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# "Growth and morpho-physiological attributes of drought-tolerance in quinoa (*Chenopodium quinoa* Willd.) under biochar-amended soil"

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# ABSTRACT

*Chenopodium quinoa* Willd. is a highly nutritious pseudocereal with the potential to address global food insecurity challenges due to current climate change scenarios. However, the water deficit significantly impacted quinoa's growth despite its inherent tolerance to drought. This research explores quinoa's morpho-physiological responses to water stress and investigates soil amendments, particularly biochar, as a potential agronomic strategy to mitigate the adverse effects of water scarcity. The current literature highlights that biochar, a carbon-rich material derived from pyrolyzed organic matter, can enhance soil physio-chemical properties, improve plant water status, and promote growth under water limitations. Similar to the reproductive phase, the vegetative growing cycle of quinoa is notably susceptible to the detrimental effects of drought. Agronomic strategies must be implemented to mitigate the negative effects of water stress during the vegetative growing cycle. Applying organic amendments, particularly biochar, becomes increasingly important in this context. A series of experiments were conducted to investigate the impact of organic amendments on the vegetative development of quinoa under water stress. These experiments were initiated by analyzing responses of two biochar types (derived from woodchips and vineyard pruning) and vermicompost (from cattle manure) on the Danish variety Titicaca, which is particularly susceptible to water stress during its early growth stages and widely cultivated around the globe particularly in Europe. The amendments were applied alone and in combination at a 2% (w/w) rate. The results revealed that among the organic amendments tested, woodchip biochar, alone and mixed with vermicompost, significantly improved quinoa's growth, specifically biomass, by 22% compared to stressed control and water use efficiency. In contrast, vineyard pruning biochar negatively affected plant growth. Successively, the same variety, Titicaca, was further analysed under different woodchip biochar rates (best performing organic amendment) (0%, 2%, and 4%) and two watering regimes (100% and 50% evapotranspiration restitution). The 2% biochar rate enhanced

vegetative growth, biomass by 23%, and panicle development by 66% compared to 4%, which negatively affected these parameters, highlighting the importance of choosing the right dose based on soil type. Subsequently, considering the 2% woody biochar rate, five quinoa varieties of different origins were compared and subjected to a water stress period starting from the 12-leaf stage. The results showed that biochar application consistently improved plant growth, particularly plant biomass, leaf nutrients, and enhanced C:N ratio, improving nitrogen bioavailability and translocation. Additionally, biochar addition in the soil positively affected root morphology, including elongation by 23% and development particularly fresh and dry biomass by 122% and 127%, respectively, and physiological attributes, i.e., chlorophyll content, plant water status and gas exchange than non-amended soil, although varietal differences were observed. The Pakistani variety UAFQ7 exhibited superior drought tolerance, while the Danish Titicaca was more sensitive to water stress. Moreover, the Italian variety Quipu significantly increased its yield-contributing traits and even doubled under biochar-amended soil than non-treated ones. Under water stress conditions, varieties positively influenced their stomatal morphology, stomatal regulation, and transpiration rates under biochar-amended soils by enhancing stomatal aperture dimensions and stomatal density, particularly for UAFQ7 by doubling the stomatal density than non-treated soil. Collectively, this research underscores the potential of biochar as a sustainable soil amendment to mitigate drought stress in quinoa by enhancing root development, physiological attributes, and overall plant performance. The findings highlight the need to select appropriate biochar types and rates and quinoa varieties to optimize growth under water-limited conditions, offering a promising agronomic strategy for improving quinoa cultivation in drought-prone regions.

# CHAPTER 1

# AIMS OF THE RESEARCH

In the current climate change scenario, agriculture mainly faces the challenge of more food with the least amount of water. Climate change continuity is characterized by irregular precipitation and an increase in global temperature (IPCC 2007), which leads to water scarcity, particularly in arid and semi-arid regions. A reduction in precipitation increases drought frequency, which strongly impacts agricultural productivity (Zarehaghi et al., 2017; Hinojosa et al., 2018). The most critical question in front of agricultural experts, is how to improve crop productivity with more nutrient foods in the current climate change scenario to face future food security challenges.

