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Effects of Sheep Manure and Di-ammonium Phosphate on Soybean Growth, Photosynthesis, and Yield under Parwan Agro-climatic Conditions, Afghanistan

Abdul Alim Osmani[1], Mohammad Wasif Amin[1,2*] Naveedullah Sediqui[3], Ijaz Ahmad Samsor[3], Zabihullah Farid[1], Shafiqullah Aryan[4], Khalid Joya[1], Habibullah Hazim[5], Hakimullah Amini[1]

ABSTRACT
With its rising summer temperatures, colder winters, and erratic rainfall, climate change severely impacts calcareous soils in arid and semi-arid regions, escalating nitrogen and phosphorus deficiencies. Limited studies investigated the effects of sheep manure (SM) and diammonium phosphate (DAP) on soybeans under calcareous soils. In this study, the fertilizer treatments (FT) were FT1 (SM = 0%, DAP= 0%), FT2 (SM= 100%, DAP= 0%), FT3 (SM = 0%, DAP= 100%), FT4 (SM = 50%, DAP= 75%), FT5 (SM = 50%, DAP= 50%), and FT6 (SM = 50%, DAP= 0%) elucidated on attributes of soybean CV. LD 04-13265 USD under calcareous soil conditions. The FT4 and FT5 treatments, combining sheep manure (SM) and diammonium phosphate (DAP), significantly enhanced net photosynthetic rate, stomatal conductance, transpiration rate, and water use efficiency compared to the control (FT1). While all fertilization treatments resulted in lower the intercellular CO_2 concentrations than the control, the combined applications of SM and DAP (FT4 and FT5) demonstrated superior growth performance, higher photosynthetic efficiency, and increased seed yield. Although plant height and nodule number remained unaffected by fertilization, other growth parameters, including leaf number, root length, and shoot biomass, were significantly influenced. There was a positive correlation between seed yield and root length (r = 0.90), photosynthetic rate (r = 0.86), shoot biomass (r = 0.82), and water use efficiency (r = 0.76). Furthermore, the FT4 and FT5 treatments showed the highest seed yields, increasing by 81.3% and 64.3%, respectively, compared to their control. The combined application of sheep manure and DAP enhanced soybean yield under calcareous conditions, offering an eco-friendly alternative to excessive chemical fertilizer use. This approach may help reduce input costs for soybean producers by optimizing fertilizer efficiency.

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Introduction

Soil infertility significantly impacts global soybean (*Glycine max* L.) production. In arid and semi-arid regions, such as Afghanistan, extreme environmental conditions exacerbate soil infertility, including fluctuating temperatures (both hot and cold) and limited water availability. These regions are often characterized by calcareous soils with low fertility, particularly low nitrogen (N) and phosphorus (P) content. Other limiting factors include low water-holding capacity, high pH, low soil organic matter content (typically 0.1 to 3%), shallow soil depth, stoniness, and other unique challenges [1,2]. Because of limited rainfall and high temperatures in these environments, there are typically insufficient inputs of organic matter and nutrients such as N and P from external resources. Discovering a practical approach that increases soybean production in dry and calcareous soils (having minimal nitrogen and phosphorus) while *simultaneously being more affordable and less hazardous can be highly beneficial.* Soybean (Glycine

[1] Faculty of Agriculture, Parwan University, Parwan, 1102, Afghanistan
[2] Faculty of Regional Environment Science, Tokyo University of Agriculture, Tokyo 156-8502, Japan
[3] Department of International Agricultural Development, Graduate School of Agriculture, Tokyo University of Agriculture, Tokyo 156-8502, Japan
[4] Faculty of Agriculture, Nangarhar University, Nangarhar, 2601, Afghanistan
[5] Faculty of Education, Parwan University, Parwan, 1102, Afghanistan
*Corresponding author: wasifamin1991@gmail.com

max L.) is a globally vital legume crop, prized for its high protein (38–42%) and oil (18–22%) content, making it a key dietary component for addressing malnutrition and food insecurity [4,20]. As a nitrogen-fixing crop, soybean also plays a critical role in sustainable cropping systems by reducing reliance on synthetic N fertilizers. However, soil infertility severely limits its productivity, particularly in arid and semi-arid regions like Afghanistan, where calcareous soils dominate. These soils are characterized by nitrogen (N) and phosphorus (P) deficiencies, high pH, low organic matter (0.1–3%), and poor water retention [1,2]. Despite soybean's cultivation on 1,188 hectares in Afghanistan, average yields remain stagnant at 1.98 t/ha—far below the global average of 2.8 t/ha—forcing reliance on imports to meet domestic demand [3,4]. Bridging this yield gap requires sustainable strategies tailored to nutrient-poor, calcareous soils. *Despite the recognized nutritional value of soybeans, domestic production remains insufficient to address malnutrition within the country. Consequently, a significant portion of soybean consumption relies on imports from other nations [4]. A primary challenge to soil* fertility in Afghanistan is the deficiency of two crucial nutrients: N and P. Nitrogen plays a pivotal role in plant growth and development. Insufficient N availability leads to stunted growth, reduced biomass production, and hampered developmental processes such as leaf expansion and the formation of new leaves. This ultimately results in decreased plant height and limited leaf number [5,6]. Poor biomass production resulted from nitrogen deficit, which also dramatically decreased leaf photosynthetic rate, transpiration rate, and chlorophyll content. Rather than the leaf chemistry's ability to carboxylate, N deficiency-induced decreased leaf photosynthetic rate was mainly associated with lower stomatal conductance.

The maximum and minimum decreases in dry weight among the plant organs were observed in leaves and roots, respectively, under N deprivation [6]. Based on estimates, roughly one-third of the world's arable soils are deficient in P and require fertilization with this element to enhance yields. Plants are frequently sensitive to P nutritional stress in both natural and agricultural environments because of the limited availability of P in the soil and its limited transportation [7]. Reduced stomatal opening can be provoked by P insufficiency. Less stomatal opening results in less CO_2 being taken and converted to triose phosphate, dramatically reducing the recycling of ATP and NADPH and reducing plants' ability to grow and perform photosynthetically [8]. Due to the feedback inhibition triggered by decreased leaf growth under P deficit, photosynthesis tends to be reduced [9]. However, excessive fertilization with N and P-based nutrients and toxic chemical contamination through runoff and leaching into surface and groundwater are some effects that result in eutrophication and a decline in soil quality [10,11].

