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ORIGINAL RESEARCH ARTICLE

Impacts of mineral and biofertilizers on rhizosphere and bulk-soil carbon, sulfur, and C/S ratio in maize for soil sustainability

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ABSTRACT

Balanced carbon–sulfur (C-S) dynamics are crucial for maintaining soil fertility and sustaining crop productivity. This study examined how mineral and biofertilizers affect total sulfur (S), organic carbon (OC), and the C/S ratio in maize rhizosphere and bulk soils. A field experiment was established with six treatments: an unfertilized control, urea (250 kg N ha^{-1}), ammonium sulfate (200 kg N ha^{-1}), BioHealth biofertilizer (4–5 kg N ha^{-1}), liquid effective microorganisms (EM, 400 L ha^{-1}), and a combined fertilizer containing one-quarter of each recommended dose. Total S, OC, and C/S ratios were measured after 70 and 100 days of maize growth. Ammonium sulfate consistently produced the highest sulfur concentrations in both rhizosphere and bulk soils, with increases of more than 40% over the control at both sampling times. The combined fertilizer treatment significantly enhanced OC content in both soil compartments, with up to a 25 % increase compared with the control. Urea yielded the greatest C/S ratio (approximately 15 % higher than the control), while all treatments showed a progressive decline in C/S as sulfur availability increased, confirming an inverse S-C/S relationship. These results demonstrate that integrating bio- and mineral fertilizers improves soil C–S balance and nutrient availability, offering a practical strategy to enhance soil fertility and support sustainable maize production.

Keywords: total sulfate; organic carbon; C/S; rhizosphere; biofertilizer; mineral fertilizer; soil sustainability

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1. Introduction

Maize (Zea mays L.) is the world's third most important food grain after wheat and rice, and serves as a major source of carbohydrates and vegetable oils[1]. In Iraq, yellow maize is a principal cereal crop cultivated extensively for both human consumption and animal feed, making its sustained productivity critical to national food security and rural livelihoods[2,3].

Despite its economic importance, maize production is constrained by declining soil fertility and nutrient imbalances that limit yield potential[4]. Excessive and poorly managed use of chemical fertilizers has contributed to soil degradation, loss of biodiversity, and contamination of surface and groundwater[5].

Continuous application of high nitrogen (N) rates accelerates nutrient leaching, ammonia volatilization, and greenhouse gas emissions, while long-term reliance on synthetic inputs disrupts soil microbial communities and reduces organic matter[6]. These problems highlight the need for integrated fertility management strategies that maintain soil health while meeting crop nutrient demands[7].

Biofertilizers—microbial inoculants such as effective microorganisms (EM), plant growth–promoting rhizobacteria (PGPR), and formulations like BioHealth—offer an eco-friendly alternative or supplement to mineral fertilizers[8].

They improve soil structure, enhance nutrient availability, and stimulate the production of plant hormones, thereby supporting vegetative growth and yield[9]. By increasing microbial diversity and enzymatic activity, biofertilizers promote the mineralization of nutrients including nitrogen and sulfur, helping sustain soil organic carbon (SOC) and nutrient cycling[10].

The rhizosphere, the thin layer of soil surrounding plant roots, is a critical ecological niche where intense biological and chemical interactions occu[11]. Rhizobacteria within this zone not only supply nutrients but also enhance plant stress tolerance, regulate phytohormone levels, and influence the synthesis of secondary metabolites that contribute to plant defense and quality traits[12]. Understanding how fertilizers both mineral and biological—affect rhizosphere dynamics is essential for developing practices that maintain soil fertility and crop resilience[13].

Sulfur (S) and organic carbon (OC) are key indicators of soil quality. Organic sulfur accounts for approximately 95 % of total soil S, while SOC represents 58–60 % of soil organic matter and supports water retention, nutrient supply, and microbial energy needs[4]. The carbon-to-sulfur (C/S) ratio reflects the quality of organic matter: lower ratios favor S mineralization and nutrient availability, whereas higher ratios may limit sulfur releas[5]. Management practices that maintain an optimal C/S ratio are therefore crucial for sustaining soil productivity[6]. Although numerous studies have examined nitrogen and carbon dynamics in cropping systems, less attention has been given to the combined effects of mineral and biofertilizers on sulfur cycling and the C/S ratio in maize rhizosphere soils.

