

Microbial Consortia and Biochar Enhance Photosynthesis, Water Relations, and Leaf Thermal Regulation in *Vigna radiata* L

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Abstract

Plant growth-promoting microorganisms (PGPMs) and biochar are increasingly recognized as sustainable strategies to enhance crop performance. However, their combined effects on photosynthesis, water relations, and leaf temperature under hydroponic conditions remain insufficiently explored. Here, we investigated the physiological responses of *Vigna radiata* L. to a bacterial biostimulant (BB), arbuscular mycorrhizal fungi (AM), their combination (BA), and a triple treatment with biochar (BiB). All treatments significantly improved plant performance compared with the control, though with differing mechanisms and magnitudes. BB markedly reduced leaf temperature (LT) through enhanced stomatal conductance and transpiration, resulting in higher net photosynthesis. AM primarily improved plant water status (RWC = 0.89) and intrinsic water-use efficiency, moderating LT via hydraulic and osmotic regulation rather than evaporative cooling. The BA treatment integrated these complementary functions, achieving the lowest LT (25.66°C), highest transpiration (4.03 mmol m⁻² s⁻¹), and maximum shoot and root biomass. Incorporation of biochar (BiB) further increased photosynthetic rate and water-use efficiency, although total biomass was slightly lower than in BA. These findings reveal a functional trade-off: bacterial inoculation promotes carbon assimilation through stomatal regulation, while AM fungi enhance hydraulic stability; their co-inoculation harmonizes both processes to optimize growth. By actively lowering leaf temperature, PGPMs mitigate heat-induced photoinhibition and sustain higher photosynthetic efficiency, offering a practical approach to improve crop resilience and productivity under controlled or resource-limited conditions.

Introduction

Global agricultural productivity is increasingly challenged by climatic extremes, resource limitations, and environmental stressors, including heat, and drought. In response, sustainable strategies that enhance plant performance while minimizing chemical inputs are urgently needed. Among these, plant growth-promoting microorganisms (PGPMs), including beneficial bacteria and arbuscular mycorrhizal (AM) fungi, have emerged as key biotic tools to improve crop growth and stress resilience through multiple physiological and biochemical pathways.

PGPB (plant growth-promoting bacteria) can stimulate plant growth by enhancing nutrient availability, producing phytohormones, and promoting stomatal conductance, thereby improving carbon assimilation and water-use dynamics (Shah et al., 2021) (Bhattacharyya & Jha, 2012). AM fungi, in contrast, enhance root hydraulic conductivity, water uptake, and nutrient foraging, contributing to improved tissue hydration and intrinsic water-use efficiency (Andrew Smith & Smith, 2011) (Begum et al., 2019). While both microbial groups individually improve growth and stress tolerance, co-inoculation may integrate these complementary mechanisms, optimizing photosynthesis and water balance in ways that single inoculants cannot.

Photosynthesis is the central process driving plant growth, with parameters such as net photosynthetic rate (P_n), intercellular CO₂ concentration (C_i), stomatal conductance (g_s), and transpiration rate (E) serving as key indicators of photosynthetic efficiency and water-use dynamics. These traits reflect the balance between carbon assimilation, water loss, and energy dissipation, directly influencing biomass accumulation and stress tolerance. Assessing how PGPMs and AM fungi modulate these processes under controlled conditions provides valuable insights into their contribution to growth promotion and stress mitigation.

In parallel, biochar has gained attention for its ability to improve substrate aeration, nutrient retention, and water-holding capacity, potentially enhancing the effectiveness of microbial inoculants (Premalatha et al., 2023) (Lehmann et al., 2011). While numerous studies have examined PGPB, AM fungi, and biochar in soil-grown plants, their combined effects in hydroponics remain insufficiently understood. Hydroponic systems provide a unique opportunity to disentangle microbial contributions to physiological processes, since root–microbe interactions occur in a precisely controlled environment rather than soil.

An underexplored dimension of microbial influence is thermal regulation at the leaf level. Leaf temperature is a critical determinant of photosynthetic performance, water conservation, and stress resilience, as it reflects the balance between absorbed radiation, transpiration, and tissue hydration (Jones, 2013) (Begum et al., 2022). While microbial inoculants are known to improve water relations and stomatal function, their potential role in modulating leaf temperature dynamics has not been systematically investigated, particularly under hydroponic conditions.

