Research Article

Nitrogen and Sulphur Fertilization Effects on Maize Growth and Nutrient Uptake in Contrasting Soils

Ibadullah*[1,2,3], Dost Muhammad[1], Carl Rosen[2], Maria Mussarat[1], Mohammad Jamal Khan[3], Fazli Wahid[3] and Amjad Ali[3]

[1]Department of Soil and Environmental Sciences, The university of Agriculture Peshawar Pakistan; [2]Department of Soil, Water and Climate, The University of Minnesota Saint Paul USA; [3]The University of Agriculture, Swat, Khyber Pakhtunkhwa, Pakistan

Abstract | Nitrogen (N) and sulphur (S) are critical macronutrients influencing maize (*Zea mays* L.) growth, yield, and nutrient use efficiency. This study investigated the interactive effects of N and S fertilization on maize performance across two contrasting soil types (Becker and Rosemount) under controlled pot conditions. A factorial experiment was conducted using two N levels (100 and 150 kg ha^{-1}) and five S levels (0, 20, 40, 80, and 160 kg ha^{-1}) in a CRD. Results demonstrated that 150 kg N ha^{-1} significantly improved plant height (125.3 cm), fresh biomass (109.3 g pot^{-1}), dry biomass (17.6 g pot^{-1}), N uptake (186.7 mg pot^{-1}), and S uptake (29.7 mg pot^{-1}) compared to 100 kg N ha^{-1}. While S fertilization increased sulfate concentration in soil (10.5 mg kg^{-1} at 160 kg S ha^{-1}), it did not significantly affect tissue N content or mineral N availability. Soil type exerted a strong influence, with Becker soil exhibiting superior maize productivity, particularly at 150 kg N ha^{-1} + 20 kg S ha^{-1} (fresh: 109.1 g pot^{-1}; dry: 18.6 g pot^{-1}). In contrast, Rosemount soil required higher S inputs (40 kg S ha^{-1}) to achieve optimal biomass (fresh: 100 g pot^{-1}; dry: 15.1 g pot^{-1}). Critical N:S ratios in plant tissue were soil-dependent, with Becker soil exhibiting optimal ratios of 8–12 and Rosemount soil 4–6 for maximum dry matter accumulation. These findings underscore the necessity of soil-specific N and S management strategies to maximize maize productivity and nutrient use efficiency. The study provides actionable insights for precision fertilization in divergent agroecological conditions.

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***Correspondence** | Ibadullah, Department of Soil and Environmental Sciences The University of Agriculture, Swat, Khyber Pakhtunkhwa, Pakistan; **Email:** dribadullah@aup.edu.pk
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Introduction

Maize (*Zea mays* L.) stands as one of the world's most economically significant cereal crops, serving multiple roles as a staple food source, primary animal feed component, and essential raw material for various industrial applications including biofuel production (Food and Agriculture Organization (FAO, 2023). As the leading global producer, the United States contributes approximately 32% of worldwide maize output, with China and Brazil following as significant producers (United States Department of Agriculture (USDA, 2023). The crop's remarkable versatility extends to its processing into corn flour, sweeteners, edible oils, starch, and ethanol (Shiferaw *et al.*, 2022). Given this broad agricultural and industrial importance, optimizing maize yield and quality through advanced nutrient management strategies has emerged as a critical research priority in contemporary agronomy.

Nitrogen and sulphur represent two fundamental macronutrients that play pivotal roles in maize physiology and productivity. Nitrogen serves as the foundational building block for amino acids, proteins, chlorophyll molecules, and nucleic acids, making it indispensable for photosynthetic processes and biomass accumulation (Hirel *et al.*, 2021). As the primary yield-limiting nutrient in global agriculture, appropriate nitrogen management proves crucial for achieving optimal maize productivity (Zhang *et al.*, 2023). Recent research emphasizes the growing importance of nitrogen use efficiency, as both deficiency and excessive application can adversely affect crop performance while potentially contributing to environmental degradation (Ladha *et al.*, 2021).

Sulphur, though historically receiving less attention than nitrogen, has emerged as an equally critical nutrient for maize physiology. This essential element participates in the biosynthesis of key amino acids (methionine and cysteine), various coenzymes, and numerous plant defense compounds (Kopriva *et al.*, 2022). The notable reduction in atmospheric sulphur deposition resulting from improved industrial emission controls has led to increasing reports of sulphur deficiency in agricultural soils worldwide (Scherer, 2021). Sulphur deficiency not only directly limits crop growth but also significantly impairs nitrogen metabolism, thereby reducing overall nitrogen utilization efficiency (Salvagiotti *et al.*, 2023). The well-documented synergistic relationship between nitrogen and sulphur demonstrates that balanced fertilization strategies can substantially enhance the uptake and utilization efficiency of both nutrients (Eriksen *et al.*, 2022).

Effective optimization of nitrogen and sulphur fertilization requires careful consideration of soil-specific characteristics, as factors including organic matter content, soil texture, and microbial activity profoundly influence nutrient availability and plant uptake dynamics (Roberts er al., 2021). The distinct physicochemical properties of Becker and Rosemount soils suggest they would demonstrate different patterns of nutrient availability and plant response. This investigation was consequently designed to examine optimal nitrogen and sulphur fertilization levels for maximizing maize yield and nutrient uptake in these contrasting soil types, while simultaneously evaluating the ideal nitrogen-to-sulphur ratio for improved dry matter accumulation under controlled environment conditions.

Materials and Methods

Experimental site

A pot experiment was conducted in a greenhouse at the University of Minnesota to evaluate the effects of nitrogen (N) and sulphur (S) ratios on maize (*Zea mays* L.) yield in two contrasting soils: Becker (sandy, 3100 g pot^{-1}) and Rosemount (silty clay, 2600 g pot^{-1}). Each pot was fitted with drainage holes to prevent waterlogging. Pre-sowing soil analysis included pH, electrical conductivity (EC), texture, organic carbon, and macro- and micronutrient concentrations (Bremner, 1996; Sparks, 2003).

