
Temporal Synchronization of Nitrogen and Sulfur Fertilization: Impacts on Nutrient Uptake, Use Efficiency, Productivity, and Relationships with Other Micronutrients in Soybean

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Article

Temporal Synchronization of Nitrogen and Sulfur Fertilization: Impacts on Nutrient Uptake, Use Efficiency, Productivity, and Relationships with Other Micronutrients in Soybean

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Abstract: Nitrogen (N) and sulfur (S) are essential macro-elements crucial for the proper development, productivity, nutrient uptake, and the building blocks of protein biosynthesis. The plant's N-metabolism is also influenced by S, and its deficiency can lead to a decline in the consumption of N from fertilizers. The objective of this research was to assess the impacts Temporal N and S applications on the nutrient uptake, productivity, use efficiencies and micronutrients content status in soybean seeds. The field trial in randomized complete block design carried out, experiment comprised fourteen treatments with varying N and S quantities. N and S were applied through starter and temporal applications in different combinations. The findings indicated that temporal application of individually N and S fertilization as 25 kg ha⁻¹ as starter and 25 kg ha⁻¹ as temporal applications at R₂ stage enhanced the highest nutrient uptake, seeds and stalks yields. The use efficiencies, as well as the Zinc (Zn) and iron (Fe) uptake by seeds, showed an increase with the temporal application of both nutrients compared to the control. Furthermore, soil Zn and Fe contents were also enhanced with the temporal applications of nitrogen and sulfur fertilization. As a result, it could be decided that the nitrogen and sulfur temporal applications not only enhanced nutrient uptake, productivity and use efficiencies but also improved the micronutrient uptake by soybean. Furthermore, this practice contributed to an improvement in soil Zn and Fe content.

Keywords: Nutrients uptake; productivity; use efficiencies; nitrogen; sulfur; soybean

1. Introduction

Soybean (*Glycine max* L.) stands as the primary legume crop globally, contributing to 56% of total global oilseed production [1]. Essential for food security, soybean serves as a vital protein source for human and animal consumption, along with being a key oil supplier for cooking and biofuel [2]. N and S are crucial macronutrients, vital for soybean's development, yield, and protein biosynthesis. S deficiency affects N metabolism, leading to a reduced uptake from fertilizers. Both N and S fertilization have been proven to enhance soybean growth and yield, emphasizing their significance in proper development, yield, and protein biosynthesis [3,4]. Numerous reports confirm that N fertilizer enhances protein content, while S fertilizer influences protein composition [5,6]. Soybean, with its substantial nutrient demand, especially requires high amounts of N as a result of seeds protein, averaging around 40% based on dry weight of seeds [7]. This elevated N requirement is crucial for achieving high seed yields, particularly in legumes, given their significant seeds protein

content [7–9]. A global challenge is the widespread deficiency of N in soil, presenting a major limitation. Meeting crop N requirements is complex, demanding temporal synchronization between seasonal indigenous N sources and crop N demand [10]. N-use efficiency is crucial, reflecting the effectiveness of crops in converting available N into seeds yield. Its main components include the relation between N absorption and N applied (recovery efficiency) and the relation between biomass and N assimilation (internal efficiency) [11]. The decrease in N-use efficiency with increasing N fertilizer rates is well-documented due to factors like nutrient availability and higher N losses [8,12]. Over a 40-year span, data from various sites revealed a linear increase in biological N uptake of 0.07 kg in per kg increase in seed yield of soybean [13]. Sulfate availability in soil emerges as a limiting factor for plant growth, as cysteine, a key S metabolism product, is vital for protein aggregation [6,14]. S-containing amino acids, crucial for human health, highlight the significance of S in nutrition [15]. The S fertilization enhances the impact of N, improving soil processes and N-use efficiency [11,16]. In poor-fertility environments with N and S deficiencies and moderate organic matter levels, S addition enhances N-recovery and agronomic efficiency [17]. Soybean, having a high N requirement during the seeds-filling period, faces potential limitations in biological N fixation meeting its needs. N demand peaks during the late growth stages (R₃–R₆), and daily observed during with extreme 3.6–4.3 kg ha⁻¹ N uptake amounts at R₄–R₅ growth stages of soybean [18]. However, studies suggest that in high yielding crops like soybean biotic N fixation during at later reproductive stage to supply N may drop back N requirement [13]. Likewise, [20] emphasizes that Zn absorption and accumulation by crops N play pivotal role, rationalizing targeted focus in biofortification initiatives [21]. The presence of high amount of N in wheat seeds layers suggests protein-rich seeds accumulate more Zn and Fe levels [22]. High N and Zn supply significantly enhanced the Zn contents in the seeds, surpassing current breeding targets [23,24]. Hence, top-dressing N during early reproductive stages proves beneficial for improving nutrient uptake and use efficiency. In this study, utilizing both N and S as starter doses and top-dressing at the R₂ stage aimed to enhance nutrient uptake, productivity, use efficiency, and the relationship with other micronutrients (Zn and Fe) in soybean. The primary objective was to determine optimal N and S fertilization practices for achieving high-quality soybean production.

