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Effect of coal ash fly zeolite and *Bacillus subtilis* on water-use efficiency of chickpea grown under water deficit

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Chickpea cultivation may be considered strategic for improving sustainability in Mediterranean cropping systems; however, global warming and drought may pose limitations to yield stability. Soil amendments, such as zeolite (Z), and microbial inoculation with plant growth-promoting bacteria (PGPB) are indicated as potential strategies to mitigate water deficiency. Still, their combined application has been little investigated, especially in pulse crops. To evaluate the effects of synthetic zeolites, PGPB inoculation, and water deficit on chickpea, a pot experiment was carried out under controlled conditions. Two chickpea genotypes (Pascià and Sultano) were subjected to a factorial combination of 2% soil-added zeolite and PGPB under optimal and limiting water conditions (100 and 50% of the water requirement, respectively). The results showed that water supply strongly influenced crop response, with a reduction of about 50% in grain yield under water deficit. Treatments with zeolite and PGPB (*Bacillus subtilis*) inoculation led to a significant increase in yield and water-use efficiency (WUE) on both chickpea genotypes under water deficit, and their combination further improved harvest index. Under non-limiting water conditions, the increase in WUE was significant with PGPB inoculation. An increase in protein content (PC) was also observed in plants treated with zeolite under water-deficit conditions, while grain total phenolic content (TPC) was negatively influenced by water supply. Overall, agronomic treatments mitigated the effects of water deficit: zeolite improved harvest index and nitrogen uptake while PGPB enhanced WUE. Their combined use appears promising as a strategy to alleviate the negative impacts of water deficit on chickpea cultivation.

KEYWORDS

drought, PGPB, pulses, soil amendment, WUE

1 Introduction

The intensification of global food production systems is increasingly constrained by limited water availability and soil degradation. Pulses represent valuable legume crops for both human nutrition and sustainable agricultural systems due to their ability to fix atmospheric N_2 (Duranti, 2006; Kumar et al., 2025). Within pulses, chickpea (*Cicer arietinum* L.) is mostly cultivated in Asia (India), Australia, and in general temperate dry environments such as the Mediterranean basin (Choudhary et al., 2024). Drought and water limitations are recurring more frequently, making crop cultivation critical in vulnerable regions (Singh et al., 2014; Tang et al., 2025). Enhancing the water-use efficiency (WUE) of chickpea is, therefore, a critical research priority to ensure food security in water-scarce environments (Amiri et al., 2021). In water-limited conditions, deficit irrigation — where crops receive reduced water input without substantial yield loss—results in a sustainable water management strategy (Pendergast et al., 2019; Choudhary et al., 2024). However, optimizing plant performance under water deficit requires agronomic approaches that mitigate water stress while maintaining crop productivity. Soil amendments and microbial bioinoculants have been explored as interesting tools, with promising outcomes in various crop species (Ma et al., 2022; De Santis et al., 2023; Garbowski et al., 2023; Caldara et al., 2024).

Zeolites (Z) are crystalline aluminosilicate minerals with a microporous framework characterized by interconnected channels and cavities. They can occur naturally or can be synthesized from pure chemical precursors in laboratory conditions (Asgar Pour et al., 2023) or can be obtained from natural raw materials and industrial by-products such as coal fly ash, a residue generated during coal combustion. Numerous studies have documented the transformation of fly ash into zeolite (Belviso et al., 2016; Belviso, 2018) and the effectiveness of these synthetic materials without the need for additional treatments or purification steps (Zhang et al., 2019; Ren et al., 2022; Buzukashvili et al., 2024). In a previous study (Belviso et al., 2015), leaching tests were also carried out to evaluate how the zeolitization process affects the mobility of potentially toxic elements present in fly ash, using solutions spanning a wide pH range. The results showed low solubility for most of these elements and, more importantly, a significant reduction in their mobility in the resulting zeolitic materials, confirming that zeolitization is an effective method for reducing the hazard associated with this type of waste. Their application as a soil amendment has been shown to modify soil physical properties, reduce water loss through evaporation, and improve nutrient retention, thereby ameliorating the negative impacts of water deficit on crop growth (Fletcher et al., 2017; Satriani et al., 2024). The role of zeolite in improving soil water-holding capacity is particularly relevant in the context of deficit irrigation, as it can extend the availability of soil moisture to crops, potentially stabilizing plant water relations during periods of reduced water supply (Mondal et al., 2020; Szerement et al., 2021; Castronuovo et al., 2023). In the agronomic field, the most commonly used dosages range from 1% to ~4–5% (Nakhli et al., 2017; Jarosz et al., 2022), with 2% as the optimal rate for crop growth based on previous studies (Belviso et al., 2022; Castronuovo et al., 2023).

Concurrently, plant growth-promoting bacteria (PGPB) have been extensively studied for their ability to enhance plant performance under abiotic stresses, including drought (Khan et al., 2019; Laranjeira et al., 2021; Bisht et al., 2024; El-Saadony et al., 2024). Among these, *Bacillus subtilis* is recognized for its multiple interactions with plants, including the enhancement of nutrient uptake, production of phytohormones such as indole-3-acetic acid (IAA), and the induction of systemic resistance mechanisms (Etesami et al., 2023). The inoculation of crops with *B. subtilis* has been shown to promote root development, increase water-use efficiency, and improve the physiological responses of plants to water stress by modulating stress-responsive genes and antioxidant enzyme activity (Etesami et al., 2023; Moraes et al., 2025).