In this study, we investigated the physiological and growth responses of *Vigna radiata* L grown in hydroponic culture to bacterial inoculants (BB), arbuscular mycorrhizal fungi (AM), their combination (BA), and a triple treatment including biochar (BiB). The specific objectives were to:

1. Assess the effects of microbial inoculation and biochar on photosynthetic performance, carbon assimilation, and water-use efficiency.
2. Examine the potential role of PGPBs and AM fungi in thermal regulation by evaluating leaf-level physiological traits.
3. Determine how these physiological changes translate into biomass accumulation and allocation.

By integrating microbial and biochar treatments under controlled hydroponic conditions, this study provides mechanistic insights into how microbial consortia influence photosynthesis, water relations, and growth. Comparing bacterial and fungal inoculants allows us to distinguish their contrasting contributions: Evaluating these mechanisms both individually and in combination offers a novel perspective on how microbial partnerships can be strategically employed to improve crop resilience and productivity under resource-limited conditions.

Materials and Methods

Seed sterilization, inoculation, and growth conditions

Seeds of *Vigna radiata* L., representing a local germplasm traditionally cultivated in the region, were collected from Ashkan Village, Alan Rural District, Sardasht County, West Azerbaijan Province, Iran. A seed sample was deposited at Zist Asa Clinic, Urmia, where seed identification and collection were confirmed (Figure 1).

V. radiata L. seeds were surface-sterilized in 5% (v/v) sodium hypochlorite for 20 minutes, then rinsed three times with distilled water. The seeds were divided into five groups: control (no inoculation), bacterial biofertilizers (BB), arbuscular mycorrhizal fungi (AM), a combination of BB and AM (BA), and a combination of BB, AM, and biochar (BiB). Seeds were transplanted into 17 × 18 cm pots containing a 1:1 mixture of river sand and perlite. River sand was washed and sterilized by autoclaving at 120 °C for 20 minutes. Plants were grown under controlled conditions with 7,000 lux light intensity from a combination of red and blue TD lamps, 2/3 strength Hoagland nutrient solution, and temperatures ranging from 17 °C (minimum) to 33 °C (maximum).

Preparation of microbial inoculants

The BB treatment included nitrogen-fixing bacteria (*Pantoea agglomerans* O4), phosphorus-solubilizing bacteria (*Pseudomonas putida* P13, *P. agglomerans* P5), potassium-solubilizing bacteria (*P. vancouverensis* S19, *P. koreensis* S14), and zinc/iron-dissolving bacteria (*P. japonica* FZ.21-1, *P. japonica* FZ.29-1). AM fungi comprised *Glomus etunicatum*, *G. mosseae*, and *G. intraradices*. BA combined BB and AM inoculations, while BiB included BB, AM, and biochar. All microbial inoculants were sourced from Green Biotech Incorporation.

Biochar preparation and analysis

Dried apple wood was pyrolyzed at 550 °C for 5 h under anaerobic conditions to produce biochar (Malaie et al., 2025). The biochar was then ground and sieved to obtain particles smaller than 2 mm for use in BiB treatment. A 1:20 (w/v) biochar:distilled water suspension was prepared and shaken for 2 h at room temperature to measure pH and electrical conductivity (EC) using a Hanna conductivity meter (HI 8819, Portugal) and a Corning pH meter 7 (UK). Ash and volatile matter were determined in a muffle furnace following standard methods, and organic matter content was calculated using the loss-on-ignition method (Abbasi et al., 2023). Total nutrients (N, P, K) were measured after acid digestion with HNO₃/HClO₄ mixture (3:1) in a perchloric-acid-rated fume hood. The resulting biochar had a pH of 8.35, EC of 0.79 dS/m, organic matter content of 53 %, total N of 0.9 %, P of 0.23 %, K of 0.41 %, and a C/N ratio of 58.8.

Physiological and growth measurements

Three weeks after planting, gas exchange parameters were measured on the terminal leaflet of the third trifoliate leaf using a Walz HCM-1000 photosynthesis system. Net photosynthesis rate (Pn, $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), stomatal conductance (gs, $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), transpiration rate (E, $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), intercellular CO₂ concentration (Ci, ppm), and leaf temperature (T leaf, °C) were recorded simultaneously. Instantaneous water-use efficiency (WUEins) and intrinsic water-use efficiency (WUEint) were calculated as Pn/E and Pn/gs, respectively. Measurements were conducted under ambient CO₂ (~380 ppm), chamber temperature of 25–26 °C, relative humidity of 50–55 %, and photosynthetically active radiation (PAR) of 1700–1800 $\mu\text{mol m}^{-2} \text{ s}^{-1}$. Leaf area (LA) of the measured leaflet was determined using a leaf area meter, and relative water content (RWC) was measured according to (Barrs & Weatherley, 1962) (Piřelová et al., 2022). Shoot and root dry weights (SDW, RDW) were recorded after oven-drying at 70 °C until constant weight.

