Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Biological strategies to mitigate the effect of water deficit on morphophysiology of soybean

Anderson dos Santos Dias

andersondias.agro@outlook.com

Universidade Federal da Grande Dourados

Luiz Otávio Montera Martins

Universidade Federal da Grande Dourados

Davi Lucas Bertolino de Alencar

Universidade Federal da Grande Dourados

Victor Garcia Holsbaque

Universidade Federal da Grande Dourados

Obadias Alves da Silva

Universidade Federal da Grande Dourados

João Pedro Assis Ozório

Universidade Federal da Grande Dourados

Cleberton Correia Santos

Universidade Federal da Grande Dourados

Dágon Manoel Ribeiro

Silvana de Paula Quintão Scalon

Universidade Federal da Grande Dourados

Research Article

Keywords: Biostimulants. Microalgae. Plant–microbe interaction, Photosynthesis, Stress tolerance

Posted Date: June 26th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9998240/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Water stress is a major constraint to soybean productivity in tropical environments, impairing physiological processes and plant growth. Growth-promoting microorganisms have been proposed as a sustainable strategy to enhance plant tolerance to drought. This study evaluated the effects of inoculation with *Bacillus subtilis* and *Trichoderma harzianum*, alone or combined with the microalga *Parachlorella* sp., on soybean responses to water deficit. The experiment was conducted in a randomized block design with a 2 × 4 factorial arrangement, considering the presence or absence of the microalga and four cultivation conditions: irrigated control, water deficit, water deficit + *B. subtilis*, and water deficit + *T. harzianum*. Gas exchange, chlorophyll fluorescence, and growth parameters were assessed 15 and 30 days after application. Water deficit significantly reduced photosynthetic rate, stomatal conductance, chlorophyll index, maximum quantum efficiency of photosystem II, and electron transport rate, while increasing the F_0/Fm ratio, indicating damage to the photosynthetic apparatus. Microbial inoculation partially alleviated these effects. The combination of *B. subtilis* and *Parachlorella* sp. enhanced photosynthetic performance and Rubisco carboxylation under stress. The microalga also increased chlorophyll content, contributed to photosystem II stability, and promoted biomass accumulation, particularly in roots. These results indicate that B. subtilis and *Parachlorella* sp. can improve soybean tolerance to water deficit by maintaining photosynthetic efficiency and supporting adaptive physiological responses.

INTRODUCTION

In tropical agriculture, irregular rainfall—which leads to water stress—is a recurring factor that significantly influences crop performance. In the agricultural context, soybean (*Glycine max* L.) stands out for its high economic importance, but it is known to be sensitive to water stress (Kodadinne et al., 2024; Ferreira et al., 2024), especially during the final stages of vegetative development and the onset of the reproductive period (Cui et al., 2019; Nguyen et al., 2024; Moseley et al., 2025).

Reduced water availability triggers a series of responses that affect photosynthetic metabolism and plant growth. At the leaf level, it promotes stomatal closure, limiting CO_2 diffusion into the mesophyll and reducing the rate of carbon assimilation (Bawa et al., 2023; Gautam et al., 2026; Wang et al., 2026). Prolonged stress can also induce constraints related to Rubisco activity, ribulose-1,5-bisphosphate regeneration, and electron transport in the photosynthetic chain (Amaral et al., 2024; Shahhoseini et al., 2025). These changes result in the degradation of photosynthetic pigments, energy imbalance, increased non-photochemical dissipation, and potential damage to photosystem II, leading to reductions in the plant's photochemical efficiency (Elsalhy et al., 2022; Basal et al., 2024).

Given this scenario, the search for strategies that promote physiological resilience in plants has received increasing attention. Among these, the use of bio-inputs based on microorganisms capable of modulating plant physiology in response to abiotic stress stands out (da Silva et al., 2026). Microorganisms such as symbiotic bacteria and fungi are capable of stimulating root growth, producing

phytohormones, modulating hormonal balance, increasing the activity of antioxidant enzymes, and improving nutrient use efficiency under stress conditions (Ren et al., 2025; Ren et al., 2026).

These microorganisms do not replace water requirements; however, evidence indicates that their use can mitigate the physiological impacts of water deficit, preserving the functional structure of the photosynthetic apparatus and enabling plant growth (Radzikowska-Kujawska et al., 2025).

Among microorganisms with biostimulant potential, *Bacillus subtilis* has been extensively studied for its ability to produce growth-regulating substances, volatile compounds, and metabolites associated with the induction of stress tolerance (Rigobelo et al., 2024a). On the other hand, the fungus *Trichoderma harzianum* is recognized not only for its role in the biocontrol of pathogens but also for promoting physiological changes, including enhanced root development and the activation of defense mechanisms in the plant. However, the magnitude and consistency of these responses may vary depending on the environmental context and the microbial interactions established in the rhizosphere (Rigobelo et al., 2024b).

More recently, microalgae have emerged as a new class of agricultural biostimulants. Species such as *Parachlorella* sp. are sources of bioactive compounds, including phytohormones, amino acids, vitamins, antioxidants, and exopolysaccharides, which can stimulate plant metabolism and increase tolerance to abiotic stresses (da Silva et al., 2024). Previous studies have demonstrated that microalgal extracts or direct applications can promote chlorophyll retention, improve photosynthetic efficiency, and stimulate plant root growth (Shi et al., 2026; Hajnal-Jafari et al., 2020). However, information regarding the use of microalgae in combination with growth-promoting bacteria and fungi remains scarce, specially under water-deficient conditions.

The interaction between different microorganisms can result in synergistic, additive, or even antagonistic effects, depending on their functional compatibility. Therefore, understanding how microbial consortia influence soybean physiology under water stress represents a significant research gap. The central hypothesis of this study posits that (i) although soybean crops are naturally sensitive to water stress, inoculation with microorganisms can mitigate the effects of this stress; however, (ii) the extent of this mitigation depends on the compatibility among the microorganisms comprising the consortium. In light of this, the present study aimed to evaluate the effect of inoculation with *Bacillus subtilis* and *Trichoderma harzianum*, either alone or in combination with the microalga *Parachlorella* sp., on the mitigation of water stress in soybeans.

MATERIALS AND METHODS

General conditions

The experiment was conducted in 4-kg plastic pots covered with 150-micron plastic sheeting to protect against rainfall at the Faculty of Agricultural Sciences, Federal University of Grande Dourados (UFGD), Dourados – MS, Brazil. The pots were previously filled with Oxisol (USDA classification), whose chemical

properties were: pH H_2O: 5.7, P: 5.82 mg dm^{-3}, K: 21.6 mmol dm^{-3}, Ca: 64.55 mmol dm^{-3}, Mg: 20.73 mmol dm^{-3}, Al: 0 mmol dm^{-3}, H + Al: 70.79 mmol dm^{-3}, sum of bases (SB) and cation exchange capacity (CEC): 106.88 mmol dm^{-3} 177.7 cmolc dm^{-3}, respectively, and base saturation (V%): 60.15.

Microalgae inoculation, water management, and application of microorganisms

The experimental design used was a randomized block design, in which treatments were arranged in a 2-factor (with or without microalgae inoculation) × 4 (cultivation conditions) factorial layout, with four replicates. Thus, each experimental unit consisted of a pot containing two soybean plants.

Initially, seeds of the Neo 610iPro cultivar were inoculated with *Bradyrhizobium elkani* and B. japonicum at 1 × 10^8 CFU mL^{-1} via seed treatment (150 mL per 50 kg of seed). Subsequently, the seeds were divided into two groups: i) without co-inoculation and ii) with co-inoculation with the microalgae culture *Parachlorella* sp. via seed at a dose of 10 mL kg seed–1 based on the work of Silva et al. (2024).

The cultivation conditions established from sowing were as follows: i) control – CK, maintaining 70–80% of the soil water-holding capacity (SWHC) according to the methodology of Souza et al. (2002), ii) water deficit – WD, characterized by maintaining 40–50% of the SWHC, iii) WDBS: water deficit + *Bacillus subtilis*, and iv) WDTH: water deficit + *Trichoderma harzianum*.

Inoculation with *B. subtilis* and *T. harzianum* was performed via foliar application at the V3 stage in the morning, using a CO_2-pressurized backpack sprayer mounted on a boom with four fan-type spray nozzles spaced 0.5 m apart, with the pressure set to 41 PSI, at rates of 45 L ha^{-1} for B. subtilis and 5.0 g L^{-1} for *T. harzianum*, in accordance with the manufacturers' recommendations, both with a spray volume adjusted to 200 L ha–1.

The microalga *Parachlorella* sp. has 5 × 10^6 cells mL^{-1} and a pH of 7.5, and contains 0.62% N and 2.02% organic carbon in the solution, in addition to amino acids and phytohormones in its molecular composition (SANTOS et al., 2024). The *B. subtilis* and *T. harzianum* inocula used were strain UVF S1 (1 × 10^8 CFU mL^{-1}) and isolate IB19/17 (2 × 10^9 viable conidia g^{-1}), respectively.

Assessments

Non-destructive assessments (visual appearance, chlorophyll index, photochemical parameters, gas exchange, stem height, and stem diameter) were conducted 15 and 30 days after application (DAA) of *B. subtilis* and *T. harzianum* via foliar spray, while destructive assessments (leaf water content, leaf area, and leaf biomass) were conducted only at 30 DAA.

Phenotypic responses

were assessed through photographic records and descriptions of the visual aspects of the plants' aboveground parts at 15 and 30 DAA.