Although the individual effects of zeolite application and *B. subtilis* inoculation on crop performance have been well documented, little research has examined their combined application, particularly in chickpea under deficit irrigation regimes. It is hypothesized that integrating these two strategies could produce synergistic effects, whereby zeolite enhances soil water retention and nutrient availability, while *B. subtilis* further supports plant growth by improving nutrient uptake, root architecture, and stress tolerance. Such synergistic effects could lead to improved water-use efficiency, enhanced drought tolerance, and increased grain yield in chickpea under water-deficit conditions, in the framework of the Climate Action Sustainable Development Goal.

This study aims to evaluate the combined effects of coal fly ash, zeolite, and PGPB on chickpea growth, yield, and water-use efficiency under both full and deficit-irrigation conditions. By elucidating the potential synergistic interactions between these two interventions, the research seeks to contribute to the development of sustainable agronomic practices that enhance crop resilience to water scarcity, offering novel insights for improving agricultural productivity in drought-prone regions.

2 Materials and methods

2.1 Experimental growing conditions

A pot experiment was conducted in a custom-made growth chamber (about 5.5 m^3, Piardi Tecnologie del Freddo s.r.l., Castenedolo, BS, Italy), under controlled conditions of temperature, humidity, and photoperiod (Giuliani et al., 2014) to evaluate the response of chickpea treated with zeolite, PGPB, and their combination under water deficit. Growing conditions are reported in Figure 1, with day length and temperature adjusted according to crop development, Rh at 70% and a photon flux density of 500 µmol/m^2/s. A complete randomized block design, with three replicates, was adopted with a factorial combination of two genotypes and four agronomic treatments consisting of the application of zeolite (Z), a PGPB strain, their combination (Z + PGPB), and an untreated control (CTR), under two irrigation levels, for a total of 48 pots. The two water regimes included a full and a deficit level, at 100 and 50% of the irrigation requirement, respectively. In particular, through gravimetric measurements of the pots, the soil was maintained at field capacity with periodic water replenishments at the full irrigation level and at

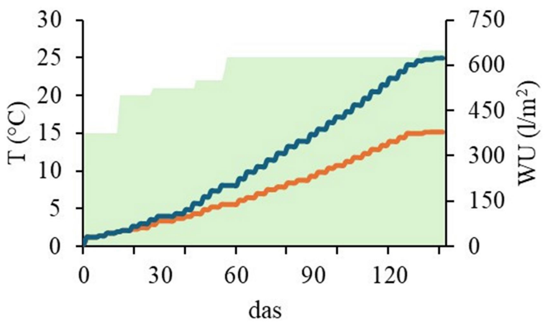

FIGURE 1
Growing conditions from sowing to maturity in terms of mean temperature (green histograms) and cumulative water use (WU) of full (blue line) and deficit water supply (orange line).

50% of the full irrigation level in the deficit level. Genetic material consisted of two genotypes: a large seed cultivar (Pascià) and a small seed one (Sultano), previously characterized in field trials and showing a good adaptability to water deficit (Ruggeri et al., 2017; De Santis et al., 2021, 2022).

The soil was classified as loam consisting of 32.5% sand, 42.3% silt, 25.2% clay, 32.0% field capacity and 14.5% wilting point, 1.18 g/cm^3 bulk density, 7.9 pH, 361 mS/cm EC, 0.191% total N, 67.2 ppm available P, 585 ppm exchangeable K, and 1.91% organic C. Treated soil was amended with 2% of synthetic zeolite (on dry weight basis) previously characterized (Belviso et al., 2022). Hydraulic parameters, including field capacity, wilting point, and available water content of the soil before and after the addition of zeolite, are reported in Table 1. The choice to add zeolite at a rate of 2% was guided by the results of previous experiments which demonstrated that larger amounts of this synthetic material (also depending on soil type) affect the porous and hydrophysical parameters of soil, resulting in a lack of water for plants and, consequently, negative effects on crop growth (Belviso et al., 2022); moreover, the previous characterization of this zeolitic material by leaching tests (Belviso et al., 2015) indicated a very low mobility of trace elements (As, Cd, Co, Cr, Cs, Cu, Mn, Ni, Pb, Se, V, and Zn), thus permitting the use in this new type of tests. A commercial PGPB was adopted (*B. subtilis* QST 713, Serenade ASO, Bayer Crop Science, Italy) through plant–soil spray inoculations at a rate of 10^8 colony-forming unit (CFU)/m^2, 2 times at 16 and 30 days after sowing (das). Sowing was carried out on 30 December 2023 with a final density of two plants per pot. Final mean water use (WU) was 622 and 356 L/m^2 for full and deficit irrigation, respectively. Nitrogen and phosphorus were supplied at a rate of 2.5 and 5.0 g/m^2 as ammonium nitrate and triple superphosphate, respectively.