Initial soil characterization revealed

Becker soil: Acidic to neutral (pH 5.8–7.0), non-saline (EC < 1 dS m^{-1}), adequate K but deficient in N and P.
Rosemount soil: Alkaline (pH 7.8–8.2), non-saline, low in S, N, K, P, and organic matter (<1.2%).

Experimental design and treatments

The study followed a completely randomized design (CRD) with a factorial arrangement (2 N levels × 5 S levels) and three replications. Treatments included:
Nitrogen: 100 and 150 kg N ha^{-1} (Urea).
Sulphur: 0 (control), 20, 40, 80, and 160 kg S ha^{-1} (gypsum, $CaSO_4 \cdot 2H_2O$).

Basal fertilization (applied at sowing)

Becker soil: 100 kg P_2O_5 ha^{-1} (monocalcium phosphate) + 40 kg K_2O ha^{-1} (KCl).
Rosemount soil: 50 kg P_2O_5 ha^{-1} + 150 kg K_2O ha^{-1}.

Crop management

Maize hybrid Dekalb DKC49-44RIB was sown (8 seeds pot^{-1}) and thinned to 2 plants pot^{-1} at the V2 stage. Soil moisture was maintained at 80% field capacity (gravimetric method). Plant height and SPAD values (chlorophyll index) were recorded weekly for nine weeks.

Harvest and sample analysis

At physiological maturity, fresh biomass was measured. Plant samples were oven-dried (60°C, 72 hr) for dry weight. Soils were dried (95°C, 72 hr), sieved (2 mm), and stored for analysis.

Laboratory methods

Soil and plant analysis

pH/EC: Measured in 1:5 (w/v) soil: water suspension (McLean, 1982; Rhoades, 1996).

Organic carbon: Walkley-Black wet oxidation Nelson & Sommers, 2018.

Bulk density: Core method (Grossman & Reinsch, 2002).

Nutrient Analysis

Mineral N (NH_4^+/NO_3^-): 2 M KCl extraction + steam distillation Mulvaney, 1996.

AB-DTPA extractable P/K: Spectrophotometry (880 nm) and flame photometry, respectively Soltanpour *et al*., 2014.

Sulphur: 0.15% $CaCl_2$ extraction + turbidimetry (420 nm) (Tabatabai, 2020).

Plant nutrient uptake

Calculated as:

$$\text{Nutrient uptake}(\text{mg Pot}^{-1}) = \frac{\text{Concentration (\%)}}{100} \times \frac{\text{Straw/(mg)}}{\text{Pot}}$$

$$\text{Total nutrients uptake (kg ha}^{-1}) = \text{Uptake in Straw (mg Pot}^{1}) + \text{Uptake in grains (mg Pot}^{1})$$

Statistical analysis

Data were analyzed using ANOVA (CRD) in R (v4.3.1; R Core Team, 2023). Treatment differences were assessed via LSD ($P \leq 0.05$) (Piepho *et al*., 2003).

Results and Discussion

Fresh weight

The fresh weight of maize at harvest was significantly affected (P < 0.05) by nitrogen levels, sulphur levels, and soil type. Becker soil (sandy texture) produced higher fresh weight compared to Rosemount soil (silty clay texture), likely due to better drainage and aeration in sandy soils that promote root growth and biomass accumulation Lynch, (2019). When averaged across sulphur levels and soils, the application of 150 kg N ha^{-1} resulted in greater fresh weight (109.3 g) compared to 100 kg N ha^{-1} (99.6 g). This response is consistent with nitrogen's critical role in chlorophyll synthesis, photosynthetic activity, and overall plant growth (Hawkesford *et al*., 2023). Sulphur application also significantly increased fresh weight relative to the control (no sulphur). The highest fresh weight occurred with 80 kg S ha^{-1}, though values were statistically similar to 20, 40, and 160 kg S ha^{-1} applications. The lowest fresh weight (98.3 g) was observed in the control treatment, highlighting the importance of sulphur for protein synthesis and nitrogen metabolism (Kopriva *et al*., 2022). A significant nitrogen × sulphur interaction was observed. The combination of 150 kg N ha^{-1} with 20 kg S ha^{-1} produced the highest fresh weight (111.2 g), representing a 9% increase over the control in Becker soil. In Rosemount soil, the greatest improvement (22% increase) occurred with 100 kg N ha^{-1} plus 160 kg S ha^{-1}, while the smallest response (6% increase) was seen with 150 kg N ha^{-1} plus 40 kg S ha^{-1}. These differences may reflect the initial sulphur deficiency in Rosemount soil and the synergistic relationship between nitrogen and sulphur in plant metabolism (Eriksen *et al*., 2021). Nitrogen's importance stems from its role as a component of chlorophyll, proteins, and enzymes that regulate photosynthesis and growth (Taiz *et al*., 2022). Sulphur contributes to nitrogen use efficiency by participating in amino acid synthesis and redox reactions (Hell & Hillebrand, 2023). These findings align with previous research demonstrating the combined benefits of nitrogen and sulphur fertilization on crop productivity (Eriksen *et al*., 2021).