Quinoa, a pseudocereal native to the Andean region, has emerged as a promising crop due to its inherent drought tolerance (Akram et al., 2021). Its unique anatomical and physiological adaptations, including tissue elasticity, thick-walled cells, and specialized epidermal structures, enable this plant to effectively conserve water under water-limited conditions (Shabala et al., 2012; Al Naggar et al., 2017). Additionally, its ability of stomatal regulation helps to maintain water potential and increase water use efficiency (Jacobsen et al., 2009; Issa Ali et al., 2018; Saddiq et al., 2021). However, the response to water limitations varies among quinoa genotypes, emphasizing the importance of selecting drought-tolerant varieties that better adapt to water-limited conditions. In light of available knowledge, research activities are needed to fully understand the mechanisms underlying quinoa's drought tolerance and to develop agronomic strategies for optimizing its cultivation.

The use of organic amendments, like biochar and compost from plants and animal origin, has the advantage of eco-friendly recycling of soil nutrients and soil remediation (Lim et al., 2016). Among these organic amendments, biochar is gaining more popularity and is categorized by its higher amount of more stable organic carbon compounds than compost, making it slowly

decomposable in soil (Eissa, 2019). Its porous structure and high surface area helps the soil increase cohesion and adhesion forces to retain more water and ensure a slow release of water to plants during water deficiency (Eissa, 2019; Abideen et al., 2020). However, biochar efficiency depends upon the feedstock, pyrolysis conditions, soil type, plant species and application rate; therefore, not every biochar is suitable for agriculture and every crop, so a comprehensive study will be needed to assess its suitability (Siedt et al., 2021). Although quinoa has a strong natural ability to cope with water-limited conditions, its early growth stages are highly sensitive to water stress despite its overall drought tolerance. Thus, the agronomic management of soil by the addition of organic amendments i.e., vermicompost and biochar, to reduce the water stress impacts on quinoa during the vegetative growing cycle is of greater importance. This approach can potentially increase yields and the cultivation area of quinoa, thereby contributing to global food security. Thus, the aims of this study were:

**1)** To evaluate the effects of different types of organic amendments (two types of biochar and compost) at same rate on the Danish variety Titicaca's growth, physiological attributes, and tolerance to water stress periods during the vegetative growing cycle.

**2)** To determine the optimal rate of the most effective organic amendment for enhancing Titicaca's growth and yield-contributing traits under water shortage conditions imposed throughout the vegetative growing cycle.

**3)** To assess the impact of the optimized organic amendment (type and rate) on water relation, gas exchange and above and below ground development of several quinoa varieties from different origins to identify the drought-tolerant varieties.

**4)** To analyse the physio-anatomical responses of the screened drought-tolerant quinoa varieties to biochar-amended soil under water stress conditions to elucidate the mechanisms underlying their tolerance.

Before starting the research activities, a comprehensive literature review was conducted on the effects of drought on quinoa, its responsive mechanisms, and the potential of biochar to mitigate drought impacts. While the bibliographic studies highlighted the importance of understanding the interplay between biochar, soil, and plants, they lacked a detailed analysis of water stress's impact on quinoa's vegetative growth. This emphasized to explore the effects of various organic amendments on soil physico-chemical properties and quinoa growth, as well as the importance of long-term field investigations and assessments of quinoa varietal responses to biochar-amended soils under water stress conditions **(Chapter 2)**.