Experimental design and statistical analysis

The experiment was conducted in a completely randomized design with four treatments (BB, AM, BA, BiB) and one control (C), each with three replicates. Data were analyzed using SAS software, and significant differences among treatments were determined by Tukey's HSD test at $\alpha = 0.05$. Figures were created in Microsoft Excel, with different letters indicating statistically significant differences.

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Results

Photosynthetic Gas Exchange

Microbial treatments significantly influenced net photosynthesis (Pn) and related gas-exchange parameters (Figure 1; Table 1). Compared with the control (8.97), BB (12.51) and BiB (13.44) showed the highest Pn, while AM alone (9.63) had a non-significant increase. Intercellular CO₂ concentration (Ci) followed a similar trend, with the highest values in BB (18.25) and BiB (18.6), positively correlating with Pn ($r \approx 0.88$), indicating enhanced photosynthetic activity under these treatments. Transpiration rate (E) was highest in BA (4.03) and BB (3.82), whereas AM showed the lowest E (2.17). Stomatal conductance (gs) mirrored these patterns, with BB and BA showing the highest values (0.14), and AM and control the lowest (0.07).

Water Relations and Water-Use Efficiency

Relative water content (RWC) varied among treatments: AM-treated plants had the highest RWC (0.89), indicating superior hydration, while BB (0.65) and BiB (0.66) showed lower values. Instantaneous water-use efficiency (WUEins) was highest in AM (4.43) and lowest in BA (2.76), reflecting a trade-off between high transpiration and carbon gain. Intrinsic water-use efficiency (WUEint) was also higher in AM (127.68) and BiB (121.45) than in BA (76.17), highlighting the contrasting water-use strategies of bacteria and AM fungi.

Leaf Temperature (LT)

All microbial treatments significantly reduced leaf temperature compared to the control. The BA combination achieved the greatest reduction (25.66 °C), followed by BB (26.34), AM (27.43), and BiB (28.03) (Figure 2). These reductions indicate improved thermal regulation through microbial inoculation.

Leaf Area and Biomass

Microbial inoculation enhanced leaf expansion and biomass accumulation (Figure 1). Leaf area (LA) was highest in BA (16.66 cm²), followed by BiB (16.16), with lower values in control (14.16) and BB (15.2). Shoot dry weight (SDW) and root dry weight (RDW) were also significantly increased under microbial treatments. BA produced the highest biomass (SDW = 3.86; RDW = 2.42), followed by BiB (SDW = 3.37; RDW = 2.26), while AM and BB alone showed moderate gains.

Table 1: Mean comparison of photosynthetic, water relation, thermal, and biomass traits of *Vigna radiata* L under microbial and biochar treatments. Values are means ± SE (n = 3). Different letters within a column indicate significant differences among treatments according to Tukey's HSD test (p < 0.05). Parameters: Pn, net photosynthesis rate; Ci, intercellular CO₂ concentration; E, transpiration rate; g_s, stomatal conductance; LT, leaf temperature; RWC, relative water content; WUE_{int}, intrinsic water-use efficiency; WUE_{ins}, instantaneous water-use efficiency; LA, leaf area; SDW, shoot dry weight; RDW, root dry weight. Treatments: BB, bacterial biostimulant; AM, arbuscular mycorrhizal fungi; BA, BB + AM; BiB, BB + AM + biochar and C, control.

Treatments	Pn	Ci	E	gs	LT	WUEins	WUEint	RWC	LA
C	8.97±0.36d	15.58±0.45b	2.51±0.2b	0.07±0.008b	29.55±0.31a	3.59±0.15ba	114.47±7.86a	0.75±0.01bc	14.16±0.12b
BB	12.51±0.42ba	18.25±0.35a	3.82±0.28a	0.14±0.006a	26.34±0.59bc	3.29±0.2b	86.5±3.18b	0.65±0.03c	15.2±0.15b
AM	9.63±0.3dc	15.66±0.17b	2.17±0.05b	0.07±0.005b	27.43±0.18bc	4.43±0.16a	127.68±5.48a	0.89±0.02a	15.8±0.14b
BA	11.14±0.43bc	17.52±0.37ba	4.03±0.21a	0.14±0.008a	25.66±0.35c	2.76±0.05b	76.17±3.18b	0.79±0.02ba	16.66±0.11a
BiB	13.44±0.55a	18.6±0.66a	3.78±0.37a	0.1±0.008b	28.03±0.15ba	3.59±0.19ba	121.45±3.7a	0.66±0.02c	16.16±0.13a

Table 2 Pearson correlation coefficients among photosynthetic, water relation, thermal, and growth traits of *Vigna radiata* L under microbial and biochar treatments. Parameters: Pn, net photosynthesis rate; Ci, intercellular CO₂ concentration; E, transpiration rate; g_s, stomatal conductance; LT, leaf temperature; WUE_{ins}, instantaneous water-use efficiency; WUE_{int}, intrinsic water-use efficiency; RWC, relative water content; LA, leaf area; SDW, shoot dry weight; RDW, root dry weight.