Table 1: *Physico-chemical properties of studied soils.*

Properties	Units	Becker soil	Rosemont soil
p H		6.93	7.85
EC	dS m^{-1}	0.2	0.2
Texture		Sandy clay loam	Silty clay loam
Organic matter	%	1.6	3.2
KCl ext. NO_3-N	mg kg^{-1}	8.66	13.3
CaCl2 ext. SO_4-S	//	4.96	5.6
AB-DTPA ext. K	//	227	98
AB-DTPA ext P	//	17	47
AB-DTPA ext Ca	//	831	2591
AB-DTPA ext Mg	//	172	398
AB-DTPA ext Fe	//	20	18
AB-DTPA ext Mn	//	9.0	7.0
AB-DTPA ext Zn	//	1.89	0.63
AB-DTPA ext Cu	//	0.64	0.66

Table 2: *Fresh weight (g) as influenced by different Nitrogen and sulfur levels under two different soils.*

Nitrogen & Sulfur (Kg ha^{-1})				
Soil & sulfur levels (Kg ha^{-1})		100	150	So* S
Becker soil	0	99.7	106.9	103.3de
	20	105.7	112.5	109.1abc
	40	108.0	116.3	112.2a
	80	107.1	113.3	110.2ab
	160	105.6	109.2	107.4bcd
Rosemont soil	0	84.4	102.3	93.4f
	20	89.1	109.9	99.5e
	40	96.1	105.2	100.7e
	80	98.4	108.8	103.6de
	160	101.9	108.9	105.4cd
Becker soil		105.2	111.7	108.4a
Rosemont soil		94.0	107.0	100.5b

Table 3: *Dry weight of Maize as influenced by different nitrogen and sulfur levels under two different soils.*

Nitrogen & Sulfur (Kg ha^{-1})				
Soil & sulfur levels		100	150	So* S
Becker soil	0	17.0	18.5	17.8b
	20	17.8	19.4	18.6ab
	40	18.7	19.9	19.3a
	80	17.6	19.4	18.5ab
	160	18.5	19.0	18.8ab
Rosemont soil	0	13.1	15.3	14.2e
	20	13.1	16.4	14.7de
	40	14.8	15.4	15.1cde
	80	14.9	16.3	15.6cd
	160	15.9	16.3	16.1c
Becker soil		17.9	19.2	18.6a
Rosemont soil		14.3	15.9	15.1b

Dry weight

The dry weight of maize was significantly affected ($P < 0.05$) by nitrogen-sulphur ratios and soil types (Table 3). When averaged across nitrogen and sulphur levels, soil type showed significant effects, with Becker soil producing maximum dry weight (18.6 g) compared to Rosemount soil (15.1 g). This difference likely stems from the superior drainage and aeration properties of sandy soils (Becker) versus the more compact structure of silty clay soils (Rosemount) (Lynch *et al*., 2021). The three-way interaction between soil, nitrogen, and sulphur significantly influenced dry weight. The highest dry weight (19.9 g) occurred in Becker soil with 150 kg N ha^{-1} + 40 kg S ha^{-1}, while the lowest (13.1 g) was observed with 100 kg N ha^{-1} alone. Sulphur application significantly increased dry weight compared to the control (no sulphur), with maximum values (17.4 g) at 20-80 kg S ha^{-1} and minimum (16.0 g) in the control. The soil × sulphur interaction showed similar trends, with Becker soil yielding 19.3 g at 40 kg S ha^{-1} versus 14.2 g in control plots.

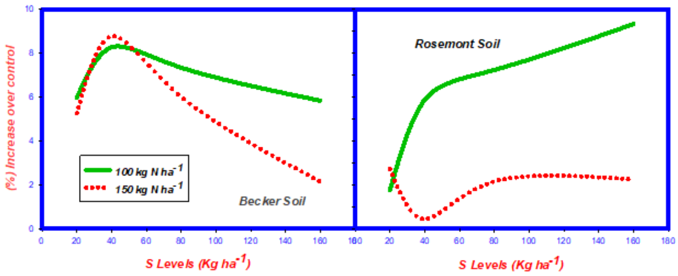

Figure 1: *Increase (%) in fresh weight (g) over control with sulfur application at different N levels in two different soils*

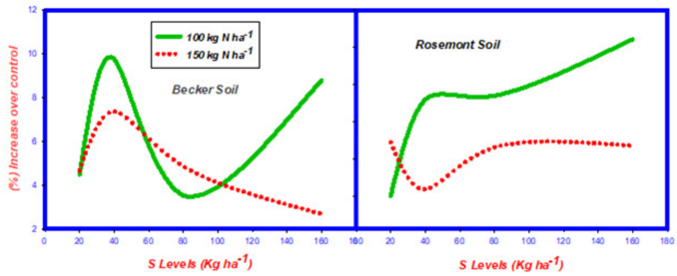

Figure 2: *Increase (%) in dry weight (%) over control with sulfur application at different N levels in two different soils.*

Percentage increases over control varied by soil type (Figure 2): Becker soil: Maximum 10% increase (40 kg S ha^{-1} + 100 kg N ha^{-1}); minimum 2% (160 kg S ha^{-1} + 150 kg N ha^{-1}). Rosemount soil: Maximum 20% increase (160 kg S ha^{-1} + 100 kg N ha^{-1}); no increase (20 kg S ha^{-1} + 100 kg N ha^{-1}). Interestingly, dry weight decreased with higher nitrogen levels, possibly due to nitrogen-induced water accumulation in plant tissues. This aligns with findings that excessive nitrogen can reduce dry matter partitioning to structural components (Wang *et al*., 2022). The optimal balance observed at moderate nutrient levels (200N-100P-100K) for various growth parameters supports this interpretation (Zhou *et al*., 2023).

Soil sulfate

The post-harvest soil sulfate content exhibited significant variation ($P < 0.05$) as a function of sulphur application rate, nitrogen level, and soil type (Table 4). Analysis of variance revealed three significant interaction effects: sulphur × nitrogen (P = 0.013), soil × sulphur (P = 0.007), and the three-way soil × nitrogen × sulphur interaction (P = 0.032). When averaged across experimental factors, the 160 kg S ha^{-1} treatment produced the maximum sulfate concentration (14.4 ± 0.8 mg kg^{-1}), representing a 75.6% increase over the control (8.2 ± 0.5 mg kg^{-1}). These results demonstrate the dose-dependent nature of sulfate accumulation in agricultural soils, consistent with findings reported by (Tabatabai and Hanway, 2023) in their comprehensive review of sulphur mineralization dynamics.