A later examination uncovered that lands used for farming should be treated with organic manures and inorganic fertilizers to give plant nutrients in readily available forms and maintain good soil health, resulting in optimal yields [12]. Farmyard manure not only serves as a source of nitrogen and other nutrients, according to research conducted by Zadeh [13], but it also improves the effectiveness of applied nitrogen. To improve organic C, improve nutrient uptake, and stimulate plant growth, manures from various animal origins (such as cow, goat, sheep, horse, chicken, and pig) may be employed as soil amendments [14]. Sheep manure is one of the most popular, affordable, and simple-to-use organic fertilizers for crop production. It has adequate levels of N, P, and K, as well as fiber components that help regulate soil temperature and moisture while inhibiting the growth of weeds on soil surfaces [15]. Organic matter maintains soil health, serves as a substrate for microbial decomposition, makes mineral nutrients available to the crop, enhances soil structure, boosts water retention, lowers the risk of heavy metal toxicity, and reduces soil-borne infections [10]. Long-term organic soil amendments boost soil quality, increase enzymatic activity in the soil, which regulates the biogeochemical nutrient cycle, and increase P availability to plants [16]. As a result of these amendments, P is more readily available to plants than it would be from synthetic fertilizers in terms of precipitation and sorption [17]. Applying chemical fertilizer is an efficient technique to ensure the wise use of chemical P fertilizers since P has poor mobility and concentration in the soil and requires more effective application to meet the plant's P requirements [18]. However, due to substantial losses from field to fork and improper P fertilizer use worldwide, a multi-criteria-based strategy should be employed to create a sustainable agricultural system. The soil fertility has been improved through various efforts to raise soybean productivity [19]. Adding organic fertilizers (sheep manure) and phosphorus fertilizer (DAP) may be a strategy used to boost soil fertility, promote growth, and increase photosynthetic output in soybean seeds. Despite the fact that the separate and combined impacts of organic and inorganic fertilizers containing nitrogen and phosphorus have been investigated on various crops, the response of soybean to sheep manure and DAP fertilizer and the thorough understanding of increasing crop yield of soybeans in infertile calcareous soils have not yet been researched. This approach represents a novel and sustainable farming practice that could provide important insights into improving soybean productivity and addressing environmental concerns associated with chemical fertilizers. It is anticipated that the combined application of sheep manure and phosphorus fertilizer will result in significant improvements in photosynthetic parameters, growth, and yield of soybeans compared to their individual applications. The findings of this research have the potential to contribute significantly to the discourse on sustainable agricultural practices aimed at optimizing soybean productivity while simultaneously mitigating environmental impacts. Additionally, this study enhances the understanding of the influences exerted by both organic and inorganic fertilizers on crop growth and development. Therefore, the objective of this research is to elucidate the potential advantages of integrating organic and inorganic fertilizer strategies to enhance soybean productivity and address the environmental concerns associated with the use of chemical fertilizers.

Materials and Methods

Site description

A short-term fertilizer pot experiment was initiated in 2021 on a soybean cropping system at the net house of Parwan University's experimental and research farm (PUERF) (35°.29'N ', 69°.17' E, and 2252.05 m above sea level), Charikar, Afghanistan. The locality of the experiment is illustrated in Figure 1. The area has a semi-arid, sub-tropical climate with cold winters and hot summers, receiving minimal monthly rainfall (less than 5 mm) during the cropping season. Figure 2 shows the average meteorological information for our test site concerning temperature and precipitation. The soils at the experimental site are inorganic and calcareous with a sandy clay loam texture and are classified as typic ustochrept. The soil samples were collected using a random sampling method from the upper 25-cm layer (where the central part of the field crops' root system exists and nutrients are mainly taken up) of 5 different sampling points of PUERF soil in Parwan University around the city of Charikar, Central Afghanistan. After soil samples were collected, they were carefully mixed to homogenize them. Then, the homogenized soil samples were labeled, sealed, and eventually transferred to the Laboratory of Soil Analysis, Directorate of Soil Science, Ministry of Agriculture, Irrigation and Livestock (MAIL) to thoroughly analyze the samples. The physical, chemical, and morphological characteristics of the surface (0–25 cm) soil layer are summarized in Table 1, based on results from laboratory analyses.

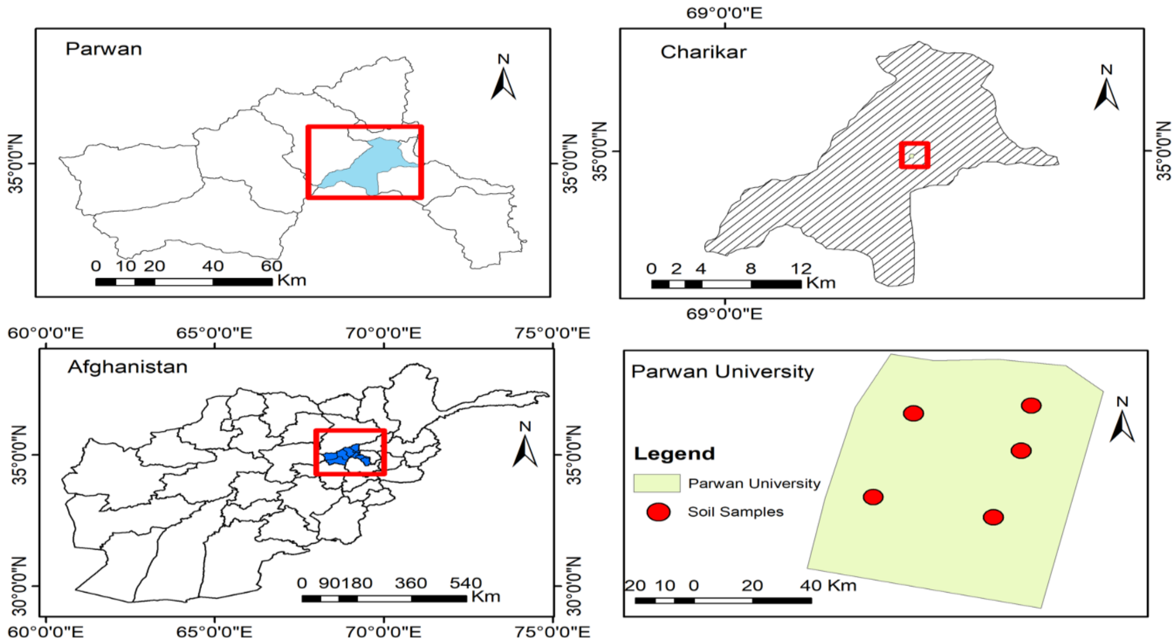

Fig 1. The map of the trial's location (Charikar City, Parwan, Afghanistan) was prepared using ArcGIS 10.8 software. The soil samples collected from Parwan University's agricultural research farm for different soil analyses are represented by the red dots.

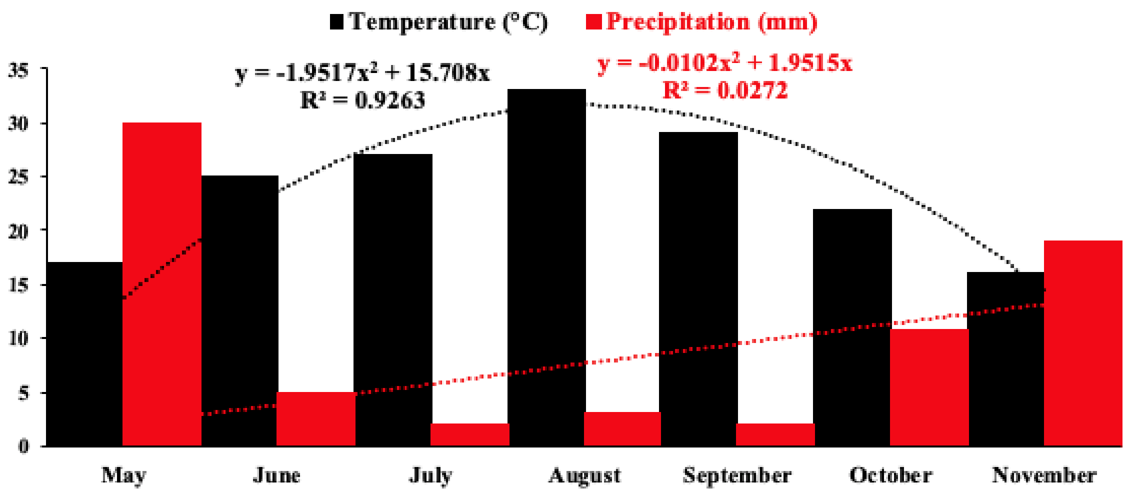

Fig 2. The experimental site's weather parameters (temperature and precipitation) during the crop-growing seasons (Parwan University, Charikar City, Afghanistan) (data with the black and grey polynomial trend lines).

