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ORIGINAL PAPER

Woody Biochar Differently Influences Plant Water Status and Growth of Five Quinoa (*Chenopodium Quinoa* Willd.) Varieties Under Water Stress

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Abstract

Only few studies have evaluated the responses of quinoa varieties to biochar amendment under water-limited conditions during the early growth stages. We examined the interaction between soil treated with and without biochar and water stress applied during the vegetative development on water relation and growth of quinoa varieties. Five varieties (Titicaca, Quipu, Regalona, UAFQ7, and Q126) were grown in soil without and with 2% (w/w) woodchip biochar and subjected to two successive water stress cycles, by withholding water until soil reached the permanent wilting point, started from 12-leaf stage. The morpho-physiological attributes, leaf nutrients and total bacterial count were assessed during the experiment. Biochar significantly improved plant growth, water use efficiency (WUE), and yield-related traits across all varieties under water-stressed conditions. Biochar under water-limited conditions enhanced plant water status, indicated by lower pre-dawn water potential and increased relative water content compared to non-treated soil. The UAFQ7 showed superior growth with biochar, producing more leaves and larger leaf areas (1517 vs. 1378 cm²/plant). Titicaca was more sensitive to drought, with a considerable reduction in leaf area (1268 vs. 1386 cm²/plant), fresh and dry biomass (6.4 vs. 7.4 g) compared to well-watered conditions. Quipu produced longer panicles, further enhanced by biochar (17 vs. 12 cm). A significant increase of 17% in WUE was observed in biochar-treated plants compared to non-amended ones. Woodchip biochar seems to mitigate the adverse effects of water stress in quinoa, but the responses vary among varieties.

Keywords *Chenopodium quinoa* Willd. · Drought Stress · Drought Mitigation · Vegetative Growth

1 Introduction

Quinoa (*Chenopodium quinoa* Willd.), a pseudocereal seed crop from the Andean region (Belguet et al. 2024), has garnered global attention as a super and functional food for its superior nutritional profile, including essential amino acids, micronutrients, vitamins, phenolic compounds, and minerals (Arguello-Hernández et al. 2024; Melini et al. 2023). Due to its genetic diversity, quinoa exhibits a high tolerance against abiotic stress, including drought (Tabatabaei et al. 2022; Akram et al. 2024a). Quinoa drought tolerance is facilitated by a combination of morpho-physiological adaptations (Tang et al. 2024). However, quinoa germplasm exhibits significant phenotypic diversity and inter-varietal variations (Hafeez et al. 2022) for drought tolerance mechanisms (Saddiq et al. 2021). Based on this diversification, quinoa is divided into five ecotypes: Valley, Altiplano, Salares, Subtropical/Yungas, and Sea level. Varieties from Valley and Altiplano ecotypes like Titicaca showed drought escape mechanisms by shortening their life cycles due to the region's dry and unpredictable climate (Taaime et al. 2022). In contrast, most of the Salare varieties, including the Italian variety Quipu (Casini 2017) and Pakistani variety UAFQ7 (Akram et al. 2021), exhibited drought avoidance strategies,

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such as leaf area reduction, possessing calcium oxalate vesicles to reduce transpiration (Infante et al. 2018) and epidermal bladder cells for moisture retention (Otterbach et al. 2021) to maintain turgor during drought. Moreover, varieties from this ecotype exhibited low osmotic potential, deeply sunken stomata in the leaf epidermis (Dizès 1992), and vigorous root systems (Ain et al. 2023). Furthermore, varieties from Subtropical and Sea level ecotypes, including Chilean varieties Regalona (Von Baer et al. 2009) and Q126 (Hafeez et al. 2022), displayed drought tolerance mechanisms through tissue elasticity and low osmotic potential. Higher proline, anti-transpiration compound accumulation other than ABA, and osmotic adjustment were observed in drought-stressed quinoa plants (Al-Naggar et al. 2017). Additionally, the induction of ornithine and raffinose pathways was observed to affect nitrogen assimilation-associated enzymes (Bascuñán-Godoy et al. 2016). This inherent diversity among quinoa germplasm offers a unique opportunity to identify quinoa genotypes with higher drought tolerance through targeted screening. In recognition of its potential contribution to global food security and poverty eradication, the United Nations (UN) declared 2013 "International Year of Quinoa" (Alandia et al. 2021). Following this global recognition, quinoa cultivation has expanded worldwide, including water scarcity regions. Although this species exhibits drought resistance, its physiological processes can be compromised by water deficit, significantly reducing growth and yield.

Drought significantly influences the soil moisture and quantity and quality of organic matter retained in the soil (Morales-Salmerón et al. 2024). Biochar addition to the soil has recently become an important agronomic strategy to address drought-related issues due to enhancing the soil's water retention capacity, particularly under water-limited conditions (El Moussaoui et al. 2024). The European Union recently approved biochar as fertilizers/soil conditioners for use in organic farming, effective from 2020. This recognition is based on its alignment with organic production principles and is outlined in Regulations 889/2008 and 2019/2164. Biochar is a carbon-rich material produced by the pyrolysis of organic materials (Bushra and Remya 2024). Studies showed that biochar addition significantly improves soil's physico-chemical properties (Getahun et al. 2020), including structure, porosity (Zanutel et al. 2023), hydraulic properties (Libutti et al. 2021) and nutrient content and availability (Antonangelo et al. 2024). However, the effectiveness of biochar depends on many factors, like feedstock, pyrolysis temperature, rate of application, soil type, and plant species (Qi et al. 2024). Soil respiration rates in biochar-amended soils demonstrated greater sensitivity to temperature variations than in non-amended soils. This temperature sensitivity was further modulated by soil texture, indicating that the interaction between biochar and soil properties plays a crucial role in shaping soil organic carbon (SOC) dynamics (Rittl et al. 2020). Besides soil physico-chemical properties, soil microbial communities are also affected by water stress due to limited substrate supply to microbial cells (Dollete et al. 2024). Microbes adapt to water limitations by shifting towards drought-tolerant populations, but this reduces their metabolic efficiency (Guevara-Hernandez et al. 2024). Biochar's porous structure and high carbon content provide a favorable environment for microorganisms to colonize and thrive. This increased microbial activity can enhance the decomposition of organic matter, leading to improved soil nutrient availability and structure (Morales-Salmerón et al. 2024). Previously, it has been reported that biochar application to the soil has a significant positive impact on microbial communities and their structures involved in C and N dynamics (Nguyen et al. 2017). However, most of the available literature is from laboratory, greenhouse or controlled conditions under short-term application, but few studies are available that evaluate the long-term biochar effects especially when applied at larger commercial scales. The available long term field trial conducted by Nguyen et al. 2017; demonstrated that one year of biochar application significantly enhanced soil bacterial diversity, promoting the enrichment of nitrifiers and pyrogenic carbon-decomposing bacteria. However, long term i.e., nine years and repeated biochar application in the soil already supplemented with biochar had no significant impact on bacterial communities and their structure.

Previous research has emphasized the beneficial effects of biochar on quinoa growth during the reproductive phase of the crop cycle (Kammann et al. 2011; Ramzani et al. 2017), with a comparatively limited focus on the vegetative development under water-limited conditions (Geerts et al. 2006). Furthermore, investigations have been restricted to a single variety, Danish Titicaca, cultivated with biochar addition under water-stressed conditions during the vegetative period (Akram et al. 2023; Rivelli et al. 2024). Consequently, knowledge concerning the responses of diverse quinoa varieties from different origins grown with biochar-amended soil under water scarcity, especially during the early growth stages, is limited. Given the critical role of the vegetative stage in plant development, this study aims to evaluate the impact of successive water stress cycles during the vegetative growth on five quinoa varieties grown in biochar-amended soil by assessing morpho-physiological attributes, leaf nutrients and soil microbial communities. We hypothesize that soil biochar addition could improve the vegetative growth of quinoa varieties under water-limited conditions and mitigate the negative effects of water stress. We expect that the impact of biochar will vary across the varieties from different origins, with those exhibiting greater drought tolerance showing more pronounced benefits. The variability may be attributed to differences in genetic traits and physiological responses to water scarcity.

2 Materials and Methods

2.1 Experimental Layout

A pot experiment was conducted with five quinoa varieties during spring-summer (April-June 2023) in the greenhouse of the University of Basilicata, Potenza, (PZ, 40°38′N-15°48′ E, 819 m a.s.l., Italy), under natural light conditions with day/night temperatures at 26/18°C. The five quinoa varieties, i.e., Titicaca (V1), Quipu (V2), Regalona (V3), UAFQ7 (V4) and Q126 (V5), were used in the experiment. Among them, three varieties Titicaca Quipu, and UAFQ7, are commercial varieties in Denmark, Italy, and Pakistan, respectively, while Regalona and Q126, are Chilean varieties and are currently under trial in Italy and Pakistan, respectively.