2. Materials and Methods

2.1. Location Description, Experimental Design and Crop Husbandry

The field trial performed during the kharif season of 2018 under rainfed conditions at the ICAR-IISR in Indore India. The aim was to assess the impact of starter and temporal N and S applications on soybean's nutrient uptake, use efficiency, and productivity. The experimental soil, classified as Vertisols, predominantly featured a soybean-wheat cropping system. Initial soil features encompassed a pH of 8.2, organic carbon (OC) content of 4.6 g kg⁻¹, and with around 56.2% clay content. The trial was in randomized complete block design (RCBD) laid out having three replications with fourteen treatments in various N and S quantities (12.5, 25 and 50 kg N and S ha⁻¹), applied through starter and temporal applications at the R₂ in various combinations (Table 1), and individually experimental plots measured 3.6 m in width and 6m in length. All additional suggested agronomic practices were adhered to in order to align with the existing norms and procedures.

Table 1. Experimental treatment combinations.

Treatment coding	Treatment details	
	N	S
N0S0	-	-
N(25)	Starter 25 kg N ha ⁻¹	-
N(50)	Starter 50 kg N ha ⁻¹	-
N(25+25)	Starter 25 kg N ha ⁻¹ + 25 kg N ha ⁻¹ at R ₂ stage	-
N(12.5+12.5)	Starter 12.5 kg N ha ⁻¹ + 12.5 kg N ha ⁻¹ at R ₂ stage	-
S(25)	-	Starter 25 kg S ha ⁻¹
S(50)	-	Starter 50 kg S ha ⁻¹
S(12.5+12.5)	-	Starter 12.5 kg S ha ⁻¹ + 12.5 kg S ha ⁻¹ at R ₂ stage
S(25+25)	-	Starter 25 kg S ha ⁻¹ + 25 kg S ha ⁻¹ at R ₂ stage
N(25+25), S(12.5+12.5)	Starter 25 kg N ha ⁻¹ + 25 kg N ha ⁻¹ at R ₂ stage	starter 12.5 kg S ha ⁻¹ + 12.5 kg S ha ⁻¹ at R ₂ stage
N(12.5+12.5), S(12.5+12.5)	Starter 12.5 kg N ha ⁻¹ + 12.5 kg N ha ⁻¹ at R ₂ stage	Starter 12.5 kg S ha ⁻¹ + 12.5 kg S ha ⁻¹ at R ₂ stage
N(12.5+12.5), S(25+25)	Starter 12.5 kg N ha ⁻¹ + 12.5 kg N ha ⁻¹ at R ₂ stage	Starter 25 kg S ha ⁻¹ + 25 kg S ha ⁻¹ at R ₂ stage
N(25+25), S(25+25)	Starter 25 kg N ha ⁻¹ + 25 kg N ha ⁻¹ at R ₂ stage	Starter 25 kg S ha ⁻¹ + 25 kg S ha ⁻¹ at R ₂ stage
N(25), S(50)	Starter 25 kg N ha ⁻¹	Starter 50 kg S ha ⁻¹

2.2. Chemical Analysis of Plant and Soil Samples

The plant specimens from the various treatments underwent dehydration in a hot-air oven at 65°C over a span of 3 days to assess the levels of macronutrients (N, S) and micronutrients (Zn, Fe) present in the plant materials. To determine the contents of S, Zn, and Fe, the finely powdered samples were subjected to digestion using a di-acid mixture comprising nitric acid and perchloric acid in a 5:4 ratio. The digestion process occurred over duration of 1 hour at a temperature of 320°C. For S in seeds was estimated by turbidimetric method and N by steam distillation Kjeldahl's method. The content that underwent digestion was directly introduced into the Atomic Absorption Spectrophotometer for the determination of Zn and Fe levels. The results were then expressed in milligrams per kilogram (mg kg⁻¹) for the plant materials. The diethylene triamine pentaacetic acid (DTPA) with pH of 7.3 extraction technique, as developed by [25] was carried out to determination the soil micronutrients, such as Zn and Fe. The extracted micronutrients were quantified in the DTPA solution using an atomic absorption spectrophotometer [26]. The calculation of nutrient uptake in soybean seeds and stalks (kg ha⁻¹) involved multiplying the yield of seeds or stalks (kg ha⁻¹) by the nutrient concentration specific to each treatment. Additionally, the total nutrient uptake (kg ha⁻¹) for each crop was determined by adding the nutrient uptake in the seeds and stalks. The analyses were carried out at the Soil-Science Chemistry/ Fertility/ Microbiology Laboratory of IISR.

Agronomic efficiency (AE) is defined as the incremental economic yield (expressed in kg) per unit of applied nutrient (expressed in kg⁻¹)

$$(AE) = \frac{SY_F - SY_C}{F \text{ kg ha}^{-1}}$$

Physiological efficiency (PE) is characterized by the seeds yield (measured in kg) per unit of nutrient uptake (measured in kg⁻¹)

$$(PE) = \frac{SY_F - SY_C}{SNU_F - SNU_C}$$

Apparent Nutrient Recovery Efficiency (ANR) is employed to indicate a plant's capability to assimilate applied nutrients from the soil.

$$(ANR) \% = \frac{SNU_F - SNU_C}{F \text{ kg ha}^{-1}} \times 100$$

where,

SY_F : Signifies the seeds yield with applied N or S.

SY_C : Represents the seeds yield for the control group.

F : Denotes the amount of applied fertilizers, whether N or S.

SNU_F: Stands for seeds nutrient uptake with applied N or S.

SNU_C : Indicates the seeds nutrient uptake for the control group.