2.2 Crop measurements

Crop growth was assessed by plant height (PH) throughout the crop cycle, with measurements taken from early development to harvest. Flowering was first recorded in both genotypes at 55 das (~1,000 °C d), according to previous observations (De Santis et al., 2021, 2022). At maturity (142 das), plant biomass was harvested, separated into root, straw, and grains, and oven dried (60 °C; 72 h). Dry weight biomass and mineral accumulation were determined in each plot and expressed as g/m^2. Harvest index (HI) was calculated as the ratio of grain yield (GY) to total above-ground biomass. Grain weight (GW) was also determined. Grains and straw were milled (Tecator, 1,093 Cyclotec; Foss Italia, Padova, Italy) using 1.0-mm sieves for analysis. N concentration was determined (elemental analyzer LECO) in grains and straw, and multiplied by their biomass to determine N uptake. The nitrogen harvest index (NHI) was determined by dividing grain N uptake by total plant uptake. Grain protein content (PC) was determined by multiplying grain N concentration × 6.25. Water-use efficiency (WUE) was determined as the ratio of GY to WU and expressed as g/m^2/l.

TABLE 1 **Soil hydraulic properties: water content at field capacity and at the permanent wilting point, and available water content of the soil before and after zeolite addition.**

Soil treatments	FC	WP	AWC
	%	%	%
CTR (0% zeolite)	32.0	14.5	17.5
Z (2% zeolite)	36.2	14.9	21.3

CTR, control; Z, zeolite; FC, field capacity; WP, wilting point; AWC, available water content.

2.3 Total phenolic compounds

Analysis of total phenolic content (TPC) was carried out on grains according to (Abou Chehade et al., 2024), with minor modifications. Briefly, 100 mg of chickpea flour, previously obtained after laboratory milling, was suspended in 1 mL of 80% (v/v) methanol. The mixture was sonicated for 30 min and centrifuged (2,000 *g* for 10 min). Total phenolic content (TPC) was determined in the extracts using the Folin–Ciocalteu method and expressed as gallic acid equivalents (mg/g).

2.4 Soil microbiological assessment

Soil samples were collected at half of the crop cycle (71 das), during stem elongation, and at harvest maturity (142 das), and soil microbial analysis was carried out according to the protocol reported in (De Santis et al., 2023). Briefly, 25 g of each sample were diluted with 225 mL of sterile saline solution (0.9% NaCl) and mixed on an orbital shaker at 300 rpm for 30 min; after that, decimal serial dilutions were carried out in saline solution and plated onto appropriate medium to select and count Mesophilic bacteria (Plate Count Agar supplemented with cycloheximide, 0.17 g L^{-1}; 30 °C for 48 h); pseudomonads (Pseudomonas Agar Base supplemented with Pseudomonas Selective Supplement; 25 °C for 48–72 h); spore-formers (Plate Count Agar, after heat-treating the dilutions at 80 °C for 10 min; the plates were incubated at 30 °C for 24 h); actinobacteria (Bacteriological Peptone, 10 g L^{-1}; Beef Extract, 5 g L^{-1}; NaCl, 5 g L^{-1}; Glycerol, 10 g L^{-1}; Agar Technical n. 3, 20 g L^{-1}; pH 7.00–7.20; 22–24 °C for 7–14 days); Enterobacteriaceae (Violet Red Bile Agar, incubated at 37 °C for 18–24 h); total soil microorganisms (PCA, 22 °C for 3–4 days); yeasts (Yeast Peptone Glucose Agar, supplemented with chloramphenicol, 0.1 g L^{-1}; 25 °C for 3–4 days); molds (Potato Dextrose Agar, PDA; 25 °C for 5 days); and nitrogen-fixing bacteria on Brown medium, incubated at 25 °C for 5 days. All media and supplements were from Oxoid (Milan, Italy). A random selection of colonies on plates, microscope examination, Gram staining, a test for spore production, catalase, and oxidase tests confirmed microbiological counts.

2.5 Data analysis

For each irrigation level, analysis of variance (ANOVA) was conducted on the factorial combination of genotype and agronomic treatments, with Tukey's test used as the *post hoc* test. Mean values between the two irrigation levels were also compared by *t*-test. Multiple regression analysis (Pearson) and principal component analysis (PCA) were performed on the correlation matrix of the traits investigated. Microbiological data were initially analyzed using a *t*-test to assess significant differences between the baseline (day 0) and the end of the trial. Subsequently, a preliminary standardization was performed, and the results were expressed as increases or decreases relative to the beginning of the experiment. When the *t*-test indicated a non-significant difference, a value of 0 was assigned as the standardized result. Standardized data were then analyzed using a multiple-correlation approach, with a two-way joining (heatmap) method employed for visualization. Raw data were also analyzed using two-way ANOVA, with the agronomic treatment (control, zeolite, PGPB, and their combination) as categorical predictors and the two water conditions (deficit or full) as the other factor. The decomposition of the statistical hypothesis was used as the output.

3 Results

3.1 Effects of genotype and agronomic treatments on crop performance

An experimental trial was conducted under two water conditions, with cumulative water supplies of 8.6 and 14.1 L/pot (depth 0.2 m), respectively 384 (water deficit) and 627 L/m^2 (full water supply). Crop growth duration was 142 days from sowing to maturity (Figure 1), with no imposed heat stress during terminal growth stages, allowing us to focus on the effect of water deficit. The growth trend, expressed as plant height (PH), under the two different water regimes is shown in Figure 2. Under both conditions, the two genotypes showed a comparable PH up to 62 days, after that the growth rate of Sultano was higher up to maturity (Figure 2), with a generally higher rate under full water supply. In contrast, the use of soil amendments and microbial biostimulants did not affect growth rate and PH under both water supplies. Mean root biomass was also higher under full irrigation. As regards productive and quality traits, analysis of variance showed a general significance of the effect of the genotype, while the effect of the agronomic treatments was more frequent under water-deficit conditions (Supplementary Table S1); the interaction between genotype and treatment was generally not significant. On average, water deficit led to a significant reduction in GY