Chlorophyll index

was measured using the Konica Minolta SPAD-502 portable chlorophyll meter.

Chlorophyll-a fluorescence and photochemical processes

The central leaflet of the last fully expanded trifoliate leaf was subjected to dark conditions, wrapped in aluminum foil for 30 minutes. After this period, the initial (F0), variable (Fv), and maximum (Fm) chlorophyll-a fluorescence emissions, and the potential photochemical quantum efficiency in photosystem II (Fv/Fm) were determined under a flash of 1,500 μmol m^{-2} s^{-1} using a portable OS30p^{+} fluorometer (OPTI-SCIENCES Chlorophyll Fluorometer, Hudson, USA). Based on these data, the maximum non-photosynthetic efficiency (F0/Fm) was calculated.

Gas exchange

Using an infrared gas analyzer (IRGA, LCIPro - SD ADC), the photosynthetic rate (*A* – μmol CO_2 m^{-2} s^{-1}), stomatal conductance (*gs* – mol H_2O m^{-2} s^{-1}), and intercellular CO_2 concentration (*Ci* - μmol CO2 mol-1), and the intrinsic carboxylation efficiency of Rubisco (*A/Ci*) and water use efficiency (*A/E* = *WUE*) were calculated. The measurements were performed according to the same criteria as for chlorophyll a fluorescence.

Leaf water content

determined according to Turner's (1981) methodology.

Growth

Plants were harvested and separated into aboveground and root parts. Thus, leaf area (LA) was determined using an area integrator (LI-COR, 3100C – Area Meter – Nebraska). The fresh mass of the aerial parts and roots was weighed immediately after plant collection. To obtain dry biomass, the plant material was placed in a forced-air oven at 60 ± 5°C for 72 hours.

Data Analysis

The data collected 15 and 30 days after the foliar application of microorganisms were analyzed individually, that is, without statistically comparing the evaluation periods. All data were subjected to analysis of variance (ANOVA), and when significant by the F-test ($p \leq 0.05$), the means based on the main effects and interactions were compared using Bonferroni's t-test ($p \leq 0.05$) for priming with microalgal culture and Tukey's test ($p \leq 0.05$) for the cultivation conditions, using SISVAR software. Information on the plants' phenotypic responses was presented descriptively.

Based on the data for the traits evaluated at 30 DAP, the similarity index between treatments was calculated using Euclidean distance, employing the Classical method with UPGMA clustering, using PAST software.

RESULTS

Gas exchange

In general, at 15 DAA, the photosynthetic rate (*A*) remained stable across treatments in the absence of *Parachlorella* sp. With microalgae present, an increase was observed in plants treated with *B. subtilis* (WDBS) (12.41 µmol CO_2 m^{-2} s^{-1}). The CK + M plants showed a decrease in the photosynthetic rate (Fig. 2-A), while at 30 DAA, without microalgae, no differences were observed among the treatments. However, upon co-inoculation with microalgae, the plants in the WDBS treatment exhibited higher A values (12.61 µmol CO_2 m^{-2} s^{-1}), which were significantly higher than the others, followed by the control (CK, 10.53 µmol CO_2 m^{-2} s^{-1}), while the lowest values were recorded in WD and WDTH (8.56 and 8.60 µmol CO_2 m^{-2} s^{-1}, respectively). Comparatively, the presence of microalgae promoted an increase in A in CK and WDBS, reduced it in WDTH, and did not affect WD (Fig. 2-B).

Overall, at 15 DAA, the application of *B. subtilis* contributed to higher stomatal conductance (0.19 mol H_2O m^{-2} s^{-1}), but did not differ from CK and WD (Fig. 2-C). At 30 DAA, however, gs was reduced by water deficit in the untreated control (WD, 0.186 mol H_2O m^{-2} s^{-1}) and WDTH (0.196 mol H_2O m^{-2} s^{-1}), while the presence of *Parachlorella* sp. resulted in higher gs (0.225 mol H_2O m^{-2} s^{-1}) than its absence (0.187) (Fig. 2-D).

Regarding intrinsic carboxylation efficiency (*A/Ci*) at 15 DAP, in + M, an increase was observed in association with *B. subtilis* (0.042 µmol mol-1), differing significantly from WD and WDTH. The CK plants presented the lowest value, differing from all others. In the absence of the microalga (–M), the treatments did not differ significantly from one another. Comparing the presence and absence of *Parachlorella* sp., there was an increase in *A/Ci* in WDBS, while for WDTH the value remained statistically similar between the conditions with and without the microalga (Fig. 2-E). At 30 DAA, the *A/Ci* value remained high (0.041 µmol mol-1) in WDBS + M, being similar to the irrigated control (CK + M, 0.032 µmol mol-1) and differing significantly from the other treatments; in the absence of microalgae, the values were lower and there were no significant differences (Fig. 1-F).

Water use efficiency (WUE) was significant only at 30 DAP; without microalgae (-M), the CK plants had a higher value (2.89 µmol CO_2/mmol H_2O/m^2), which was higher than that of the WD (1.96 units), which had a lower value (Fig. 2-G). With microalgae, no significant differences were observed among the treatments.

Chlorophyll index and photochemical processes

The chlorophyll index was influenced by the treatments and the presence of *Parachlorella* sp. at 15 DAA (Fig. 3-A), with lower values in plants in the WD treatment, differing from those in the WDBS treatment (36.08) and in plants without microalgae. At 30 DAA, the lowest chlorophyll index was observed in WD, differing from the other treatments, especially WDBS. During this period, plants with *Parachlorella* sp. maintained the highest value (Fig. 3-B).

For the F0/Fm ratio, at 15 DAA, plants in the WD treatment had the highest value (0.45), differing from the other treatments; similarly, analysis of the effect of the microalga *Parachlorella* sp. showed that its absence resulted in a higher F0/Fm ratio (0.45) compared to its presence (0.34) (Fig. 3-C). At 30 DAA, the F0/Fm ratio was also higher in WD (-M: 0.35), being higher than CK and the inoculated treatments, which presented similar values (Fig. 3-D).

The maximum quantum efficiency of photosystem II (Fv/Fm) was significantly affected by water deficit and modulated by microbial inoculation at 15 DAA; the highest Fv/Fm values were observed in the CK, WDBS, and WDTH plants, differing from those in the WD treatment, which exhibited a lower value (< 0.600) (Fig. 3-E); at 30 DAA, the WD treatment under the + M condition exhibited the lowest values (0.681), whereas *B. subtilis* and *T. harzianum* maintained high quantum efficiency (0.723 and 0.686, respectively) under the same condition. In the absence of microalgae (-M), no significant differences were observed among the treatments (Fig. 3-F).

The Electron Transport Rate (ETR) was only significant at 30 DAA, with the lowest value in WD without microalgae (207.38 µmol elétrons m^{-2} s^{-1}), differing from the other treatments under this condition, including those with microalgae in the same treatment. With the microalgae, no differences were observed between the treatments ($p > 0.05$) (Fig. 3-G).

Growth

The dry mass of the aerial parts was drastically affected by water stress, with CK being the only treatment to exhibit the highest dry mass (0.976 g plant^{-1}), which was statistically significantly higher than that of the WDBS and WDTH inoculated plants (0.65 and 0.59 g plant^{-1}, respectively) (Fig. 4-A). Furthermore, the presence of *Parachlorella* sp. significantly increased the dry mass of the aerial part (0.72 g plant^{-1}) compared to its absence (0.55 g plant^{-1}). Root dry mass was influenced by the microalgae effect; CK + M recorded the highest mass (1.52 g plant^{-1}), exceeding all others. Under the -M condition, the same was observed, with a similar effect of WDBS (0.67 g plant^{-1}) in inducing greater root growth compared to CK -M (Fig. 4-B).

Figure 4. Leaf dry weight (A), root dry weight (B), leaf area (C), and specific leaf area (D) in soybean plants grown under different water regimes, depending on inoculation with *Trichoderma harzianum* or *Bacillus subtilis*, with or without application of *Parachlorella* sp. Uppercase letters compare the effects of co-inoculation methods using Tukey's test ($p \leq 0.05$), and lowercase letters compare the effect of Parachlorella sp. foliar application using Bonferroni's test ($p \leq 0.05$), both ± standard error. CK: no co-

inoculation, WD: water deficit, WDBS: water deficit + foliar application of *B. subtilis*; WDTH: water deficit + foliar application of *T. harzianum*.

Leaf area was drastically reduced by water stress, with the CK-M treatment having the statistically highest value (348.68 cm^2), while all other treatments showed significantly lower values. The presence of microalgae (+ M) reduced the leaf area in the CK (276.10), but in WDBS it showed the highest value in the presence of microalgae (112.80) among the water-deficient treatments (Fig. 4-C). Specific leaf area (Fig. 4-D) was also higher in the CK (-M, 269.37 $cm^2\ g^{-1}$), being reduced by water stress in the other treatments. Under the + M condition, CK and WD showed the same values (217.33 and 205.16 $cm^2\ g^{-1}$, respectively). Furthermore, WDBS and WDTH exhibited the lowest values of specific leaf area (106.92 and 57.32 $cm^2\ g^{-1}$, respectively), which were significantly similar.

Phenotypic responses of plants

Regarding the observed phenotype, at 15 DAP, control plants showed normal development, while those under water deficit (WD) exhibited reduced vigor, lower leaf expansion, and the onset of chlorosis, which was more evident in the absence of microalgae. The application of *B. subtilis* and *T. harzianum* partially mitigated these effects, particularly when combined with the microalgae (Fig. 5).