Plant material and experimental design

Soybean seeds of the LD 04-13265 USD variety were obtained from the NEI gene bank. The experiment was designed with four replications in a fully randomized manner. Before planting, soil samples from PUERF were collected and sent to the soil analysis laboratory at the Directorate of Soil Science, Ministry of Agriculture, Irrigation, and Livestock (MAIL) for physical and chemical analysis. Each pot was filled with 5 kg of topsoil, with a depth ranging from 0 to 25 cm. On May 20, 2021, four healthy LD 04-13265 USD seeds were planted in black plastic containers (18" diameter x 25" height). The seeds were covered with soil and thinned to three plants per pot 10 days after sowing (DAS). After thinning, the soils in the pots were covered with green mulch to retain moisture. Sheep manure with a pH of 6.5 was procured from Parwan University's animal husbandry farm, dried in the sun to remove excess moisture, mixed by hand with the soil, and then placed into the pots. One month before planting, the pots were filled with a mixture of soil and sheep manure to enhance the decomposition process; at that time, DAP was added. Except for the absolute control treatment, both fertilizers were applied according to the doses that had been previously planned for each treatment. The combination of fertilization treatments and the nutrient contents of the sheep manure are displayed in Table 2. When sowing, urea fertilizer containing 25 kg of N was applied to all experimental pots. Plant protection measures were implemented according to the specifications, and the pots were watered with farm water cans.

Soil physical and chemical analysis

Soil samples were collected from the experimental site (0-25 cm depth) using a randomized sampling design, then air-dried, homogenized, and sieved through a 2-mm mesh prior to analysis. Particle size distribution was determined using the hydrometer method (Bouyoucos, 1962), with results classified according to the USDA textural triangle as sandy clay loam. Soil pH and electrical conductivity (EC) were measured in a 1:2.5 soil-to-water suspension using a pH meter and conductivity meter, respectively. Cation exchange capacity (CEC) was determined through ammonium acetate (NH4OAc) extraction at pH 7.0. Available phosphorus was extracted using Olsen's bicarbonate method (0.5 M NaHCO3). Organic matter content was quantified via the Walkley-Black wet oxidation method. Exchangeable potassium and sodium were measured using ammonium acetate extraction followed by flame photometry, respectively. Calcium carbonate content was determined by calcimetry and classified. Available sulfur was extracted using calcium phosphate and analyzed by turbidimetry. All soil property ratings (low, medium, high) were based on established thresholds for calcareous soils from FAO guidelines, the Afghanistan Soil Information System, and critical limits for crop production. The soil was characterized as calcareous mountain soil derived from mountainous parent material, with moderately drained conditions on highland topography.

Table 1. Physical, chemical, and morphological properties of the soil surface of experimental soil (0-25 cm).

Characteristics	**Value**	**Rating**
Sand (%)	53.76	High
Silt (%)	26.00	Medium
Clay (%)	20.24	Low
pH	8.65	Alkaline
EC (dS/m)	0.20	Low
CEC ($cmol_{(+)}$/kg)	9.00	Low
P_2O_5 (ppm)	106.00	Low
OM (%)	0.81	Low
Potassium (ppm)	45.00	Medium
Na (ppm)	47.00	High
$CaCO_3$	12.75	Medium
Sulphur (ppm)	11.05	Low
Textural class	Sandy clay loam	
Parent material	Mountainous	
General soil type	Calcareous mountain soil	
Topography	High land	
Drainage	Moderately drained	

Table 2. Combination of fertilization treatments and nutrient contents of sheep manure on a dry matter basis.

Fertilization Treatment (FT)	Sheep manure (SM)	DAP (P_2O_5)	Description
FT1	0	0	Control (without any fertilizer use)
FT2	100%	0	10 t/ha Sheep manure
FT3	0	100%	45 kg/ha DAP
FT4	50%	75%	5 t/ha Sheep manure + 33.75 kg/ha DAP
FT5	50%	50%	5 t/ha sheep manure + 22.5 kg/ha DAP
FT6	50%	0	5 t/ha sheep manure

SM nutrient contents	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)
	1.70	0.80	1.80	0.70	0.30	0.30	500.00	50.00	15.00	5.00

DAP: Diamonium phosphate; SM: Sheep Manure; ppm: parts per million. Source: Laboratory of Soil Analysis, Directorate of Soil Science, Ministry of Agriculture Irrigation and Livestock (MAIL) [21]. All nutrient concentrations in sheep manure are expressed on a dry weight basis.

Growth parameters and yield attributes

Based on plant height (PH), leaf number (NL), and root length (RL), the vegetative growth was assessed. A tape meter was used to measure the height of the plant from the bottom to the top of the plant at 60 DAS. From the soil surface to the top of the plant in each pot, NL was frequently counted at 60 DAS. One plant was chosen for each pot, and the plants' shoot biomass (SB) was collected at 60 DAS. The samples were then sun-dried, and the hot air oven was used to dry them for approximately 48 hours at 65 °C. The SB data was measured as grams per plant after drying. At 90 DAS, the selected plants were carefully uprooted from each container, and their roots were rinsed with water to remove soil. Nodules were manually excised and counted, with active (nitrogen-fixing) nodules distinguished from inactive ones based on interior color: pink/red nodules (indicating leghemoglobin presence) were classified as active, while white/gray nodules were considered inactive. Following harvest, the total number of pods per plant (PN) and the number of seeds in each pod (NSP) were counted separately. The seed yield per plant (SY) was calculated by weighing the pods per pot and expressed in grams per pot. A meter rod measured RL after the plants were finally plucked from all the trial pots [22].

Photosynthetic-related traits

To measure photosynthetic indices during the reproductive phases, specifically from the beginning of blooming (R1) to full blooming (R2), we assessed parameters such as net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs), and intercellular CO2 concentration (Ci). These measurements were taken on fully expanded leaves located at the upper, middle, and lower parts of the plant. We used a Portable Photosynthesis Measuring System LCi-SD (ADC Bioscientific, Hoddesdon, UK) for this purpose, conducting the measurements between 10:00 a.m. and 1:00 p.m.The light intensity used in the measurement process was 1500 µmol m^{-2} s^{-1} the humidity was 20%, and the leaf chamber temperature was 30°C. For each pot, three leaves per plant (upper, middle, and lower) were randomly selected and examined. The calculation of the water use efficiency percentage (WUEp) was performed using the leaf net photosynthetic rate (Pn) divided by transpiration rate (Tr) as described by Shenglan et al. [23] and Amin et al [24] in the following formula.

$$WUEp=Pn/Tr$$

Statistical analysis

The data were examined using ANOVA, correlation matrix analysis, and principal component analysis (PCA) with R 3.6.2 statistical software. Python was used to visualize the parameters examined. Tukey's test was employed to differentiate treatment means at a 5% significance level.