The five quinoa varieties (V1-V5) were cultivated without biochar and served as a control (B0) and in soil amended with 2% (w/w) woodchip biochar (B2) based on soil dry weight. Before initiating the trial, a comprehensive physicochemical characterization of the soil was conducted following the official analytical methods outlined in the Italian Official Gazette No. 248 (Decree 1999). According to the USDA soil classification system, the soil was characterized as sandy loam, with a particle size distribution of 66.1% sand, 11.5% silt, and 22.4% clay. The soil exhibited a field capacity (−0.03 MPa) of 22.8% (dry weight basis) and a wilting point (−1.5 MPa) of 11.4% (dry weight basis). The initial soil chemical properties included a pH of 7.6, an electrical conductivity (EC) of 600 µS cm⁻¹, an organic carbon content of 5.9 mg g⁻¹, organic matter of 1%, total nitrogen content of 1.5% and C: N 3.9. The biochar was purchased from Nerabiochar Company (Ivrea, Torino, Italy), which produces biochar from wood wastes from cleaned green areas. The biochar used in the current study is certified as Class 1 biochar, according to the European Biochar Certificate (EBC) (EBC 2012) and the International Biochar Initiative (IBI) standards (IBI 2015) based on its properties ($C>50\%$, $C_{org} > 60\%$, $H/C_{org} \leq 0.7$, $O/C_{org} \leq 0.4$). The biochar used in the experiment has the following particle size: 1% dw < 5 mm, 70% dw < 2 mm and 29% dw < 0.5 mm, pH (8.9), EC (52 mS m^{-1}), total C (68.3%) and C: N (67.2). However, detailed properties of woodchip biochar used in the experiment can be found in our previous study (Akram et al. 2023). Furthermore, soil hydraulic properties, as field capacity and permanent wilting point, were determined after the biochar amendment. The soil demonstrated a field capacity of 23.98% (dry weight basis) at -0.03 MPa and a permanent wilting point of 11.91% (dry weight basis) at -1.5 MPa.

Ten quinoa seeds were sown in 7.5 L plastic pots filled with 5 kg of either B0 or B2. At the 4-leaf stage, plants were thinned to one plant per pot. Then, the soil surface was covered by a 3 cm layer of polythene beads to minimize evaporation. Plants were maintained in well-watered conditions (WW: by restoring 100% evapotranspiration losses) from emergence to the twelve-leaf stage (t_1). Evapotranspiration losses were measured on a daily basis by weighing the pots at the same hour (08:00−09:00 am), and the intervention limit for watering was set at 30% depletion of the soil's available water content (*AW*). At this stage (t_1), half of the pots were kept under well-watered conditions. In contrast, others were subjected to two successive water stress (WS) cycles by withholding water until the soil reached the permanent wilting point (*PWP*) one after another. Different growth-related parameters were recorded at the start and end of each water stress cycle (t_1, t_2, and t_3, respectively). After the water stress period (completion of two water stress cycles), plants were rewatered to recover from water stress (t_4). They were well-watered until flowering initiation, when the experiment was stopped, and different growth-related parameters were measured. The experiment followed a factorial, completely randomized experimental design (three-way) with three replications × five varieties (*V*) × two biochar rates (*B*) × two watering conditions (*W*), resulting in 60 experimental units.

2.2 Plant Measurements and Analyses

2.2.1 Growth-related Parameters

At t_1, t_2, and t_3, plant height (*PH*, cm) and leaf number (*NL*, n^0) were recorded. Additionally, the length (*L*) and width (*W*) of each leaf were recorded and subsequently used for calculating the leaf area (*LA*, cm^2/plant) by using Eq. (1) (Talebnejad and Sepaskhah 2016; Akram et al. 2023):

$$LA = 0.64\,(L \times W) \tag{1}$$

Similarly, leaf greenness was assessed simultaneously using a handheld SPAD-502 m (Konica-Minolta Corporation Ltd., Osaka, Japan) by recording SPAD readings on the three youngest fully expanded leaves per plant. The same parameters were measured at the flowering initiation as well as the numbers of branches (*NB*, n^0), main panicle length (*PL*, cm), sub-panicle numbers per plant (*NSP*, n^0), and the above-ground plant's fresh (*TFW*, g) and dry biomass (*TDW*, g).

2.2.2 Water-Related Parameters

Total water consumption (*TWC*, L) throughout the experiment was determined by cumulating the amount of water applied during each watering event. Water use efficiency (*WUE*, g L^{-1}) was calculated using the final plant-dried biomass and the total water consumption. Leaf relative water content (*RWC*, %) was assessed at t_3 i.e., at permanent wilting point and t_4. For that purpose, a small section of the youngest fully expanded leaf was collected from each plant. The fresh weight (*FW*, g) was recorded, and afterwards, the leaf was placed in an ice bucket, and the leaf segment was submerged in distilled water to determine the turgid weight (*TW*, g). The leaf tissue was then dried to measure the dry weight (*DW*, g) in a ventilated oven at 70 °C until constant weight was achieved. Finally, Eq. (2) was used to calculate leaf *RWC* (%):

$$RWC = \frac{FW - DW}{TW - DW} \times 100 \quad (2)$$

Furthermore, the turgid weight to dry weight ratio (*TW:DW*) and pre-dawn leaf water potential (Ψ, MPa) were determined. Plants were kept in a dark chamber overnight, and measurements of leaf Ψ were made at 6 am using a Scholander pressure chamber (PMS model 1000, Corvallis, OR, USA) on the youngest fully expanded leaf from each plant.

2.2.3 Total Bacterial Communities Count in Soil

The total bacterial count (*TBC*) in soil samples from control (B0) and biochar-treated pots (B2) was measured as colony-forming units per millilitre (CFU/mL) at three times: t_1, t_2, and t_3. Soil samples were collected at t_1 from all pots and at t_2 and t_3 from water-stressed pots only for both B0 and B2 treatments. Sample processing followed the standard protocol (Olsen and Bakken 1987). First, 1 gram of each soil sample was mixed with 9 mL of sterile distilled water to create the original suspension. The samples were homogenized for 2 min, and four serial decimal dilutions were prepared (10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}). Plate Counting Agar (*PCA*) was prepared with 5 g/L tryptone, 2.5 g/L yeast extract, 1.0 g/L glucose, and 12.0 g/L bacteriological agar, all dissolved in distilled water at room temperature using a magnetic stirrer. The pH of the medium was adjusted to 7.0±0.2 before autoclave at 121 °C for 20 min. For *TBC* analysis, 1 mL of the two highest dilutions (10^{-4} and 10^{-5}) was pipetted into 90 mm Petri dishes, followed by the addition of PCA medium at 45 °C. The plates were incubated at 30±2 °C for 24–48 h under aerobic conditions. After incubation, bacterial colonies were counted using a stereomicroscope, and the total number of colony-forming units per millilitre (CFU/mL) of soil was calculated by multiplying the colony count by the dilution factor.

2.2.4 Total Leaf Nitrogen and Carbon Content

At the end of the experiment, dried leaf samples were grounded to a homogenous fine powder with a fine milling machine. Subsequently, powdered leaves were dried in a ventilated oven at 70 °C, and the total leaf nitrogen (*N*) and carbon (*C*) contents were measured as a percentage of dry weight (% *DW*) by a CHNO/S Analyzer (Flash 2000, Thermo Fisher Scientific Cambridge UK) that operated with the dynamic flash combustion analysis, according to the modified Dumas method (Dumas 1831).

2.3 Statistical Analysis

All the experimental data were checked for normality and homogeneity of variance and processed by two- and three-way analysis of variance (ANOVA) for a completely randomized design. When significant differences among means were detected, the latter were compared by Tukey's honest significant difference (HSD) at a 0.05% significance level. Similarly, principal component correlation analysis (PCA) and correlation matrix (mantel graph) were conducted to check the correlation between measured traits and varietal response. All the analyses were carried out by using the statistical program RStudio (Team 2013).