The initiation of experimental activities for the doctoral study, based on the knowledge gap identified by bibliographic research, focused on evaluating the impact of different organic amendments on quinoa growth and water use under drought conditions. Two types of biochar (i.e., from woodchip and vineyard pruning) and vermicompost were applied to the Danish variety Titicaca during the vegetative growing cycle, both individually and in combination. The selection of Titicaca was based on its wide cultivation and most importantly its drought sensitivity that could lead us to evaluate the effectiveness of amendments under water limitations. The study comprehensively monitored various plant growth parameters, water-related attributes, and yield-related traits and soil’s properties to assess the influence of these amendments on plant development and water consumption. The experiment exhibited that woodchip biochar alone and in combination with vermicompost was the best performing amendment that was used for further studies **(Chapter 3, Chapter 5).**

After selecting the best performing organic amendment, i.e., woodchip biochar, the second phase involved the investigation of the interactive effects of different biochar rates (0%, 2% and 4% based on dry weight of soil) and water regimes (W100 and W5, restitution of 100 and 50% of evapotranspiration, respectively) on plant phenology, growth and water-related traits,

and yield-contributing characteristics of the same variety, Titicaca, in addition to the soil properties, during the vegetative growing cycle **(Chapter 4, Chapter 5)**.

After biochar dose optimization, the next step in this study was to evaluate the behaviour of several quinoa varieties from different ecotypes to the optimized dose under water limitations. These varieties were selected based on their tolerance to drought and cultivating locations, i.e., Italian and Pakistani grown varieties along with already tested Titicaca variety. Given the limited knowledge available on the response of diverse quinoa varieties to biochar-amended soil, particularly during the early growth stages, this research contributes significantly to our understanding of the potential benefits of biochar based on varietal response. The research findings highlighted the potential of biochar soil amendment as a sustainable strategy to improve quinoa productivity and adaptability under water limitations (**Chapter 6)**.

Following the screening of quinoa varieties, the subsequent phase of the study focused on a more in-depth evaluation of the three selected varieties, with particular emphasis on their root development. Given the critical role of root traits in drought tolerance, the study aimed to explore the potential benefits of biochar application in enhancing quinoa's root growth **(Chapter 7)**. The final phase of the doctoral study focused on investigating the mechanisms underlying the beneficial effects of biochar on selected quinoa varieties by examining stomatal morphology, water potential, and gas exchange parameters. The study elucidated biochar improves plant physiological responses to drought stress. The results contribute significantly to our understanding of how quinoa varieties maintain their stomatal morphology and plant water status under soil amendment with biochar to mitigate the impacts of water-limited conditions occurring during the vegetative growing cycle **(Chapter 8)**.

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# CHAPTER 2

# INTRODUCTION

# DROUGHT STRESS IN QUINOA: EFFECTS, RESPONSIVE MECHANISMS, AND MANAGEMENT THROUGH BIOCHAR AMENDED SOIL: A REVIEW

*This chapter is a state-of-the-art chapter, and its content has been already published:*

**Akram, M.Z.,** Libutti, A., & Rivelli, A.R. (2024). Drought stress in quinoa: effects, responsive mechanisms, and management through biochar amended soil. A review. *Agriculture, 14(8), 1418.*

## 2.1. ABSTRACT

*Chenopodium quinoa* Willd. (quinoa), a highly nutritious pseudocereal, is a promising crop to address global food insecurity challenges intensified by population growth and climate change. However, drought stress remains a significant constraint for quinoa cultivation. The plant exhibits several morphophysiological adaptations to water stress conditions, including root system modifications, reduced growth rate, leaf abscission, and stomatal closure. While these adaptations enhance drought tolerance, they can also negatively impact plant growth, potentially through alterations in root architecture, physiological changes, e.g., stomatal regulations, and anatomical changes. Different studies have suggested that soil amendment with biochar, a pyrolyzed organic material, can improve quinoa growth and productivity under drought stress conditions. Biochar application to the soil significantly enhances soil physiochemical characteristics and maintains plant water status, thereby promoting plant growth and potentially mitigating the negative consequences of drought on quinoa production. This review focuses on the current understanding of quinoa behavior under drought stress and the potential of soil amendment with biochar as a management strategy. We summarize existing research on applying biochar-amended soil to alleviate quinoa drought stress.