Traits	Pn	Ci	E	gs	LT	WUEins	WUEint	RWC	LA	SDW	RDW
Pn	1	0.98	0.81	0.62	-0.41	-0.37	-0.25	-0.74	0.49	0.53	0.55
Ci	0.98	1	0.91	0.75	-0.5	-0.55	-0.43	-0.77	0.49	0.59	0.57
E	0.81	0.91	1	0.90	-0.63	-0.83	-0.72	-0.66	0.53	0.70	0.61
gs	0.626	0.75	0.90	1	-0.83	-0.81	-0.90	-0.47	0.47	0.66	0.56
LT	-0.41	-0.5	-0.62	-0.83	1	0.48	0.74	-0.02	-0.71	-0.79	-0.77
WUEins	-0.37	-0.55	-0.83	-0.81	0.48	1	0.89	0.47	-0.25	-0.51	-0.32
WUEint	-0.25	-0.43	-0.72	-0.90	0.74	0.88	1	0.26	-0.26	-0.5	-0.35
RWC	-0.74	-0.77	-0.66	-0.47	-0.03	0.47	0.26	1	0.16	0.02	0.08
LA	0.49	0.49	0.53	0.47	-0.71	-0.25	-0.26	0.16	1	0.96	0.99
SDW	0.53	0.59	0.70	0.66	-0.78	-0.51	-0.49	0.02	0.96	1	0.97
RDW	0.55	0.57	0.61	0.56	-0.77	-0.32	-0.34	0.08	0.99	0.97	1

Correlation Analysis

Correlation analysis revealed several significant relationships among physiological traits (Table 2). Net photosynthesis (Pn) was positively correlated with Ci (r≈0.88), E (r≈0.82), gs (r≈0.80), LA (r≈0.85), and SDW (r≈0.84), but negatively correlated with LT (r≈-0.73), RWC (r≈-0.65), and WUE_{ins} (r≈-0.60). WUE_{ins} and WUE_{int} were inversely related to transpiration and leaf temperature, indicating a balance between carbon assimilation and water conservation. These patterns indicate that bacterial inoculation primarily promotes photosynthesis and evaporative cooling, whereas AM fungi enhance hydraulic stability and water-use efficiency, with BA and BiB combining these complementary mechanisms.

Discussion

This study demonstrates that bacterial biostimulants (BB), arbuscular mycorrhizal fungi (AM), their combination (BA), and the triple combination with biochar (BiB) produce distinct, and in some cases complementary, effects on photosynthesis, water relations, leaf temperature, and biomass of *V. radiata* L. grown in hydroponic culture. Key patterns are: (1) BiB and BB produced the highest instantaneous photosynthetic rates (Pn: BiB 13.44, BB 12.51), (2) AM greatly improved tissue hydration and water-use efficiency (RWC 0.89, WUE_{ins} 4.43, WUE_{int} 127.7), (3) BA delivered the largest gains in leaf area and both shoot and root dry weight (LA 16.66 cm², SDW 3.86 g, RDW 2.42 g), and (4) Clear trade-offs emerged, as treatments that maximized biomass did not necessarily optimize water-use efficiency. Together, the responses indicate complementary functional roles for bacteria, AM fungi, and biochar: bacteria drive stomatal and gas-exchange stimulation, AM fungi improve hydraulic status and efficiency, and biochar shifts physiology toward higher photosynthetic capacity and intrinsic WUE.