3 Results

3.1 Water-related Attributes at the End of the Water-stress Period (t_3) and After the Recovery (t_4)

Plant water status, assessed by leaf's pre-dawn total water potential (Ψ), relative water content (*RWC*), and turgid weight to dry weight ratio (*TW*:*DW*), was significantly affected by all experimental factors, including variety (V), biochar (B) and watering condition (W) and their two- and three-way interactions during the water stress period (Table 1). At t_3, Ψ and *RWC* were significantly affected by all experimental factors and interactions ($p \leq 0.001$). Biochar application improved plant water status, as evidenced by less negative Ψ and higher *RWC* values than the control plants (Fig. 1). The varieties V4 and V5 were affected most by water stress, resulting in reductions in *RWC* of 65% and 59%, respectively, compared to well-watered conditions. *TW*:*DW* was influenced by variety (V) ($p \leq 0.001$), variety × biochar (V × B) ($p \leq 0.001$), and variety × watering condition (V × W) ($p \leq 0.001$), aligning with Ψ and *RWC* trends. The

Table 1 Leaf's pre-dawn water potential (*Ψ*), relative water content (*RWC*) and turgid weight to dry weight ratio (*TW: DW*) of five Quinoa varieties (V1-V5) grown without (B0) and with Biochar (B2) under well-watered (WW) and water-stressed (WS) conditions

Experimental Factors	End of water stress period (t_3)			Recovery from water stress (t_4)		
	Ψ (MPa)	RWC (%)	TW:DW (-)	Ψ (MPa)	RWC (%)	TW:DW (-)
Variety (V)						
V1	-1.33 a	67.2 a	12.8 a	-0.55	75.5 a	12.5 b
V2	-1.52 b	63.2 b	10.4 b	-0.47	75.2 a	11.3 c
V3	-1.66 d	66.5 a	10.4 b	-0.49	76.7 a	11.2 c
V4	-1.60 c	60.3 c	10.6 b	-0.51	73.9 ab	11.3 c
V5	-1.75 e	62.7 c	12.6 a	-0.53	70.4 b	14.2 a
Biochar (B)						
B0	-1.62 b	62.1 b	11.4	-0.47 a	74.7	11.6 b
B2	-1.52 a	65.0 a	11.3	-0.55 b	74.0	12.6 a
Watering (W)						
WW	-0.58 a	77.0 a	11.1 b	-0.45 a	76.9 a	11.3 b
WS	-2.56 b	50.2 b	11.6 a	-0.58 b	71.8 b	12.9 a
Variety × Biochar						
V1-B0	-1.43 b	65.4 ac	12.7 ab	-0.51	75.7	12.3 ce
V1-B2	-1.23 a	68.9 a	12.9 ab	-0.60	75.3	12.7 bd
V2-B0	-1.51 c	62.1 ce	9.4 e	-0.42	76.3	9.7 g
V2-B2	-1.53 c	64.3 bd	11.5 bd	-0.52	74.0	12.8 bc
V3-B0	-1.69 d	65.6 ac	10.5 ce	-0.41	74.9	11.1 ef
V3-B2	-1.62 d	67.4 ab	10.3 ce	-0.57	78.6	11.4 ef
V4-B0	-1.66 d	57.2 f	11.1 cd	-0.48	75.0	11.0 e.g.
V4-B2	-1.53 c	63.4 ce	10.1 de	-0.54	72.8	11.5 df
V5-B0	-1.80 e	60.2 ef	13.6 a	-0.55	71.4	13.9 ab
V5-B2	-1.69 d	61.2 de	11.6 bc	-0.51	69.4	14.4 a
Variety × Watering						
V1-WW	-0.53 a	77.3 ab	12.8 ab	-0.43	77.8	12.9 bc
V1-WS	-2.12 b	57.0 c	12.7 ac	-0.68	73.2	12.1 c
V2-WW	-0.61 a	78.3 ab	9.6 f	-0.44	75.9	10.3 d
V2-WS	-2.44 c	48.0 d	11.3 cd	-0.50	74.4	12.3 c
V3-WW	-0.58 a	79.5 a	9.6 ef	-0.43	79.1	10.0 d
V3-WS	-2.74 e	53.5 c	11.1 de	-0.55	74.3	12.5 bc
V4-WW	-0.56 a	75.2 b	9.7 ef	-0.47	77.0	9.7 d
V4-WS	-2.63 d	45.4 d	11.5 bd	-0.55	70.7	12.9 bc
V5-WW	-0.60 a	74.6 b	13.6 a	-0.45	74.6	13.6 ab
V5-WS	-2.89 f	46.8 d	11.5 bd	-0.61	66.2	14.7 a
Biochar × Watering						
B0-WW	-0.60 a	75.9	11.3	-0.45 a	76.3 a	11.1 c
B0-WS	-2.64 c	48.3	11.6	-0.50 a	73.1 b	12.1 b
B2-WW	-0.55 a	78.0	10.8	-0.44 a	77.5 a	11.5 bc
B2-WS	-2.49 b	52.0	11.7	-0.65 b	70.5 b	13.6 a
Level of Significance						
V	***	***	***	ns	***	***
B	***	***	ns	*	ns	***
W	***	***	ns	***	***	***
V × B	***	*	***	ns	ns	***
V × W	***	***	***	ns	ns	***
B × W	***	ns	ns	**	*	**
V × B × W	***	*	ns	ns	ns	***

The different letters in the same column indicate differences among treatments. F-test significant at *: (*$p \leq 0.05$*), **: (*$p \leq 0.01$*), ***: (*$p \leq 0.001$*), ns: not significant

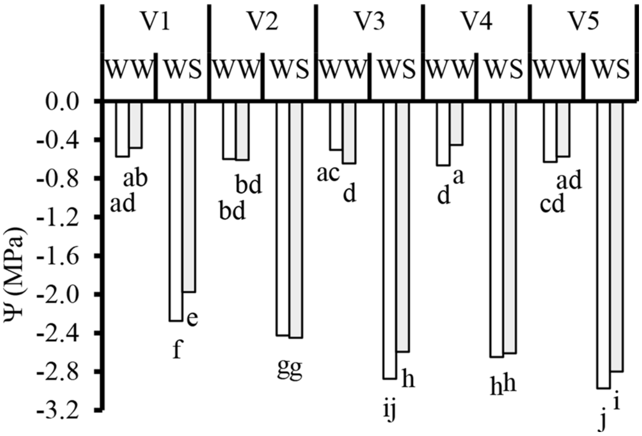

Fig. 1 Leaf's pre-dawn water potential (Ψ) of five quinoa varieties (V1-V5) grown without (B0) and with biochar (B2) under well-watered (WW) and water-stressed (WS) conditions recorded at the end of water-stress period (t_3). Values are means ($n=3$) ± S.E. In each graph, different letters indicate significant differences among treatments ($p \leq 0.05$, Tukey's test). V1: Titicaca, V2: Quipu, V3: Regalona, V4: UAFQ7, V5: Q126; white bars: no biochar, grey bars: 2% woodchip biochar

same varieties, V4 and V5, had significantly lower *TW*:*DW* ratio in biochar-amended soil than in non-treated soil.

At t_4, Ψ was significantly affected by biochar ($p \leq 0.05$) and watering conditions ($p \leq 0.001$) and their interaction (B × W) ($p \leq 0.01$), while *RWC* was influenced by variety ($p \leq 0.001$) and watering condition ($p \leq 0.001$) and (B × W) ($p \leq 0.05$), but not by biochar alone, two-way (V × B and V × W) and three-way interactions (V × B × W). Notably, V2 and V4 demonstrated superior *RWC* recovery by 55% and 49% under water stress compared to other varieties. The *TW*:*DW* was significantly influenced by all experimental factors at t_4 ($p \leq 0.001$), with water-stressed plants exhibiting higher values, except for V1.

3.2 Growth-Related Attributes during the Water Stress Period

Plant growth attributes, including plant height (*PH*), leaf number (*NL*) and area (*LA*), were measured three times, i.e., t_1, t_2, and t_3 (Table 2). Significant main effects were observed for variety ($p \leq 0.001$) and biochar ($p \leq 0.001$) at all time points, while the watering condition significantly affected growth attributes at t_2 and t_3 ($p \leq 0.001$) when plants were water-stressed. At t_1, V4 showed the highest *PH*, *NL* and *LA* values. At t_1, *PH* was also influenced by the interaction V × B ($p \leq 0.001$). Mainly, V4 exhibited higher *PH* when grown with biochar, although, at t_2 and t_3, it was overcome by V2 grown with biochar.