*Keywords: Drought; Chenopodium quinoa* Willd.*; morphology; physiology; anatomy; biochar; soil properties; plant growth*

## 2.2. INTRODUCTION

Current agricultural practices include excessive use of chemical fertilizers, pesticides, herbicides, untreated sewage, and industrial wastes that cause pollution and severe soil degradation (Bisht and Chauhan, 2020). Thus, while thinking about food production, the attention must also be towards the reclamation of soil-degraded properties, the efficient usage of environmental resources, and sustainability to avoid further damage (Khan et al., 2023). Under natural field conditions, plants can face adverse environmental conditions like excess salts, water deficiency, heavy metal accumulation, high temperature, or light that can alter average plant growth (Kapazoglou et al., 2023). Global warming causes water deficiencies through changes in rainfall patterns that significantly impact sustainable crop production (Begna, 2022).

Quinoa (*Chenopodium quinoa* Willd.) is a pseudocereal, nutrient-diversified crop known as a super and functional food with promising potential to meet the global food demand and aid in reducing poverty due to population growth and global warming (Nazeer et al., 2022). It originated from the Andean region, where it is consumed as a staple food, and is ranked as a climate-proof crop due to its ability to withstand adverse climate conditions (Bazile, 2023). Its remarkable agronomic adaptation to adverse climatic conditions makes it appropriate for cultivating in regions susceptible to climate change's effects (Cui et al., 2024). Quinoa's superior adaptability enables its cultivation in a diverse range of environments, including lowlands, marginal lands, deserts, and areas above 4000 m of sea level (Yadav et al., 2023).

Recently, it has been gaining global attention because of its gluten-free and superior nutrition profile (Liu et al., 2022). The year 2013 was declared by the United Nations the "International Year of Quinoa" due to the importance of this crop in ensuring food security and quality. A significant increase in quinoa production worldwide was evident after that (Agarwal et al., 2023).

In 2020, Peru was the leading quinoa producer, yielding 134,400 metric tons, followed by Bolivia with a production of 88,500 metric tons (Patel et al., 2024).

Quinoa's remarkable adaptability to diverse environments is attributable to its extensive genetic variability (Ain et al., 2023). The plant shows numerous adaptations to drought conditions through its morphology and physiology by exhibiting different drought responses, including escape, avoidance, and tolerance (Ain et al., 2023). The different responses involve changes in root architecture and growth, leaf dropping, and stomatal closure and might include changes in gene expression (Hinojosa et al., 2018). Quinoa is important for current and future challenges and is a source of genes with major biotechnological applications (Alandia et al., 2021).

Drought appears to be the most recurring natural disaster whose impacts on available water resources depend upon timing, duration, frequency and intensity (de Vries et al., 2023). The Mediterranean region has faced these trends since the 1960s and is also known as the region most vulnerable to climatic changes and global warming (Legg, 2021). Recently, drought has spread globally in two-thirds of all cultivation area, which is foreseen to increase due to global warming (Kim et al., 2023). Drought stress significantly affects plant growth (Shiade et al., 2022), physiology (stomatal conductance, respiration) (Babar et al., 2023), morphology (leaf dropping, root architecture) (Ahmad et al., 2022) and biochemistry (increased production of reactive oxygen species) (Munsif et al., 2022), which ultimately creates serious threats for future food security (Wen et al., 2021). The current focus is on using potential organic amendments, specifically biochar, that can simultaneously enhance crop growth, productivity, and soil properties under drought conditions and contribute to future food security.