This study addresses that gap by evaluating how mineral and biological fertilizers influence total sulfur, organic carbon, and the C/S ratio in maize rhizosphere and bulk soils, with the goal of identifying strategies that enhance soil fertility and long-term sustainability.

2. Materials and methods

2.1. Study area

The field experiment was conducted in the Al-Nuriyah subdistrict of the Al-Shafi'iyah district, Diwaniyah Governorate, during the autumn of 2024, on a silty clay soil.

2.2. Experimental design

A randomized complete block design (RCBD) was employed with three replications. Each replication consisted of six randomly assigned treatments, resulting in a total of 18 experimental units. The experiment comprised the subsequent treatments:

1. Control treatment
2. Urea fertilizer (46% N) applied 10 days post-planting at a level of 250 kg ha^{-1}.
3. Ammonium sulfate fertilizer (21% N, 24% S) applied 10 days post-planting at a level of 200 kg ha^{-1}.
4. Bio Health fertilizer, 10 days post-planting, at a rate of 4-5 kg ha^{-1}.
5. Effective Microorganisms Liquid Biofertilizer: The spraying process was carried out manually, diluting one liter of this fertilizer in four liters of water. This process was carried out using a 16-liter hand sprayer, with five sprays, each spraying (400 l ha^{-1}).
6. Fertilizer Mixture (a quarter of the recommended amount of each fertilizer).

Bio Health fertilizer, manufactured by Humintech Germany, is a mixture of fungi, bacteria, humic acid, and seaweed. It is in the form of water-soluble granules and contains the ingredients listed in **Table 1**.

Table 1. Components of Bio Health fertilizer as specified by the manufacturer, Humintech Germany.

	Contents	***Quantity***
Fungi	Trichoderma	5%
Bacteria	Bacillus subtilis	5%
Organic matter	Humic acid	75%
Organic matter	Seaweed	5%
Organic matter	Other	65%
Elements	Water-soluble potassium K2O	11%
Elements	Boron	15 mg kg^{-1}

2.3. Crop establishment and management

Yellow corn (*Zea mays* L.) Seeds were sown in rows on August 17, 2024. The inter-row distance was 75 cm, and the spacing between holes was 25 cm, with four rows allocated per plot. Three seeds were sown in each hole and subsequently thinned to one following germination. Each row had 12 plants, totaling 48 plants in the experimental unit. The corn stem borer was managed with granular diazinon herbicide, applying one grain at the center of each leaf. Manual weeding was conducted five times throughout the growing season. The yellow corn crop was harvested on December 19, 2024, with maturity signs indicated by ear drying.

2.4. Soil and plant analyses

Total sulfate: Sulfate was estimated by precipitation as barium sulfate[14].

Organic carbon: Soil organic carbon was estimated based on organic matter values using a conversion constant of 1.72[15]. Organic matter was estimated by wet digestion using 1N K2Cr2O7, according to the Walkley and Black method described in[16].

C/S ratio: This ratio was calculated by dividing organic carbon by total sulfur.

2.5. Statistical analysis

The SAS Statistical Analysis System (SAS) was used to analyze the data, which were collected in a randomized complete block design (RCBD). Substantial differences between means were evaluated using the least significant difference (LSD) test at a significance threshold of 0.05.