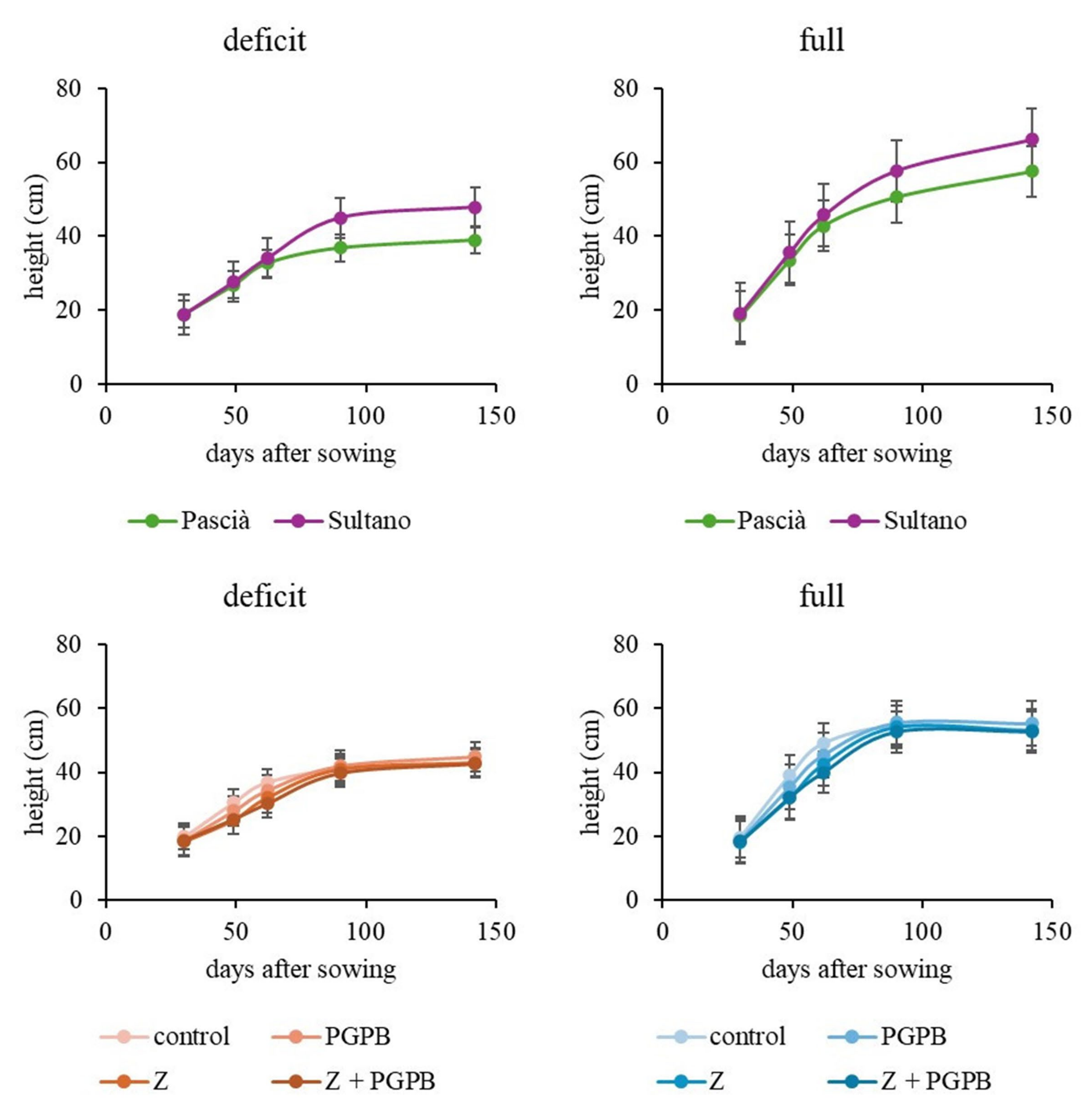

FIGURE 2
Plant height from leaf expansion to harvest in response to genotype, zeolite (Z) and plant growth-promoting bacteria (PGPB) treatments under deficit and full water supply.

(−50%), N uptake and water-use efficiency (WUE); in fact, GY was about 2 times higher under full irrigation, in a range comparable to other observations in open field trials (from 73 to 254 g/m^2); in contrast, a higher total phenolic content in grains (TPC) was observed under water deficit (Table 2).

3.1.1 Genotype response

While under water deficit, no significant difference between the two genotypes was observed in GY, in full water supply, Sultano showed a significantly higher productivity than Pascià (Table 3). A complementary variation between the two major yield components considered was observed, with Pascià characterized by higher grain weight (GW) and lower harvest index (HI), under both water conditions. As regards root, a higher mean biomass was observed in Pascià than Sultano (0.344 g/plant, equivalent to 30.6 g/m^2 in Pascià vs. 0.196 g/plant, equivalent to 17.4 g/m^2 in Sultano, $p < 0.05$), while no significant differences were found at full irrigation (0.516 g/plant, equivalent to 45.9 g/m^2 in Pascià vs. 0.503 g/plant, equivalent to 44.7 g/m^2 in Sultano, ns). Regarding nitrogen (N) assimilation, the same genotype showed higher N uptake under water-deficit conditions, whereas under non-limiting water conditions, Sultano showed markedly higher N uptake, in accordance with the higher GY. The same cv. Sultano showed a higher N partitioning in grain (NHI), under both water conditions. In accordance with yield, water-use efficiency (WUE) showed a significant genotypic difference only at full water supply, while under water deficit, the response of the two genotypes was comparable. As regards quality, grain protein content (PC) was higher in Pascià only under water deficit, while, in contrast, total phenolic content (TPC) resulted higher in Sultano under full water supply.