Photosynthetic Performance and Carbon Assimilation

The physiological responses of *V. radiata* L. to microbial inoculation revealed two complementary routes to improved carbon gain. BiB (13.44) and BB (12.51) exhibited the highest photosynthetic rates, whereas AM alone maintained lower Pn (9.63) but higher intrinsic water-use efficiency (WUEint). The application of PGPMs can enhance photosynthetic performance in plants (Haider et al., 2022) (Li et al., 2024). This improvement is often attributed to the stimulation of stomatal opening, increasing the uptake of CO₂ from the atmosphere (Del Amor & Cuadra-Crespo, 2012). For example, inoculating sweet pepper with *Azospirillum brasilense* and *Pantoea dispersa* maintained higher stomatal conductance under salt stress (Del Amor & Cuadra-Crespo, 2012), while *Sphingobacterium changzhouense* enhanced photosynthesis and stomatal conductance in maize under drought conditions (Hagaggi & Abdul-Raouf, 2022).

Elevated Pn in BB and BiB aligns with bacterial stimulation of stomatal opening and nutrient-driven enhancement of photosynthetic biochemistry, including improved nitrogen and phosphorus acquisition (Bhattacharyya & Jha, 2012) (Shah et al., 2021). Similarly, microbial inoculants have been reported to enhance the biochemical aspects of photosynthesis by improving the plant's nutritional status (Pérez-Moncada et al., 2024) (Wang et al., 2025). A consortium of AMF, yeasts, and bacteria significantly improved N, P, and K concentrations in strawberry plants, correlating with higher photosynthetic rates under drought (Pérez-Moncada et al., 2024).

In our previous study, PGPMs, except AM alone, significantly increased chlorophyll *a* and *b* contents, indicating that enhanced pigment levels underlie the higher photosynthetic capacity observed in these treatments (Malaie et al., 2024). Other microbial treatments have similarly increased chlorophyll content, providing a larger capacity for light harvesting and contributing to higher photosynthetic efficiency (Li et al., 2024) (Hagaggi & Abdul-Raouf, 2022).

The addition of biochar, particularly in combination with microbial inoculants, can further amplify these positive effects on photosynthesis (Haider et al., 2022). Biochar improves the soil environment by increasing porosity, water availability, and nutrient retention, thus creating more favorable conditions for both root development and microbial activity (Liu et al., 2025). The co-application of biochar and microbes often produces synergistic effects that are greater than either treatment alone (Haider et al., 2022). In our experiment, the BiB treatment further amplified microbial effects by improving nutrient retention, strengthening root–rhizosphere interactions, and increasing substrate aeration (de Souza et al., 2015) (Premalatha et al., 2023).

Hormonal regulation may also play a role in this synergy. Recent work demonstrated that biochar–microbe synergy enhanced auxin-mediated root development and nutrient uptake, thereby boosting photosynthetic efficiency (Mustafa et al., 2025). The combined use of biochar and auxin-producing microbes improved root architecture, leading to more efficient nutrient acquisition and overall enhancement of photosynthetic performance (Mustafa et al., 2025).

Stomatal vs. Non-Stomatal Mechanisms

Different microbial treatments can enhance photosynthesis through distinct mechanisms, which can be broadly categorized as stomatal and non-stomatal (or biochemical) factors.

Stomatal-driven enhancement: Some microbial inoculants primarily increase CO₂ uptake by promoting higher stomatal conductance (Del Amor & Cuadra-Crespo, 2012). While effective, this mechanism makes the plant's carbon gain more reliant on environmental factors such as water status and vapor pressure deficit.

Non-stomatal enhancement: Other treatments improve photosynthesis by targeting biochemical limitations. For instance, a study on *Aronia melanocarpa* found that a microbial inoculant helped the plant avoid a “noon break” in photosynthesis caused by non-stomatal factors, boosting the intrinsic efficiency of the photosynthetic apparatus (Shan et al., 2021). Enhancements in chlorophyll content, nutrient uptake, and photosynthetic enzyme activity also contribute to this category (Li et al., 2024) (Pérez-Moncada et al., 2024).

In our findings, BiB achieved high Pn without the highest stomatal conductance ($g_s = 0.10$ vs. 0.14 for BB and BA), indicating that non-stomatal (biochemical) factors such as increased mesophyll conductance, Rubisco activity, or chlorophyll content significantly contributed to photosynthetic enhancement. In contrast, BB and BA primarily relied on stomatal-driven CO₂ uptake. These mechanistic distinctions suggest that stomatal-driven gains are more sensitive to water status and vapor pressure deficit, whereas non-stomatal enhancements are more resilient under transient stomatal closure. The combination of biochar and microbes, therefore, appears to achieve a balance between these two mechanisms, leading to improved and more stable photosynthetic performance under varying environmental conditions (Mustafa et al., 2025) (Haider et al., 2022).

