjee, A. Additions of Ammonium Sulfate and Urease Inhibitor with Urea to Improve Spring Wheat and Sugar Beet Yield. *Arch. Agron. Soil Sci.* 2018, 64, 1459–1464. [CrossRef]


87. Ibadullah; Muhammad, D. Enhancement in Maize-Wheat Productivity and N Use Efficiency through Sulfur Application in Two 


92. Peters, J.B.; Nathan, M.V., Gelderman, R., Eds.; North Central Regional Research Publication No. 221 (Revised). SB 1001; Missouri Agricultural Experiment Station: Columbia, MO, USA, 2012; Chapter 4; pp. 4.1–4.7.

93. Peters, J.B.; Nathan, M.V., Gelderman, R., Eds.; North Central Regional Research Publication No. 221 (Revised). SB 1001; Missouri Agricultural Experiment Station: Columbia, MO, USA, 2012; Chapter 4; pp. 4.1–4.7.


114. Dick, W.A.; Kost, D.; Chen, L. Availability of Sulfur to Crops from Soil and Other Sources. In *Grains as a Sustainable Source of Protein for Health*, 156–164. [CrossRef]


118. Javid Iqbal, M.; Shams, N.; Fatima, K. Nutritional Quality of Wheat. In *Amino Acid Composition and Protein Quality in Winter Wheat*, e0178494. [CrossRef] [PubMed]


**Disclaimer/Publisher’s Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.