At t_2 and t_3, *NL* was affected by all experimental factors (t_2, t_3: $p \leq 0.001$), as well as the two-way interactions V × B (t_2: $p \leq 0.001$, t_3: $p \leq 0.05$) and V × W (t_2: $p \leq 0.001$, t_3: $p \leq 0.001$) (Table 4). All varieties treated with biochar consistently exhibited higher *NL*, and similarly, when they were subjected to well-watered conditions. For leaf area (*LA*), a significant variation was observed among variety (*LA*: t_1, t_2, t_3: $p \leq 0.001$), biochar (*LA* t_1, t_3: $p \leq 0.001$, t_2: $p \leq 0.05$) and watering condition (*LA* t_1: $p \geq 0.05$, t_2,t_3: $p \leq 0.001$) as well as two-factor interactions V × B (*LA* t_1, t_2, t_3: $p \leq 0.001$) and V × W (*LA* t_1: $p \leq 0.01$, t_2, t_3: $p \leq 0.001$) (Table 3). In line with *NL*, the variety V4 grown with biochar exhibited a bigger leaf area at all stages. Additionally, the SPAD index (Fig. 2) was also significantly influenced by variety (t_1, t_2, t_3: $p \leq 0.001$), biochar (t_1: $p \leq 0.05$, t_2, t_3: $p \leq 0.001$) and watering condition (t_2, t_3: $p \leq 0.001$). Furthermore, the two-way V × B and the three-way V × B × W interactions were significant at t_2 and t_3 (t_2: $p \leq 0.001$, t_3: $p \leq 0.01$). At t_3 (Fig. 2), under water-stress conditions, biochar significantly enhanced SPAD index in all varieties, but a more pronounced increment was recorded in V1, V2 and V3 compared to not-amended soil.

3.3 Total Bacterial Count

At t_1 (sampling all pots), t_2 and t_3 (sampling from water-stressed treatments), total bacterial count (*TBC*) was affected by variety ($p \leq 0.001$), biochar ($p \leq 0.001$) and their interaction (V × B) ($p \leq 0.001$) (Fig. 3). At t_1 (Fig. 3a), biochar-treated soils have more bacterial communities than non-amended soil except for the variety V1 and V3. On the contrary, when the same counting was done at t_2 (Fig. 3b) and t_3 (Fig. 3c) under water-stressed conditions, V4 and V5 grown in biochar recorded a significant decrease in bacterial communities than soils without biochar (B0). At t_2, V1 grown on biochar-amended soil showed a remarkable increase in bacterial colony counts compared to B0 (220 vs. 11.5) (the analysis was repeated three times for results confirmation).

3.4 Growth and Yield-Related Parameters at Flowering Initiation (the end of the experiment)

Plant height (*PH*) was significantly affected by experimental factors and two-way interactions, i.e., V × B and V × W ($p \leq 0.001$) (Table 4). V4 and V5 grown in biochar-amended soil reached the highest *PH*. As to the leaf number, the same level of significance was observed for the three experimental factors (V, B and W) as well as their two-way interactions (V × B, V × W, and B × V) ($p \leq 0.001$) (Table 3). V4 consistently had the highest leaf numbers. Leaf area varied significantly between the varieties, biochar, and watering condition ($p \leq 0.001$) and their two-factor interaction V × B ($p \leq 0.001$) and V × W ($p \leq 0.01$) (Table 3). Similar to the *NL*, branch numbers (*NB*) were also significantly affected among

Table 2 Plant height (*PH*), leaf number (*NL*) and area (*LA*) of five Quinoa varieties (V1−V5) grown without (B0) and with Biochar (B2) under well-watered (WW) and water-stressed (WS) conditions

Experimental Factors	Start of water stress cycle (t_1)			End of 1st water stress cycle (t_2)			End of 2nd water stress cycle (t_3)		
	PH (cm)	NL (n^0)	LA (cm^2)	PH (cm)	NL (n^0)	LA (cm^2)	PH (cm)	NL (n^0)	LA (cm^2)
Variety (V)									
V1	17.0 d	32 d	430 d	29.8 d	76 d	689 d	39.1 c	105 d	993 c
V2	24.5 b	65 b	623 b	40.2 a	106 b	968 b	54.3 a	132 c	1221 b
V3	22.2 c	59 c	590 c	35.9 c	76 d	737 d	48.3 b	107 d	1177 b
V4	27.0 a	76 a	684 a	36.1 c	122 a	1052 a	49.0 b	156 a	1305 a
V5	24.1 b	58 c	588 c	37.8 b	103 c	870 c	53.0 a	136 b	1157 b
Biochar (B)									
B0	22.0 b	56 b	570 b	34.3 b	90 b	852 b	46.4 b	120 b	1058 b
B2	23.9 a	60 a	595 a	37.6 a	102 a	875 a	51.0 a	134 a	1283 a
Watering (W)									
WW	22.9	59	585	39.6 a	101 a	893 a	52.7 a	135 a	1208 a
WS	23.0	57	581	32.3 b	92 b	833 b	44.7 b	119 b	1133 b
Variety × Biochar									
V1-B0	17.7 d	32	431 e	28.0 f	70 f	686 d	35.8 g	96 g	927 g
V1-B2	16.3 d	32	429 e	31.5 e	81 e	692 d	42.3 f	113 e	1059 ef
V2-B0	24.5 bc	63	617 bc	39.8 ab	100 c	1020 a	52.5 ac	126 d	1113 de
V2-B2	24.5 bc	68	628 bc	40.5 a	112 b	916 b	56.0 a	138 c	1330 ab
V3-B0	21.5 c	56	595 cd	33.8 d	73 f	709 d	48.7 ce	102 f	998 fg
V3-B2	22.8 bc	62	584 cd	38.0 b	80 e	766 cd	47.8 de	111 e	1356 a
V4-B0	23.7 bc	74	660 ab	33.7 d	115 b	1019 a	44.7 ef	147 b	1179 cd
V4-B2	30.3 a	77	708 a	38.5 b	128 a	1085 a	53.3 ab	164 a	1430 a
V5-B0	22.7 bc	55	549 d	36.0 c	94 d	825 c	50.3 bd	129 d	1074 df
V5-B2	25.5 b	60	626 bc	39.7 ab	112 b	916 b	55.7 a	143 bc	1241 bc
Variety × Watering									
V1-WW	16.7	32	428 e	32.8 ef	80 e	707 d	42.8 e	112 d	1006 e
V1-WS	17.3	32	432 e	26.7 g	72 f	671 d	35.3 f	97 f	980 e
V2-WW	24.3	67	636 bc	43.8 a	116 b	1049 ab	58.3 a	148 b	1361 a
V2-WS	24.7	64	609 cd	36.5 d	96 d	887 c	50.2 cd	116 d	1082 de
V3-WW	22.7	59	583 d	40.3 bc	79 e	737 d	55.0 ab	110 d	1124 cd
V3-WS	21.7	58	596 cd	31.5 f	73 f	737 d	41.5 e	103 e	1230 bc
V4-WW	26.3	78	706 a	39.5 c	124 a	1113 a	52.2 bc	158 a	1369 a
V4-WS	27.7	73	662 ab	32.7 ef	119 ab	991 b	45.8 de	153 ab	1240 b
V5-WW	24.3	57	571 d	41.7 bc	106 c	860 c	57.8 a	147 b	1182 bd
V5-WS	23.8	58	605 cd	34.0 e	99 d	881 c	48.2 cd	125 c	1133 cd
Level of Significance									
V	***	***	***	***	***	***	***	***	***
B	***	***	***	***	***	*	***	***	***
W	ns	ns	ns	***	***	***	***	***	***
V × B	***	ns	***	***	***	***	***	*	***
V × W	ns	ns	**	*	***	***	***	***	***
B × W	ns	ns	ns	ns	ns	*	ns	ns	*
V × B × W	ns	ns	ns	ns	*	ns	ns	ns	ns

The different letters in the same column indicate differences among treatments. F-test significant at *: ($p \leq 0.05$), **: ($p \leq 0.01$), ***: ($p \leq 0.001$), ns: not significant

the experimental factors (data not shown), which was also reflected in the number of sub-panicles counts. Notably, V4, regardless of watering conditions, exhibited higher leaf area than the other varieties, showing pronounced recovery ability from water-stressed conditions, resulting in more leaf expansion.