Thermal Regulation and Water Relations

Microbial inoculations exerted clear effects on leaf temperature (LT), transpiration (E), stomatal conductance (g_s), and relative water content (RWC), revealing distinct, and sometimes complementary, strategies of thermal regulation. RWC, a reliable indicator of tissue hydration and cellular water balance, reflects the leaf's capacity to maintain turgor and sustain metabolic activity (Schonfeld et al., 1988). In our experiment, BB and BA treatments exhibited high g_s and E ($\approx 0.14 \text{ mol m}^{-2} \text{ s}^{-1}$ and $3.8\text{--}4.0 \text{ mmol m}^{-2} \text{ s}^{-1}$, respectively), which translated into lower LT, especially in BA (25.66°C). These results support the role of PGPM in promoting stomatal opening, enhancing evaporative cooling and short-term carbon assimilation (Shah et al., 2021) (Del Amor & Cuadra-Crespo, 2012). At the same time, the high transpiration observed in BB coincided with reduced tissue hydration ($\text{RWC} \approx 0.65$), illustrating the trade-off between evaporative cooling and maintenance of leaf water status.

By contrast, AM-inoculated plants sustained high RWC (0.89) despite low g_s and E ($\approx 0.07 \text{ mol m}^{-2} \text{ s}^{-1}$ and $2.17 \text{ mmol m}^{-2} \text{ s}^{-1}$) and a moderate LT (27.43°C), suggesting that AM-mediated thermal regulation relies less on stomatal evaporative cooling and more on improved whole-plant water relations, e.g., enhanced root hydraulic conductivity and osmotic adjustment (Augé, 2001). AM symbioses have been shown to upregulate aquaporin expression and alter root hydraulic architecture, thereby sustaining leaf water potential and allowing more conservative stomatal behavior without a catastrophic loss of photosynthetic capacity (Choudhary et al., 2019) (Quiroga et al., 2017) (Zhang et al., 2021).

Co-inoculation (BA) integrated these complementary strategies: BA achieved the lowest LT (25.66 °C) while maintaining relatively high RWC (0.79) and elevated transpiration (4.03 mmol m⁻² s⁻¹), indicating a synergistic balance between stomatal cooling and hydraulic support. This synergy likely reflects coordinated improvements in nutrient status, hormonal signalling, and root–microbe interactions that allow stomata to operate more effectively without excessively draining tissue water reserves (Lopes et al., 2019) (Begum et al., 2022).

Mechanistically, PGPB and AM fungi regulate g_s through multiple, partly overlapping pathways. PGPBs modulate phytohormones (ABA, cytokinins, auxins), produce ACC-deaminase, solubilize minerals, and stimulate osmolyte synthesis, all of which can shift stomatal sensitivity and plant water use toward a more favourable carbon–water balance under stress (Liu et al., 2025) (Gupta et al., 2022) (Timofeeva et al., 2024) (Fiodor et al., 2021) (Fanai et al., 2024) (Tiwari et al., 2017) (Riseh et al., 2021). Some reports further suggest that certain bacteria alter ABA signaling or its sensitivity in leaves, giving plants a finer control of stomatal aperture under fluctuating water availability (Tiwari et al., 2017) (Riseh et al., 2021). At the guard-cell level, microbial partners promote osmolyte accumulation and antioxidant activity that maintain guard-cell turgor and responsiveness. Microbial inoculation has also been associated with changes in stomatal anatomy (density and pore traits) and improved leaf thermal regulation via enhanced transpiration cooling (Chandrasekaran et al., 2014).

The regulation of leaf temperature by microbial inoculants is an emerging and rapidly developing field. While transpiration is the primary driver of leaf cooling (Schymanski et al., 2013), recent studies using thermal imaging and physiological assays have directly shown that PGPB consortia can lower leaf temperature under water stress by sustaining stomatal opening and improving plant water status (Zhang et al., 2021) (Mahreen et al., 2023). Conversely, AM fungi appear to buffer thermal extremes by improving osmotic adjustment (e.g., accumulation of glycine betaine and other compatible solutes) and by increasing water uptake capacity, which together maintain tissue hydration during heat and drought episodes (Mamun et al., 2024) (Singh et al., 2023). The complementary functions of PGPB (evaporative cooling) and AMF (hydration-based buffering) explain why co-inoculation frequently produces additive or synergistic benefits for leaf energy balance, photosynthesis, and growth across taxa (He et al., 2024) (Fiodor et al., 2021).