At 30 DAP, water stress intensified the negative effects on growth, resulting in reduced plant size, fewer leaves, and increased chlorosis in plants under WD. In contrast, treatments with microorganisms showed better vegetative performance, with greater leaf area and better coloration. Co-inoculation with *Parachlorella* sp. enhanced these effects, resulting in more vigorous plants, especially in the WD + *B. subtilis* treatment (Fig. 5).

At 15 DAP, CK plants exhibited normal vegetative development, with expanded leaves and uniform green coloration, regardless of co-inoculation with microalgae. On the other hand, WD plants showed initial symptoms of stress, characterized by reduced leaf expansion and mild chlorosis, with these effects being more evident in the absence of microalgae. When grown in WD + *B. subtilis* and WD + *T. harzianum*, the plants exhibited better development compared to WD alone, with greater leaf retention and lower chlorosis intensity. These effects were more pronounced when associated with the microalgae, indicating a possible positive interaction already in the early stages of evaluation.

At 30 DAP, the differences between the treatments became more evident. Plants under WD showed a marked reduction in growth, fewer leaves, increased chlorosis, and signs of premature senescence. In contrast, the WD + *B. subtilis* and WD + *T. harzianum* treatments maintained better vegetative health, with greater leaf area, better turgor, and intense green coloration. Plants co-inoculated with microalgae exhibited greater vegetative growth, greater leaf expansion, and fewer visible signs of stress, both under control conditions and under water deficit.

Analysis of the dendrogram using Euclidean distance (coefficient of co-phenetic: 0.7804) demonstrated the formation of distinct groups among the evaluated treatments. The WDTH and WDTH + M treatments

showed the greatest proximity, forming the most cohesive cluster, which indicates high similarity in their behaviors. Next, WDBS was associated with this group, showing an intermediate level of similarity. Subsequently, WD + M and WD were incorporated into the cluster at greater Euclidean distances, indicating less proximity to the more cohesive treatments (Fig. 6).

The C + M and WDBS + M treatments formed another group with high internal similarity, but were more distant from the other treatments. On the other hand, treatment C showed the greatest distance from the others and was considered the most dissimilar within the analysis. Overall, the dendrogram reveals cohesive regions represented mainly by WDTH and WDTH + M, while WD and WD + M occupy an intermediate position in the clustering structure.

DISCUSSION

The water deficit imposed throughout the experiment triggered significant morphophysiological responses in the soybean crop, highlighting its sensitivity to water limitation. Given this, our initial hypothesis that the use of biostimulant microorganisms would induce tolerance to water deficit was validated, since these biological agents were able to activate the soybean plants' resistance mechanisms to abiotic stress by modulating physiological and biochemical processes.

Taken together, this behavior indicates physiological impairment of the photosynthetic apparatus, especially at the level of photosystem II, associated with metabolic limitation and photochemical damage induced by water stress (Adireddy et al., 2025; Qiao et al., 2024). Similar results have been reported by other authors, not only for soybean but also for other species, where water limitation triggered changes primarily in chlorophyll degradation and the induction of PSII photoinhibition, which have been identified as the main physiological limiting mechanisms in water-limited environments (Karami et al., 2025; Peco et al., 2025; Du et al., 2023).

The decrease in Fv/Fm and the increase in the F0/Fm ratio observed in the WD treatment are consistent with studies linking the effect of water stress to increased non-photochemical quenching and structural disorganization of PSII reaction centers (Sommer et al., 2023). Under water stress, the limitation of carbon assimilation leads to the accumulation of NADPH and ATP, promoting the over-reduction of the electron transport chain. This imbalance favors electron transfer to oxygen and the consequent formation of reactive oxygen species (ROS), which are directly associated with damage and photoinhibition of photosystem II (Didaran et al., 2024; Duan et al., 2024). In response, proton gradient-dependent photoprotective mechanisms, such as non-photochemical dissipation, are activated to attempt to dissipate the excess energy, resulting in heat generation (Yang et al., 2024). However, when these mechanisms are insufficient, a reduction in maximum photochemical efficiency (Fv/Fm) and structural disorganization of PSII reaction centers occur (Guo et al., 2024; Julián et al., 2024).

The combination of *B. subtilis* and *Parachlorella* sp. in soybean plants subjected to water stress, although insufficient to restore physiological parameters to levels similar to those of the irrigated control (CK), helped mitigate the negative effects of water restriction. This behavior indicates that, even under

adverse conditions, the plant–microorganism association was able to modulate physiological responses during stress, reducing potential damage to molecular structure, promoting metabolic maintenance, and ensuring plant survival under limiting conditions.

Microorganisms of the genus *Bacillus* are known to mitigate biotic and abiotic stresses through the production of phytohormone-like substances, such as auxins and cytokinins. Furthermore, these bacteria can excrete enzymes associated with nutrient cycling, such as phosphatases and proteases, contributing to nutrient availability in the soil. More importantly, they act as modulators of plant metabolism, promoting the activation of the antioxidant system by increasing the activity of enzymes such as superoxide dismutase, catalase, and peroxidases, which contributes to reducing the accumulation of reactive oxygen species under stress conditions (Buqori et al., 2025; Ali et al., 2025).

The maintenance of higher Fv/Fm values and the reduction in the F_0/F ratio in the treatments inoculated with *Bacillus subtilis* and *Parachlorella* sp. indicate partial protection of PSII, since, although these parameters show improvement compared to the treatment under stress, they do not reach the levels observed under non-limiting conditions. This behavior suggests that the microorganisms mitigate, but do not completely eliminate, the effects of water stress on the photosynthetic apparatus, possibly through the activation of antioxidant mechanisms and the stabilization of thylakoid membranes, reducing photoinhibition without fully restoring maximum photochemical efficiency, as reported by Chen et al. (2026) and Lin et al. (2022).

The association with the microalga Parachlorella sp. played a central role in mitigating the effects of water deficit; however, its action as a biostimulant was complementary to the treatments. The consistent increase in chlorophyll index, PSII quantum efficiency, and photosynthetic rate may be related to the microalga's ability to produce bioactive compounds, including amino acids, osmoprotectants, and plant growth regulators such as auxins, cytokinins, and gibberellins, as well as antioxidants and extracellular polysaccharides, which contribute to metabolic balance and cellular protection (Tajti et al., 2026). Furthermore, the reduction in the F_0/F ratio at 15 DAA suggests less damage to PSII reaction centers, since lower F_0 values are associated with greater structural integrity of the photosynthetic complexes and improved energy coupling in the thylakoid membranes (Zait et al., 2024). This effect can be attributed to reduced oxidative stress and greater membrane stability, promoted by the antioxidant and osmoprotective action of the compounds released by the microalgae. However, this effect did not persist at 30 DAA, indicating a possible limitation in the persistence or activity of the microalgae throughout the culture cycle.

In addition, the effects of *Parachlorella* were not limited to physiological protection alone, but also influenced the allocation of photoassimilates and, most notably, the plant architecture. The reduction in leaf area and other parameters associated with increased root biomass may suggest a redistribution of carbon assimilated during photosynthesis to favor the development of other organs, such as the root system, thereby supporting the plant's survival under adverse conditions. Furthermore, limiting leaf area development under water stress conditions may be related to a survival strategy, given the reduction in

the active transpiration area rate, which may be compensated by an increase in photosynthetic activity, as observed in our results and by Dayoub et al. (2022).

Among the combinations, the pairing of *B. subtilis* and *Parachlorella*. sp. stood out as the most efficient and consistent across the two evaluation periods (15 and 30 DAA). The increase in the photosynthetic rate, Rubisco carboxylation efficiency, and maintenance of PSII quantum efficiency observed in this treatment are consistent with studies reporting the role of B. subtilis in the production of phytohormones, in modulating the REDOX balance, and in inducing systemic tolerance to water stress (Ren et al., 2025; Oliveira et al., 2025; Barghi and Etemadzedeh, 2025).

With regard to the interaction observed between *B. subtilis* and *Parachlorella* sp., functional compatibility between these two microorganisms is suggested, possibly related to the complementary nature of their mechanisms of action. Some authors highlight that microbial consortia well-matched in terms of density and population compatibility can enhance the individual effects of the microorganisms, provided there is no direct competition for niche or resources (Wang et al., 2026; Rawat et al., 2025; Parmar et al., 2024; Zaytsev et al., 2025; Solomon et al., 2023).

Interaction with *T. harzianum* showed variable responses, especially when associated with the microalga. Although the fungus is widely recognized for its role in inducing resistance and biocontrol of plant pathogens, its effectiveness in promoting growth may depend on the environmental context and interactions with other microorganisms present in the cultivation system, which can alter the nature of the ecological relationship (Contreras-Cornejo et al., 2024; Tan et al., 2022; Alwadai et al., 2024). Therefore, the absence of synergistic responses with *Parachlorella* sp. suggests that compatibility between these microorganisms must be carefully evaluated during the formulation of mixed bio-inputs, requiring prior knowledge of the plant-microbial consortium interaction for their use in other crops.

The results regarding biomass and associated parameters reinforce the notion that water stress limited vegetative growth, particularly in terms of leaf area and leaf area ratio, regardless of inoculation. However, the presence of the microalgae promoted a significant increase in the dry mass of the aboveground parts and, particularly, of the root system, corroborating studies demonstrating that microalgal biostimulants favor root development and increase water absorption capacity under stress (Molina-Favero et al., 2026; Memeli et al., 2025; Arenas et al., 2025;). The increased root growth observed typically suggests an adaptive strategy of the plant that contributes to greater physiological resilience under water-limited conditions.