Considering the plant growth attributes, total fresh (*FW*) and dry biomass (*DW*) were influenced by V, B, W and their two-way interactions ($p \leq 0.001$) (Table 4). Biochar

Table 3 Plant height (*PH*), leaf number (*NL*) and area (*LA*), fresh (*FW*) and dry biomass (*DW*) and water use efficiency (*WUE*) of five Quinoa varieties (V1-V5) grown without (B0) and with Biochar (B2) under well-watered (WW) and water-stressed (WS) conditions

Experimental Factors	PH (cm)	NL (n^0)	LA (cm^2)	FW (g)	DW (g)	WUE (g/L)
Variety (V)						
V1	64.9 b	144 c	1327 d	51.2 c	6.9 d	14.5 b
V2	75.0 a	174 b	1481 b	61.9 a	9.5 b	16.1 a
V3	74.7 a	142 c	1453 b	58.8 ab	9.4 b	15.0 ab
V4	67.8 b	214 a	1580 a	61.2 a	10.4 a	15.8 ab
V5	68.0 b	172 b	1397 c	55.3 b	8.6 c	14.9 ab
Biochar (B)						
B0	68.4 b	159 b	1378 b	53.0 b	8.3 b	14.1 b
B2	71.7 a	179 a	1517 a	62.3 a	9.6 a	16.4 a
Watering (W)						
WW	75.8 a	177 a	1505 a	61.9 a	9.7 a	13.6 b
WS	64.3 b	162 b	1390 b	53.4 b	8.3 b	16.9 a
Variety × Biochar						
V1-B0	64.2 c	137 f	1243 e	46.5 e	6.2 g	13.1 c
V1-B2	65.7 bc	152 e	1410 cd	55.9 cd	7.6 f	15.8 ab
V2-B0	74.8 a	166 d	1391 cd	59.4 bc	8.9 de	15.6 ab
V2-B2	75.2 a	183 c	1571 b	64.4 ab	10.1 bc	16.6 a
V3-B0	75.2 a	133 f	1443 c	51.0 de	8.4 e	13.6 bc
V3-B2	74.2 a	152 e	1463 c	66.5 a	10.4 bc	16.3 a
V4-B0	62.3 c	200 b	1469 c	59.1 bc	9.6 cd	15.2 ac
V4-B2	73.2 a	229 a	1691 a	63.3 ab	11.2 a	16.3 a
V5-B0	65.7 bc	162 d	1345 d	49.0 e	8.4 e	12.9 c
V5-B2	70.3 ab	182 c	1450 c	61.5 ac	8.9 e	16.9 a
Variety × Watering						
V1-WW	68.8 de	148 ef	1386 ef	55.4 ce	7.4 e	12.9 e
V1-WS	61.0 f	140 fg	1268 g	47.0 f	6.4 f	16.1 ad
V2-WW	85.0 a	194 c	1561 ac	69.4 a	10.3 b	14.9 ce
V2-WS	65.0 ef	155 e	1401 df	54.3 de	8.7 d	17.4 ab
V3-WW	78.7 b	147 ef	1505 bc	61.1 bc	10.4 b	13.1 e
V3-WS	70.7 cd	138 gj	1402 de	56.4 be	8.4 d	16.9 ac
V4-WW	74.3 bc	223 a	1598 ac	63.0 ab	11.4 a	13.3 e
V4-WS	61.2 f	206 b	1562 ab	59.4 bd	9.4 c	18.2 a
V5-WW	72.3 cd	174 d	1477 cd	60.5 bd	8.8 cd	14.0 de
V5-WS	63.7 ef	171 d	1317 fg	50.0 ef	8.5 d	15.8 bd
Level of Significance						
V	***	***	***	***	***	**
B	***	***	***	***	***	***
W	***	***	***	***	***	***
V × B	***	**	***	***	***	*
V × W	***	***	**	**	***	*
B × W	ns	***	ns	***	***	***
V × B × W	ns	ns	ns	ns	***	ns

The different letters in the same column indicate differences among treatments. F-test significant at *: ($p \le 0.05$), **: ($p \le 0.01$), ***: ($p \le 0.001$), ns: not significant

application enhanced *FW* and *DW*, with V4 showing the highest dry weight. Dry weight was also influenced by the three-way interaction (V × B × W) (Fig. 4). All varieties grown with biochar-treated soil increased their *DW* than non-amended ones (Fig. 4). Water use efficiency (*WUE*) was affected by the main factors (V, B, W) and their interactions, except for V × B × W. The water use efficiency was higher in V5 grown on biochar-amended soil, and it was consistently higher in all varieties under water-stressed conditions than under well-watered ones (Table 4).

In line with the growth-related traits, yield contributing parameters, i.e., panicle length (*PL*) and the number of sub-panicles (*NSP*), were affected by V, B, and W (Table 4) and their two-factor interaction V × B and V × W (*PL*, *NSP*:

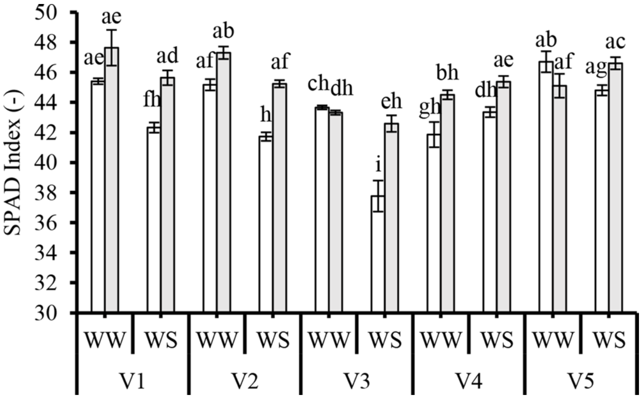

Fig. 2 SPAD index of five quinoa varieties (V1−V5) grown without (B0) and with biochar (B2) under well-watered (WW) and water-stressed (WS) conditions recorded at t_3. Values are means ($n=3$) ± S.E. In each graph, different letters indicate significant differences among treatments ($p \leq 0.05$, Tukey's test). V1: Titicaca, V2: Quipu. V3: Regalona, V4: UAFQ7, V5: Q126; white bars: no biochar, grey bars: 2% woodchip biochar

Table 4 Panicle length (*PL*) and the number of sub-panicles (*NSP*) of five Quinoa varieties (V1-V5) grown without (B0) and with Biochar (B2) under well-watered (WW) and water-stressed (WS) conditions

Experimental Factors	PL (cm)	NSP (n^0)
Variety (V)		
V1	10.1 b	15.7 c
V2	14.3 a	17.1 b
V3	5.8 d	14.9 c
V4	8.4 c	22.0 a
V5	3.4 e	17.6 b
Biochar (B)		
B0	7.0 b	16.1 b
B2	9.8 a	18.8 a
Watering (W)		
WW	9.1 a	19.2 a
WS	7.7 b	15.7 b
Level of Significance		
V	***	***
B	***	***
W	***	***
V × B	***	***
V × W	***	*
B × W	ns	ns
V × B × W	ns	ns

The different letters in the same column indicate differences among treatments. F-test significant at *: (*$p \leq 0.05$*), **: (*$p \leq 0.01$*), ***: (*$p \leq 0.001$*), ns: not significant

$p \leq 0.001$) (Fig. 5). The variety V2 produced the longest panicles, while V4 produced the largest number of sub-panicles (Table 4) because it also exhibited higher branch numbers (data not shown); in other words, we can also compare the number of sub-panicles and branch numbers.

The Italian variety Quipu (V2) exhibited the longest panicles also when grown on biochar-amended soil (Fig. 5a). In contrast, the Pakistani variety UAFQ7 (V4) accounted for the highest number of sub-panicles both when grown on