Results

Combined effect of sheep manure and DAP fertilizer on growth attributes

Results regarding growth parameters, including both above- and below-ground parts of the plant, are shown in Figure 3. The combined application of fertilization treatments (SM and DAP fertilizer) significantly increased NL, RL, and SB of soybean compared to the control and individual treatments ($p<0.05$). However, PH and NN did not show statistically significant differences between the fertilization treatments (Figures 1A and 1E). Correlation matrix analysis indicated that PH and RL are highly correlated ($r=0.88^*$, $p<0.05$), suggesting that the longer a plant's roots, the more water and nutrients it can access, contributing to greater plant height (Figure 6). The application of SM (FT2), DAP fertilizer (FT3), and their combination (FT4) increased NL in soybeans (Figure 3B). SM is an organic fertilizer that contains nutrients such as nitrogen, phosphorus, potassium, and micro-nutrients. When applied to the soil, it can enhance soil fertility and increase the availability of nutrients for plants. This improvement can lead to a rise in NL in soybean plants as more nutrients support the growth of additional leaves. In contrast, DAP fertilizer is a synthetic option containing high N and P levels. Its use in the soil supplies the essential nutrients for plant growth and development. Consequently, a strong positive correlation exists between NL and SB ($r=0.85^*$, $p<0.05$) as well as Tr ($r=0.83^*$, $p<0.05$) (Figure 6), indicating that plants receiving both fertilizers can enhance NL and SB, providing the necessary nutrients for growth. However, the impact on the transpiration rate may vary based on the application rate and timing of the fertilizers. If the fertilizer application rate is excessively high (FT4), it may induce water stress in plants and increase Tr (Figure 4C). Similarly, except for the control condition (FT1), root length (RL) improved significantly across all fertilization treatments. The root length of soybean showed a significant positive correlation with Pn ($r=0.85^*$, $p<0.05$) and Tr ($r=0.95^{**}$, $p<0.01$) (Figure 6), indicating that longer roots can explore a greater volume of soil, thereby absorbing more water and nutrients. This can enhance the plant's overall health and, in turn, boost its ability to carry out photosynthesis. The length of roots can indirectly influence Pn by affecting the plant's capacity to absorb water and nutrients. While plants with longer roots typically have a larger surface area for water uptake from the soil, which allows them to absorb more water and consequently transpire more. The outcome in Figure 3D indicates that with FT4 treatment, a significant amount of SB was produced, followed by FT5, FT3, and FT2. The use of SM and DAP fertilizer resulted in a significant positive correlation between SB and SY ($r=0.81^*$, $p<0.05$) (Figure 6). This is due to a balanced amount of SM and DAP meeting the crop's nutrient requirements, which can promote healthy plant shoot growth and enhance SY. The addition of SM and DAP fertilizer could influence NN in soybean plants. However, our results demonstrated that none of the treatments significantly affected NN (Figure 3E).

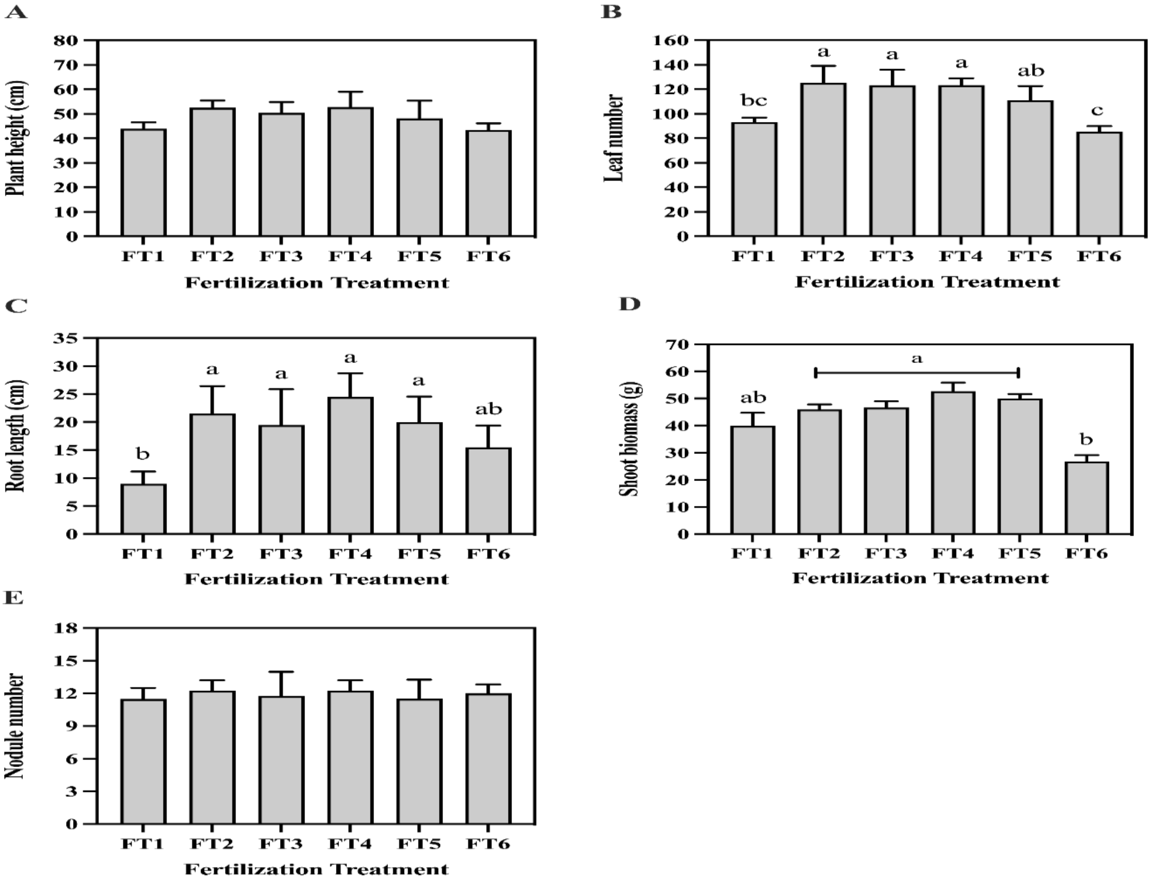

Fig 3. The combined effect of sheep manure and DAP fertilizer on growth attributes of soybean. Plant height (A), leaf number (B), root length (C), shoot biomass (D), and nodule number (E) grown in soils with different fertilization treatments, FT1 (Sheep manure= 0%, DAP= 0%), FT2 (Sheep manure= 100%, DAP= 0%), FT3 (Sheep manure= 0%, DAP= 100%), FT4 (Sheep manure= 50%, DAP= 75%), FT5 (Sheep manure= 50%, DAP= 50%), and FT6 (Sheep manure= 50%, DAP= 0%).