Taken together, the results demonstrated that the mitigation of water deficit in soybean crops through the use of bio-inputs is directly related to the preservation of photosynthetic activity, increasing metabolic efficiency and modulating carbon allocation in plants—processes that contribute to maintaining growth and productivity under water-restricted conditions.

CONCLUSION

Water stress significantly impaired the morphophysiological performance of soybeans, primarily due to reduced photosynthetic efficiency, highlighting the crop's high sensitivity to water limitation. Inoculation with *B. subtilis* and *Parachlorella* sp. resulted in the maintenance of the photosynthetic rate, improved carbon allocation, and preservation of photosystem II functionality, leading to greater biomass accumulation, particularly in the root system.

DECLARATIONS

Conflicts of Interest:

The authors declare no conflicts of interest.

Funding:

This research received no external funding. **Institutional Review Board Statement**: Not applicable.

Author Contribution

Author Contributions: Conceptualization, A.d.S.D., C.C.S. and S.d.P.Q.S; methodology, A.d.S.D., C.C.S. and S.d.P.Q.S, formal analysis, A.d.S.D., L.O.M.M. and D.L.B.d.A.; investigation e resources, A.d.S.D., C.C.S., S.d.P.Q.S and D.M.R, S.d.P.Q.S.; data curation, A.d.S.D. e C.C.S.; writing—original draft preparation, A.d.S.D.; writing—review and editing, A.d.S.D. and C.C.S.; supervision, C.C.S. and S.d.P.Q.S. All authors have read and agreed to the published version of the manuscript.

Acknowledgement

Acknowledgments: The National Council for Scientific and Technological Development (CNPq) and Coordination for the Improvement of Higher Education Personnel (CAPES) for the granting of scholarships, and the Foundation for Support to the Development of Education, Science, and Technology of the State of Mato Grosso do Sul (FUNDECT) for financial support.

Data Availability Statement:

The data presented in this study are available in the graphs and tables provided in the manuscript.

REFERENCES

1. Adireddy RG, Anapalli SS, Ojha M, Puppala N, Reddy KN (2025) Photosynthetic responses to light levels in drought-tolerant novel peanut (Arachis hypogaea L.) genotypes. Scientific Reports 15:e25032 DOI: https://doi.org/10.1038/s41598-025-10978-z
2. Alwadai AS, Wahibi MSA, Alsayed MF, Alshaikh NA, Perveen K, Elsayim R (2024) Molecular characterization of plant growth-promoting Trichoderma from Saudi Arabia. Scientific Reports 14:e23236 DOI: https://doi.org/10.1038/s41598-024-73762-5
3. Amaral J, Lobo AKM, Carmo-Silva E (2024) Regulation of Rubisco activity in crops. New Phytologist 241:35–51 DOI: https://doi.org/10.1111/nph.19369
4. Andreata EC, Molinari MDC, Kafer JM, Marin SRR, Marin DR, Fuganti-Paglinarini R, Vanzela ALL, Rech EL, Nepomuceno AL, Mertz-Henning LM (2025) Molecular Mechanisms and Crosstalk Signaling in Soybean's Response to Water Deficit and Excess: Implications for Stress Resilience and Productivity. Plants 14:e3245 DOI: https://doi.org/10.3390/plants14213245
5. Arenas Colarte C, Balic I, Díaz Ó, Cortes I, Moreno AA, Amenabar MJ, Retamal MC, Fuentes NC (2025) Microalgae-based biostimulants: effects on growth and stress resistance in agricultural crops. Plants 14:e3488 DOI: https://doi.org/10.3390/plants14223488
6. Basal O, Munkhbat U, Veres S (2024) Enhancing Drought Tolerance in Two Soybean Genotypes with Varied Susceptibilities Through Foliar Application of Acetic Acid. Journal of Plant Growth Regulation 43:1304–1315 DOI: https://doi.org/10.1007/s00344-023-11184-9
7. Brawa G, Yu X, Liu Z, Zhou Y, Sun X (2023) Surviving the enemies: regulatory mechanisms of stomatal function in response to drought and salt stress. Environmental and Experimental Botany 209:e105291
8. Chen YH, Yu X, Liu Z, Zhou Z, Sun X (2026) Biofilms of Bacillus subtilis as biostimulants for plant resilience to drought and flooding. Scientific Reports 16:e6113 DOI: https://doi.org/10.1038/s41598-026-36767-w
9. Contreras-Cornejo HA, Schmoll M, Esquivel-Ayala BA, González-Esquivel CE, Rocha-Ramírez V, R, Larsen J (2024) Mechanisms for plant growth promotion activated by Trichoderma in natural and managed terrestrial ecosystems. Microbiological Research 281:e127621 DOI: https://doi.org/10.1016/j.micres.2024.127621
10. Cui Y, Jian S, Jin J, Ning S, Feng P (2019) Quantitative assessment of soybean drought loss sensitivity at different growth stages. Quantitative assessment of soybean drought loss sensitivity at different growth stages based on S-shaped damage curve. Agricultural Water Management 213:821–832 DOI: https://doi.org/10.1016/j.agwat.2018.11.020
11. Da Silva DMM, Santos CC, Wagner FE, Martins LOM, Ozório JPA, Da Silva OA, Ribeiro DM, Scalon SDPQ (2024) Seed biopriming with Parachlorella, Bacillus subtilis and Trichoderma harzianum alleviates salinity effects in soybean. BMC Plant Biology 24:e1149 DOI: https://doi.org/10.1186/s12870-024-05646-9
12. Da Silva MDGC, De Medeiros AO, Roque BAC, Dos Santos MR, Bezerra KGO, De Almeida FCG, Sarubbo LA (2026) Next-Generation Bioinputs: A Systematic Review of Biosurfactants in

Sustainable Agriculture and Research Frontiers with Bacterial Cellulose. Processes 14:e398 DOI: https://doi.org/10.3390/pr14030398

13. Dayoub E, Lamichhane JR, Debaeke P, Maury P (2022) Genotypic differences in root traits to design drought-avoiding soybean ideotypes. OCL 29:e26
14. Du XB, Zhang X, Wei Z, Lei W, Hu G, Huang Z, Kong L (2023) Photosynthetic characteristics of subtending leaves and their relationships with soybean pod development under heat, drought and combined stresses. Journal of Agronomy and Crop Science 209:204–215 DOI: https://doi.org/10.1111/jac.12616
15. Elsalahy HH, Reckling M (2022) Soybean resilience to drought supported by recovery of photosynthetic traits. Frontiers in Plant Science 13:e971893 DOI: https://doi.org/10.3389/fpls.2022.971893
16. Ferreira RC, Sibaldelli RNR, Crusiol LGT, Neumaier N, Farias JRB (2024) Soybean Yield Losses Related to Drought Events in Brazil: Spatial–Temporal Trends over Five Decades and Management Strategies. Agriculture 14:2144 DOI: https://doi.org/10.3390/agriculture14122144
17. Gautam M, Peissel A, Kariyat R (2026) Under Concurrent Drought and Herbivory, Drought Dominates Herbivory on Morpho-Physiological Responses in Soybean. Physiologia Plantarum 178:e70748 DOI: https://doi.org/10.1111/ppl.70748
18. Hajnal-Jafari T, Seman V, Stamenov D, Duric S (2020) Effect of Chlorella vulgaris on Growth and Photosynthetic Pigment Content in Swiss Chard (Beta vulgaris L. subsp. cicla). Polish Journal of Microbiology 69:235–238 DOI: https://doi.org/10.33073/pjm-2020-023
19. Karami S, Shiran B, Ravash R (2025) Molecular investigation of how drought stress affects chlorophyll metabolism and photosynthesis in leaves of C3 and C4 plant species: A transcriptome meta-analysis. Heliyon 11:e42368
20. Kodadinne Narayana N, Wijewardana C, Alsajri FA, Reddy KR, Stetina SR, Bheemanahalli R (2024) Resilience of soybean genotypes to drought stress during the early vegetative stage. Scientific Reports 14:e17365 DOI: https://doi.org/10.1038/s41598-024-67930-w
21. Lin S-Y, Chen P-A, Zhuang B-W (2022) The Stomatal Conductance and Fv/Fm as the Indicators of Stress Tolerance of Avocado Seedlings under Short-Term Waterlogging. Agronomy 12:e1084 DOI: https://doi.org/10.3390/agronomy12051084
22. Marcos AW, Tomaz JP, Nogueira AF, Mosela M, Alves DS, Dos Santos Neto J, Watanabe LS, Afonso L, Faria MV, Scislowski L, Fagundes DFV, Cava HB, Cabral PDS, Wisniewski DCR, De Assis R, De Azevedo SV, Candido LS, Gonçalves LSA (2026) Bacillus velezensis Ag129 and Ag132: two novel probiotics enhancing drought tolerance and agronomic performance in maize and soybean. Front. Plant Sci. 7:e1711687 DOI: https://doi.org/10.3389/fpls.2026.1711687
23. Memeli İ, Tüzel Y, Uysal Ö, Durdu T, Uysal FÖ, Harouna OS, Ekinci K, Gruda NS (2025) Microalgae enhanced plant growth and yield, and improved leaf color in soilless lettuce. Scientific Reports 15:e40313 DOI: https://doi.org/10.1038/s41598-025-24004-9