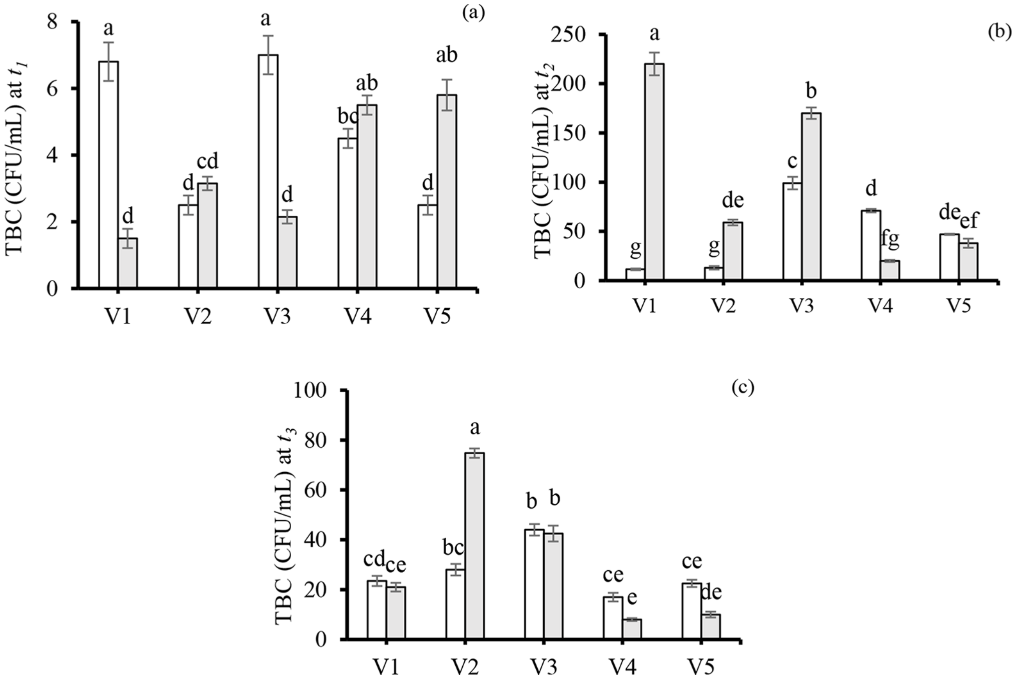

Fig. 3 Total bacterial count (*TBC*) at t_1 (**a**), t_2 (**b**) and t_3 (**c**) in soil without (B0) and with biochar (B2). Values are means ($n=3$) ± S.E. In each graph, different letters indicate significant differences among treatments ($p \leq 0.05$, Tukey's test). t_1: start of water stress, t_2: end of first water stress cycle, t_3: end of second water stress cycle. V1: Titicaca, V2: Quipu, V3: Regalona, V4: UAFQ7, V5: Q126; white bars: no biochar, grey bars: 2% woodchip biochar

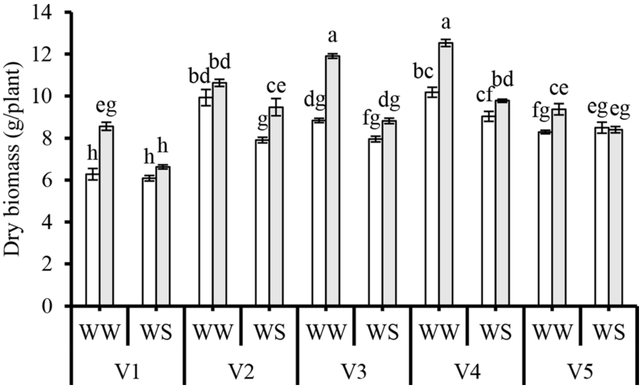

Fig. 4 Dry biomass (*DW*) of five quinoa varieties (V1-V5) grown without (B0) and with biochar (B2) under well-watered (WW) and water-stressed (WS) conditions. Values are means ($n=3$) ± S.E. In each graph, different letters indicate significant differences among treatments ($p \leq 0.05$, Tukey's test). V1: Titicaca, V2: Quipu, V3: Regalona, V4: UAFQ7, V5: Q126; white bars: no biochar, grey bars: 2% woodchip biochar

biochar amended and non-amended soil (Fig. 5b).

The SPAD index, recorded at the end of the experiment, was significantly affected by all experimental factors ($p \leq 0.001$) (Table 5). More specifically, biochar application enhanced the SPAD index, with V1 showing the highest value. Considering the watering condition, V4 and V5 under water-stressed conditions maintained the same high values as well-water ones. Moreover, nitrogen content, which was only influenced by the main factors, V and W ($p \leq 0.001$), reached the highest value in V1 and V5 ($p \leq 0.001$), aligning with the similarly highest value of SPAD index (Table 5). Water-stressed plants exhibited increased leaf nitrogen content ($p \leq 0.001$). Consequently, V1 and V5 displayed a lower *C: N* ratio ($p \leq 0.001$), indicating more nitrogen uptake than carbon in line with the SPAD index trend (Table 5). A significant decrease in *C: N* was evident in plants grown under water-stressed compared to well-watered conditions.

3.5 PCA Correlation Analysis at t_3

A principal component analysis (PCA) was conducted to explore the relationships among the measured plant parameters at the end of the water-stress period (t_3) (Fig. 6). The two principal components (F1 and F2) explained 85.92% of the total variance. Factor F1 was primarily associated with plant water status, as indicated by its strong correlations with *RWC*, Ψ, and *TW: DW* ratio, with V1 showing higher values. In contrast, F2 was more closely related to plant growth, with high loadings for *NL*, *NB*, *PH*, and *LA*, displaying V5 positive values. The analysis suggests that the two factors impacted plant water status and growth, with distinct patterns for the different plant parameters.

3.6 PCA Correlation Analysis at the End of the Experiment

At the end of the experiment, the PCA showed that the first principal component (F1), explained 59.2% of the total variance and was positively correlated with leaf-related traits, including nitrogen (*N*%) and carbon (*C*%) contents and SPAD index (Fig. 7). It showed that the varieties V1 and V5 performed better regarding these parameters. Conversely, the second principal component (F2) demonstrated stronger associations with plant growth parameters, such as leaf area (*LA*), total dry weight (*TDW*), and water use efficiency (*WUE*), with V4 showing the highest values. These findings suggest that F1 primarily captured variations in leaf nutrient composition under biochar application and watering conditions, while F2 reflected treatment-induced changes in plant growth and water use strategies.

3.7 The Mantel Graph Based on Harvest Data

The correlation matrix (mantel graph) revealed several important relationships between key plant traits (Fig. 8). Strong positive correlations were observed between total fresh weight (*TFW*) and leaf area (*LA*), as well as between

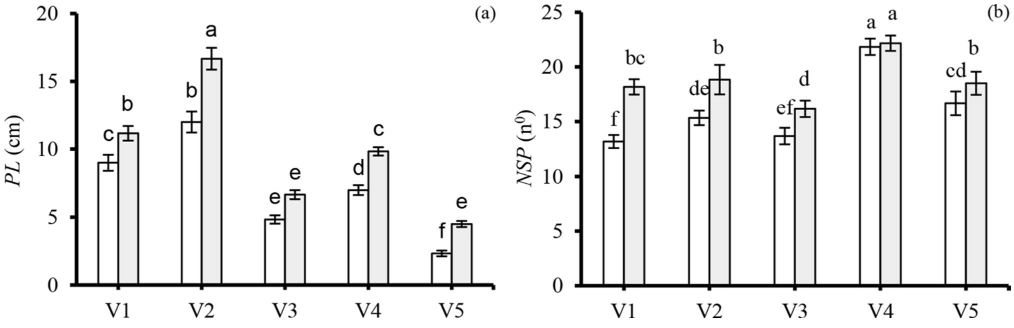

Fig. 5 (**a**) Panicle length (*PL*) and (**b**) the number of sub-panicles (*NSP*) of five quinoa varieties (V1-V5) grown without and with biochar. Values are means ($n=3$) ± S.E. In each graph, different letters indicate significant differences among treatments ($p \leq 0.05$, Tukey's test). V1: Titicaca, V2: Quipu, V3: Regalona, V4: UAFQ7, V5: Q126; white bars: no biochar, grey bars: 2% woodchip biochar

Table 5 SPAD, nitrogen (*N*%), carbon (*C*%) and carbon to nitrogen ratio (*C:N*) in leaves of five Quinoa varieties (V1-V5) grown without and with Biochar under well-watered (WW) and water-stressed (WS) conditions. The different letters in the same column indicate differences among treatments. F-test significant at *: ($p \leq 0.05$), **: ($p \leq 0.01$), ***: ($p \leq 0.001$), Ns: not significant

Experimental Factors	SPAD (-)	*N* (%)	C (%)	C:*N* (-)
Variety (V)				
V1	45.7 a	2.7 a	35.5 c	13.5 b
V2	42.1 c	1.8 b	35.6 c	19.7 a
V3	40.8 d	2.0 b	36.1 bc	19.1 a
V4	44.4 c	1.9 b	37.7 ab	20.4 a
V5	46.2 a	2.6 a	38.2 a	15.4 b
Biochar (B)				
B0	42.6 b	2.1	34.9 b	17.6
B2	45.1 a	2.3	38.3 a	17.6
Watering (W)				
WW	44.4 a	1.9 b	36.6	20.0 a
WS	43.3 b	2.5 a	36.6	15.3 b
V × B				
V1-B0	43.9 de	2.6	33.9 bc	13.6
V1-B2	47.5 a	2.8	37.1 ab	13.3
V2-B0	40.0 f	1.7	33.3 c	19.3
V2-B2	44.2 ce	1.9	37.9 a	20.1
V3-B0	40.2 f	1.8	33.4 c	19.7
V3-B2	41.5 f	2.2	38.8 a	18.5
V4-B0	43.4 e	1.8	36.1 ac	20.6
V4-B2	45.4 bd	2.0	39.3 a	20.3
V5-B0	45.7 bc	2.7	37.9 a	14.8
V5-B2	46.8 ab	2.5	38.4 a	16.0
V × W				
V1-WW	47.3 a	2.3	35.1	15.1 cd
V1-WS	44.1 c	3.0	35.8	11.9 d
V2-WW	42.2 d	1.7	35.3	21.4 ab
V2-WS	42.0 d	2.0	35.9	18.1 bc
V3-WW	42.3 d	1.6	36.9	22.6 ab
V3-WS	39.3 e	2.3	35.2	15.6 cd
V4-WW	44.3 bc	1.5	37.5	24.7 a
V4-WS	44.5 bc	2.3	37.9	16.2 cd
V5-WW	45.8 ab	2.5	38.1	16.0 cd
V5-WS	46.6 a	2.6	38.3	14.8 cd
Level of Significance				
V	***	***	***	***
B	***	ns	***	ns
W	***	***	ns	***
V × B	***	ns	*	ns
V × W	***	ns	***	**
B × W	ns	ns	ns	ns
V × B × W	ns	ns	ns	ns