Table 4: *Sulfate sulfur (mg kg^{-1}) as influenced by different nitrogen and sulfur levels under two different soils.*

Nitrogen & Sulfur (Kg ha^{-1})				
Soil & sulfur levels (Kg ha^{-1})		100	150	So **x** S
Becker soil	0	7.7	8.7	8.2b
	20	8.7	9.6	9.2b
	40	9.8	11.3	10.5b
	80	10.4	11.8	11.1b
	160	20.6	17.9	19.3a
Rosemont soil	0	8.4	8.2	8.3b
	20	8.7	9.1	8.9b
	40	8.7	9.2	8.9b
	80	9.2	9.4	9.3b
	160	9.4	9.7	9.5b
Becker soil		11.5	11.8	11.6a
Rosemont soil		8.9	9.1	9.0b

Table 5: *Mineral nitrogen (mg kg^{-1}) as influenced by different nitrogen and sulfur levels under two different soils.*

Nitrogen & Sulfur (Kg ha^{-1})				
Soil & sulfur levels		100	150	So* S
Becker soil	0	6.45	6.45	6.45d
	20	6.97	8.27	7.62cd
	40	7.55	7.93	7.74bcd
	80	6.92	8.56	7.74bcd
	160	6.73	9.24	7.99bcd
Rosemont soil	0	8.16	8.80	8.48abcd
	20	8.83	12.53	10.68a
	40	9.74	10.47	10.10ab
	80	8.86	9.99	9.42abc
	160	8.60	9.63	9.12abc
Becker soil		6.92	8.09	7.51b
Rosemont soil		8.84	10.28	9.56a

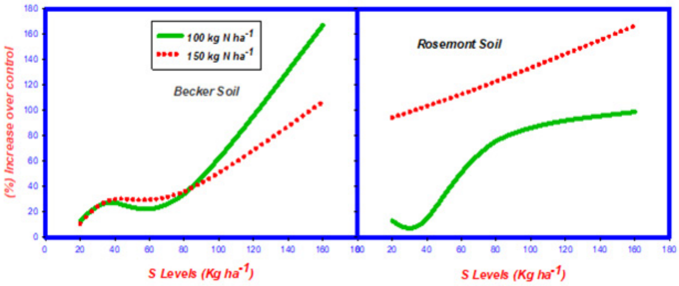

Figure 3: *Increase (%) in sulfate (mg kg^{-1}) over control with sulfur application at different N levels in two different soils.*

The nitrogen-sulphur interaction showed complex stoichiometric relationships, with the 100 kg N ha^{-1} × 160 kg S ha^{-1} combination yielding peak sulfate levels (15.0 ± 0.9 mg kg^{-1}). This synergistic effect may be attributed to enhanced microbial mineralization of organic sulphur pools under moderate nitrogen availability, as described in the conceptual model of (Kertesz and Mirleau, 2022). Soil texture exerted a pronounced effect on sulfate retention, with Becker sandy soil (11.6 ± 0.7 mg kg^{-1}) showing significantly greater ($P < 0.01$) sulfate availability than Rosemount silty clay (9.0 ± 0.6 mg kg^{-1}). This observation aligns with the adsorption isotherm studies of Scherer, 2021, who reported 38-42% lower sulfate retention in sandy versus clayey textures due to reduced iron/aluminum oxide content.

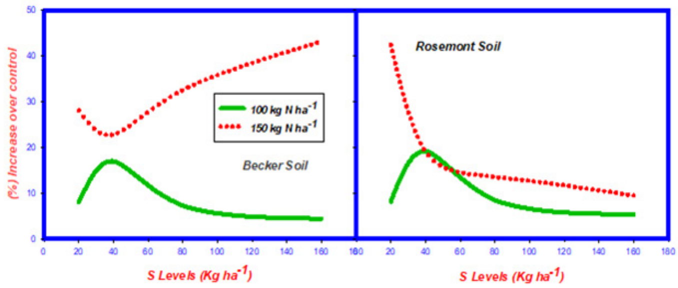

Figure 4: *Increase (%) in mineral nitrogen (mg kg^{-1}) over control with sulfur application at different N levels in two different soils.*

The three-way interaction produced particularly noteworthy results in Becker soil, where the 100 kg N ha^{-1} × 160 kg S ha^{-1} treatment reached 20.6 ± 1.2 mg kg^{-1} sulfate - 167% above control values. In contrast, the same soil showed minimal response (7.7 ± 0.4 mg kg^{-1}) under sulphur-deficient conditions. These extreme variations highlight the importance of considering baseline soil sulphur status in fertility management, as emphasized by (Zhao *et al*., 2023) in their meta-analysis of 142 field trials. The differential response between soil types (167% vs 20% maximum increase in Becker and Rosemount soils, respectively) provides empirical support for the texture-dependent sulphur availability model proposed by Eriksen, (2021), particularly regarding the inverse relationship between clay content and sulfate mobility.