24. Molina-Favero C, Rizza LS, Olano AMG, Maroniche G, Polizzi M, Gerónimo E, Creus C, Curatti L, Pagnussat LA (2026) Eukaryotic microalgae-bacteria synthetic consortia boost crop productivity and drought tolerance in bread wheat (Triticum aestivum). Frontiers in Plant Science 16:e1726084 DOI: https://doi.org/10.3389/fpls.2025.1726084
25. Moseley D, Casteel S, Conley S, Holshouser D, Lee C, Licht MA, Lindsey LE, Kandel H, Kleinjan J, Knott C, Naeve S, Nafziger E, Pumblee M, Ross J, Singh M, Vann R, Ortmeier-Clarke HJ (2025) The soybean growth cycle: Important risks and management strategies. Crop, Forage & Turfgrass Management 11:e70086 DOI: https://doi.org/10.1002/cft2.70086
26. Parmar P, Kumar R, Neha Y, Srivatsan V (2023) Microalgae as next generation plant growth additives: Functions, applications, challenges and circular bioeconomy based solutions. Frontiers in Plant Science 14:e1073546 DOI: https://doi.org/10.3389/fpls.2023.1073546
27. Peco JD, Centeno A, Moratiel R, Villena J, López-Perales JA, Moreno MM, Pérez-López D (2025) Intermittent versus continuous drought: chlorophyll a fluorescence reveals photosystem resilience in tomato. Frontiers in Plant Science 16:e1699777 DOI: https://doi.org/10.3389/fpls.2025.1699777
28. Santini G, Biondi N, Rodolfi L, Tredici MR (2021) Plant Biostimulants from Cyanobacteria: An Emerging Strategy to Improve Yields and Sustainability in Agriculture. Plants 10:e643 DOI: https://doi.org/10.3390/plants10040643
29. Qiao M, Hong C, Jiao Y, Hou S, Gao H (2024) Impacts of Drought on Photosynthesis in Major Food Crops and the Related Mechanisms of Plant Responses to Drought. Plants 13:e1808 DOI: https://doi.org/10.3390/plants13131808
30. Radzikowska-Kujawska D, Piechotta T, Jarzyniak K, Kowalczewski PL, Wojewódzki P (2025) Effects of biopreparations based on Bacillus and Trichoderma, combined with mineral and organic fertilization and a Pisum sativum L. forecrop on improving the tolerance of Maize plants to drought stress. PLoS One 20:e0322718 DOI: https://doi.org/10.1371/journal.pone.0322718
31. Rawat A, Han B, Patel N, Allehaibi H, Rosado AS, Hirt H (2025) Symbiotic plant–bacterial–fungal interaction orchestrates ethylene and auxin signaling for optimized plant growth. The Plant Journal 122:e70174 DOI: https://doi.org/10.1111/tpj.70174
32. Ren P, Zhou B, Bi Y, Chen X, Yao S (2025) Study on the Microbial Mechanism of Bacillus subtilis in Improving Drought Tolerance and Cotton Yield in Arid Areas. Agronomy 15:e1932 DOI: https://doi.org/10.3390/agronomy15081932
33. Ren P, Zhou B, Bi Y, Chen X, Yao S (2026) Bacillus subtilis enhances the drought resistance of plants through the aggregation of rhizosphere microbiota mediated by root exudates. Agricultural Water Management 327:e110287 DOI: https://doi.org/10.1016/j.agwat.2026.110287
34. Rigobelo EC, De Andrade LA, Santos CHB, Frezarin ET, Sales LR, De Carvalho LAL, Pinheiro DG, Nicodemo D, Babalola OO, Verdi MCQ, Mondin M, Desoignies N (2024) Effects of Trichoderma harzianum and Bacillus subtilis on the root and soil microbiomes of the soybean plant INTACTA RR2 PRO™. Frontiers in Plant Science 15:e1403160 DOI: https://doi.org/10.3389/fpls.2024.1403160

35. Rigobelo EC, De Carvalho LAL, Santos CHB, Frezarin ET, Pinheiro DG, Nicodemo D, Babalola OO, Desoignies N (2024) Growth promotion and modulation of the soybean microbiome INTACTA RR PRO with the application of the fungi Trichoderma harzianum and Purpureocillum lilacinum. Scientific Reports 14:e21004 DOI: https://doi.org/10.1038/s41598-024-71565-2
36. Shahhoseini R, Farhadi N, Angourani HR (2025) Metabolic activities, biochemical, and physiological processes in Mentha pulegium L. induced by drought stress and application of 5-aminolevulinic acidf. BMC Plant Biology 25:1410 DOI: https://doi.org/10.1186/s12870-025-07489-4
37. Shi Y, Chen R, Jian K, Jiang A, Yang J, Cui H, Chen M (2026) Key metabolites secreted by Chlorella vulgaris alleviate salt stress in soybean seedlings. Journal of Integrative Plant Biology PRÉ-PRINT DOI: https://doi.org/10.1111/jipb.70207
38. Solomon W, Mutum L, Janda T, Molnár Z (2023) Potential benefit of microalgae and their interaction with bacteria to sustainable crop production. Plant Growth Regulation 101:53–65 DOI: https://doi.org/10.1007/s10725-023-01019-8
39. Sommer SG, Han E, Li X, Rosenqvist E, Liu F (2023) The Chlorophyll Fluorescence Parameter Fv/Fm Correlates with Loss of Grain Yield after Severe Drought in Three Wheat Genotypes Grown at Two CO2 Concentrations. Plants 12:e436 DOI: https://doi.org/10.3390/plants12030436
40. Tajti K, Farkas A, Farkas M, Bíró T, Ordog V, Maróti G (2026) Synergistic Plant Biostimulatory Effects of an Inter-Kingdom Interaction: Chlorella sp. and Kocuria rhizophila Algal–Bacterial Co-Culture for Sustainable Crop Production. Plants 15:e292 DOI: https://doi.org/10.3390/plants15020292
41. Tan B, Li Y, Deng D, Pan H, Zeng Y, Tan X, Zhuang W, Li Z (2022) Rhizosphere inoculation of Nicotiana benthamiana with Trichoderma harzianum TRA1-16 in controlled environment agriculture: Effects of varying light intensities on the mutualism-parasitism interaction. Frontiers in Plant Science 13:e989155 DOI: https://doi.org/10.3389/fpls.2022.989155
42. Nguyen TC, Jo H, Tran HA, Lee J, Lee J-D, Kim JH, Seo HS, Song JT (2024) Assessment of Drought Responses of Wild Soybean Accessions at Different Growth Stages. Agronomy 14:e471 DOI: https://doi.org/10.3390/agronomy14030471
43. Wang C, Sun A, Zhu LJ, Liu M, Zhang Q, Wang L, Gao X (2025) Drought and rewatering effects on soybean photosynthesis, physiology and yield. PeerJ 13:e19658 DOI: http://doi.org/10.7717/peerj.19658
44. Wang D, Yang M, Kiu Y, Hu X, Hao F, Sun D, Wang N, Dong Y, Liu W, Li X, Wang F (2026) GmPLC8 enhances drought resistance in soybean by regulating stomatal closure. iScience 29:e114546 DOI: http://doi.org/10.1016/j.isci.2025.114546
45. Wang Y, Xue X, Schagerl M, Hu X, Srinuanpan S, Rehman O, Yu Z, Mi C, Zhu F, Huo S (2026) Combined application of microalgae and plant growth-promoting rhizobacteria: Synergistic effects in improving soil health and crop root disease resistance. Applied Soil Ecology 218:e106684 DOI: https://doi.org/10.1016/j.apsoil.2025.106684
46. Xia C, Huang Z, Lu B, Geng Z, Wang S, Xu C (2026) Physiological and molecular response of soybean to combined drought and heat stresses during flowering. BMC Plant Biology 26:319 DOI:

https://doi.org/10.1186/s12870-025-08081-6

47. Yang J, Tang M, Zhao H (2026) Physiological Mechanisms of Plant Growth-Promoting Rhizobacteria in Enhancing Abiotic Stress Tolerance of Vegetable Crops: A Review. Plants 15:e686 DOI: https://doi.org/10.3390/plants15050686
48. Zait Y, Shemer OE, Cochavi A (2024) Dynamic responses of chlorophyll fluorescence parameters to drought across diverse plant families. Physiologia Plantarum 176:e14527 DOI: https://doi.org/10.1111/ppl.14527
49. Zaytsev PA, Rodin VA, Zaytseva AA, Zvereva MI, Solovchenko AE (2024) Advances of high-throughput sequencing for unraveling biotechnological potential of microalgal-bacterial communities. Journal of Applied Phycology 36:1901–1919 DOI: https://doi.org/10.1007/s10811-024-03267-1
50. Didaran F, Kordrostami M, Ghasemi-Soloklui AA, Pashkovskiy P, Kreslavski V, Kuznetsov V, Allakhverdiev SI (2024) The mechanisms of photoinhibition and repair in plants under high light conditions and interplay with abiotic stressors. Journal of Photochemistry and Photobiology B: Biology 259:e113004 DOI: https://doi.org/10.1016/j.jphotobiol.2024.113004
51. Duan H, Shao C, Zhao N, Wang D, De Dios VR, Tissue DT (2024) The role of leaf superoxide dismutase and proline on intra-specific photosynthesis recovery of Schima superba following drought. Scientific Reports 14:e8824 DOI: https://doi.org/10.1038/s41598-024-59467-9
52. Yang Q-Y, Wang X-Q, Yang Y-J, Huang W (2024) Fluctuating light induces a significant photoinhibition of photosystem I in maize. Plant Physiology and Biochemistry 207:e108426 DOI: https://doi.org/10.1016/j.plaphy.2024.108426
53. Guo Y, Ren H, Wang HZ, Zhang JW, Zhao B, Ren BZ, Liu P (2024) High Temperature and Drought Combined Stress Inhibited Photosystem □ Performance and Decreased Grain Yield of Summer Maize. Scientia Agricultura Sinica 57:4205–4220 DOI: https://doi.org/10.3864/j.issn.0578-1752.2024.21.004
54. Julián C, Villagangos S, Jené L, Pasques O, Pintó-Marijuan M, Munné-Bosch S (2024) Biological outliers: essential elements to understand the causes and consequences of reductions in maximum photochemical efficiency of PSII in plants. Planta 260:e32 DOI: https://doi.org/10.1007/s00425-024-04466-3
55. Buqori DMAI, Sugiharto B, Siswoyo TA, Hariyono K (2025) Mitigating drought stress by application of drought-tolerant Bacillus spp. enhanced root architecture, growth, antioxidant and photosynthetic genes expression in sugarcane. Scientific Reports 15:e5259 DOI: https://doi.org/10.1038/s41598-025-89457-4
56. Ali Q, Khan TA, Ali M, Gururani MA, Mundra S (2025) Bacillus paranthracis alleviates saline stress in tomato by reprogramming physiology, photosynthesis, and antioxidant activity. BMC Plant Biology 25:e1362 DOI: https://doi.org/10.1186/s12870-025-07456-z
57. Li Y, Xia L, Meng Y, Shen X, Wan X, Gan F, Zhang R (2026) Bacillus velezensis SQR9-Emitted Volatiles Enhance Arabidopsis Salt Tolerance via ROS Scavenging and Ion Transport Regulation. Plants