TFW and plant height (*PH*), indicating that plants with larger leaf areas and greater heights tend to accumulate more biomass. Similarly, the SPAD index positively correlated with the number of leaves (*NL*), suggesting that higher leaf counts are associated with increased photosynthetic capacity. Conversely, leaf *N*% and *C*% contents displayed a weak negative correlation, implying that these traits are not closely linked. Mantel's test identified significant correlations between *PH* and carbon-nitrogen ratio (*C: N*) and between panicle length (*PL*) and sub-panicle number (*NSP*), highlighting potential physiological and reproductive interactions. These results suggest that leaf area (*LA*), plant height (*PH*), and SPAD are vital in influencing plant growth and biomass accumulation.

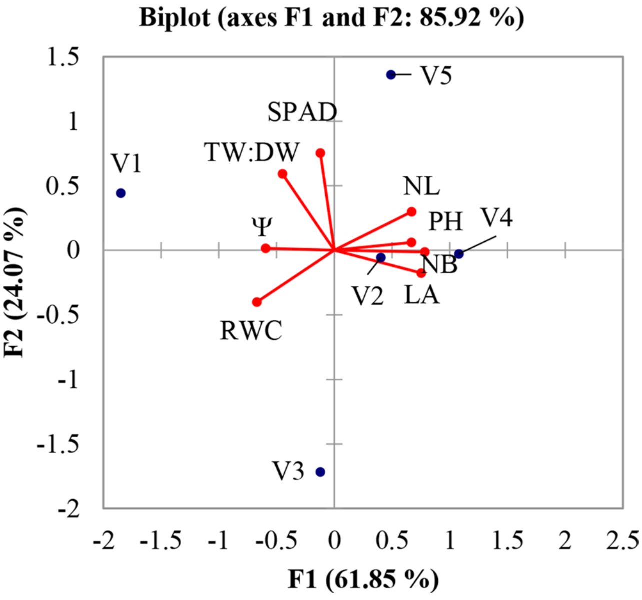

Fig. 6 PCA correlation analysis of growth and water-related attributes recorded at the end of the water stress period. *RWC*: Leaf relative water content, *Ψ*: pre-dawn water potential, *TW: DW*: Turgid weight to dry weight ratio, SPAD: SPAD index (leaf chlorophyll), *NL*: number of leaves, *NB*: number of branches, *PH*: plant height, *LA*: leaf area. V1: Titicaca, V2: Quipu, V3: Regalona, V4: UAFQ7, V5: Q126

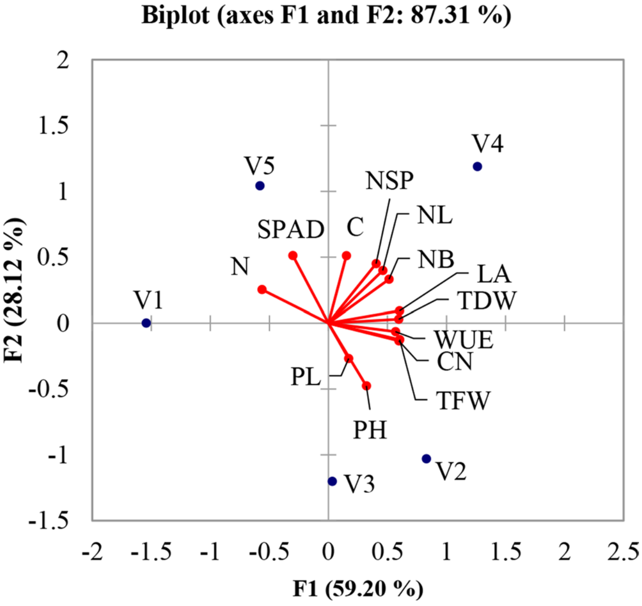

Fig. 7 PCA correlation analysis of growth-related attributes recorded at the end of the experiment. N: leaf nitrogen%, C: leaf carbon%, *C: N*: leaf carbon to nitrogen ratio, SPAD: SPAD index (leaf chlorophyll), *NSP*: number of sub panicles, *NL*: number of leaves, *NB*: number of branches, *LA*: leaf area, *TFW*: total fresh weight, *WUE*: water use efficiency, *PL*: panicle length, *PH*: plant height. V1: Titicaca, V2: Quipu, V3: Regalona, V4: UAFQ7, V5: Q126

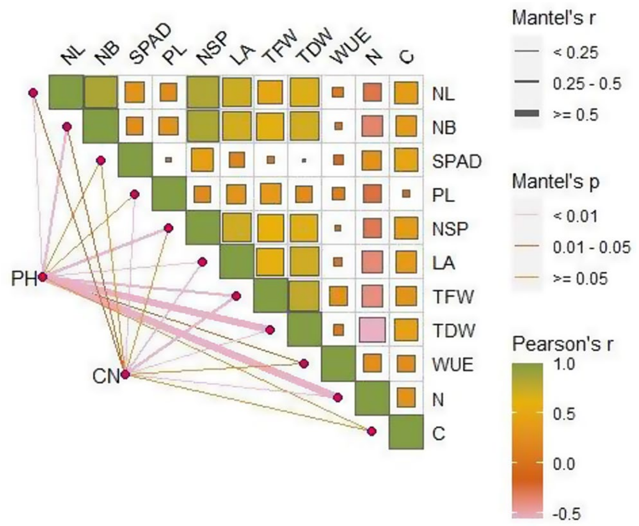

Fig. 8 Mantel graph of growth-related traits measured at the end of the experiment. N: leaf nitrogen content, C: leaf carbon content, *C: N*: carbon to nitrogen ratio, SPAD: SPAD index (leaf chlorophyll), *NSP*: number of sub panicles, *NL*: number of leaves, *NB*: number of branches, *LA*: leaf area, *TFW*: total fresh weight, *WUE*: water use efficiency, *PL*: panicle length, *PH*: plant height

4 Discussion

Drought imposed during the vegetative phase significantly reduced the growth of all five quinoa varieties. However, soil amendment with biochar has been shown to alleviate adverse effects of water stress, thereby promoting growth and water use efficiency in quinoa. Previous studies have indicated that the Danish variety Titicaca is notably susceptible to prolonged water stress periods (Akram et al. 2023) and water shortage during the vegetative growing cycle (Rivelli et al. 2024). Despite this, woodchip biochar has been effective in helping the Titicaca to avoid the negative consequences of water stress (Akram et al. 2023, 2024b; Issa Ali et al. 2019; Akram et al. 2025).

Similarly, in the present study, biochar addition enhanced plant development across different quinoa varieties, though the extent of its effectiveness varied. Significant differences were observed in growth parameters such as plant height, leaf numbers and area, and fresh and dry biomass among the varieties tested (Tables 2 and 3; Fig. 4). These differences align with quinoa's diverse distribution, which has resulted from different quinoa ecotypes' behaviours (Risi and Galwey 1989; Hafeez et al. 2022). The varieties in this experiment, representing different ecotypes (collected from different origins), exhibited varied responses to biochar addition. Correlation analysis (Figs. 6, 7 and 8) revealed that varieties with higher leaves and branching characteristics tend to develop more inflorescence (Table 4; Fig. 5). Inflorescence length was also positively associated with plant height, indicating that varieties with greater plant height developed longer panicles, which was also reported in another study (Yang et al. 2016). Our study results, in line with a previous study (Rivelli et al. 2024), observed that woody biochar application under water shortage conditions improved the plant height, number of leaves and branches and leaf area of quinoa. Similar trends were also noted in other crops, such as stevia (Ahmadzai et al. 2024), spinach (Kausar et al. 2023), tomato (Obadi et al. 2023), and canola (Ullah et al. 2024).