2.3. Statistical Analysis

The data were analyzed using SAS statistical software. The one-way ANOVA was performed through the ANOVA procedure in SAS Enterprise and the least significant differences (LSD) test at p = 0.05 was employed to distinguish between treatment means. To conduct multivariate analysis and ascertain robust Pearson correlations among various features, Biplot and correlation plots were created utilizing the Origin Pro 2023b software.

3. Results

3.1. Nutrients Uptake (kg ha⁻¹)

Seeds, stalks and total N and S absorption by soybeans markedly elevated with various starter and temporal N and S synchronizations (Figures 1 and 2). The results revealed that the maximum seeds, stalks and total N assimilation of soybeans were highest where each N and S were applied in two split at 25 kg ha⁻¹ as starter and 25 kg ha⁻¹ temporal application at R₂ stage of soybean, which did not statistically varied from treatments 50 kg N ha⁻¹ application as the starter dose; 25 kg N ha⁻¹ as starter and temporal application at R₂ stage with 12.5 kg S ha⁻¹ as starter dose and temporal application at R₂ stage; 25 kg N ha⁻¹ plus 50 kg S ha⁻¹ as starter doses treatments. However, they were considerably varied from others treatment combinations, including the control group in this study. The lowest Seeds, stalks and total N and S absorption were determined with no N and S uses (control). Similarly, results also indicated that S uptake by seeds, stalks and total absorption significantly varied with the various starter and temporal synchronizations of N and S applications. The highest seeds, stalks and total absorption of S were observed with each N and S were applied in two split at 25 kg ha⁻¹ as starter dose and 25 kg ha⁻¹ temporal application at R₂ stage, this was similar to 25 kg N ha⁻¹ plus 50 kg S ha⁻¹ as starter doses; 12.5 kg N ha⁻¹ as starter and split application at R₂ stage with 25 kg S as starter and split at R₂ stage; 25 kg N ha⁻¹ as starter and split application at R₂ with 12.5 kg S as starter and split at R₂ stage, while significantly different from other treatments and the control. The lowest seeds, stalks and total absorption of S were determined in control plots.

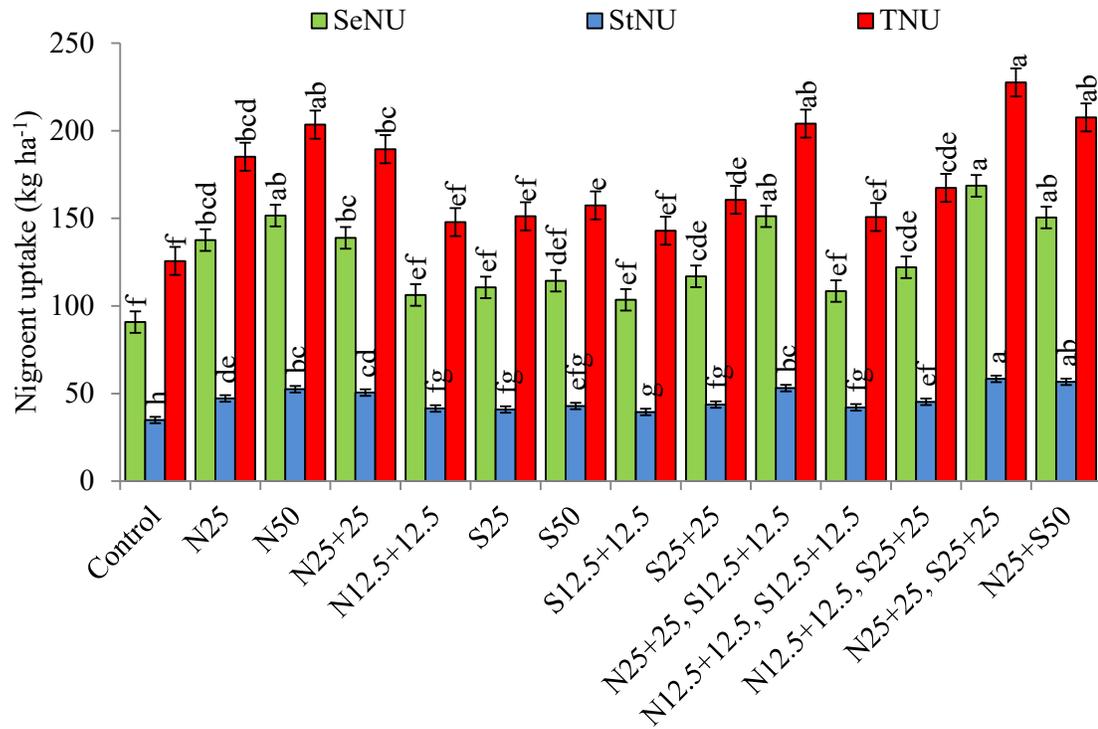


Figure 1. illustrates the impact of different starter and temporal N and S applications on nutrient uptake. The presented data represent the mean values with standard error bars, and distinctions between means are indicated by dissimilar letters at $P=0.05$, as established by Least Significant Difference (LSD) test. The abbreviations used in the figure are as follows: SeNU for seeds N uptake, StNU for stalks N uptake, and TNU for total N uptake.