The combined effect of sheep manure and DAP fertilizer on photosynthesis related traits

Figure 4 illustrates that the application of combined organic and inorganic fertilizers can significantly enhance the net photosynthetic rate (Pn), stomatal conductance (Gs), and transpiration rate (Tr) of soybean. FT4 exhibited the highest Pn, Gs, and Tr. The Ci values for each fertilization were lower than those of FT1. The concentration of Ci in treatments FT2, FT3, and FT6 was the highest. The Tr for FT4 peaked, possibly linked to a greater leaf area index (LAI). Additionally, the application of SM can effectively improve soybean's instantaneous water use efficiency, with the FT5 treatment achieving the highest efficiency. The differences in fertilization treatments compared to FT1 were statistically significant ($P<0.05$). The water use efficiency potential (WUEp) of leaves with FT5, FT4, FT2, FT6, and FT3 was greater than that of FT1, showing increases of 43.70%, 30.04%, 21.20%, 19.70%, and 8.90%, respectively, compared to FT1. Overall, the combined application of SM and DAP fertilizer significantly increased the rates of photosynthesis (Pn), stomatal conductance (Gs), and transpiration (Tr) in soybean compared to the control and single treatments. Pn had a significant positive correlation with Gs ($r=0.96^{**}$, $p<0.01$) and Tr ($r=0.69^{**}$, $p<0.01$), suggesting that a higher Pn rate is associated with increased Gs and Tr (Figure 6). Additionally, Pn showed a significant positive correlation with soybean yield (SY) ($r=0.86^{**}$, $p<0.01$), indicating that a higher Pn rate correlates with greater yield. Interestingly, Ci exhibited a significant negative correlation with other photosynthesis-related traits. Consequently, even though the Ci is lower, the plant can still maintain a high Pn rate by regulating the opening and closing of its stomata. Therefore, the correlation analysis results indicate that soybean's photosynthesis-related traits were positively correlated with one another, except for Ci, and were positively associated with yield. This underscores the importance of enhancing soil fertility through the combined application of SM and DAP fertilizers to improve the photosynthesis efficiency of soybean, ultimately leading to increased yield.

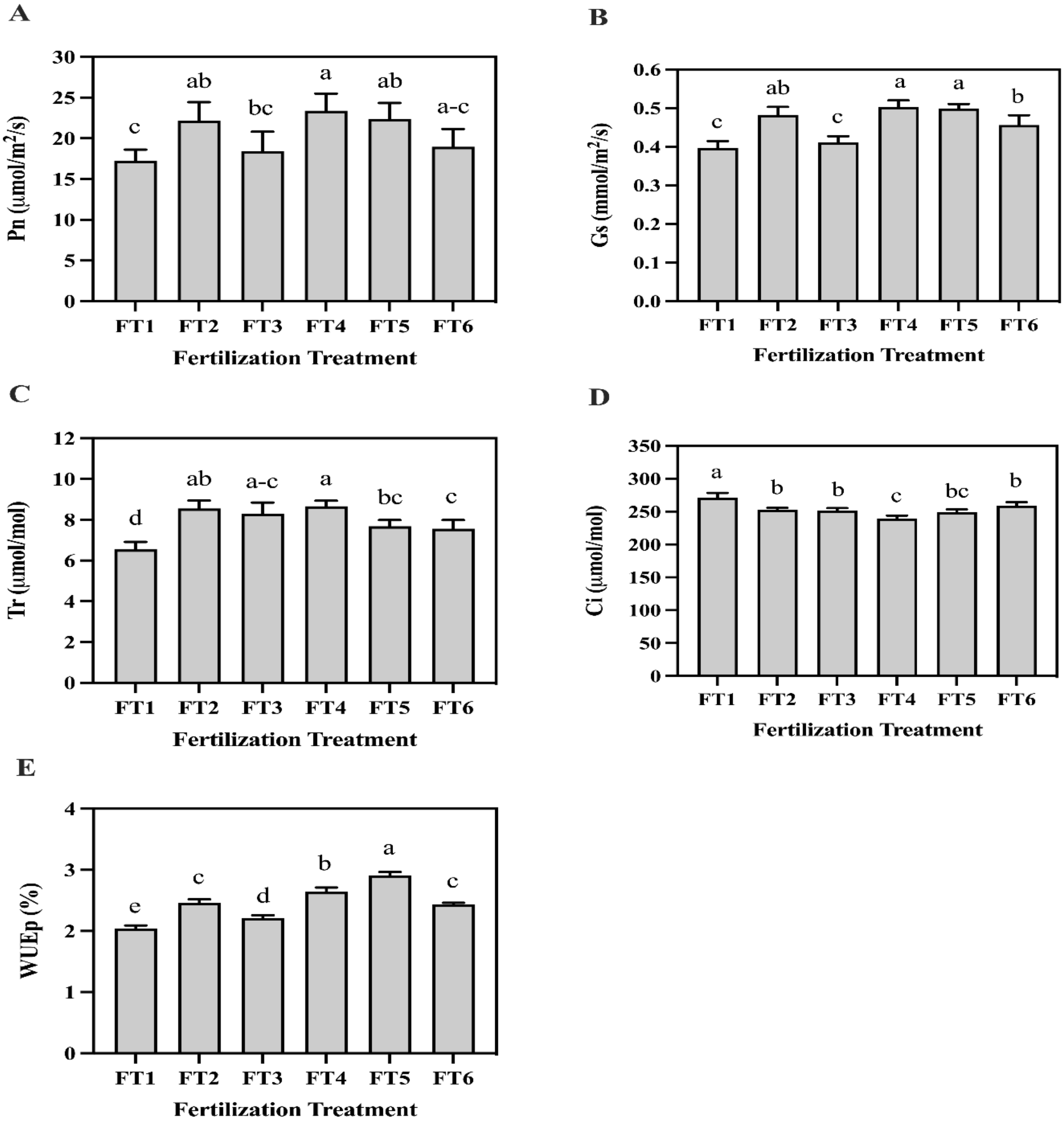

Fig 4. The combined effect of sheep manure and DAP fertilizer on photosynthetic-related traits of soybean. Photosynthetic rate (A), stomatal conductance (B), transpiration rate (C), intercellular CO_2 concentration (D), and water use efficiency percentage (E) grown in soils with different fertilization treatments, FT1 (Sheep manure= 0%, DAP= 0%), FT2 (Sheep manure= 100%, DAP= 0%), FT3 (Sheep manure= 0%, DAP= 100%), FT4 (Sheep manure= 50%, DAP= 75%), FT5 (Sheep manure= 50%, DAP= 50%), and FT6 (Sheep manure= 50%, DAP= 0%).

The combined effect of sheep manure and DAP fertilizer on yield and its components

Neither single fertilization nor combined application significantly affected PN and NSP (Figure 5A and 5B). However, there was a significant difference ($p<0.05$) between fertilization treatments regarding soybean SY. The integrated use of SM and DAP fertilizers (FT4 and FT5) considerably increased SY compared to the single treatment and FT1 (control). FT4 and FT5 treatments raised the SY by 81.30% and 64.32%, respectively, compared to FT1 (Figure 5C). The correlation analysis results suggest that soybean growth and photosynthesis-related traits were positively correlated with one another, and both were positively correlated with seed yield (SY) (Figure 6). We developed a descriptive model based on the relationship between growth, photosynthetic traits, and seed yield per plant by applying SM and DAP fertilizer, as illustrated in Figure 7. This model demonstrates that combining fertilizers increased the growth rate and photosynthetic activity, resulting in a higher seed yield.