3. Results and discussion

3.1. Total sulfur concentration in the experimental soil 70 days post-planting

Table 2 presents the effect of biofertilizer, mineral fertilizer, and their combination on total sulfur concentration in rhizosphere and bulk soils 70 days after planting, revealing significant differences ($p \leq 0.05$) among treatments. All fertilizer treatments except urea produced significantly higher sulfur levels than the control, with ammonium sulfate recording the greatest concentrations (442.7 mg kg^{-1} in the rhizosphere and 474.0 mg kg^{-1} in bulk soil). These findings reflect the direct sulfur contribution of ammonium sulfate (24 % S, 21 % N)[17] and highlight its dual role as a nitrogen and sulfur source, which supports the formation of sulfur-containing amino acids and proteins crucial for maize growth. The biofertilizer–mineral mixture produced moderately high sulfur values (334 mg kg^{-1} rhizosphere; 352 mg kg^{-1} bulk), whereas the control and urea treatments remained low (60–72 mg kg^{-1}). The negligible sulfur response under urea is expected because urea supplies only nitrogen (46 % N) and no sulfur[18]. This pattern parallels the report of[19], who found that urea-treated soils had reduced molybdenum levels, a nutrient that often follows sulfate dynamics.The BioHealth biofertilizer yielded intermediate sulfur contents (205 mg kg^{-1} rhizosphere; 220 mg kg^{-1} bulk). Rather than adding sulfur directly, BioHealth likely enhanced microbial activity that mineralized organic-S compounds, gradually releasing sulfate[20,21]. Overall, these results demonstrate that supplying sulfur either directly (ammonium sulfate) or indirectly via microbial mineralization (biofertilizers) is critical for sustaining rhizosphere sulfur pools. Enhanced sulfur availability can improve enzyme activity and chlorophyll synthesis, thereby contributing to greater soil fertility and crop productivity compared with nitrogen-only fertilization.

Table 2. Effect of adding fertilizer type on the total sulfur concentration (mg kg^{-1}) in the rhizosphere soil and bulk soils 70 days post-planting.

TREATMENTS	SOIL ZONE	
	Rhizosphere soil	**Bulk soil**
Control Treatment	59.684	72.351
Urea	75.332	83.332
Ammonium Sulfate	442.704	474.037
Biohealth	204.881	219.881
Effective Microorganisms	131.454	144.454
Bio-Mineral Fertilizer Combination	333.621	352.288
Lsd 0.05	37.6876	39.0651

Total sulfur concentration in the experimental soil 100 days post-planting

Table 3 presents the effects of biofertilizer, mineral fertilizer, and their combination on total sulfur (S) concentration in rhizosphere and bulk soils 100 days after planting. Statistical analysis ($p \leq 0.05$) revealed significant treatment differences. As at 70 days, all fertilizer treatments except urea significantly exceeded the control in S concentration, confirming that external S inputs or stimulated mineralization are essential to sustain soil sulfur over time. The ammonium sulfate treatment produced the highest S levels (417.37 mg kg^{-1} in rhizosphere; 448.37 mg kg^{-1} in bulk soil). This outcome reflects the dual nutrient supply of ammonium sulfate (21 % N and 24 % S) and its ready solubility, which enables rapid sulfur availability for plant uptake[22]. In contrast, the urea treatment maintained the lowest S concentrations (64.67 mg kg^{-1} rhizosphere; 72.67 mg kg^{-1} bulk), values that were not statistically different from the control. Although urea is a concentrated nitrogen source (46 % N) devoid of sulfur, its application can slightly stimulate microbial activity, accelerating mineralization of native organic sulfur and causing only a marginal increase relative to unfertilized soil[23, 24]. The liquid EM biofertilizer yielded moderate S levels (118.12 mg kg^{-1} rhizosphere; 133.12 mg kg^{-1} bulk soil). This reflects its dependence on soil organic matter as a substrate for microbial decomposition; with limited organic matter, EM activity and subsequent S release remain lower than with mineral fertilizers[25]. When data from **Tables 2 and 3** are compared, a clear decline in total sulfur is observed from 70 to 100 days across all treatments. This downward trend likely results from increased sulfur demand by maturing maize plants, coupled with biological immobilization of S within microbial biomass as the crop approaches reproductive stages[26, 27]. These findings underscore the necessity of balanced S management particularly the inclusion of S-containing fertilizers such as ammonium sulfate to maintain soil sulfur availability throughout the maize growth cycle.

Table 3. Effect of fertilizer type on total sulfur concentration (mg kg^{-1}) in the rhizosphere and bulk soils 100 days post-planting.