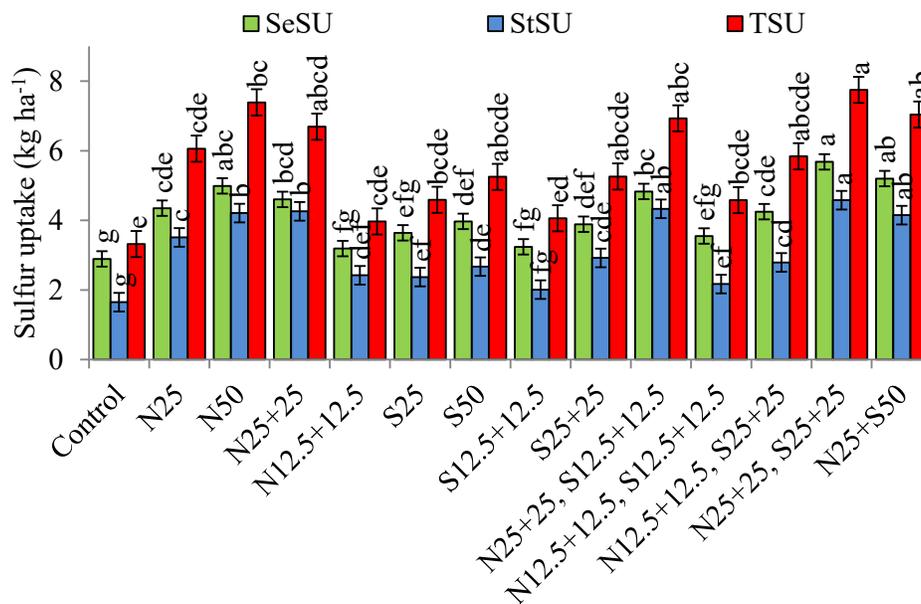


Figure 2. illustrates the impact of different starter and temporal N and S applications on nutrient uptake. The presented data depict the mean values with standard error bars, and distinctions between means are indicated by distinct letters at $P=0.05$, as determined by Least Significant Difference (LSD)

test. The abbreviations used in the figure are SeSU for seeds S uptake, StSU for stalks S uptake, and TSU for total S uptake.

3.2. Seeds and Stalks Yield ($t\ ha^{-1}$)

The data indicated that the seeds and stalks yield in soybeans were affected significantly by starter and temporal N and S applications alone, as well as by joined application treatments (Figure 3). The application of N and S at $25\ kg\ ha^{-1}$ each in two split as starter doses and temporal applications at R_2 stage treatment produced the highest seeds yield which was statistically similar to $25\ kg\ N\ ha^{-1}$ as starter dose and split application at R_2 stage with $12.5\ kg\ S$ as starter dose and split at R_2 stage; $25\ kg\ N\ ha^{-1}$ plus $50\ kg\ S\ ha^{-1}$ as starter doses; $50\ kg\ N\ ha^{-1}$ as starter dose alone, but significantly changed from others starter and temporal N and S applications, along with no N and S applications. However, control plots produced the lowest seeds yield. Similarly, the lowest stalks yield of soybeans was obtained from the control treatment where no N and S fertilizers were applied (Figure 3). N and S fertilization significantly increased the stalks yield of soybean. The most beneficial effects were detected in the treatments where N and S were applied as $25\ kg\ ha^{-1}$ each in two split as starter doses and temporal applications at R_2 stage treatment which was statistically similar to $25\ kg\ N\ ha^{-1}$ plus $50\ kg\ S\ ha^{-1}$ as starter doses; $50\ kg\ N\ ha^{-1}$ as starter dose alone treatments, but significantly different from other treatment combinations in the study, including the control.

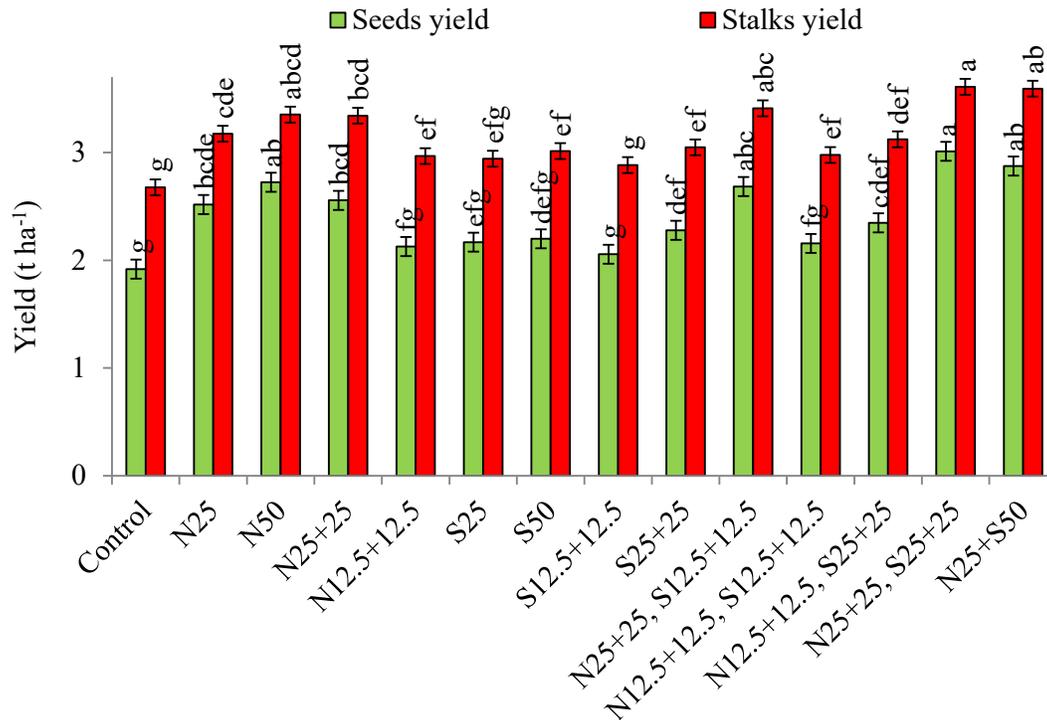


Figure 3. illustrates the impact of different starter and temporal N and S applications on seeds and stalks yield. The presented data depict the mean values with standard error bars, and distinctions between means are indicated by distinct letters at $P=0.05$, as determined by Least Significant Difference (LSD) test.