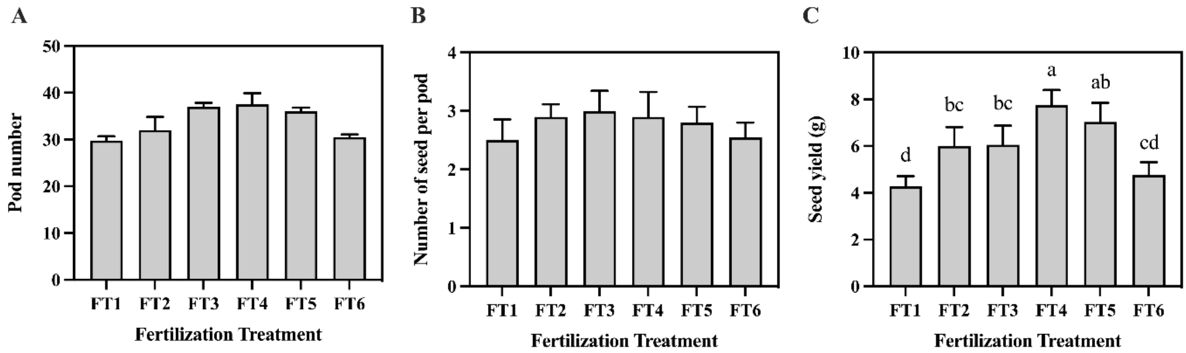

Fig 5. The combined effect of sheep manure and DAP fertilizer on yield and the components of soybean. Pod number (A), number of seed per pod (B), and seed yield (C) grown in soils with different fertilization treatments, FT1 (Sheep manure= 0%, DAP= 0%), FT2 (Sheep manure= 100%, DAP= 0%), FT3 (Sheep manure= 0%, DAP= 100%), FT4 (Sheep manure= 50%, DAP= 75%), FT5 (Sheep manure= 50%, DAP= 50%), and FT6 (Sheep manure= 50%, DAP= 0%).

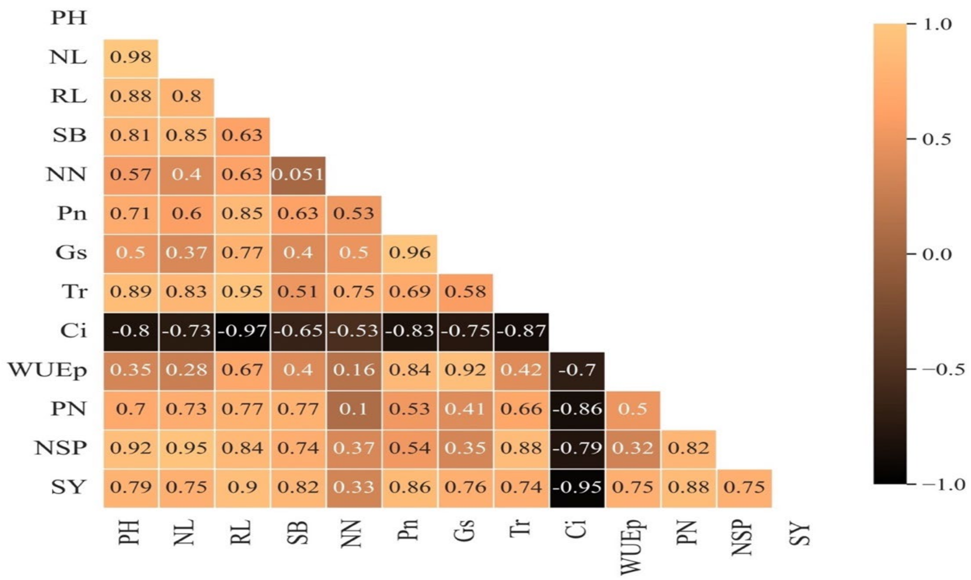

Fig 6. Correlation coefficients (matrix) between different parameters of LD 04-13265 USD variety as affected by sheep manure and DAP fertilizer. ns: not significant; ** $P < 0.01$, * $P < 0.05$. PH: Plant height, NL: Number of leaves, RL: Root length, SB: Shoot biomass, NN: Number of nodules, Pn: Photosynthetic rate, Gs: Stomatal conductance, Tr: Transpiration rate, Ci: Intercellular CO_2 concentration, WUEp: Water use efficiency percentage, PN: Number of Pods, NSP: Number of seeds per pod, SY: Seed yield.

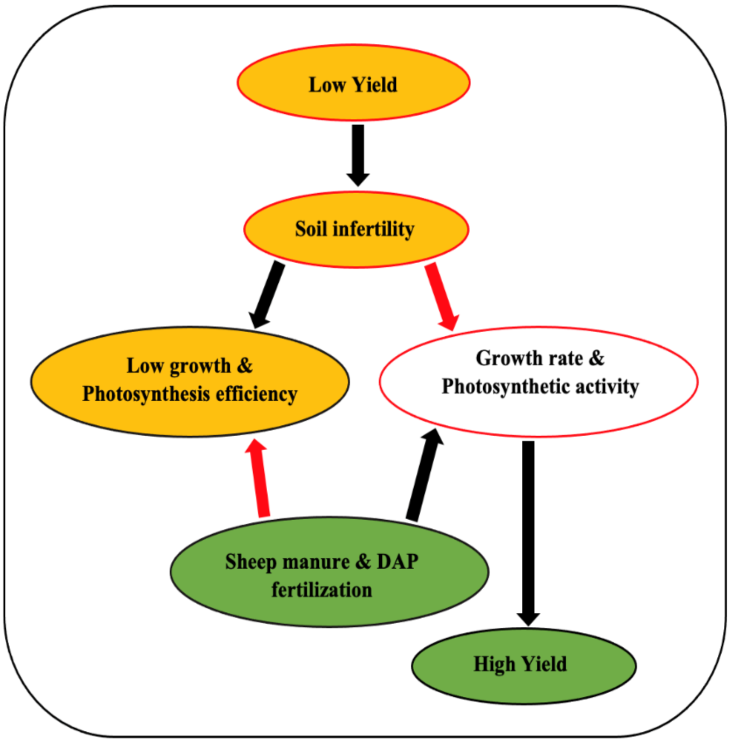

Fig. A conceptual model illustrating the combined effects of sheep manure and DAP fertilizer on soybean growth and photosynthetic function. The application of both fertilizers improves soil fertility, which subsequently enhances plant growth and photosynthetic activity, ultimately contributing to higher yield. Black arrows indicate positive effects (enhancement), while red arrows indicate negative effects (reduction)

Principal Component Analysis

A principal component analysis (PCA) was performed using the study's data to examine further the relationships between soybean growth, photosynthesis-related traits, and yield. According to the PCA, the first principal component (PC1) and the second principal component (PC2) accounted for 71.1% and 13.5% of the variance in the data, respectively. Therefore, these two components are sufficient to explain the variance. Gs, WUEp, and Pn exhibit the most significant loadings on PC1. This suggests that PC1 encompasses all features related to photosynthetic activity. NSP, NL, and SB exhibited the highest loadings on PC2, indicating that PC2 reflects soybean growth and yield traits. RL, SY, and Ci show a strong correlation with PC1 in the loadings plot (values close to 1 or -1) but a slight correlation with PC2, as illustrated by the loadings plot. Overall, the PCA analysis indicates that the combined application of SM and DAP fertilizer positively affected soybean crop yield and growth, and the characteristics associated with photosynthesis processes. The high Gs, WUEp, and Pn loadings on PC1 and the high NSP, NL, and SB loadings on PC2 further support this. According to the findings, applying DAP fertilizer in combination with SM may enhance the growth and characteristics related to photosynthetic attributes in soybeans, ultimately contributing to a higher yield. Additionally, a strong positive correlation among WUEp, Gs, Pn, NN, SY, and RL indicates that photosynthesis-related parameters promote plant growth. However, Ci negatively correlates with photosynthesis and growth attributes (Figure 8).