Biochar is a carbonaceous material obtained from pyrolysis conducted at high/moderately high temperatures (250 to 900 °C) (Mašek et al., 2024). Historical perspective gives the main reason for manufacturing activated carbon (biochar) as improving crop productivity through soil application (Kamarudin et al., 2022). Biochar is highly recalcitrant in soil, and its persistence in

soil is approximately 10–1000 times longer than most soil organic amendments (Lehmann et al., 2024). In recognition of its potential to improve soil's physicochemical properties and crop production, biochar has become a significant focus for scientists and farmers in modern agriculture (Khan et al., 2023). Plant growth stimulation through biochar addition is attributed to enhanced soil water retention capacity and mineral content (Whalen et al., 2024), increased soil cation exchange capacity (Antonangelo et al., 2024), neutralized phytotoxic compounds in soil (Kumari et al., 2024), decreased soil bulk density (Das and Ghosh, 2024), improved soil hydraulic conductivity (Libutti et al., 2021), drainage and aeration (Kabir et al., 2023), modified soil pH (Akram et al., 2024a; Hailegnaw et al., 2019), flourishing soil microbiota, and induced systemic resistance in plants against diseases (Arshad et al., 2024). Various studies have shown biochar's capacity to immobilize natural and inorganic contaminants in soil (Long et al., 2024). It has been reported that biochar significantly enhances biomass, growth, physiology, water use efficiency, and drought tolerance in quinoa (Kammann et al., 2011; Ramzani et al., 2017; Akram et al., 2023). A study previously revealed that soil amendment with biochar increased quinoa biomass and yield-contributing traits up to 21% and 50%, respectively (Akram et al., 2023). In this review, we will discuss: (1) quinoa botanical characteristics and responses to drought stress; (2) recent advancements (from the last decade) in using biochar to alleviate drought stress through soil property improvements and enhance plant growth under water shortage conditions; and (3) quinoa's responses to biochar application under drought conditions.

## 2.3. QUINOA'S BOTANICAL CHARACTERISTICS AND GERMPLASM DIVERSITY

A quinoa plant ranges between 0.3 to 3 m in height, while its stems are thick, erect, and hollow. It exhibits various colors, i.e., white, yellow, purple, pink, and black. At the start, its leaves show a green color, turning yellow, red, or purple with time, depending on the genotype (Tabatabaei et al., 2022). The leaves are alternate, generally pubescent, of different sizes on the same plant, and have a goosefoot shape with a lanceolate to triangular outline (El-Harty et al.,

2021. The seeds are cylindrical-lenticular-shaped, with two flat surfaces, and are round on both sides, with a diameter of 0.28–2.1 cm (Apaza et al., 2015). Like the plants, the seeds are also diverse in color, varying from yellow to orange, pink, red, and black (Coronado et al., 2022). Its panicle is of three different shapes, i.e., Glomerulate, Intermediate, and Amarantiform (Voronov et al., 2023). The panicle density is classified into three types: lax, intermediate, and compact. From this, cultivars with compact panicle density usually yield high (Habib et al., 2024). Pollen grains are monads, medium in size, from 42.99 to 51.30 µm, polypantoporate, scabrate (microechinate), and have an exine thickness of 2.08–2.40 µm (Sarwar et al., 2023). Mature quinoa plants can have a main panicle length of 30 to 60 cm (Voronov et al., 2023) and 100-seed weights ranging from 0.2 to 0.5 g based on the different genotypes (Maestro-Gaitán et al., 2023). Flowering typically occurs after 40–50 days, with some quicker varieties reaching this stage in just 25–30 days. Commonly known quinoa varieties, including the Italian variety Quipu (Casini, 2017), Chilean variety Regalona (Vásquez et al., 2024), and Pakistani variety UAFQ7 (V7) (Akram et al., 2021), take 150 to 180 days to mature, while some faster-growing varieties, like the Danish varieties Titicaca and Baer, can be ready in just 90–100 days (Taaime et al., 2022). It has a pivotal, vigorous, deep root system reaching up to 1.8 m deep in the soil. The roots are well-branched and play an essential role against drought and in the stability of the plants (Ain et al., 2023). Akram et al. (2021) tested 13 superior genotypes from 128 USDA lines under agroecological conditions in Faisalabad. They reported that UAFQ7 was recorded to have longer root growth and higher yield than the other genotypes studied. UAFQ7 was later approved as the first quinoa variety in Pakistan.