3.3. Zn and Fe Content of Seeds and Soil ($mg\ kg^{-1}$)

Seeds and soil Zn and Fe content were significantly enhanced with different starter and temporal applications of N and S (Table 2). The results indicate that soil Zn and Fe content at both R_2 and R_5 were significantly highest with $25\ N$ and $S\ kg\ ha^{-1}$ each in two split were applied as starter doses and temporal applications at R_2 stage of soybeans, which was statistically similar to N & S fertilization as $25\ kg\ N\ ha^{-1}$ as starter dose and split application at R_2 stage with $12.5\ kg\ S\ ha^{-1}$ as starter dose and

split at R₂ stage; 12.5 kg N ha⁻¹ as starter dose and split application at R₂ stage with 25 kg S ha⁻¹ as starter dose and split at R₂ stage in this study. However, the lowest soil Zn and Fe content were estimated from the control. Likewise, the temporal N and S application significantly increased seeds Zn and Fe content as 25 N and S kg ha⁻¹ as starter doses and split applications at R₂ stage individually in two split were applied, followed by 25 kg N ha⁻¹ along with 12.5 kg S ha⁻¹ as starter dose and split at R₂ stage. While the lowest seeds Zn and Fe contents were found in control group.

3.3. Nutrient Use Efficiency

The starter and temporal N and S applications significantly influenced the efficiency indices during the study year (Table 3). The highest agronomic use efficiency was measured with 25 kg N ha⁻¹ plus 50 kg S ha⁻¹ as starter dose at time of sowing treatment and the highest use efficiency for S was recorded with the 25 kg N ha⁻¹ along with 12.5 kg S ha⁻¹ as starter dose and split at R₂ stage treatment. The maximum apparent N recovery was observed with the application of 25 kg N ha⁻¹ plus 50 kg S ha⁻¹ as starter doses, followed by 25 kg N ha⁻¹ as starter dose alone and for S, it was 25 kg N ha⁻¹ along with 12.5 kg S ha⁻¹ each in two split as starter doses and split at R₂ stage, followed by 25 N and S kg ha⁻¹ individually in two split were applied as starter doses and temporal applications at R₂ stage; 25 kg N ha⁻¹ plus 50 kg S ha⁻¹ as starter doses applications treatments. A parallel trend was observed regarding physiological use efficiencies. The highest physiological efficiency for N and S as well was calculated with 25 kg N ha⁻¹ plus 50 kg S ha⁻¹ as starter doses. The results obtained on crop productivity, uptake, and use efficiencies can be attributed to the synergistic effect of N and S fertilizer applications, as elaborated in the previous paragraphs.

3.4. Pearson Correlation and Principal Component Analysis (PCA)

The correlation analysis results revealed a significant strong positive correlation between numerous N, S, Zn and Fe uptake through seeds, stalks of soybean and as well as Zn and Fe content in the soil (Figure 4). Notably, strong positive correlations are observed between seeds N uptake and both stalks N uptake (0.98) and total N uptake (0.99), indicating a high degree of association between these variables. Similarly, there are substantial positive correlations within S and Zn-related variables. For instance, seeds S uptake shows a notable positive correlation with stalks S uptake (0.95) and total S uptake (0.98). Furthermore, the strong positive correlation among seeds Zn content and soil Zn content (0.94), reflecting the interconnectedness of plant and soil Zn levels. These correlation coefficients provide valuable insights into the interdependencies among different nutrient content and uptake parameters. PCA for the studied traits revealed that strong association exists among nutrients uptake by both plant and soil (Figure 5). In this case, PC1 is primarily influenced by positive contributions from seeds N uptake, Stalks N uptake, Total N uptake, seeds S uptake, stalks S uptake, total S uptake, seeds Zn content, soil Zn content, and stalks Fe content. Among these, the highest positive coefficients are associated with seeds N uptake, total N uptake, and seeds S uptake. These variables contribute positively to the overall variability captured by PC1. PC2, on the other hand, is characterized by negative contributions from most variables, with notable positive contributions from seeds Fe content and seeds Zn content. Specifically, seeds Fe content have the highest positive coefficient, indicating its strong influence on the second principal component. PC2 captures additional variability in the dataset, orthogonal to PC1, and is dominated by the contrasting effects of seeds Fe content and seeds Zn content. In summary, the PCA results suggest that PC1 is influenced by a broad range of variables, while PC2 is particularly sensitive to the contrasting effects of seeds Fe content and seeds Zn content. These principal components provide a more concise representation of the original variables, capturing the essential patterns and relationships within the data.