3.1.2 Response to agronomic treatments

The 2% coal fly ash zeolite (Z) soil mixture showed an increase in field capacity (36.2% vs. 32.0%) and wilting point (14.9% vs. 14.5%), with an increase in soil available water from 17.5 to 21.3%. A significant increase in GY was observed for samples treated with Z with respect to the untreated control; however, this effect was significant only under water deficit (Table 3). In contrast, the application of microbial plant biostimulant (PGPB), that is, *B. subtilis*, was associated with a significant increase in GY under both water supplies, as also supported by the increase in aerobic spore-forming bacteria. The increase in yield also occurred in combination with zeolite. While GW was generally unaffected by agronomic treatments, except for a reduction in the Z thesis under full water supply, HI was more influenced. In fact, an increase in HI due to soil Z application was observed, under water deficit, both with and without PGPB; further, in non-limiting water conditions, no significant differences in HI were observed between PGPB and Z + PGPB treatments. WUE, indeed, was generally improved with the application of microbial biofertilizer, in both combinations with Z. No significant effects were observed on root biomass from agronomic treatments. As regards quality, the only effects were relative to PC, which was found to be higher in the Z + PGPB combination with respect to only PGPB, under water deficit; furthermore, no significant effects on TPC were recorded under both water supplies (Table 3).

3.2 Microbial characteristics

Figure 3a shows the viable counts for five representative soil group at the end of the trial. Microbial abundance was generally higher under well-watered conditions, for most groups, except spore-forming bacteria. However, for a better understanding of microbial dynamics, data were further analyzed through a multivariate analysis after standardization and reported an increase/decrease compared to the beginning of the trial (Figure 3b). The color gradient reflects the degree of variation from the initial state, with green indicating increased abundance, red indicating a reduction, and white indicating a non-significant variation.

Under deficit irrigation (Figure 3b), the response of the microbial groups exhibited a clear treatment-dependent pattern. Pseudomonads were among the most negatively affected taxa in the control sample, as indicated by a strong reduction in the viable count (approximately −1.5 log CFU/g), consistent with their known sensitivity to osmotic stress and reduced soil moisture. This decline was considerably attenuated when PGPB were applied (reduction in the range 0.5–1 log CFU/g). In addition, the use of zeolite, either alone or combined with PGPB, counteracted the negative effect of water deficit, as evidenced by values close to zero.

Actinobacteria also showed a decrease under deficit irrigation in the control (≈ -0.5 log CFU/g), confirming the vulnerability of several non-sporulating taxa to low soil water levels. In contrast, the treatments, including zeolite, with or without the application of PGPB, increased in this microbial group (0.5–1 log CFU/g). This suggests that zeolite may enhance aeration and water-holding capacity in the root zone, providing microenvironments suitable for Actinobacteria.

Under full irrigation, the microbial community exhibited greater overall stability than under deficit conditions. Both mesophilic and spore-forming bacteria did not show great changes; instead, they remained largely stable in the control samples, with differences near zero. In several treatments (particularly PGPB and Z + PGPB), slight decreases (0.5 log CFU/g) were observed, suggesting that optimal moisture availability alone may not be sufficient to stimulate these groups beyond baseline levels.

TABLE 2 Mean comparison of the investigated productive and quality traits subjected to deficit (50%) and full (100%) irrigation.

Water	Root	GW	HI	GY	PC	N uptake	NHI	WUE	TPC
	g/plant	mg	%	g/m^2	%	g/m^2	%	mg/m^2/l	mg/g
Deficit	0.270	331	31.9	99	15.4	5.5	48.0	256	1.16
Full	0.509	346	27.0	187	15.2	10.3	48.6	295	0.96
p level	*	ns	ns	***	ns	***	ns	*	*

RB, root biomass; GW, grain weight; HI, harvest index; GY, grain yield; PC, grain protein content; NHI, nitrogen harvest index; WUE, water-use efficiency; TPC, total phenolic content. Level of significance: ns, not significant. *Significant at $p \leq 0.05$; **Significant at $p \leq 0.01$; ***Significant at $p \leq 0.001$.

TABLE 3 Effect of genotype and agronomic treatments on the investigated productive and quality traits of chickpea grown under water deficit and full water supply.