The maintenance of leaf water content is of paramount importance in plants (Farooq et al. 2024). However, leaf water potential depends on the severity and duration of water stress (Hájíčková et al. 2024; Alomari-Mheidat et al. 2024; Pereira et al. 2024) and the type of varieties used for the trial (Geleta et al. 2024; Pappula-Reddy et al. 2024). In the current study, water-related attributes, including predawn leaf water potential and RWC, were adversely affected by water stress. However, adding woody biochar to the soil improved plant water status and maintained growth under water-limited conditions (Table 1; Fig. 1). For instance, the Danish variety Titicaca (V1) maintained higher water content due to its smaller leaf area that might experience reduced transpiration.

In contrast, the Pakistani variety UAFQ7 (V4) was most affected due to its bushy canopy with numerous leaves and branches. The improved plant water status might be due to the increased cation exchange (CEC) following biochar amendment that led to reduced Na^+ uptake and increased K^+ uptake, which are also supported by another study (Yang et al. 2020). Biochar's porous structure and high surface area are known to contribute to increased CEC, improved soil aeration, and reduced soil bulk density, thereby facilitating enhanced root penetration (Yu et al. 2019). In response to severe water stress, quinoa plants exhibited the capacity to maintain positive turgor pressure up to zero turgor, as previously reported (Jenson et al. 2000). Importantly, a reduction in the turgid weight to dry weight ratio (TW: DW) was observed exclusively in quinoa (cv. Titicaca) amended with woodchip biochar (Akram et al. 2023). Andersen et al. (1996), including a low osmotic potential, a diminished TW: DW, reduced tissue elasticity and the ability to sustain positive turgor pressure at low leaf water potential. The observed decrease in the turgid weight to dry weight ratio in biochar-amended plants suggests a potential enhancement of drought tolerance through alterations in plant water relations. Notably, after water stress conditions diminished, the Pakistani variety UAFQ7 recovered its water status more rapidly than other varieties, with biochar further enhancing this recovery. The observed drought response mechanisms varied among the different varieties tested. The Danish variety displayed a drought escape mechanism by shortening its life cycle (by phenology observations, data not shown), also reported by another study (Taaime et al. 2022).

In contrast, the Italian variety Quipu and Pakistani UAFQ7 exhibited drought avoidance through stomatal regulation and more developed root systems (Akram et al. 2021, 2024c). Further investigations of stomatal morphology and root development of tested varieties are needed to elucidate these mechanisms. The Pakistani variety appeared to be the most efficient in biochar response, likely due to its ability to develop longer roots based on its ecotype properties, while biochar can further enhance the development of its roots under water-limited conditions (Xiao et al. 2016). Further decrease in the bulk density due to highly porous biochar enhanced the total porosity of the soil that ultimately increased the soil aeration properties improves root respiration and soil microbial communities to facilitate a better plant growth (Hossain et al. 2020). The latter also showed a significant leaf expansion during post-water stress recovery, evident by no significant differences between well-watered and water-stressed conditions at the experiment's end (Table 3).

Water stress lowers the rate of photosynthesis by stomatal closure, which prevents leaves from absorbing CO_2. It decreases the photochemical efficiency of photosystem PSII by reducing electron transport and releasing reactive oxygen species (ROS) (Zahra et al. 2023). Biochar application can enhance WUE through multiple physiological mechanisms, primarily by influencing stomatal regulation and root hydraulic conductivity (Murtaza et al. 2024). The porous structure of biochar improves soil water retention and availability, thereby reducing plant water stress. This can lead to a reduction in stomatal conductance, minimizing transpiration water loss while maintaining sufficient CO_2 uptake for photosynthesis (Gharred et al. 2022). Furthermore, biochar influences hormonal signalling, particularly that of abscisic acid, enabling more efficient stomatal regulation (Wu et al. 2023). As a result, plants optimize their carbon assimilation per unit of water transpired, thereby improving WUE. It is likely that it also occurred in our experiment because of the low leaf carbon-to-nitrogen ratio, which could result from stomatal closure that conserved water while limiting photosynthesis, as also reported by another study (Zhao et al. 2020). In the current experiment, the SPAD index value of the Danish variety Titicaca (V1) was the most adversely affected by water stress (Fig. 2). However, biochar addition mitigated these adverse effects, resulting in less growth reduction and SPAD index values than non-amended soil. The SPAD index, monitored throughout the experiment, showed pronounced variations under water stress, correlating with leaf nitrogen percentages and carbon-to-nitrogen ratio. Varieties with higher SPAD indices had higher leaf nitrogen percentages and responded better to nitrogen uptake and translocation. Enhanced SPAD index values with biochar were attributed to the large surface area, better water adhesion and cohesion, increased soil acidity, and heightened production of photosynthetic pigments. Similar improvements in SPAD indices were observed in wheat grown under biochar-amended soil (Anee et al. 2022). Another study reported significant improvements in the root morphology of okra under woody biochar application (Jabborova et al. 2021), likely due to enhanced nutrient acquisition and improved microbial communities (Elad et al. 2010; Joseph et al. 2021).

Variations in the SPAD index, notably lower SPAD values among quinoa varieties tested, particularly Regalona, suggest that differences in SPAD are not only due to leaf nitrogen content but may also be influenced by the soil microbiome (Fig. 3). This finding points to the potential of specific quinoa varieties to interact differently with soil microbial communities, possibly affecting their nutrient uptake and growth. While total bacterial counts provide a preliminary view, a detailed examination of the microbial community composition and function is essential to understand these interactions fully. Distinct rhizosphere microbiomes could significantly impact nutrient cycling, especially nitrogen mineralization and availability, which supports nutrient acquisition by the plant (Bhowmik et al. 2017). Additionally, biochar has a multifaceted influence on the soil microbiome. Its porous structure, nutrient retention capabilities, pH moderation, and long-term stability can increase microbial diversity, enhanced microbial activity, improved enzymatic functions, and more resilient microbial communities (Singh et al. 2024). The overall effect highly depends on biochar's properties and the specific soil environment. Still, its positive impact on microbial life makes it a valuable tool for sustainable soil management and ecosystem restoration. Microorganisms introduced with biochar may enhance microbe survival rates, improving microbial integration and proliferation within the soil and the plant rhizosphere (Azeem et al. 2021). Further research is needed to investigate the specific microbial taxa associated with each quinoa variety and to elucidate their functional roles in nutrient acquisition and plant performance. Examining how variety selection and agricultural practices influence these microbial interactions will be crucial for optimizing quinoa production systems.

5 Conclusion

This study demonstrates that biochar soil amendment significantly enhances quinoa's resilience to water stress by modulating key physiological processes, thereby validating our hypothesis that biochar improves vegetative growth of the quinoa plants by influencing key morpho-physiological traits, accordingly, improving water-use efficiency and biomass production under limited water availability. Specifically, biochar facilitated an improved plant water status across all quinoa varieties subjected to water stress, likely due to enhanced root hydraulic conductivity and more efficient stomatal regulation. This resulted in a notable increase in biomass accumulation, particularly in the Pakistani variety UAFQ7, which exhibited the highest overall productivity. Furthermore, the Italian variety Quipu displayed a substantial enhancement in yield-contributing traits by doubling panicle length, suggesting that biochar mitigates stress and optimizes resource allocation for reproductive development. These findings underscore the mechanistic link between biochar's influence on soil properties, plant physiological responses, and subsequent yield improvements. The observed varietal differences, with UAFQ7 and Quipu demonstrating superior performance, highlight the importance of varietal selection for maximizing biochar's benefits in water-limited environments. Furthermore, the Danish variety Titicaca exhibited more pronounced adverse effects under water stress conditions, as evidenced by a significant reduction in SPAD values and biomass accumulation compared to the other varieties. The current study offers a compelling agronomic strategy for enhancing quinoa production in regions experiencing water scarcity, emphasizing the potential of biochar to promote sustainable agriculture and food security. Given these considerations, while the significant benefits of biochar in our study are well-documented, its economic feasibility should be evaluated in a case-specific manner, taking into account production costs, application rates and local agricultural conditions. Future studies incorporating life cycle assessments and cost-benefit analyses could further support the economic viability of biochar as a soil amendment, reinforcing its role in sustainable agriculture and circular economy frameworks. The differential responses between varieties with the following ranking: UAFQ7 > Quipu > Regalona > Q126 > Titicaca, illustrate that biochar's effectiveness is strongly dependent on the variety's genetic makeup, opening new avenues for future breeding programs that aim to maximize crop performance in combination with biochar soil amendments.

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Declarations

Conflict of Interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

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