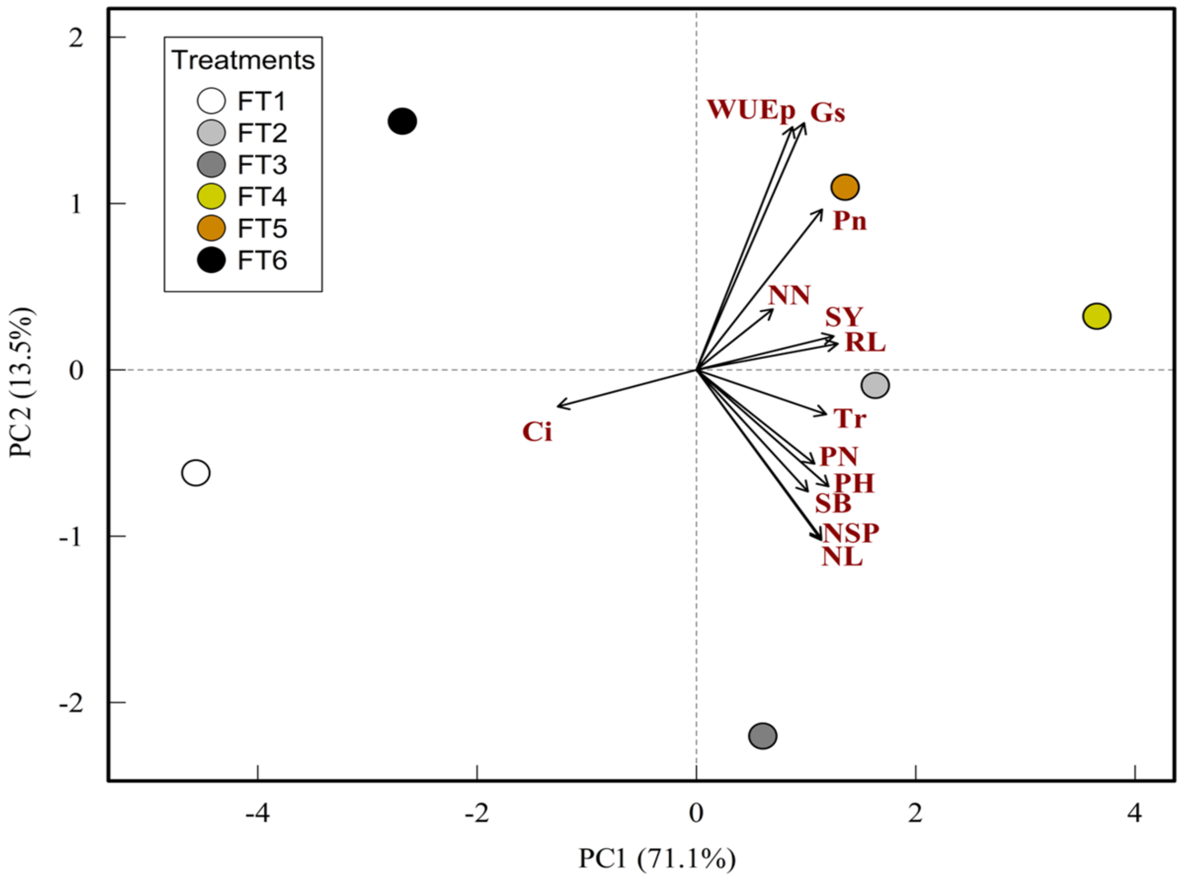

Fig 8. Principal component analysis of growth parameters, photosynthesis-related traits, and yield attributes of LD 04-13265 USD variety as affected by sheep manure and DAP fertilizer. FT1 (Sheep manure= 0%, DAP= 0%), FT2 (Sheep manure= 100%, DAP= 0%), FT3 (Sheep manure= 0%, DAP= 100%), FT4 (Sheep manure= 50%, DAP= 75%), FT5 (Sheep manure= 50%, DAP= 50%), and FT6 (Sheep manure= 50%, DAP= 0%). PH: Plant height, NL: Number of leaves, RL: Root length, SB: Shoot biomass, NN: Number of nodules, Pn: Photosynthetic rate, Gs: Stomatal conductance, Tr: Transpiration rate, Ci: Intercellular CO_2 concentration, WUEp: Water use efficiency percentage, PN: Number of Pods, NSP: Number of seeds per pod, SY: Seed yield.

Discussion

Interpretation of growth attributes trend

The experiment produced several results related to the fertilization of organic and inorganic resources. Despite the study's limitations, our findings suggest that combined interventions of chemical and non-chemical fertilizers can significantly contribute to managing and enhancing low yields. Utilizing organic additions that enhance the effectiveness of manufactured phosphorus fertilizers presents one potential solution. In this study, the combination of solid manure and diammonium phosphate improved the growth parameters of the soybean plant (Figure 3), indicating that the combined use of organic and inorganic sources provides more adequate nutrients to the soybean plant than any single source. The enhanced growth may be linked to improved physical and chemical properties, such as increased soil organic matter and available nutrients promoted by SM and DAP fertilizer, which supported root development and nutrient uptake. An increase in plant growth and physiological attributes was observed, likely associated with improved soil characteristics and nutrient availability from organic and chemical amendments [25]. Enhanced soil characteristics and efficient nutrient uptake have boosted the rates of cell division, expansion, and elongation [26,27]. Rhizobium and phosphate-solubilizing bacteria (PSB) found in organic manures, particularly SM, enhance plant growth by increasing the availability of soluble phosphate [28,29,30]. According to the study's findings, both DAP and SM, whether used alone or together, were insufficient to promote taller plant growth. However, it is important to note that results reported by Khan et al. [22] and their team indicated that combining organic manure with DAP fertilizer improved plant height. As shown in Figure 3B, applying various amounts of DAP fertilizer and SM, whether together or separately, could positively affect NL, which may ultimately increase SB production. Generally, a plant with more leaves tends to have a greater SB and an increased Tr. Each leaf features tiny openings called stomata, allowing gas exchange and water vapor to escape. Thus, the larger the surface area of the leaves, the more stomata a plant possesses, resulting in a higher transpiration rate. Additional research by Usmani et al. [31] and their group also demonstrated that applying SM alongside DAP fertilizer significantly impacted NL.The positive correlation between SB and PH ($r=0.81^*$, $p<0.05$) and NL ($r=0.85^*$, $p<0.05$) (Figure 6) also supports the idea that a balanced application of SM and DAP fertilizer can promote healthy plant shoot growth. Plants fertilized with both resources (FT4) showed a greater increase in RL (Figure 3C). The increase in RL resulted from using organic manure combined with chemical fertilizers, which provided better aeration and lower surface soil bulk density, thereby facilitating improved root proliferation [32,33]. The diminished growth response in treatments involving only organic and inorganic fertilizers indicated that single sources were insufficient to provide adequate phosphorus. Additionally, the organic amendment released nutrients slowly, which was not enough to fulfill the crop's requirements, resulting in reduced growth [34]. Although applying SM and DAP fertilizers could influence NN in soybean plants, our results indicated that none of the treatments significantly affected NN (Figure 3E). This may be because nodulation in soybeans mainly depends on environmental factors such as temperature, moisture, and soil pH, and might not be solely affected by fertilizer application.