Undoubtedly, the center of origin for quinoa is the Andean Altiplano and its extended areas, i.e., Bolivia, Peru, Ecuador, Northern Chile, and Colombia (Bazile, 2023). Huge phenotypic diversity and inter-varietal variations were observed among quinoa germplasm (Romero et al., 2019; El-Harty et al., 202; Manjarres-Hernández et al., 2021; Hafeez et al., 2022). Given the

vast genetic variability of the different ecotypes, there is a need to screen the germplasm for specific objectives, like drought tolerance. The germplasm evaluation is the first step to assessing quinoa suitability and adaptability potential to new environmental conditions (Hafeez et al., 2022). According to a study conducted by Gomez-Pando et al. (2019a), greater diversity was found between the Cuzco, Peru, and Bolivia ecotypes, with the largest number of landraces located in the Andean area and its extensions, including Lake Titicaca in Bolivia and Peru. Evaluating germplasm collected from various locations is essential for the effective agricultural management of this crop (Kilian et al., 2021).

Based on ecotypes, quinoa is classified into five classes: (i) Valley, (ii) Altiplano, (iii).Salares, (iv) Subtropical/Yungas, and (v) Sea level. These ecotypes may contribute to unique and remarkable adaptations to drought (Bazile, 2023). Depending on the ecotype, quinoa exhibits different drought-responsive mechanisms, i.e., drought escape, avoidance, and tolerance. Most ecotypes from Valley and Altiplano are of short-duration varieties, like Titicaca (Taaime et al., 2022). They complete their growth cycle earlier to avoid the negative effects of drought at later growth stages, called terminal drought, exhibiting drought-escape mechanisms. On the contrary, ecotypes from Salares outlined deeper and more well-established root systems (Ain et al., 2023) and stomatal regulations than other ecotypes, exhibiting drought avoidance. Commonly used varieties from these ecotypes are UAFQ7 and Quipu. On the other hand, Subtropical and Sea level ecotypes exhibited drought-tolerance mechanisms through tissue elasticity and low osmotic potential. They accumulated higher proline levels along with the induction of ornithine and raffinose pathways that affect nitrogen-associated enzymes (Bascuñán-Godoy et al., 2016). Notable varieties from these ecotypes are Regalona (Von Baer et al., 2009) and PI665283 accession (Hafeez et al., 2022).

Currently, quinoa evaluation for its adaptability under diverse environmental conditions is undergone globally, especially in the U.S., Australia, Canada, China, E.U., India, Israel, Middle

East, Pakistan, and Turkey, by collection of its germplasm all over the world (Alandia et al., 2020). Expanding the cultivation of this crop by identifying high-yielding quinoa cultivars adaptable to different agroecological zones is a primary approach to meeting the increasing global demand for food (Angeli et al., 2020).

## 2.4. QUINOA RESPONSES TO DROUGHT

Insufficient soil moisture content in the soil, referred to as agricultural drought, significantly reduces plant production (Walia et al., 2024). Drought frequency has increased for the past few years, with detrimental impacts on crop production and the agricultural system, which is the backbone of food security. Globally, quinoa is considered a drought-tolerant crop that can grow in arid and semi-arid regions receiving less than 200 mm of annual rainfall, including Chile, Northwest Argentina, Peru, and Bolivia (Cruz et al., 2024). Apart from these areas, studies conducted outside of the Andean region have shown that quinoa has the potential for adaptation and seed production in other arid and semi-arid environments, such as the Mediterranean area, Asia, and North Africa (Abidi et al., 2024; Matías et al., 2024). Its exceptional ability to grow under water limitations is due to its low water requirements and capacity to maintain gaseous exchanges during the occurrence of water stress conditions (Grenfell-Shaw and Tester, 2021). Quinoa confronts these conditions and produces substantial yields (Pathan et al., 2023).