Treatments	Soil Zone	
	Rhizosphere soil	**Bulk soil**
Control Treatment	47.684	58.684
Urea	64.665	72.665

Treatments	Soil Zone	
	Rhizosphere soil	Bulk soil
Ammonium Sulfate	417.370	448.370
Biohealth	189.547	202.881
Effective Microorganisms	118.121	133.121
Bio-Mineral Fertilizer Combination	306.621	324.955
Lsd 0.05	33.8660	30.0090

Table 3 (*Continued*)

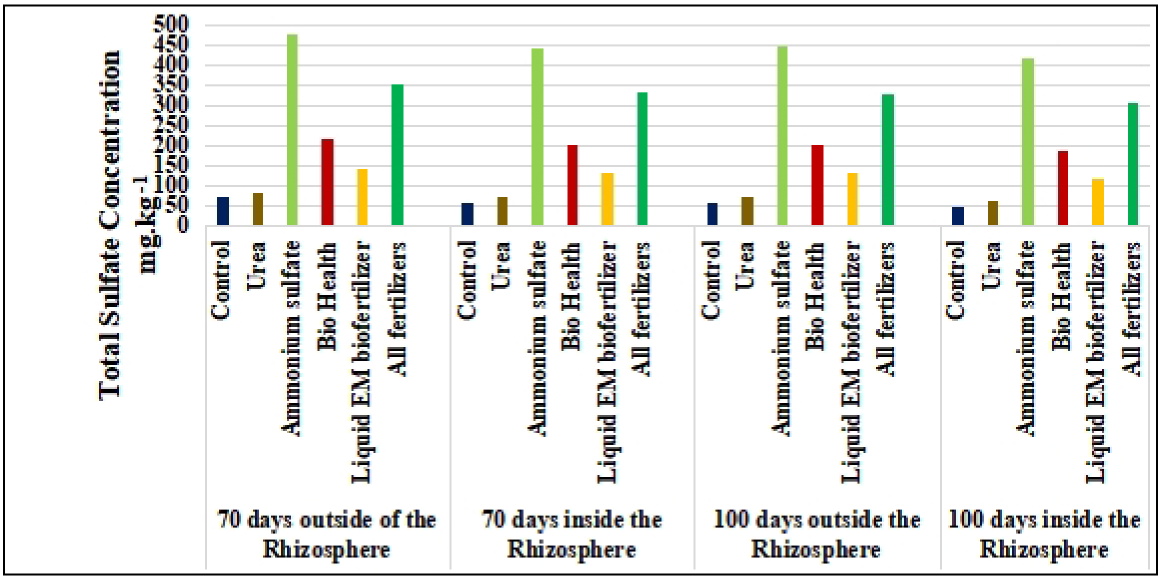

Figure 1. Impact of fertilizer type on total sulfur concentration in the rhizosphere and bulk soils at 70- and 100-days post-planting (mg kg^{-1}).

Sulfur is a vital nutrient for plants, contributing to the sustainability of the soil. Ammonium sulfate is a crucial nitrogen-sulfur fertilizer. It serves as an accessible source of sulfur (SO4 sulfate) while concurrently supplying nitrogen. This helps preserve the equilibrium of essential nutrients, a cornerstone of sustainable soil management (**Figure 1**).

Organic carbon concentration in the experimental soil 70 days post-planting

Table 4 shows the influence of mineral fertilizers, biofertilizers, and their combined application on soil organic carbon (OC) concentrations in the rhizosphere and bulk soils 70 days after planting. Analysis of variance ($p \leq 0.05$) confirmed significant differences among treatments. All fertilizer applications increased OC relative to the control (5.14 g kg^{-1} in rhizosphere; 5.33 g kg^{-1} in bulk), indicating that both mineral and biological inputs effectively enhance soil carbon availability. The highest OC levels were recorded when biofertilizers were integrated with mineral fertilizers, reaching 15.82 g kg^{-1} in the rhizosphere and 18.71 g kg^{-1} in the bulk soil. Such enrichment reflects a synergistic interaction between added nutrients and stimulated microbial activity. Previous studies have reported that co-application of bio- and mineral fertilizers improves the decomposition of organic matter, promotes humus formation, and supports microbial biomass, thereby increasing OC stocks[28,29]. Individual treatments with either mineral or biofertilizers alone produced intermediate OC values, underscoring the importance of balanced nutrient and microbial management. Biofertilizers enhance microbial diversity and root–microbe interactions, while mineral fertilizers provide readily available nutrients that fuel microbial metabolism. This dual action not only raises organic carbon but also improves soil structure and aggregate stability, leading to better water retention and nutrient cycling. Collectively, these findings highlight that combining mineral fertilizers with biofertilizers creates an optimal environment for sustaining organic carbon in both rhizosphere and bulk soils, thereby improving soil fertility and long-term productivity of maize cropping systems.