Interpretation of variation in photosynthesis related traits

This research confirms that, compared to the control and single treatments, applying SM and DAP fertilizers significantly increased soybeans' Pn rate, Gs, and Tr (Figure 4). This enhanced photosynthetic capacity may be attributed to soybean leaves' increased chlorophyll content and chlorophyll fluorescence parameters, indicating higher photosynthetic efficiency.The enhanced chlorophyll content and fluorescence characteristics of soybean leaves suggest that improved photosynthetic capacity indicates greater photosynthetic efficiency. According to research by Li et al. [35] and colleagues, organic fertilizers can potentially influence leaf transpiration rates. They can also vary the photosynthetic rate (Pn) of jujube leaves. This aligns with the findings of this study. The Pn values in the leaves were ranked as FT4, FT5, FT2, FT3, FT6, and then FT1, sequentially. Pn represents the accumulation of photosynthetic products in plants, while increasing photosynthetic efficiency can produce more organic matter, which benefits plant growth and development [23]. Additionally, a sufficient phosphorus supply may promotes the photosynthetic phosphorylation process in plants, providing energy, increasing CO_2 consumption in the leaf tissue, and lowering the photosynthetic CO_2 compensation point. This increases photosynthetic activity and stomatal conductance in the leaf tissue [36,37], significantly improving photosynthesis. Our research contributes to the growing literature on how DAP fertilizer and SM influence growth and photosynthetic attributes. The changes in leaf chlorophyll concentration corresponded with the fluctuating patterns of photosynthetic rate (Pn) and stomatal conductance (Gs). This could be because various organic and inorganic fertilizers improve soil fertility [23], promoting plant photosynthesis and enabling plants to accumulate more photosynthetic products. The inter-cellular CO_2 concentration (Ci) did not change in sync with the Gs and Pn of the leaf. It is likely that non-stomatal factors limit CO_2 uptake and contribute to CO_2 buildup [38,39]. Ci significantly correlated negatively with other photosynthetic features, including Pn, Gs, and Tr. This may occur because plants can control the stomata's partial or total closure to reduce Ci, which limits the amount of CO_2 entering the plant while simultaneously decreasing water loss. The regulation of stomatal closure and opening enables the plant to maintain a high Pn rate even when Ci is reduced. For instance, the reason for a higher Pn rate despite lower Ci in plants lies in the process of stomatal regulation. Stomata are tiny pores on the surface of leaves that facilitate gas exchange between the plant and the environment, including the uptake of CO_2 for photosynthesis and the release of O_2. Plants control the opening and closing of stomata to optimize the balance between CO_2 uptake and water loss through transpiration. More CO_2 can enter the plant when the stomata are open, facilitating a higher photosynthesis rate. However, this also increases the amount of water lost through transpiration. To maintain the balance between CO_2 uptake and water loss, plants can decrease the Ci by partially or fully closing the stomata, limiting the amount of CO_2 entering the plant and reducing water loss. This strategy is especially crucial for plants in arid or drought-prone environments where water is a limiting factor. Since WUEp is calculated as the difference between the photosynthetic rate and stomatal conductance, it indicates how effectively plants utilize water (high internal utilization in CO_2 assimilation and low losses due to transpiration). The water use efficiency of soybeans significantly improved with the combined application of SM and DAP fertilizer. This is likely because organic fertilizers can enhance the soil's overall porosity and nutritional status, which benefits plant growth and water use. Additionally, increasing P availability positively affects crop yield and WUEp [40]. Applying SM to soybeans enhanced

WUEp, confirming that this method can effectively increase water use efficiency. FT5 exhibited the highest WUEp, and the fertilization treatment showed significant differences from FT1 ($P < 0.05$).

Inference on yield and its components

This study revealed that both manuring and the application of DAP had a significant ($P < 0.05$) effect on NSP and the growth of pods per plant. There was also a notable difference in soybean SY among the fertilization treatments ($p < 0.05$). This underscores the significance of enhancing soil fertility through the combined application of sheep manure (SM) and diammonium phosphate (DAP) fertilizer to improve soybean growth and photosynthesis-related traits, ultimately leading to increased yield. Our findings are corroborated by Iqbal et al. [41] and colleagues, who stated that combining organic and inorganic fertilizers can lead to substantial yield increases in soybeans.

Numerous authors have reported positive effects from integrating DAP with SM and sole phosphorus. Arjumand Banu et al. [42] and her colleagues found that fertilizing snap beans with phosphorus enhanced phosphorus mobilization and increased photosynthetic activity, which subsequently improved PH, the number of branches (NB), NL, the number of pods per plant (NPP), and SY. DAP application stimulates phosphorus mobilization and boosts the accumulation of photosynthates in the plant's economic part [43], likely contributing to the enhanced growth and SY of soybeans in treatments FT4 (sheep manure=50%, DAP=50%) and FT5 (sheep manure=50%, DAP=75%) (Figure 5C). The low growth and yield of soybeans in the control treatment indicate a recognition of nutrient deficiency. Increased soybean production may also result from the biological nitrogen fixation process inherent in most legume species, which supplies nitrogen to the crop. Higher yields stem from improved photosynthetic and metabolite resources.

Furthermore, enhanced yield characteristics contributed to increased soybean production, complementing the balanced application of organic manures and inorganic fertilizers. These findings correspond with the results observed by [28,44,45]. Our research has significant implications for agricultural land conservation and crop producers, underscoring the urgent need for sustainable and chemically-based fertilizer strategies to maximize crop output for the growing global population.

Conclusions

Our study demonstrates that the combination of sheep manure (SM) and diammonium phosphate (DAP) (FT4 and FT5) was the most effective fertilization strategy for soybeans. This combination significantly enhanced growth, photosynthesis, and yield compared to sole applications. Given the rising costs of chemical fertilizers and the environmental benefits of organic inputs, the integrated use of SM and DAP offers a promising approach to improve soybean productivity while maintaining soil health. Further research is necessary to fully understand the underlying mechanisms and optimize this integrated fertilization strategy.

Authors' contribution

Conceptualization, A.A.O, M.W.A and S.A.; methodology, A.A.O, M.W.A, Z.F and H.A.; software, M.W.A, K.J, S.A.; validation, H.H, N.S and MWA.; formal analysis, A.A.O, M.W.A, I.A.S, S.A.; investigation, A.A.O and M.W.A.; resources, A.A.O and M.W.A.; data curation, M.W.A, S.A, NS and KJ.; writing-reviews and editing, M.W.A, Z.F, S.A, H.A and H.H.; visualization, M.W.A, I.A.S and S.A.; project administration, A.A.O and M.W.A. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare that they have no conflicts of interest related to this research study.

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