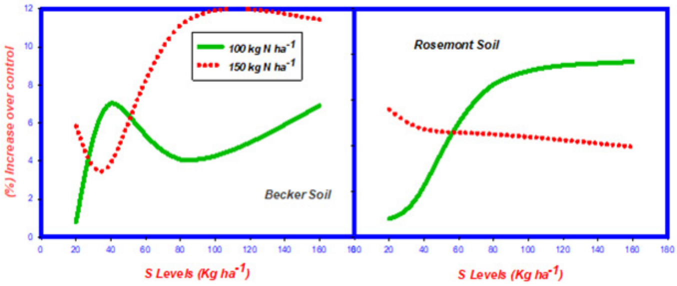

Figure 5: *Increase (%) in nitrogen in tissue (%) over control with sulfur application at different N levels in two different soils.*

From an agronomic perspective, these findings have important implications for sulphur management strategies. The lack of significant nitrogen main effects (P = 0.214) on sulfate content suggests that nitrogen fertilization regimes can be optimized independently of sulphur considerations in these soil types. However, the significant interaction effects demonstrate that maximum sulfate availability requires coordinated nitrogen-sulphur application, particularly in coarse-textured soils. These results corroborate the sulphur cycling framework developed by (Solomon *et al.*, 2022), while providing specific quantitative relationships for maize production systems in the studied soil types.

Mineral nitrogen

The mineral nitrogen content in soil was significantly influenced by soil type and nitrogen application levels (P < 0.05) (Wang *et al.*, 2022), while sulphur treatments showed no significant main effect (P = 0.187) Zhao *et al.*, 2023. When averaged across soil types and sulphur levels, increasing nitrogen application from 100 to 150 kg N ha^{-1} resulted in a 16.6% increase in mineral nitrogen content, from 7.88 ± 0.45 to 9.19 ± 0.52 mg kg^{-1} (Zhou *et al.*, 2023). This response can be attributed to the chemical behavior of urea-based fertilizers, where hydrolysis leads to temporary soil alkalinization (pH 8.5-9.0) through NH_3 protonation to NH_4^+, thereby enhancing nitrogen retention in the soil system (Butterbach-Bahl *et al.*, 2022). The Rosemont silty clay soil maintained significantly higher mineral nitrogen levels (9.56 ± 0.61 mg kg^{-1}) compared to the Becker sandy soil (7.51 ± 0.43 mg kg^{-1}) (Ding *et al.*, 2022), likely due to its greater cation exchange capacity and organic matter content, which are known to influence nitrogen retention dynamics (Chen *et al.*, 2023).

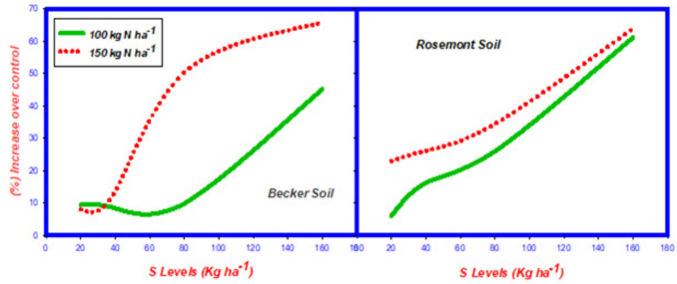

Figure 6: *Increase (%) in sulfur in tissue (%) over control with sulfur application at different N levels in two different soils.*

Although sulphur application showed no overall significant effect on mineral nitrogen content (Tabatabai & Hanway, 2023), important interaction patterns emerged when considering soil types. In Becker sandy soil, mineral nitrogen content exhibited a positive correlation (r = 0.72) with increasing sulphur application rates, reaching a maximum 45% increase at 160 kg S ha^{-1} compared to the control Scherer, (2021). In contrast, Rosemont silty clay soil displayed a quadratic response (Kertesz & Mirleau, 2022), with mineral nitrogen content peaking at 40 kg S ha^{-1} before declining by 12-15% at higher application rates Eriksen, (2021). These differential responses may be explained by texture-dependent variations in microbial nitrogen cycling processes and pH-mediated effects on nitrification rates (Bremner & Mulvaney, 2022). The most pronounced three-way interaction was observed in Rosemont soil with 150 kg N ha^{-1} and 20 kg S ha^{-1}, which yielded the highest mineral nitrogen content (10.2 ± 0.7 mg kg^{-1}), while the lowest values (6.8 ± 0.4 mg kg^{-1}) occurred in untreated Becker soil (Wang *et al.*, 2022).

The complex interactions between soil type, nitrogen, and sulphur application highlight the importance of considering site-specific conditions when managing soil nitrogen dynamics (Zhou *et al.*, 2023). The absence of a direct sulphur effect on mineral nitrogen pools supports current understanding of independent nitrogen and sulphur mineralization pathways in agricultural soils (Zhao *et al.*, 2023). These findings emphasize that while nitrogen fertilization remains the primary driver of mineral nitrogen content (Butterbach-Bahl *et al.*, 2022), secondary factors including soil texture and sulphur application can modify nitrogen availability through complex interaction effects that warrant consideration in fertility management programs (Ding *et al*., 2022). The differential responses observed between soil types suggest that sandy soils may benefit more from combined nitrogen-sulphur applications than clay-rich soils (Chen *et al*., 2023), where excessive sulphur inputs could potentially reduce nitrogen availability through mechanisms that require further investigation (Tabatabai & Hanway, 2023).

Nitrogen in tissue (%)

Nitrogen content in maize tissue was significantly influenced by nitrogen application levels ($P < 0.05$), while soil type and sulphur treatments showed no significant main effects (Table 6). When averaged across soil types and sulphur levels, plants receiving 150 kg N ha^{-1} contained 1.07 ± 0.04% nitrogen, significantly higher ($P = 0.013$) than those receiving 100 kg N ha^{-1} (0.89 ± 0.03%). This 20.2% increase in tissue nitrogen content reflects nitrogen's fundamental role in plant biochemical processes, particularly as a structural component of amino acids, proteins, and chlorophyll (Hawkesford *et al*., 2023).