Water	Level	GW	HI	GY	PC	N uptake	NHI	WUE	TPC
		mg	%	g/m^2	%	mg/m^2	%	$g/m^2/l$	mg/g
Deficit	Pascià	421 a	26.6 b	93 a	17.3 a	6.6 a	40.3 b	241 a	1.18 a
	Sultano	241 b	37.3 a	104 a	13.4 b	4.5 b	55.8 a	270 a	1.13 a
	CTR	329 a	21.2 c	76 b	14.3 b	5.1 b	35.9 b	198 b	1.17 a
	PGPB	338 a	27.9 bc	105 a	14.0 b	4.7 b	45.2 ab	272 a	1.20 a
	Z	331 a	37.3 ab	100 a	17.4 a	6.2 a	50.0 ab	258 ab	1.08 a
	PGPB + Z	326 a	41.3 a	114 a	17.7 a	6.0 a	61.0 a	294 a	1.18 a
Full	Pascià	443 a	19.2 b	142 b	15.6 a	11.8 a	30.7 b	224 b	0.90 b
	Sultano	249 b	35.7 a	232 a	14.8 a	8.8 b	66.5 a	366 a	1.02 a
	CTR	385 a	23.0 b	164 b	14.4 a	8.5 b	51.0 a	259 b	0.97 a
	PGPB	340 ab	27.3 ab	202 a	16.4 a	12.0 a	46.1 a	318 a	0.96 a
	Z	312 b	29.6 a	189 ab	15.2 a	11.4 a	46.1 a	298 ab	0.97 a
	PGPB + Z	348 ab	30.1 a	193 a	14.7 a	10.3 a	51.2 a	304 a	0.94 a

CTR, control; PGPB, sample treated with plant promoting-growth bacteria; Z, zeolite; GW, grain weight; HI, harvest index; GY, grain yield; PC, grain protein content; NHI, nitrogen harvest index; WUE, water-use efficiency; TPC, total phenolic content. Different letters indicate significant differences at $p < 0.05$ according to Tukey's test.

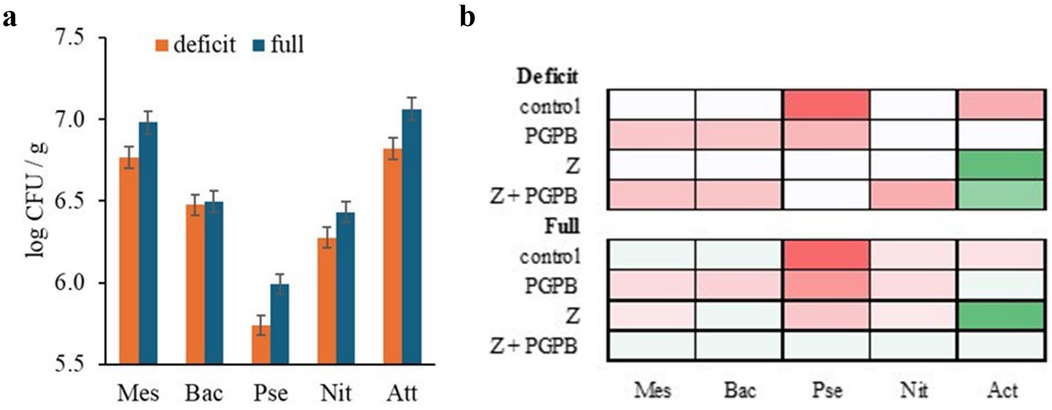

FIGURE 3
(a) Soil microbiological profile as affected by water supply, reported as viable count of colony-forming units (CFU)/g of soil; **(b)** heat map for the increase/decrease of microbiological counts (log CFU/g). Red, decrease in the viable count; green, increase; white, no significant changes. Mes, mesophilic bacteria; Bac., aerobic spore-forming bacteria (bacilli); Pse, pseudomonads; Act, actinobacteria; Nit, nitrogen-fixing bacteria. PGPB, plant growth-promoting bacteria; Z, coal fly ash zeolite.

Actinobacteria displayed a more distinct treatment response under full irrigation. In the control condition, this group slightly decreased (0.5 log CFU/g), possibly reflecting competitive exclusion by faster-growing taxa under high-moisture conditions. However, the presence of zeolite resulted in a marked increase (2 log CFU/g), the highest recorded among all combinations, while the joint application of zeolite and PGPB also promoted a substantial rise (1 log CFU/g).

3.3 Multivariate analysis

Multiple regression analysis was performed among the investigated traits, as reported in Supplementary Table 2. In general, WU showed a significant negative correlation with TPC and a positive correlation with GY, N uptake, PH, and the group of N-fixing bacteria (Nfb); in fact, Nfb showed a good correlation with GY; furthermore, WUE was significantly correlated with HI, NHI, and the soil Pseudomonas bacteria (Pse) content at maturity.

Principal component analysis, performed on the correlation matrix, identified two major components that together explained 74.2% of the observed variability (Figure 4). Component 1 was associated with the variations in yield components and WUE due to the investigated agronomic treatments and referred to "*effect of agronomic treatments on productivity.*" In particular, samples treated with both zeolite and PGPB were distributed in the right part of the biplot, associated with an increase in both HI and NHI, and then GY, especially in cultivar Sultano, characterized by lower GW. In contrast, component 2 was mainly associated with variations in TPC and, secondarily, PC, due to water use (WU) and was referred to as "*effect of water supply on quality.*" In this framework, the co-application of the two investigated treatments