Table 4. Impact of fertilizer type on organic carbon concentration (g kg^{-1}) in the rhizosphere and bulk soils 70 days post-planting.

Treatments	Soil Zone	
	Rhizosphere soil	**Bulk soil**
Control Treatment	5.14	5.33
Urea	10.21	12.67
Ammonium Sulfate	9.13	12.02
Biohealth	15.66	17.46
Effective Microorganisms	9.35	12.88
Bio-Mineral Fertilizer Combination	15.82	18.71
Lsd 0.05	3.249	3.434

The findings indicated a rise in organic carbon concentrations due to urea treatment, with levels of 12.67 g kg^{-1} in the rhizosphere and 10.21 g kg^{-1} in the bulk soils, respectively. This is due to urea providing a substantial nitrogen supply, which promotes root development and subsequently enhances the production of root exudates, including amino acids and sugars, which are considered carbon-rich[30]. The BioHealth treatment produced a greater total carbon content than the EM biofertilizer treatment, as the latter included organic matter (75% humic acid, 5% seaweed, and 65% other materials), which are abundant in carbon.

Organic carbon concentration of the experimental soil 100 days post-planting

Table 5 illustrates the impact of mineral fertilizers, biofertilizers, and their combined application on the organic carbon concentration in both rhizosphere and bulk soils, measured 100 days post-planting. The statistical analysis results indicated significant differences at the 0.05 significance level. All treatments surpassed the control treatment in both rhizosphere and bulk soils, with carbon contents attaining 3.79 and 4.35 g kg^{-1} for the rhizosphere and bulk soils, respectively. The urea treatment resulted in the most significant organic carbon concentrations in the rhizosphere and bulk soils, measuring 11.65 and 15.10 g kg^{-1}, respectively. This aligns with the findings of[31] in their research on maize plants in the maturity stage. The ammonium sulfate treatment resulted in reduced carbon concentrations in both the rhizosphere and bulk soils relative to other fertilizer types, achieving values of 8.22 and 12.94 g kg^{-1}, respectively. Ammonium sulfate may enhance the decomposition of organic matter in the soil, leading to increased microbial activity that consumes carbon as an energy source.

Table 5. Impact of fertilizer type on organic carbon concentration (g kg-1) in the rhizosphere and bulk soils 100 days post-planting.

Treatments	Soil Zone	
	Rhizosphere soil	**Bulk soil**
Control Treatment	3.79	4.35
Urea	11.65	15.10
Ammonium Sulfate	8.22	12.94
Biohealth	11.02	18.21
Effective Microorganisms	7.29	15.48
Bio-Mineral Fertilizer Combination	10.75	13.08
Lsd 0.05	1.841	7.610

The results displayed in **Tables (5, 6)** and **Figure (2)** indicate that the content of organic carbon in the rhizosphere is markedly lower than in the bulk soil across all treatments, except for urea, as growth periods increase. Simultaneously, organic carbon levels rose in all treatments beyond the rhizosphere, as research suggests that the rhizosphere constitutes a biologically active milieu. Microorganisms utilize carbon as an energy source, resulting in a reduction of its concentration in this area[32].