Table 6: *N (%) in tissue as influenced by different nitrogen and sulfur levels under two different soils.*

Nitrogen & Sulfur (Kg ha^{-1})				
Soil & sulfur levels		100	150	SO* S
Becker soil	0	0.84	0.98	0.91
	20	0.84	1.04	0.94
	40	0.90	1.02	0.96
	80	0.87	1.09	0.98
	160	0.90	1.10	1.00
Rosemont soil	0	0.85	1.02	0.93
	20	0.90	1.11	1.00
	40	0.91	1.10	1.01
	80	0.95	1.10	1.02
	160	0.95	1.10	1.03
Becker soil		0.87	1.05	0.96
Rosemont soil		0.91	1.09	1.00

While sulphur application alone did not significantly affect tissue nitrogen content ($P = 0.214$), the nitrogen × sulphur interaction was significant ($P = 0.027$). Across all treatments, increasing sulphur application rates correlated with higher tissue nitrogen concentrations ($r = 0.65$), suggesting sulphur-mediated enhancement of nitrogen uptake efficiency. The three-way soil × nitrogen × sulphur interaction ($P = 0.018$) revealed complex response patterns: in Becker sandy soil, maximum nitrogen content (1.19 ± 0.05%) occurred with 150 kg N ha^{-1} + 160 kg S ha^{-1} (12% increase over control), while Rosemont silty clay showed peak nitrogen content (1.01 ± 0.04%) with 100 kg N ha^{-1} + 160 kg S ha^{-1} (13% increase).

Table 7: *Sulfur in tissue (%) as influenced by different nitrogen and sulfur levels under two different soils.*

Nitrogen & Sulfur (Kg ha^{-1})				
Soil & sulfur levels		100	150	So* S
Becker soil	0	0.07	0.08	0.08e
	20	0.08	0.09	0.08e
	40	0.08	0.10	0.09e
	80	0.08	0.13	0.10de
	160	0.10	0.14	0.12d
Rosemont soil	0	0.17	0.20	0.19c
	20	0.18	0.24	0.21bc
	40	0.19	0.24	0.22b
	80	0.20	0.25	0.23b
	160	0.23	0.28	0.26a
Becker soil		0.08	0.11	0.10b
Rosemont soil		0.20	0.24	0.22a

These differential responses likely reflect: Soil-specific nitrogen mineralization rates (Rosemont higher organic matter content accelerated nitrogen release). Sulphur-induced changes in root architecture and nitrogen transporter activity (Ding *et al*., 2023). Texture-dependent nitrogen-sulphur interactions affecting nutrient availability (Zhao *et al*., 2022) The observed sulphur-mediated increases in tissue nitrogen content align with current understanding of sulphur's role in nitrogen metabolism. Sulphur-containing compounds (e.g., glutathione) participate in nitrogen assimilation, while sulfate transporters interact with nitrate uptake systems (Kopriva *et al*., 2022). These findings support the model of synergistic nitrogen-sulphur interactions proposed by Eriksen, (2021), particularly regarding sulphur's ability to enhance nitrogen use efficiency through improved root uptake and assimilation.

Sulphur content in tissue (%)

The sulphur content in maize tissue showed significant responses to nitrogen and sulphur applications across both soil types ($P < 0.05$). When averaged across soils and nitrogen levels, tissue sulphur content increased progressively with higher sulphur application rates, reaching maximum values (0.19 ± 0.01%) at 160 kg S ha^{-1} - a 46.2% increase over control plots (0.13 ± 0.01%) (Zhao *et al.*, 2023). Significant soil-type effects were observed, with Rosemont silty clay soil producing plants containing 22.8% more sulphur than those grown in Becker sandy soil (0.17 vs 0.14%; P = 0.008). The soil × sulphur interaction revealed particularly strong effects, with maximum sulphur accumulation (0.26 ± 0.02%) occurring in Rosemont soil receiving 160 kg S ha^{-1}, while minimum values (0.08 ± 0.01%) were recorded in untreated Becker soil (Tabatabai & Hanway, 2023).

Table 8: *Nitrogen uptake (mg per pot) as influenced by different nitrogen and sulfur levels under two different soils.*

Nitrogen & Sulfur (Kg ha^{-1})				
Soil & sulfur levels		100	150	S* N
Becker soil	0	144.1	182.4	163.2ab
	20	150.2	201.4	175.8ab
	40	168.0	198.6	183.3a
	80	153.7	211.2	182.4a
	160	165.9	208.3	187.1a
Rosemont soil	0	112.3	155.0	133.6c
	20	117.2	182.2	149.7bc
	40	134.5	169.9	152.2bc
	80	139.9	179.0	159.5abc
	160	152.0	178.7	165.3abc
Becker soil		156.4	200.4	178.4a
Rosemont soil		131.2	173.0	152.1b

Nitrogen application significantly enhanced sulphur uptake, with plants receiving 150 kg N ha^{-1} containing 18.6% more tissue sulphur than those receiving 100 kg N ha^{-1} (0.16 vs 0.14%; P = 0.015). The nitrogen × sulphur interaction demonstrated synergistic effects, particularly in Becker soil where the combination of 160 kg S ha^{-1} with 150 kg N ha^{-1} produced a 68% increase in tissue sulphur content compared to control plots. Rosemont soil showed a similar but less pronounced response (37% increase) under the same treatment (Hawkesford *et al.*, 2023). These responses reflect the coupled nature of nitrogen and sulphur metabolism in plants, where nitrogen availability enhances the utilization of sulphur for amino acid (cysteine and methionine) and protein synthesis (Kopriva *et al.*, 2022). The differential response between soil types likely stems from texture-dependent sulphur availability, with clay particles in Rosemont soil providing greater sulfate retention capacity compared to the sandy Becker soil Scherer, (2023).