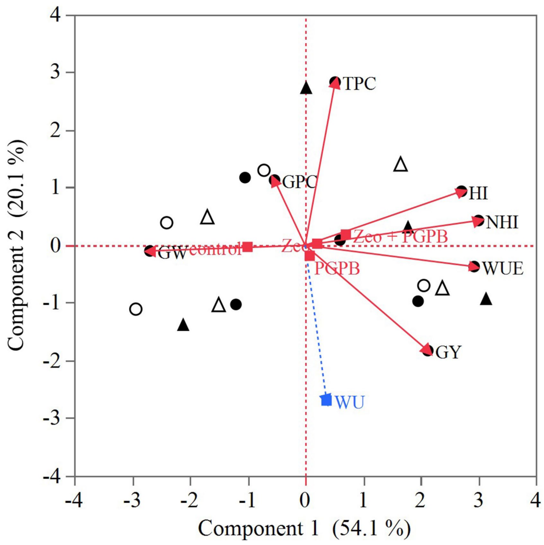

FIGURE 4
Biplot of the principal component analysis (PCA). GW, grain weight; HI, harvest index; GY, grain yield; PC, grain protein content; PGPB, treatment with *B. subtilis*; NHI, nitrogen harvest index; WU, water use; WUE, water-use efficiency; TPC, total phenolic content; Zeo, treatment with coal fly ash zeolite.

emerged, under the current growing conditions, as generally more impactful on productivity traits rather than quality, regardless of water supply, which influenced quality in both genotypes.

4 Discussion

4.1 Crop yield in response to water deficit

Chickpea is generally considered a drought-tolerant pulse crop (Abderemane et al., 2024); however, the response to water supply can affect yield and quality traits, which are favorably welcomed by consumers, such as grain size. The levels of grain productivity observed in the current experiment, even if pot scale, were in a range comparable to the productivity level of chickpea under field conditions, that is, 0.7–2.5 t/ha (López-Bellido et al., 2004; Oweis et al., 2004; Singh et al., 2014), with water deficit causing severe yield loss (Choudhary et al., 2024). Previous observations indicated good adaptability in the two investigated genotypes, although they differed in grain dimensions (De Santis et al., 2021, 2022). However, water deficit limited the genotypic differences in productivity, which resulted in clearer differences under non-limiting water conditions (Samarah et al., 2009; Choudhary et al., 2024). Under the current experimental conditions, without high temperature stress—since the post-anthesis period was set at around 25 °C—genotypic differences in grain productivity were observed only under optimal water supply. This is in accordance with other literature data, which confirms the productive genotype differences between the Pascià and Sultano genotypes, under good growing conditions (Ruggeri et al., 2017). The differences in yield were, in general, correlated with other morphological and crop physiological traits, in particular plant height and harvest index, while grain weight is generally more influenced by genotype effect (Kashiwagi et al., 2006; Keerthi Sree et al., 2023; Tiwari et al., 2023; Pappula-Reddy et al., 2024).

4.2 The effect of zeolite in water deficit mitigation

The interest in the use of zeolite from coal fly ash is also to promote a circular economy by converting a problematic industrial waste into a valuable product, reducing landfill dependency and resource depletion (Querol et al., 2002; Mlonka-Mędrala, 2023). Results obtained in the current study indicated a good efficacy of synthetic zeolite to modify the coarse-textured soil and improve the hydraulic properties, also in loam soil, despite a well-documented ability, especially in sandy soils (Lima et al., 2021; Comegna et al., 2023; Satriani et al., 2024). The trait that benefited the most from zeolite application was the harvest index, suggesting a good mitigation of water deficit, especially in the critical stages defining yield (Pappula-Reddy et al., 2024). A comparable behavior was preliminary observed in other horticulture crops, in which the application of zeolite did not lead to an increase in fresh and dry biomass in the vegetative parts (Castronuovo et al., 2023). The hypothesis by which the use of coal ash fly zeolite might show the best performance under water-deficit conditions seems confirmed in the current experiment. In contrast, under well-watered conditions, the addition of the soil amendment was associated with an increase in N uptake. In terms of soil microbial dynamics, in those conditions, the increase in Actinobacteria, generally linked to nitrate assimilation, might have contributed to promoting the increase in crop N uptake (Zhang et al., 2024). In contrast, zeolite applications in the field have recently been reported to improve marketable yield in tomato, possibly indicating a higher benefit on crop reproductive parts (Conversa et al., 2024).

4.3 The use of PGPB to improve water productivity

The effects on water-use efficiency or crop productivity are, however, more complex, with the biofertilization resulting more promising, both under different water conditions. In fact, the inoculation of PGPB, in particular *bacilli*, has been recently reported to improve crop productivity in chickpea (Elkoca et al., 2007; Yadav and Verma, 2014; Khan et al., 2019, 2021), and in our study, this resulted in higher harvest index and water-use efficiency. From a microbial ecology standpoint, the application of *B. subtilis* QST 713 significantly modulated soil microbial communities, as demonstrated by multivariate analyses. The use of PGPB resulted in an increase of spore-forming bacilli, mainly with zeolite, across both irrigation regimes, indicating successful colonization and persistence, which are basic requisites for a biofertilizer (Etesami et al., 2023). A result of concern was found for deficit irrigation conditions, as in this case, the use in combination of zeolite and PGPB exerted a sort of "stabilizing effect" on microbial communities, mainly on actinobacteria and Pseudomonas; these taxa are often associated with stress mitigation and root health (Martins et al., 2023; Chen et al., 2024; Mikiciuk et al., 2024). In addition, zeolite appeared to counteract declines in some groups, suggesting that it could act as a physical buffer by improving water retention and microhabitat stability, and, as a consequence, could protect and/or enhance the ecological niches of beneficial microbes. This evidence confirms the suitability of zeolite for soil health (Hernández et al., 2024).