15:e218 DOI: https://doi.org/10.3390/plants15020218

58. Ren P, Zhou B, Bi Y, Chen X, Yao S (2025) Study on the Microbial Mechanism of Bacillus subtilis in Improving Drought Tolerance and Cotton Yield in Arid Areas. Agronomy 15:e1932 DOI: https://doi.org/10.3390/agronomy15081932
59. Oliveira JAdosS, Leite EF, Matos GGF, Corniani NJM, Coneglian CF, Rocha MEdeS, Viero P, Marcusso LTV, Da Silva CAC (2025) Dose-dependent effects of Bacillus subtilis SB008 on root development, photosynthetic efficiency, and antioxidant activity in soybean plants. Biocatalysis and Agricultural Biotechnology 70:e103879 DOI: https://doi.org/10.1016/j.bcab.2025.103878
60. Barghi A, Etemadzadeh SS (2025) Role of *Bacillus subtilis* W1L in Mitigating Oxidative and Osmotic Stress in Lettuce under Drought and Salt Conditions. The Plant Pathology Journal 41:709–722 DOI: https://doi.org/10.5423/PPJ.OA.05.2025.0063

Figures

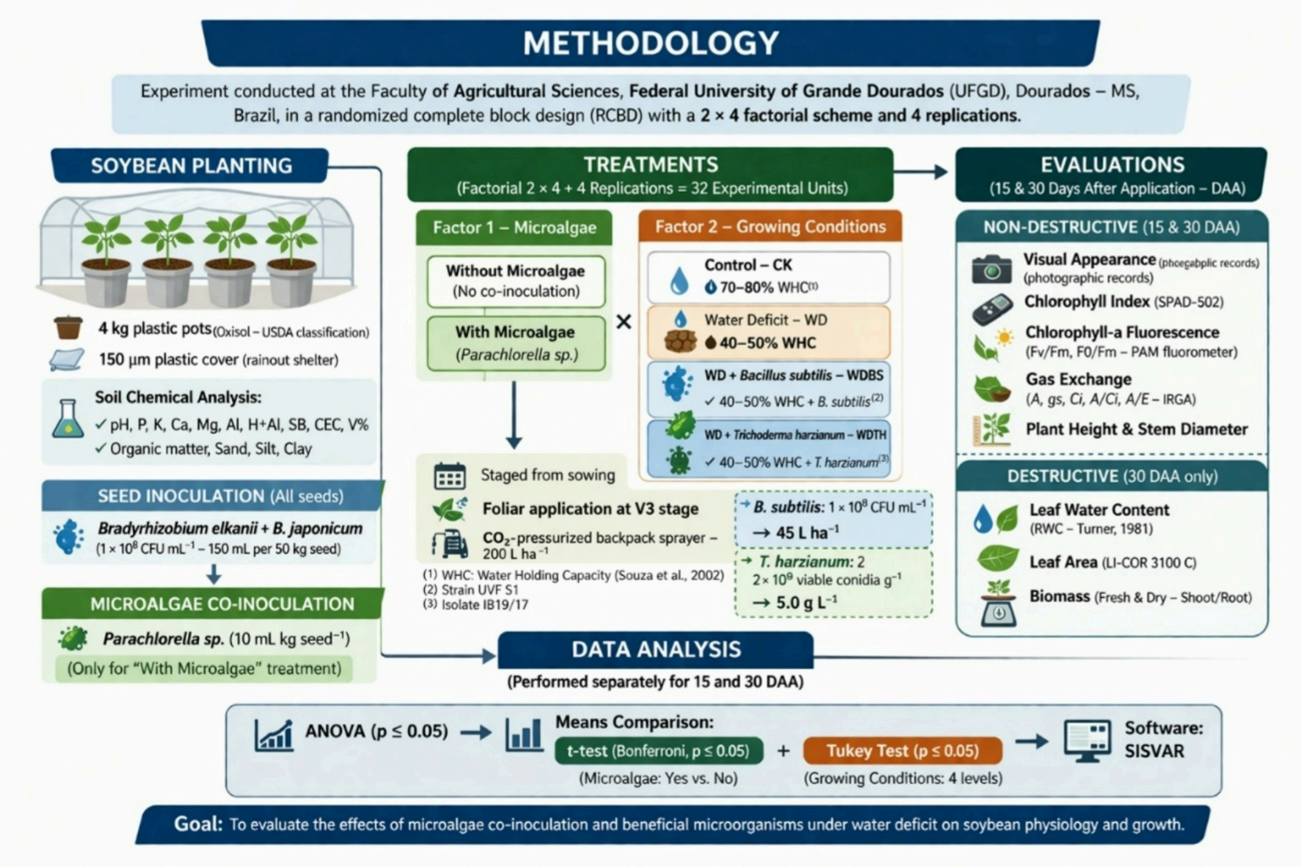


Figure 1

Schematic overview of the experimental design, treatments, application timing, physiological and growth evaluations, and statistical analyses performed to assess the effects of microalgae co-inoculation and beneficial microorganisms under water deficit conditions in soybean.