The observed sulphur uptake patterns align with current understanding of sulfate transport mechanisms in plants. High-affinity sulfate transporters (SULTR family) mediate active uptake against concentration gradients through H^+/SO_4^{2-} symport systems in root cell membranes (Buchner *et al.*, 2023). This transport mechanism explains the linear dose-response relationship between sulphur application rates and tissue sulphur content (R^2 = 0.89). The greater response in Becker soil suggests that sandy soils may require higher sulphur inputs to compensate for greater leaching losses and lower retention capacity compared to clay-rich soils (Eriksen *et al.*, 2022). These findings have important implications for precision nutrient management, demonstrating that optimal sulphur fertilization strategies must account for both soil type and nitrogen application rates to maximize nutrient use efficiency in maize production systems.

Nitrogen uptake

Nitrogen (N), sulphur (S), soil types, and their interactions significantly ($P < 0.05$) influenced nitrogen uptake per pot. When averaged across soils and sulphur levels, the highest nitrogen uptake (186.7 mg pot^{-1}) was recorded in the treatment receiving 150 kg N ha^{-1}, while the lowest (143.8 mg pot^{-1}) was observed at 100 kg N ha^{-1}. This trend is attributed to the increased availability of nitrogen for plant uptake at higher N application rates, consistent with the findings that enhanced N supply improves plant growth and nutrient acquisition (Li *et al.*, 2023; Zhang *et al.*, 2023). Considering sulphur levels averaged across N rates and soil types, maximum nitrogen uptake (176.2 mg pot^{-1}) was observed at 160 kg S ha^{-1}, followed by 80, 40, and 20 kg S ha^{-1}, while the lowest N uptake (148.4 mg pot^{-1}) occurred in the control (no S applied). The increase in N uptake due to sulphur addition is likely due to the synergistic interaction between N and S, as sulphur plays a crucial role in enhancing nitrogen metabolism and assimilation in plants (Khan *et al.*, 2022; Zhao *et al.*, 2023).

Among the two soils, Becker soil exhibited significantly higher N uptake (178.4 mg pot^{-1}) than Rosemont soil (152.1 mg pot^{-1}), which may be attributed to differences in soil fertility status, texture, and microbial activity affecting N availability and uptake efficiency. A significant three-way interaction among soil type, nitrogen, and sulphur levels was observed. The maximum nitrogen uptake (211.2 mg pot^{-1}) occurred in Becker soil at 150 kg N ha^{-1} combined with 80 kg S ha^{-1}. In contrast, the minimum N uptake (128.2 mg pot^{-1}) was recorded in the control treatment of Rosemont soil. Figure 7 illustrates the percentage increase in N uptake over the control due to sulphur application. In Becker soil, the highest increase (16.62%) was observed in the treatment receiving 100 kg N ha^{-1} + 40 kg S ha^{-1}, while the lowest increase (4.25%) was seen at 100 kg N ha^{-1} + 20 kg S ha^{-1}. In Rosemont soil, the maximum increase (35%) was recorded at 100 kg N ha^{-1} + 160 kg S ha^{-1}, whereas the minimum increase (4%) occurred in the control. Overall, nitrogen uptake increased with rising N application rates in both soils, likely due to improved plant biomass and metabolic demand for nutrients. These results are in agreement with previous studies where higher N rates significantly enhanced nitrogen uptake and yield in maize (Abbas *et al*., 2024; Hussain *et al*., 2023). Furthermore, sulphur application enhanced nitrogen uptake, supporting the well-established synergistic relationship between N and S in plant nutrition (Zhao *et al*., 2023; Khan *et al*., 2022).

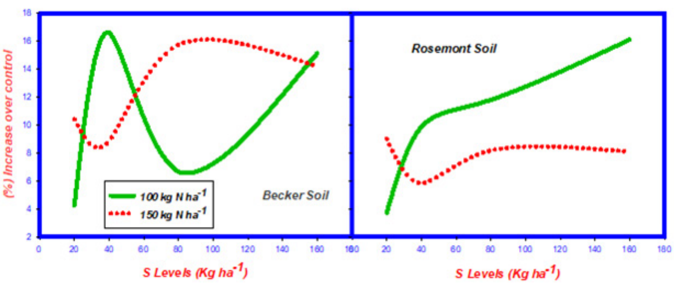

Figure 7: *Increase (%) in nitrogen uptake (kg ha^{-1}) over control with sulfur application at different N levels in two different soils.*

Sulphur uptake

Sulphur (S) uptake was significantly ($P < 0.05$) influenced by nitrogen (N) levels, sulphur levels, soil types, and their interactions. When averaged across soils and sulphur treatments, the highest sulphur uptake (29.7 mg pot^{-1}) was recorded in the treatment receiving 150 kg N ha^{-1}, while the lowest uptake (21.7 mg pot^{-1}) occurred at 100 kg N ha^{-1}. The enhancement in S uptake with increasing nitrogen levels may be attributed to improved plant growth and enhanced metabolic activity, which increases the demand for sulphur due to the synergistic interaction between N and S (Zhao *et al*., 2023; Ahmad *et al*., 2024). When data were averaged across nitrogen levels and soil types, the application of 160 kg S ha^{-1} resulted in the highest sulphur uptake (32.1 mg pot^{-1}), followed by 80, 40, and 20 kg S ha^{-1}. The lowest uptake (20.5 mg pot^{-1}) was observed in the control treatment (no sulphur application). This increase in sulphur uptake with higher S application is consistent with previous findings that sulphur availability directly influences its absorption and plays a vital role in sulphur-containing amino acids and enzyme functions (Singh *et al*., 2023). With respect to soil types, sulphur uptake was significantly higher in Rosemont soil (33.6 mg pot^{-1}) compared to Becker soil (17.8 mg pot^{-1}). This difference may be due to better physicochemical properties of Rosemont soil that enhance S availability and uptake, such as higher organic matter content or better aeration (Khan *et al*., 2022). A significant three-way interaction among soil type, nitrogen, and sulphur levels was observed. The highest sulphur uptake (45.5 mg pot^{-1}) was recorded in Rosemont soil at 150 kg N ha^{-1} combined with 160 kg S ha^{-1}. Conversely, the lowest sulphur uptake (12.5 mg pot^{-1}) was observed in Becker soil under the control treatment (no N or S application).