The findings validate that biological and organic treatments, particularly when combined with mineral fertilizers, represent optimal techniques for enhancing the concentration of organic carbon in the soil, thus fostering soil fertility and sustainability. Biofertilizers enhanced organic carbon levels, illustrating their capacity to bioactivate and accumulate organic matter in the soil. Conversely, mineral fertilizers exhibited fluctuating values, but demonstrated reduced stability over time. The incorporation of biofertilizers into the agricultural system significantly enhances organic matter accumulation and carbon cycling, hence directly bolstering the sustainability attributes of agricultural soils.

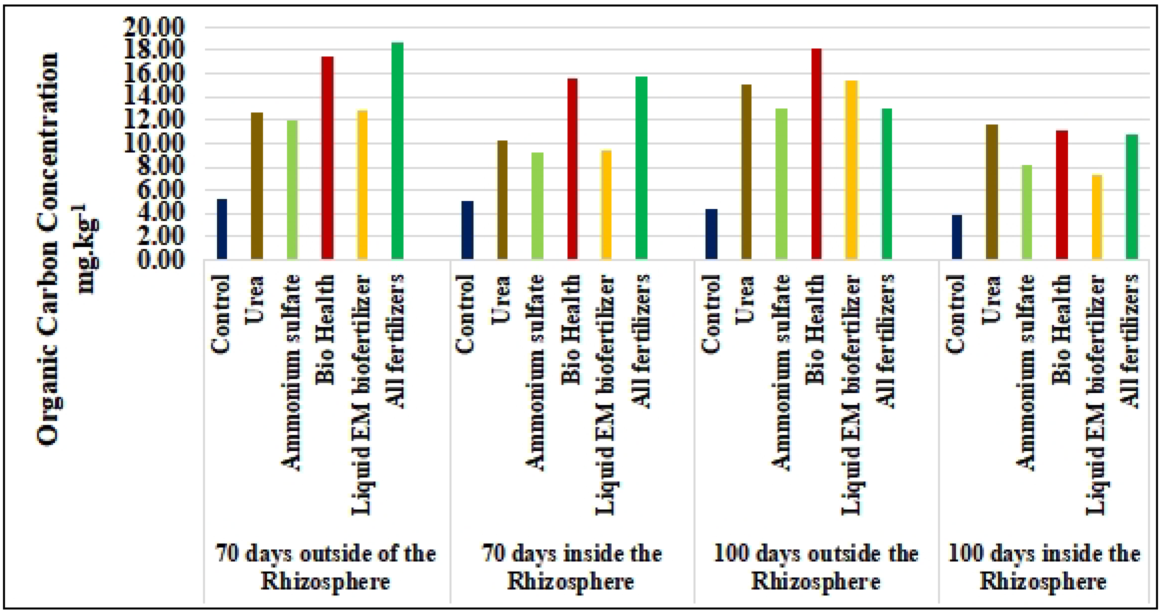

Figure 2. Impact of fertilizer type on organic carbon concentration in the rhizosphere and bulk soils at 70- and 100-days post-planting (g kg-1).

3.2. Carbon to Sulfur Ratio (C/S)

Carbon to Sulfur Ratio in the Experimental Soil 70 Days Post-Planting

Table 6 presents the effects of mineral fertilizers, biofertilizers, and their combined application on the carbon-to-sulfur (C/S) ratio in rhizosphere and bulk soils 70 days after planting. Analysis of variance ($p \leq 0.05$) revealed significant differences among treatments, reflecting the distinct impacts of nutrient sources on soil C and S dynamics. The lowest C/S ratio occurred with ammonium sulfate, measuring 20.72 in the rhizosphere and 25.41 in the bulk soil. This decline is expected because ammonium sulfate supplies 24 % sulfur, elevating soil S while modestly affecting organic carbon, which drives the ratio downward. A lower C/S ratio is typically associated with enhanced sulfur mineralization and improved availability of plant-available sulfate, as also reported by similar fertilization studies[33]. Conversely, urea application produced the highest C/S ratio (137.04 and 153.42 for rhizosphere and bulk soils, respectively), indicating a relative accumulation of carbon compared with sulfur. Urea provides nitrogen but no sulfur, and it can stimulate microbial growth and root exudation, indirectly increasing soil organic matter and carbon inputs while leaving sulfur levels largely unchanged[33]. The combined bio- and mineral fertilizer treatment achieved intermediate ratios (48.11 and 53.65 for rhizosphere and bulk soil), suggesting a more balanced nutrient environment. This equilibrium reflects the synergistic effect of adding both organic and inorganic nutrient sources, which supports microbial activity and organic matter stabilization while supplying sufficient sulfur to prevent excessive C accumulation.