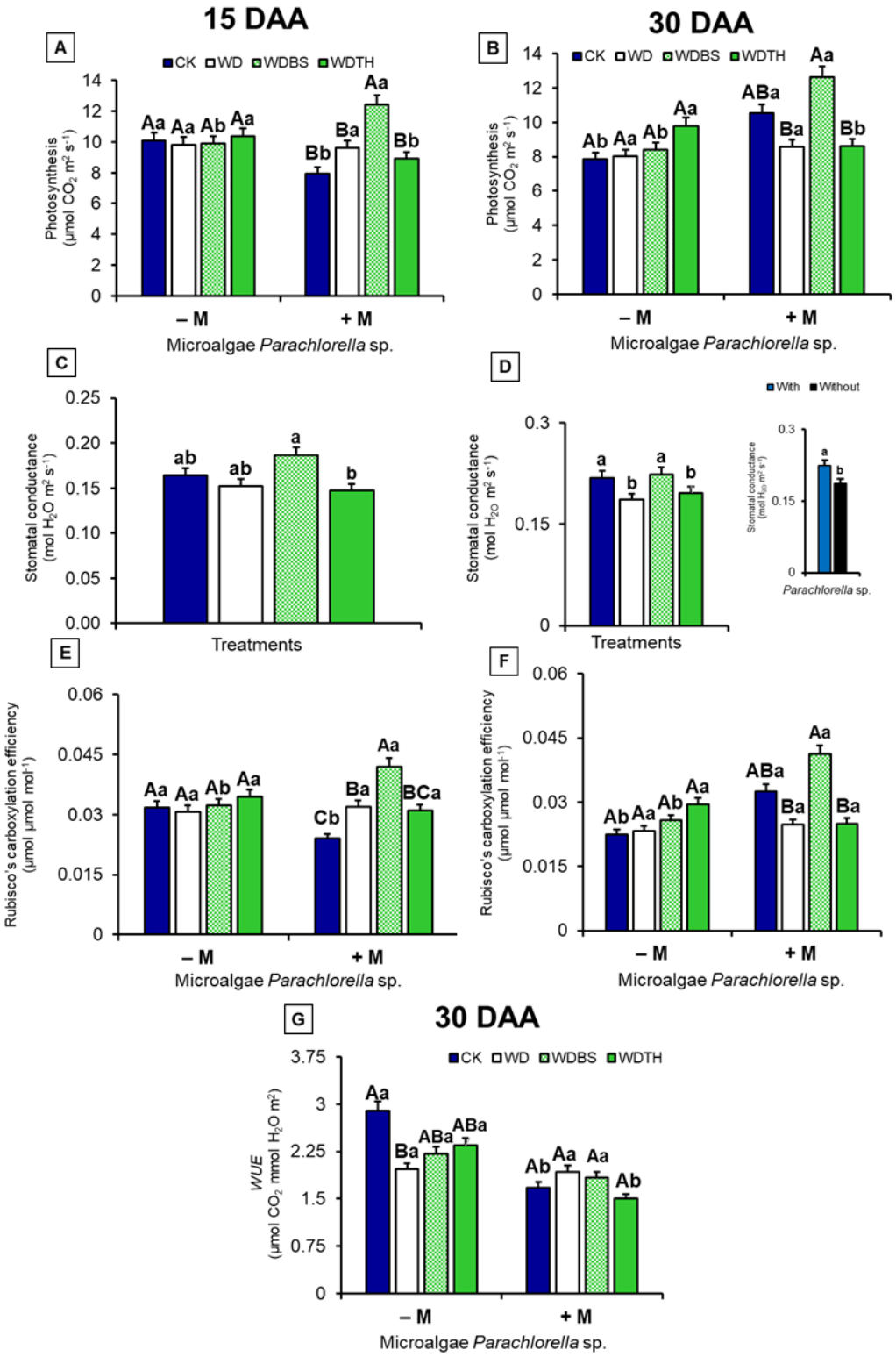


Figure 2

Photosynthesis (A, B), stomatal conductance (C, D), and intrinsic carboxylation efficiency (C, D) at 15 and 30 days after application (DAA), respectively, and water use efficiency (WUE) at 30 DAA in soybean plants grown under different water regimes depending on inoculation with *Trichoderma harzianum* or *Bacillus subtilis*, with or without application of *Parachlorella* sp. Uppercase letters compare the effects of co-inoculation methods using Tukey's test ($p \leq 0.05$), and lowercase letters compare the effect of *Parachlorella* sp. foliar application using Bonferroni's test ($p \leq 0.05$), both ± standard error. CK: no co-inoculation, WD: water deficit, WDBS: water deficit + foliar application of *B. subtilis*; WDTH: water deficit + foliar application of *T. harzianum*

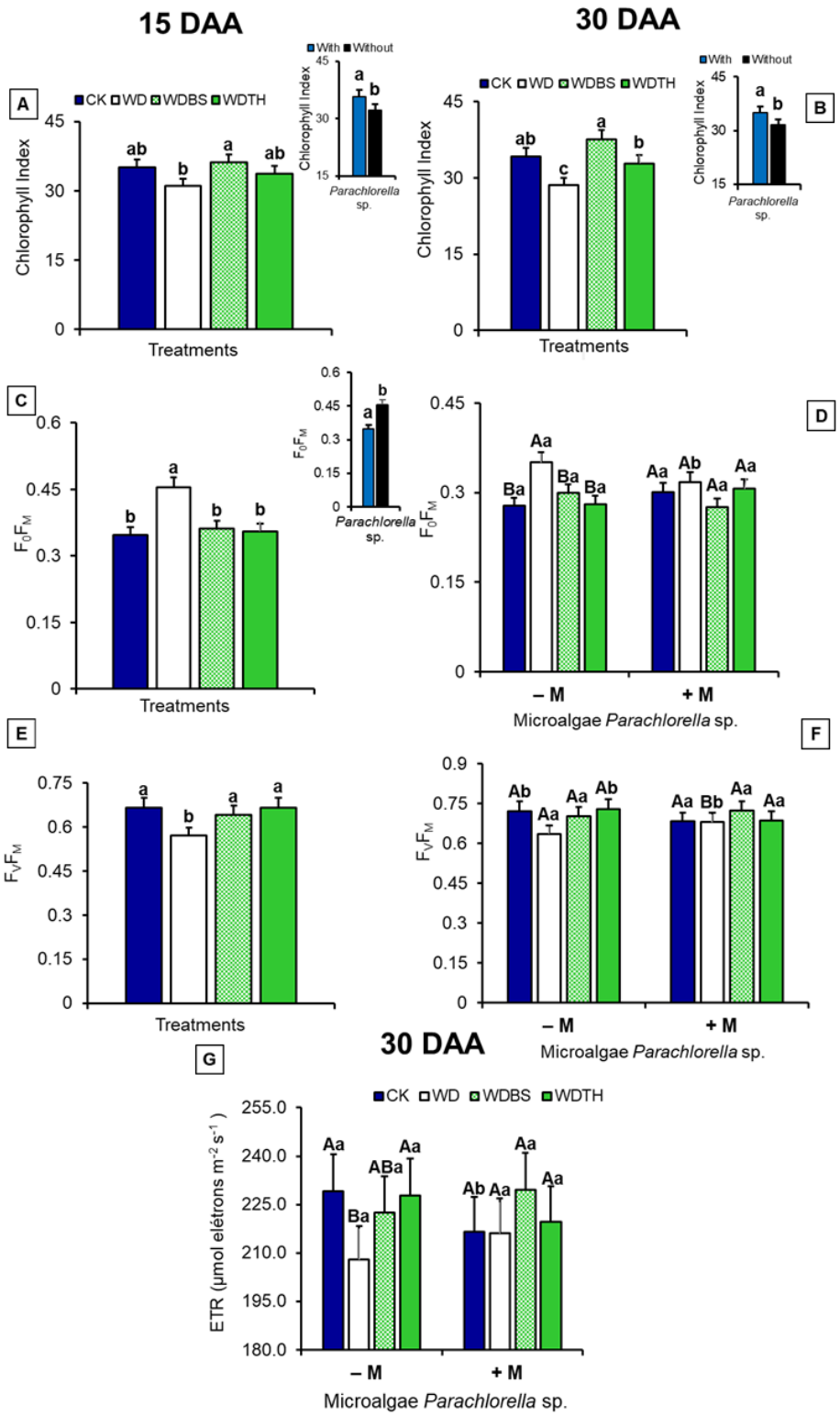


Figure 3

Chlorophyll index (A, B), maximum basal yield of non-photochemical processes—F0/Fm (C, D), and maximum quantum efficiency of photosystem II – Fv/Fm (E, F) at 15 and 30 days after application (DAA) and electron transport rate (ETR) at 30 DAA in soybean plants grown under controlled irrigation conditions following inoculation with *Trichoderma harzianum* or *Bacillus subtilis*, with or without application of *Parachlorella*sp. Uppercase letters compare the effects of co-inoculation methods using

Tukey's test ($p \leq 0.05$), and lowercase letters compare the effect of *Parachlorella*sp. foliar application using Bonferroni's test ($p \leq 0.05$), both ± standard error. CK: no co-inoculation, WD: water deficit, WDBS: water deficit + foliar application of *B. subtilis*; WDTH: water deficit + foliar application of *T. harzianum*.

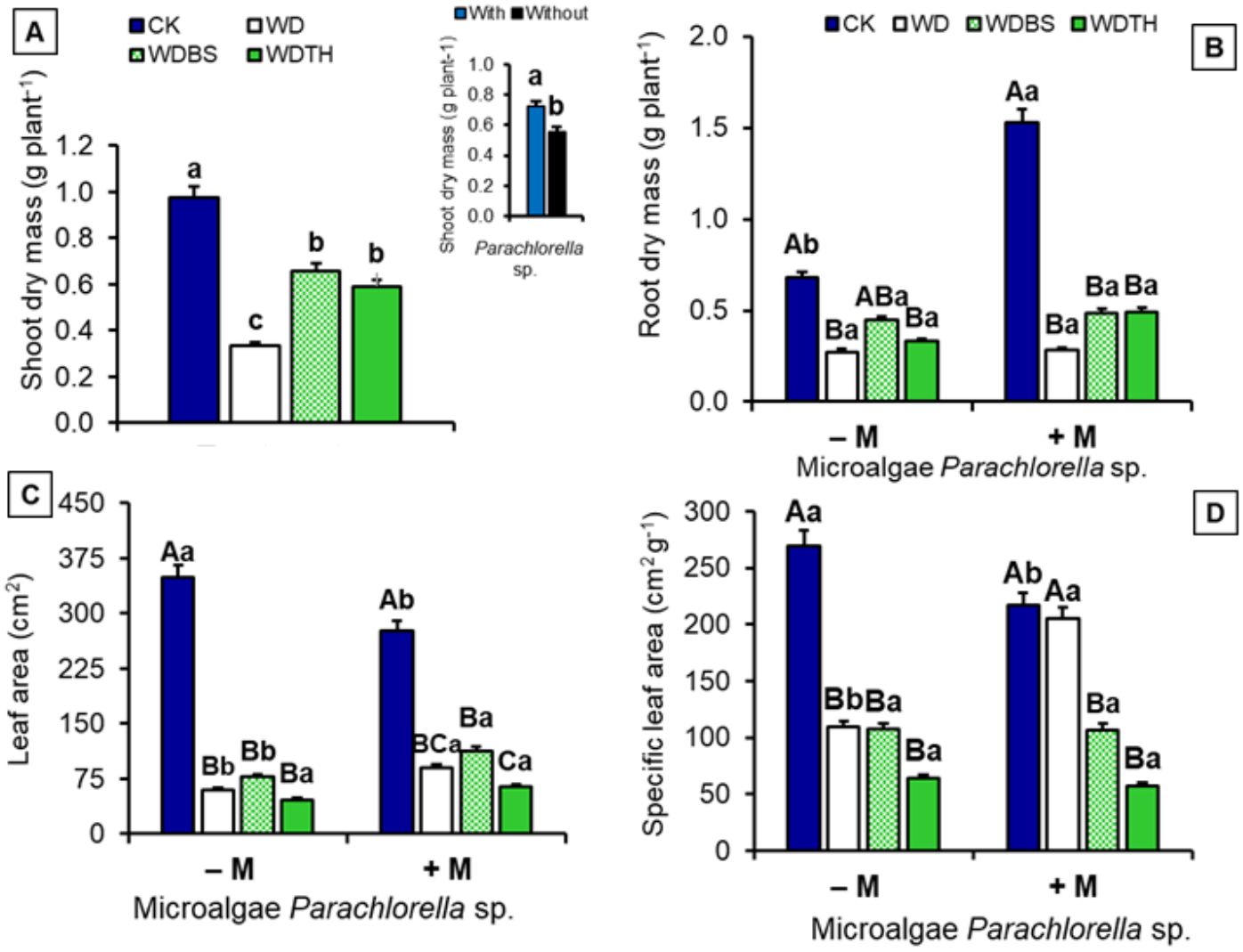


Figure 4

Leaf dry weight (A), root dry weight (B), leaf area (C), and specific leaf area (D) in soybean plants grown under different water regimes, depending on inoculation with *Trichoderma harzianum*or *Bacillus subtilis*, with or without application of *Parachlorella*sp. Uppercase letters compare the effects of co-inoculation methods using Tukey's test ($p \leq 0.05$), and lowercase letters compare the effect of Parachlorella sp. foliar application using Bonferroni's test ($p \leq 0.05$), both ± standard error. CK: no co-inoculation, WD: water deficit, WDBS: water deficit + foliar application of *B. subtilis*; WDTH: water deficit + foliar application of *T. harzianum*.