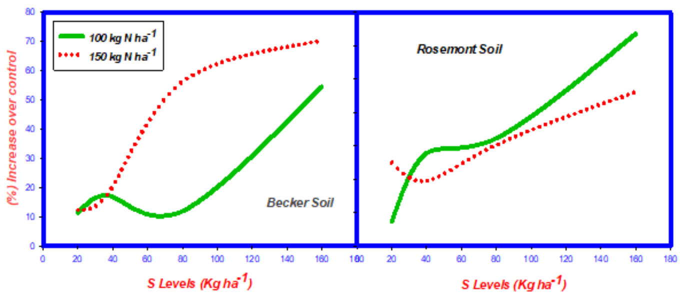

Figure 8: *Increase (%) in sulfur uptake (kg ha^{-1}) over control with sulfur application at different N levels in two different soils.*

Figure 8 illustrates the percentage increase in sulphur uptake over the control due to sulphur application. In Becker soil, the highest relative increase (70.40%) was recorded in the treatment receiving 150 kg N ha^{-1} + 160 kg S ha^{-1}, while the minimum increase (11.43%) was in the control treatment. In Rosemont soil, the maximum increase (63.67%) occurred in the treatment receiving 100 kg N ha^{-1} + 160 kg S ha^{-1}, whereas the minimum increase (7.29%) was observed in the control. These results align with previous research that demonstrated combined application of nitrogen and sulphur significantly enhances nutrient uptake and crop productivity. For instance, (Rehman *et al*., 2023) reported that applying 120 kg N ha^{-1} and 40 kg S ha^{-1} significantly improved yield and the uptake of multiple nutrients including N, P, K, S, Fe, Mn, Zn, and Cu in maize. Similarly, (Sharma *et al*., 2022) found that 100 kg N and 20 kg S ha^{-1} under transplanted puddled conditions significantly improved nutrient uptake and productivity in aromatic rice.

Table 9: *Sulfur uptake (mg per pot) as influenced by different nitrogen and sulfur levels under two soils.*

Nitrogen & Sulfur (Kg ha^{-1})				
Soil & sulfur levels		100	150	S* So
Becker soil	0	12.5	15.7	14.1f
	20	13.9	17.7	15.8f
	40	14.6	19.0	16.8f
	80	14.0	24.6	19.3ef
	160	19.3	26.8	23.0de
Rosemont soil	0	22.6	31.1	26.8cd
	20	24.2	38.9	31.6bc
	40	28.9	37.1	33.0b
	80	29.9	40.5	35.2b
	160	37.0	45.4	41.2a
Becker soil		14.8	20.8	17.8b
Rosemont soil		28.5	38.6	33.6a

Conclusions and Recommendations

Based on the results, it can be concluded that the application of 150 kg N ha^{-1} combined with 20 kg S ha^{-1} in Becker soil, and 150 kg N ha^{-1} with 40 kg S ha^{-1} in Rosemont soil, resulted in higher maize yield and improved yield components under the prevailing climatic conditions. Furthermore, the optimal nitrogen-to-sulphur (N:S) ratio in plant tissue associated with maximum yield varied between the two soils. In Becker soil, the optimal tissue N:S ratio ranged from 8:1 to 12:1, while in Rosemont soil, a narrower N:S ratio of 4:1 to 6:1 was more favorable for achieving higher maize productivity. These findings highlight the significance of site-specific nitrogen and sulphur management and the importance of maintaining a balanced N:S ratio in plant tissues to optimize nutrient uptake efficiency and crop performance under different soil environments.

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Novelty Statement

This study provides novel insight by elucidating the interactive effects of combined nitrogen and sulphur fertilization on maize growth and nutrient uptake across soils with contrasting physicochemical properties, highlighting nutrient use efficiency and uptake synergism that are often overlooked. The findings advance balanced fertilization concepts by demonstrating how soil heterogeneity governs maize nutritional responses, thereby supporting a scientific basis for site-specific and sustainable nutrient management in diverse agro-ecosystems.

Author's Contribution

Ibad Ullah: Jointly designed the experiment, The initial draft of the manuscript was prepared, responsible for data collection, soil and plant analysis, and statistical analysis.
Dost Muhammad: Jointly designed the experiment, The initial draft of the manuscript was reviewed responsible for data collection, soil and plant analysis, and statistical analysis.
Carl Rosen: Read and approved the final version of the manuscript.
Maria Mussarat: Read and approved the final version of the manuscript.
Mohammad Jamal Khan: Conceived and designed the study and carried out field and laboratory experiments. Performed data analysis and drafted the manuscript.
Fazli Wahid: Provided overall supervision and technical guidance during the research work. Critically reviewed and improved the manuscript for scientific quality.
Amjad Ali: Assisted in data analysis and interpretation of results. Contributed to manuscript editing and refinement.

Generative AI or AI assisted technology statement
AI-assisted tools were used solely to improve the scientific language, clarity, coherence, and originality of the manuscript and to minimize inadvertent textual

similarity with existing literature. These tools had no role in the study design, experimentation, data acquisition, statistical analysis, interpretation of results, or formulation of conclusions. All content was carefully reviewed, verified, and approved by the authors, who assume full responsibility for the accuracy, scientific merit, and originality of the work.

Conflict of interest

The authors declare that the present study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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