Overall, these results underscore the importance of selecting fertilizer regimes that balance carbon and sulfur cycling. Maintaining a moderate C/S ratio through integrated bio-mineral fertilization can improve sulfur mineralization, sustain microbial diversity, and promote long-term soil fertility.

Table 6. Impact of fertilizer type on the carbon-to-sulfur ratio 70 days post-planting.

Treatments	Soil Zone	
	Rhizosphere soil	Bulk soil
Control Treatment	86.859	75.132
Urea	137.036	153.415
Ammonium Sulfate	20.724	25.411
Biohealth	76.227	79.193
Effective Microorganisms	71.093	89.627
Bio-Mineral Fertilizer Combination	48.106	53.648
Lsd 0.05	22.5936	35.9174

The C/S ratios for the EM biofertilizer treatment were 71.093 for the rhizosphere soil and 89.627 for the bulk soil. The elevated ratios signify a substantial concentration of carbon relative to sulfur, as the microbes in this fertilizer break down organic materials.

Carbon-to-sulfur ratio in the experimental soil 100 days post-planting

Table 7 summarizes the effects of mineral fertilizers, biofertilizers, and their integrated application on the carbon-to-sulfur (C/S) ratio in rhizosphere and bulk soils 100 days after planting. Analysis of variance ($p \leq 0.05$) revealed significant treatment effects, indicating that fertilizer type strongly regulates the relative dynamics of carbon and sulfur as maize matures. The lowest C/S ratios were recorded in soils receiving ammonium sulfate, with values of 19.74 in the rhizosphere and 28.62 in the bulk soil. This decline reflects the direct sulfur enrichment from ammonium sulfate, which supplies ~24 % sulfur and consequently narrows the carbon-to-sulfur balance. A lower ratio is generally associated with improved sulfur mineralization and greater sulfate availability for plant uptake, consistent with previous fertilization studies[34]. In contrast, urea application produced the highest C/S ratios, reaching 182.22 in the rhizosphere and 213.08 in the bulk soil. Because urea is a nitrogen fertilizer devoid of sulfur, its application promotes plant growth and stimulates the release of carbon-rich root exudates, increasing soil organic carbon while leaving sulfur levels relatively unchanged[35]. This imbalance elevates the C/S ratio and may slow sulfur mineralization over time. The combined bio- and mineral fertilizer treatment maintained intermediate C/S ratios, reflecting a balanced input of carbon and sulfur. Such integrated management fosters microbial activity and steady organic matter turnover while supplying adequate sulfur to prevent excessive carbon accumulation. Overall, these findings indicate that balanced fertilization using both biofertilizers and mineral sources helps maintain a moderate C/S ratio, promoting efficient sulfur cycling and sustaining long-term soil fertility in maize production systems.

Table 7. Impact of fertilizer type on the carbon-to-sulfur ratio 100 days post-planting.

TREATMENTS	SOIL ZONE	
	Rhizosphere soil	Bulk soil
Control Treatment	81.012	74.842
Urea	182.216	213.080
Ammonium Sulfate	19.743	28.619
Biohealth	58.084	89.474
Effective Microorganisms	61.669	116.106

TREATMENTS	SOIL ZONE	
	Rhizosphere soil	Bulk soil
Bio-Mineral Fertilizer Combination	35.572	41.088
Lsd 0.05	25.6859	64.2801

Table 7. (*Continued*)

The C/S ratio for the BioHealth treatment was 85.084 in both rhizosphere and non-rhizosphere soils. This is a comparatively elevated ratio. This is attributable to the presence of organic materials in this fertilizer (**Table 7**).