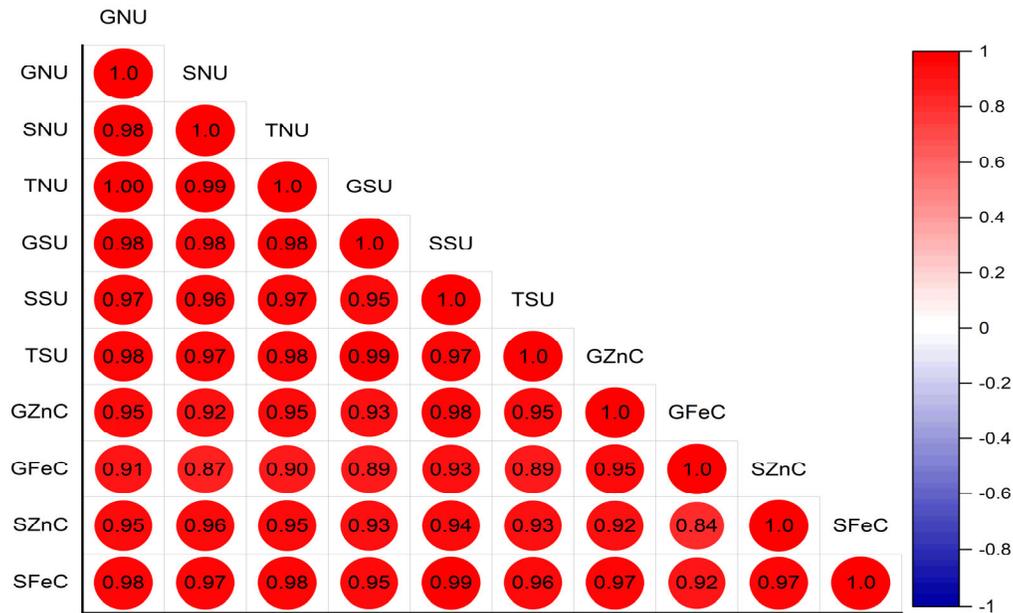


Figure 4. depicts the Pearson correlations analysis among different nutrient uptake variables of soybean; significance level for correlation is set at $P=0.05$. The abbreviations used in the figure are as follows: SeNU for seeds N uptake, StNU for stalks N uptake, TNU for total N uptake, SeSU for seeds S uptake, StSU for stalks S uptake, TSU for total S uptake, SeZnC for seeds Zn content, SeFeC for seeds Fe content, SZnC for soil Zn content, and SFeC for soil Fe content.

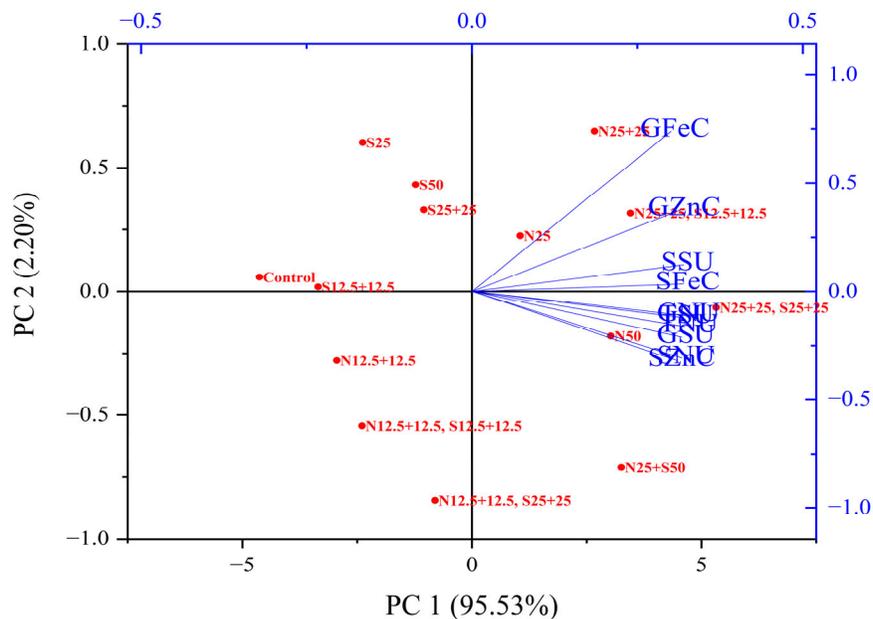


Figure 5. presents a Principal Component Analysis (PCA) illustrating the relationships among various nutrient uptake variables of soybean. The abbreviations used in the figure are as follows: SeNU for seeds N uptake, StNU for stalks N uptake, TNU for total N uptake, SeSU for seeds S uptake, StSU for stalks S uptake, TSU for total S uptake, GZnC for seeds Zn content, GFeC for seeds Fe content, SZnC for soil Zn content, and SFeC for soil Fe content.