Limitations remain: the magnitude and direction of microbial effects depend on strain identity, host genotype, environmental context, and the timing of inoculation (Meza et al., 2025) (Lopes et al., 2019). Some studies report no synergy under certain conditions, underscoring the importance of matching microbial consortia to crop and environment. Nevertheless, our hydroponic demonstration that a combined AMF + PGPB treatment (BA) lowers leaf temperature more effectively than single inoculants, while also protecting tissue hydration and supporting transpiration-driven cooling, represents a novel contribution to the literature (Begum et al., 2019). It provides direct evidence that microbial consortia can re-balance the leaf energy budget through integrated hydraulic and stomatal mechanisms, offering a promising strategy to enhance crop resilience to concurrent heat and water stress.

Growth Responses: Leaf Area, Shoot and Root Biomass, and Allocation

Growth responses of *V. radiata* L paralleled the observed physiological and thermal adjustments. Co-inoculation with AM fungi and PGPB consistently produced the greatest leaf area and total biomass, surpassing single inoculants, while uninoculated controls remained lowest. The addition of biochar further strengthened these effects, yielding substantial increases in both shoot and root dry matter. These findings highlight the synergistic benefits of combining microbial agents, which integrate complementary mechanisms to maximize biomass accumulation (Begum et al., 2022; Naik et al., 2019; Zeng, 2025; Sabannavar, 2011; Subhashini, 2013).

PGPB-mediated growth promotion was primarily driven by nutrient mobilization, phytohormone production (e.g., auxins), and enhanced carbon assimilation, which directly promoted leaf expansion and shoot biomass accumulation (Maheshwari & Dheeman, 2019) (Begum et al., 2022) (Naik et al., 2019) PGPBs can also improve water-use efficiency and modulate stress-responsive hormone signaling, further supporting shoot growth under adverse conditions (Gupta et al., 2022) (Fanai et al., 2024).

AM Fungi-mediated growth promotion contributed through improved water uptake, enhanced root hydraulic conductivity, and extensive hyphal networks that increased nutrient foraging, particularly for less mobile elements such as phosphorus (Latef et al., 2016) (Chandrasekaran et al., 2014) (Varma et al., 2017). These mechanisms supported root development, maintained tissue hydration under stress, and facilitated more stable physiological performance.

When AMF and PGPB were combined, their effects were often additive or synergistic, leading to both large leaf area and balanced shoot–root allocation (Lopes, 2019; Pierre, 2014; Primieri, 2021). The inclusion of biochar further amplified these benefits by improving soil physical and chemical properties, such as porosity, nutrient retention, and rhizosphere health, which enhanced microbial activity, nutrient uptake, and photosynthetic performance (Premalatha et al., 2023) (Mustafa et al., 2025) (Liu et al., 2025). For example, biochar combined with auxin-producing microbes significantly increased biomass and grain yield in canola by up to 203% and 212%, respectively (Mustafa et al., 2025).

Overall, the observed strong correlations among photosynthesis, transpiration, water-use efficiency, and growth parameters confirm that microbial treatments influence biomass production through integrated physiological, biochemical, and structural adjustments rather than through isolated effects. These findings reinforce the concept that co-inoculation strategies, particularly when paired with biochar, provide a robust and synergistic avenue to enhance crop growth and resilience under variable environmental conditions.

Conclusion

This study demonstrates that co-inoculation of PGPB and AM fungi (BA), with or without biochar (BiB), enhances photosynthesis, water-use efficiency, and biomass in *V. radiata* L. through complementary and synergistic mechanisms, with a pronounced effect on thermal regulation. BA achieved the largest leaf area and shoot and root dry weights, integrating stomatal-driven CO₂ assimilation with improved hydraulic support. PGPB (BB) and BiB maximized net photosynthesis, while AM fungi independently improved tissue hydration and intrinsic water-use efficiency.

Importantly, co-inoculation effectively lowered leaf temperature (25.66°C) by integrating stomatal cooling via PGPB with hydration-based buffering by AM fungi, demonstrating a novel microbial-mediated pathway for thermal regulation. Correlations among photosynthesis, transpiration, leaf area, and biomass confirm that growth gains arise from integrated physiological adjustments rather than isolated traits. Biochar further amplified these effects by enhancing rhizosphere function and nutrient availability. Overall, microbial consortia, particularly with biochar, optimize carbon gain, water balance, and leaf energy management, providing a mechanistic framework for improving legume productivity under variable thermal conditions.