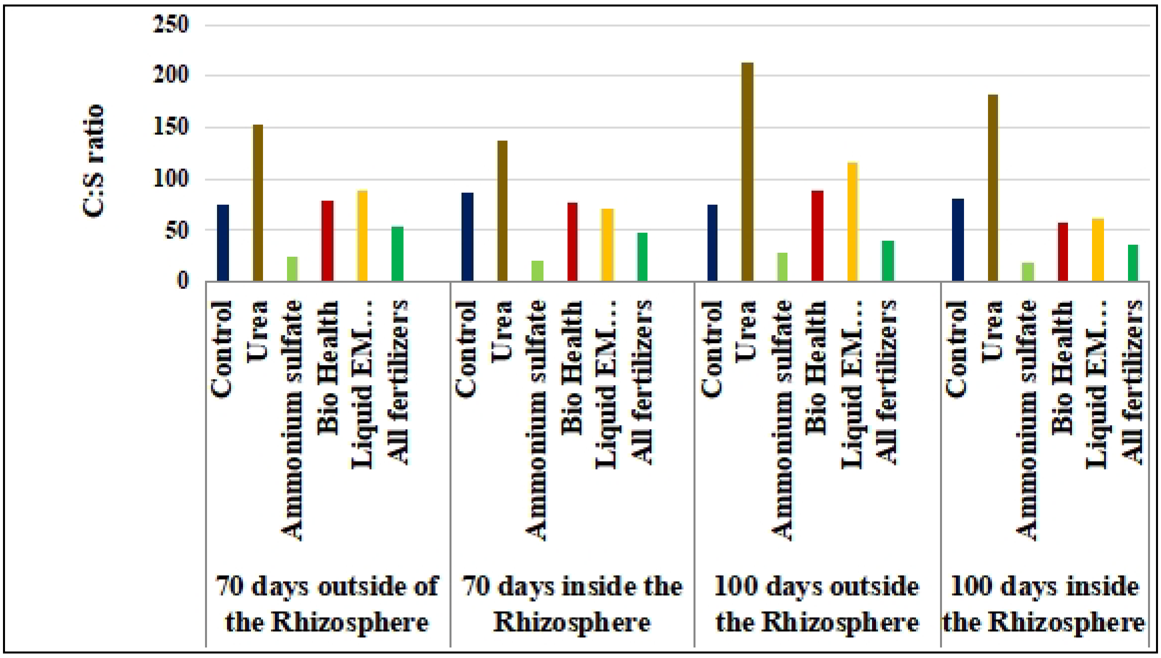

Figure 3. Impact of fertilizer type on the carbon-to-sulfur ratio in the rhizosphere and bulk soils at 70- and 100-days post-planting (g kg^{-1}).

The results presented in **Tables 6 and 7**, as well as **Figure 3**, indicate that for most treatments, the carbon-to-sulfur (C/S) ratio in the bulk soil was higher than in the rhizosphere. This is attributed to root absorption of nutrients, accumulation of carbon from root exudates, and distinct microbial activity near the roots. The urea treatment showed a progressive increase in this ratio at both 70 and 100 days in rhizosphere and bulk soils because urea contains no sulfur, causing carbon to accumulate without a parallel rise in sulfur. Although urea treatment effectively raises soil carbon content, it significantly increases the C/S ratio, particularly after 100 days. In contrast, the combination of liquid EM treatments achieved a balanced relationship between carbon and sulfur, supporting the soil's biological processes. Consequently, the integration of organic and mineral fertilizers provides a sustainable approach to maintaining soil structure and fertility.

4. Conclusion

The study aimed to evaluate the effects of biofertilizers and chemical fertilizers on total sulfur, organic carbon, and the carbon-to-sulfur (C/S) ratio in both rhizosphere and bulk soils after 70 and 100 days of planting. Results revealed that ammonium sulfate treatment recorded the highest sulfur concentration, which decreased as plant age increased. Biofertilizers and mineral fertilizers enhanced soil organic carbon, while urea treatment showed the highest C/S ratio after 100 days. Overall, the findings indicated that increasing sulfur led to a reduction in the C/S ratio, with the most significant decrease observed under ammonium sulfate treatment.

Conflict of interest

The authors declare no conflict of interest.

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