4.4 Factors affecting chickpea quality

Quality traits showed greater sensitivity to growing conditions, particularly water availability. Under the current experimental conditions, water supply and, secondarily, genotype, showed an impact on the investigated quality traits, with the agronomic treatment showing a major effect on productivity. Protein content in chickpea is generally a stable trait, whereas most of the changes occur in protein composition (De Santis et al., 2021). Regarding bioactive compounds, it has been reported that drought-tolerant chickpea genotypes accumulate higher total phenolic content in leaves under water-limited conditions (Kasbi et al., 2024). In fact, secondary metabolites, including phenolic compounds, are generally produced by plants in response to biotic or abiotic stress (Jameel et al., 2021; Quiroz-Figueroa et al., 2022). Hyperosmotic stresses are generally responsible for the increase in phenolic compound accumulation, as also observed in the current study (Asati et al., 2024), while heat temperature may lead to a reduction of phenolic compounds (Jameel et al., 2021); heat and drought might occur especially in Mediterranean regions, increasing the complexity of the response of quality traits to environmental stress (Awasthi et al., 2024; Chimphango et al., 2025). Furthermore, the genotypic variation in phenolic acid content might be explained by the reported higher accumulation of phenolic compounds in small grain chickpea genotypes (Quiroz-Figueroa et al., 2022).

4.5 Factors affecting soil microbiota

The effect on the soil microbiota was linked to agronomic outputs and to the positive modulation of some parameters; these outcomes could result from a combination of factors, including a more favorable rhizosphere environment for plants. It is well known that *B. subtilis* can stimulate root exudation and auxin production, enhancing root architecture and water uptake efficiency (Hakim et al., 2021). Moreover, the positive effect on Pseudomonas and actinobacteria could, in turn, promote a higher synthesis of siderophores or stress-related metabolites (Chen et al., 2024).

However, when focusing on the influence of agronomic inputs on the soil microbiota, it is worth noting that the changes resulted from a combination of factors. In particular, the heatmap reveals clear taxon-specific responses driven by moisture availability and amendment type. Under deficit irrigation, the decrease of pseudomonads and Actinobacteria in the control condition is in line with literature reports on the sensitivity of non-sporulating bacterial groups to reduced water potential and osmotic constraints (Naylor and Coleman-Derr, 2018). However, the application of zeolite—either alone or in combination with plant growth-promoting bacteria (PGPB)—substantially attenuated these negative effects, yielding profiles that approached stability or even positive deviations from the baseline. This pattern could be due to the ability of zeolite to enhance water retention, regulate ion exchange, and buffer fluctuations in soil microenvironments (Belviso, 2025). In addition, the mitigation of the negative effects on soil microorganisms by PGPB could be due to microbial network resilience, resulting from stimulation of rhizosphere activity, although their effects were less pronounced than those of zeolite. Under full irrigation, the overall stability of mesophilic and spore-forming bacteria is in line with the hypothesis that water deficit could be a major driver of microbiota in soil; thus, in well-watered conditions, microbiota tends to be more stable (Manzoni et al., 2012). However, differences were found for Actinobacteria, which experienced an increase with zeolite and PGPB also under full irrigation. This result suggests that some amendments modifying soil structure and aeration could generally favor some taxa (Lehmann and Kleber, 2015), as in the case of Actinobacteria, which are, to the authors' knowledge, slow-growing taxa. The additive effect of PGPB in the zeolite-amended treatment further confirms the importance of multi-component soil amendments in shaping microbial communities.

5 Conclusion

The current study investigated the potential of the single or combined use of two promising tools, such as zeolite from waste coal fly ash and a natural microbial biofertilizer, to improve water-use efficiency under water deficit in chickpea. Both agronomic applications modulated soil microbial characteristics and positively influenced yield response. The use of PGPB improved grain yield under both water-deficient and non-limiting water conditions, suggesting a potential smart use to improve chickpea adaptability across a range of growing conditions. In contrast, the use of zeolite more effectively improved yield only under water deficit, showing its best performance in water-use efficiency in combination with PGPB inoculation. These preliminary results are mainly due to variations in harvest index, more than grain weight. The effects on quality were mostly dependent on water supply and genotype, in general with higher total phenolic content in grains under water deficit. The soil microbial resilience under combined treatments suggests synergy, or at least an additive effect, between the use of zeolite and PGPB. This connection should be further explored using functional metagenomics or metabolomics to elucidate microbial-driven pathways involved in drought adaptation. Overall, the data support the hypothesis that integrated approaches that combine microbial inoculants with soil conditioners might offer a promising strategy for sustaining soil functionality and crop productivity in water-limited environments. However, further field experiments across soils, climates, and longer-term assessments are necessary to validate preliminary observations to improve chickpea environmental and economic sustainability, while also taking into account potential metal accumulation, especially in long-term assessments.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

MD: Supervision, Formal analysis, Resources, Writing – original draft, Visualization, Data curation, Conceptualization, Investigation, Methodology. AS: Writing – review & editing, Conceptualization, Visualization, Methodology. CB: Writing – review & editing, Resources. AL: Resources, Writing – review & editing. AB: Writing – review & editing, Funding acquisition, Formal analysis, Methodology, Data curation, Visualization. Ad'A: Investigation, Writing – review & editing. MC: Funding acquisition, Project administration, Writing – review & editing. LG: Investigation, Writing – review & editing. DT: Investigation, Writing – review & editing. ZF: Funding acquisition, Conceptualization, Project administration, Writing – review & editing.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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