Declarations

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Figures



Figure 1

Seeds of *Vigna radiata* L. used in this study

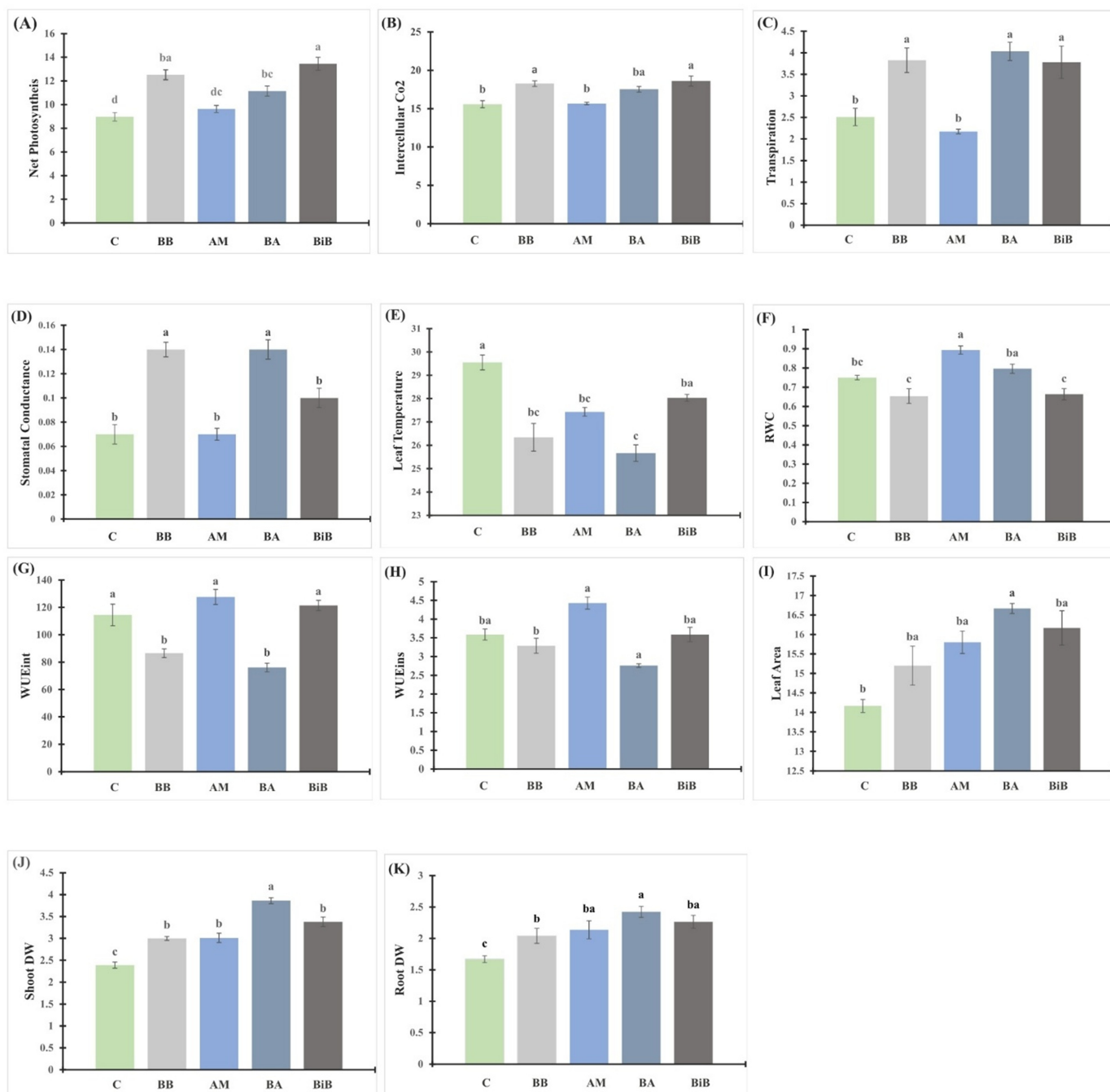


Figure 2

Photosynthesis and growth responses of *Vigna radiata* L under different treatments. Parameters: (A) net photosynthesis rate (Pn, $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$); (B) intercellular CO_2 concentration (Ci, $\mu\text{mol mol}^{-1}$); (C) transpiration rate (E, $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$); (D) stomatal conductance (g_s , $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$); (E) leaf temperature (LT, $^{\circ}\text{C}$); (F) relative water content (RWC); (G) intrinsic water use efficiency (WUEint, $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$); (H) instantaneous water use efficiency (WUEins, $\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$); (I) leaf area (LA, cm^2); (J) shoot dry weight (SDW, g plant^{-1}); (K) root dry weight (RDW, g plant^{-1}). Treatments: BB = bacterial biofertilizers; AM = arbuscular mycorrhiza; BA = BB + AM; BiB = BB + AM + biochar; and C = Control.