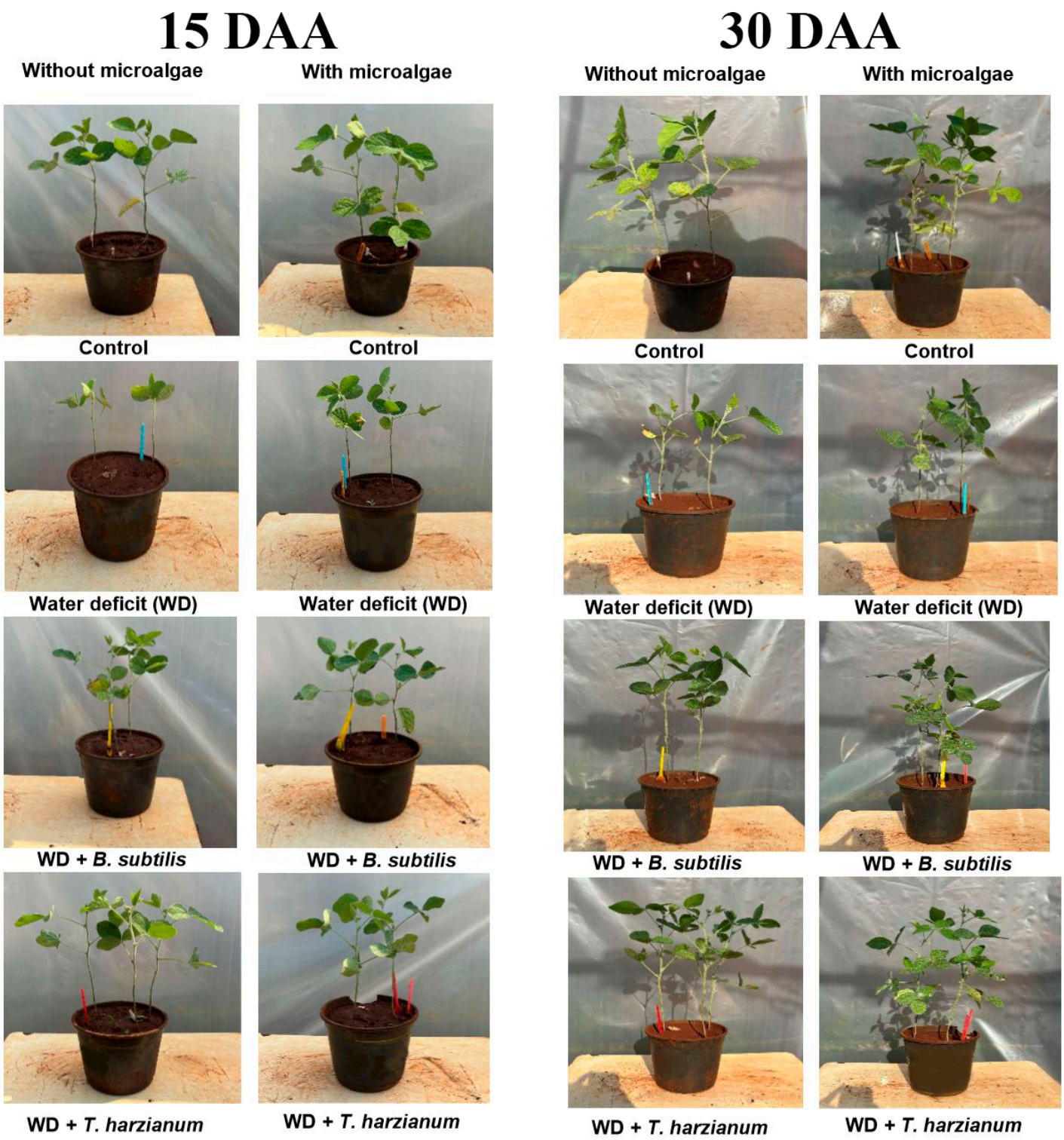


Figure 5

Phenotypic responses of soybean plants 15 and 30 days after application under different growing conditions, with and without the addition of microalgae (*Parachlorella* sp.).

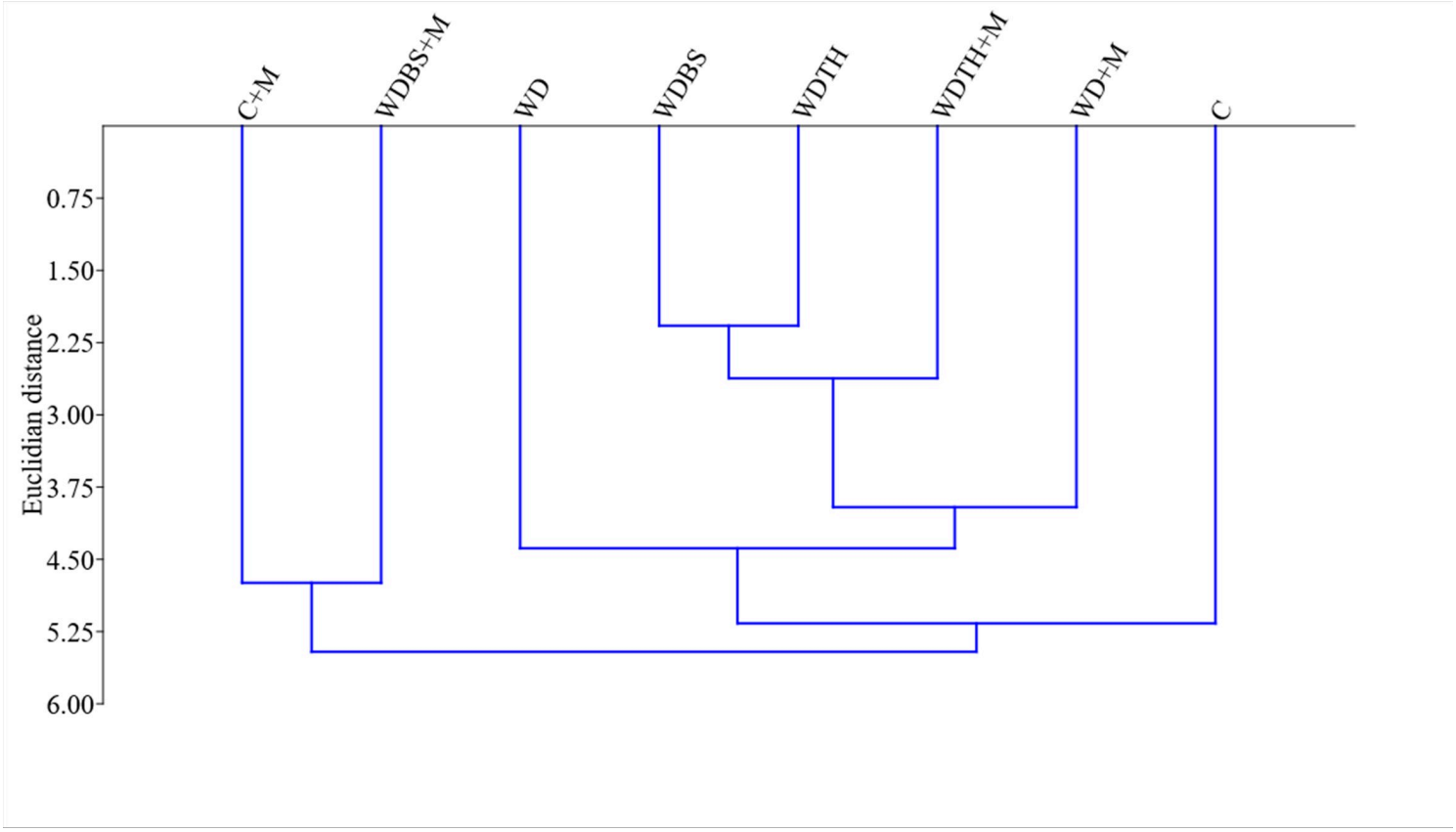


Figure 6

Similarity dendrogram showing hierarchical groups based on Euclidean distance for the characteristics of soybeans derived from seeds inoculated with isolated or combined bio-inputs, evaluated 30 days after application.