4. Discussion

The experiment was based on the hypothesis that starter and temporal applications of N and S increase nutrient uptake through seeds, stalks, and total uptake, productivity, use efficiencies, and micronutrient uptake in soybeans. Soybeans require substantial amounts of N and S due to their high protein and oil content. They meet their N requirement through atmospheric N fixation and mineral fertilization. The findings showed that the temporal N and S applications of fertilizers significantly enhanced the seeds, stalks, and total absorption of N and S of soybeans (Figures 1 and 2). The positive results of temporal application at a later crop stage indicated improved nutrient content and uptake. The reported findings align with those of [27] and [28], who observed increased total uptake of N and S in soybeans with the applications of N and S. The maximum daily N uptake rates during the R₄ to R₅ growth stages [18], while [19] measured at the R₄ growth stage a higher daily (4.6 kg ha⁻¹) N uptake rate. According to [29], in soybeans only up to 52% of total N uptake originates from symbiotic N fixation, with the remaining N requirement coming from nitrates taken up from the soil. The plants remobilize N from leaves to seeds, reducing photosynthesis and limiting yield potential, If total N supply does not meet soybean needs [30]. Many researchers, including [3,31–33], found that starter and temporal N and S application increased soybean seeds yield, a result confirmed by the current study. Our findings agreed with [28], who reported significantly higher soybean seeds yield with N application. Temporal N and S applications enhanced seeds yield as compared to control group, attributed to increased root system activity, photosynthesis rate, and maximum leaf area index [4,34–36]. Soybeans have a high N demand, particularly during the seeds-filling stage. N supply from existing N resources supplements preventing premature aging during this stage of plants and as result enhanced seeds yield [37,38]. The experiment also indicated that temporal N and S application also considerably improved soil Zn and Fe content at R₂ and R₅ stages of soybean growth. Seeds and stalks Zn and Fe content were enhanced with the temporal application of N and S over no N and S applications (Table 2). N fertilization is acknowledged to increase grain yield of wheat and enable grains more Fe and Zn uptake [22,39]. Similarly, [20] emphasized N's critical role in the absorption and uptake of Zn in crops, emphasizing its importance in food crops biofortification especially with Zn. During the anthesis stage, when Zn supply is withheld, the process of Zn remobilization from sources existing prior to anthesis becomes highly reliant on N supply, contributing significantly to the zinc content in nearly all seeds [21]. The embryo and aleurone layers protein, Fe, and Zn contents of wheat seeds indicating that highest grain protein content stored higher amounts of Zn and Fe were identified in grains [22]. Augmenting the availability of Zn and N exhibited a substantial effect on the accrual of zinc within the endosperm, achieving concentrations that exceeded the current benchmarks established in breeding objectives [23,24]. This suggests that N and S application can biofortify soybean seeds. Agronomic biofortification, acknowledged as a potent strategy for addressing micronutrient deficiency in plants [23], underscores the significance of N supply as a crucial element in augmenting the levels of Zn and Fe in crops [40]. The highest agronomic use efficiency, apparent N recovery, and physiological efficiency were also found with the temporal application of N and S in this study (Table 3). N-use efficiency, involving the transformation of available N into seeds yield, showed decreasing efficiency with increasing N fertilizer rates [8,12,41], likely due to limiting factors like the presence of additional nutrients or increased N fertilizer losses can influence the agricultural system. Sulphur fertilization not only amplifies the impact of N but also plays a role in soil processes, enhancing the crop's nitrogen-use efficiency. This enhancement is credited to a higher nitrogen recovery rate without alterations in internal efficiency [11,16]. The apparent interconnectedness of nitrogen and sulphur underscores the rationale for a comprehensive examination of the combined effects of these essential nutrients. The addition of S improved N-recovery efficiency and agronomic efficiency of available N in poor-fertility environments characterized by deficiency of N and S [17].

Table 2. displays the impact of various starter and temporal N and S applications on soil Zn and Fe content at R₂ and R₅ growth stages, as well as seeds Zn and Fe content.

Treatment	Soil Zn content (mg kg ⁻¹)	Soil Fe	Soil Zn content (mg kg ⁻¹)	Soil Fe content (mg kg ⁻¹)	seeds Zn	seeds Fe
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	content (mg kg ⁻¹)			R ₅	content (mg kg ⁻¹)	content (mg kg ⁻¹)
	R ₂					
Control	0.68±0.04 ^g	3.58±0.04 ^g	0.76±0.02 ^g	3.80±0.02 ⁱ	34.8±1.7 ^h	78.9±2.4 ^f
N(25)	0.79±0.03 ^{cd}	3.80±0.05 ^c	0.84±0.03 ^c	4.09±0.03 ^d	42.5±1.3 ^{cd}	112.1±3.9 ^c
N(50)	0.85±0.02 ^b	3.91±0.04 ^b	0.88±0.02 ^b	4.14±0.06 ^{cd}	46.3±1.5 ^b	113.9±5.6 ^c
N(25+25)	0.81±0.02 ^c	3.76±0.03 ^{cd}	0.89±0.06 ^b	4.19±0.03 ^{bc}	46.4±1.2 ^b	125.3±7.1 ^b
N(12.5+12.5)	0.72±0.02 ^f	3.69±0.04 ^{ef}	0.77±0.02 ^{efg}	3.91±0.03 ^{fgh}	36.4±1.0 ^{fgh}	81.0±2.9 ^{ef}
S(25)	0.73±0.04 ^{ef}	3.71±0.06 ^{de}	0.74±0.02 ^g	3.88±0.05 ^{gh}	37.1±1.2 ^{fg}	102.4±2.5 ^d
S(50)	0.76±0.01 ^{d^e}	3.73±0.02 ^{d^e}	0.79±0.01 ^{d^{ef}}	3.94±0.04 ^{efg}	39.7±1.3 ^e	104.8±1.4 ^d
S(12.5+12.5)	0.71±0.02 ^{fg}	3.64±0.02 ^f	0.75±0.04 ^g	3.88±0.03 ^{ef}	35.3±0.6 ^{gh}	86.8±3.4 ^e
S(25+25)	0.76±0.02 ^{d^e}	3.79±0.04 ^c	0.79±0.03 ^{d^{ef}}	3.95±0.04 ^{ab}	40.8±1.3 ^{d^e}	100.5±4.7 ^d
N(25+25),S(12.5+12.5)	0.88±0.04 ^{ab}	3.87±0.05 ^b	0.91±0.03 ^{ab}	4.21±0.07 ^{ab}	46.6±0.7 ^b	126.9±2.7 ^{ab}
N(12.5+12.5),S(12.5+12.5)	0.78±0.05 ^{cd}	3.73±0.03 ^{d^e}	0.81±0.03 ^{d^e}	3.91±0.04 ^{fgh}	36.8±0.9 ^{fg}	84.08±7.7 ^{ef}
N(12.5+12.5),S(25+25)	0.77±0.03 ^d	3.79±0.04 ^c	0.82±0.05 ^{cd}	3.98±0.03 ^e	37.7±0.8 ^f	88.2±6.0 ^e
N(25+25),S(25+25)	0.90±0.05 ^a	3.99±0.07 ^a	0.94±0.04 ^a	4.27±0.04 ^a	49.1±0.9 ^a	133.2±2.8 ^a
N(25),S(50)	0.87±0.03 ^b	3.91±0.05 ^b	0.91±0.04 ^{ab}	4.16±0.05 ^{ab}	43.6±1.51 ^c	115.2±2.0 ^c
LSD(<i>p</i> =0.05)	0.04	0.06	0.04	0.06	1.9	7.2

The data provided represent the mean values obtained from three replicate samples, and the standard deviation (SD) is specified. In the same column, distinctions between means are indicated by distinct letters at a significance level of *P*=0.05, as determined by Fisher's Least Significant Difference (LSD) test.

Table 3. presents the influence of different starter and temporal N and S applications on agronomic, recovery, and physiological use efficiency in soybean crop.

Treatment	N Agronomic efficiency kg kg ⁻¹	N		S		S recovery efficiency kg kg ⁻¹
		physiological efficiency kg kg ⁻¹	recovery efficiency %	agronomic efficiency kg kg ⁻¹	physiological efficiency kg kg ⁻¹	
N(25)	23.96	10.07	238	-	-	-
N(50)	16.12	10.36	156	-	-	-
N(25+25)	16.12	10.00	129	-	-	-
N(12.5+12.5)	8.36	9.41	89	-	-	-
S(25)	-	-	-	10.00	196.85	5.1
S(50)	-	-	-	5.62	145.60	3.9
S(12.5+12.5)	-	-	-	5.52	186.49	3.0
S(25+25)	-	-	-	7.20	185.57	3.9
N(25+25),S(12.5+12.5)	15.32	9.77	157	30.64	210.44	14.4
N(12.5+12.5),S(12.5+12.5)	9.52	9.48	101	9.52	146.91	5.0
N(12.5+12.5),S(25+25)	17.16	10.29	167	8.58	170.24	5.0
N(25+25),S(25+25)	21.86	10.73	204	21.86	246.73	8.9
N(25),S(50)	38.28	11.68	333	19.14	257.26	7.4

5. Conclusion

In conclusion, the study revealed that the combined N and S applications, particularly as 25 kg ha⁻¹ each in two splits as starter doses and temporal application at R₂ stage of soybean treatment resulted in the highest total N uptake and S uptake. The above the ground biomass N uptake did not significantly differ between the 25 kg N ha⁻¹ as starter dose and split application at R₂ stage with 12.5 kg S as starter dose and split at R₂ stage; 50 kg N ha⁻¹ alone as starter dose; and 25 kg N ha⁻¹ plus 50 kg S ha⁻¹ as starter doses. Likewise, the application of N and S, whether through starter or temporal applications, led to the highest seeds and stalks yields. Notably, the 25 kg N ha⁻¹ plus 50 kg S ha⁻¹ as starter doses application demonstrated the highest agronomic use efficiency, while the temporal N and S applications each in two split were applied as 25 N and S kg ha⁻¹ as starter doses and temporal

applications at R₂ stage treatments showed the highest S use efficiency. The apparent N recovery was notably high with the application of 25 kg N ha⁻¹ alone as starter dose and 25 kg N ha⁻¹ plus 50 kg S ha⁻¹ as starter doses, whereas for S, starter application as 25 kg N ha⁻¹ plus 50 kg S ha⁻¹ as starter doses; temporal application as 25 N and S kg ha⁻¹ each in two split were applied as starter doses and temporal applications at R₂ stage exhibited the highest recovery rates. The study identified a consistent pattern in physiological use efficiencies; these findings underscore the significance of specific nutrient management practices in optimizing crop yield and nutrient use efficiency, wherein the temporal application of N and S significantly enhanced soil Zn and Fe content during the R₂ and R₅ stages of soybean crop growth. Both starter and temporal applications contributed to increased seeds Zn and Fe content, suggesting the potential of N and S application in biofortifying soybean seeds. This agronomic biofortification approach held promise for addressing micronutrient deficiencies in soybean, contributing to improved nutritional content. The findings emphasize the efficacy of temporal N and S application, specifically each in two splits as 25 kg N and S ha⁻¹ as starter doses and temporal applications at R₂ stage treatments, in enhancing nutrient uptake, nutrient use efficiency, and overall productivity. Furthermore, the study highlighted the positive impact of this approach on enhancing the micronutrient content in soybean seeds, showcasing its potential for agronomic biofortification in soybean cultivation.

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