Drought response in quinoa is attributed to its deep extended root system, which can penetrate up to 1.5 m in sandy soils (Ain et al., 2023), and the presence of calcium oxalate vesicles on leaves, which help to reduce transpiration (Infante et al., 2018). The plant can also avoid the negative consequences of drought through leaf-area reduction and small cells with thick walls that can preserve water and release it during the dry period to maintain its water status (Otterbach et al., 2021). Additionally, due to its immense diversity in genotypes and ecotypes, quinoa's drought responses are divided into drought escape, avoidance, and tolerance (Raney, 2012). Usually, short-duration cultivars develop drought escape mechanisms by shortening

their life cycle through precocity (Zurita Silva et al., 2015). This phenomenon is essential in those areas where the drought instance risk is higher at the end of the growing season, called terminal drought, such as the Altiplano and Valley regions (Tapia, 2015).

Alternatively, drought avoidance creates an equilibrium between water uptake and loss (Walia et al., 2024). Plants accumulate excess osmolytes to lower the tissue water potential and enhance water uptake (Iqbal et al., 2023). Moreover, for water conservation, stomatal closure reduces transpiration (Ain et al., 2023). Drought avoidance mechanisms become insufficient under severe stress conditions, and the stress tolerance mechanisms aim to protect against cell damage. They usually involve the detoxification of reactive oxygen species (ROS) and the accumulation of late embryogenesis abundant (LEA) proteins, as well as other solutes, especially proline, that act as osmolytes and osmoprotectants simultaneously (Sachdev et al., 2023). Both mechanisms follow the ABA-dependent pathway that includes DREB proteins (Zhang et al., 2022). As drought conditions diminish, quinoa can resume its former photosynthetic level and leaf area (Jacobsen et al., 2009).

### *2.4.1. Morphological Responses of Quinoa*

Several morphological adaptations were reported for quinoa under drought stress, significantly reducing leaf area through leaf shedding (Kohan et al., 2023) and extending the root system (Nguyen et al., 2022). The inhibition of leaf growth and reduction of leaf area appeared to reduce water loss through transpiration to ensure plant survival under water limitations (Ain et al., 2023). However, in severe drought, leaves wilt and are shed to reduce transpiration and avoid direct exposure to sunlight (Maestro-Gaitán et al., 2023). A rapid and sharp decrease in leaf elongation in quinoa has been reported due to drought conditions termed acute growth inhibition. In contrast, after drought termination, the recovery of new steady-state growth, referred to as acclimation, has been observed. Moreover, quinoa's leaf expansion rate is reduced

by 30–50% under water-limited conditions, compared to well-watered plants (Zurita Silva et al., 2015).

Quinoa root architecture and morphology are important in determining its ability to face drought conditions. The root architecture is generally classified into two types: dichotomic, i.e., without any predominant root axis, and herringbone, with the main root axis supporting lateral roots (Fitter et al., 1991; Bouma et al., 2001). Under drought conditions, quinoa reveals a herringbone root architecture pattern (Singh et al., 2022) that helps the plant to efficiently explore deep soil layers for water and nutrients (Gandarillas et al., 2013). Under limited water availability, a rapid primary root elongation was observed that slowed down afterward, giving good support to the above-ground plant part (Alvarez-Flores et al., 2018). However, quinoa root development is strongly influenced by ecotypes (Singh et al., 2022). Compared to Salares ecotypes, coastal ecotypes show slower root growth (Zurita Silva et al., 2015). A study revealed that when both ecotypes were at 6 to 9 cm of shoot length, the Salares ecotypes reached 1 m deep in the soil, as compared to the coastal ecotype that attained the same length after 1 to 2 weeks (Flores, 2012). When drought conditions arise at early plant growth stages (vegetative growing cycle), primary roots are significantly longer, which is helpful in the exploitation of deeper and more reliable water resources through deeper penetration in the soil (Issa Ali et al., 2019).

Water limitations undoubtedly reduce the total root length, but less reduction has been recorded in the Salares ecotype than in other ecotypes. As a response to drought, primary root elongation is stimulated in all ecotypes but more pronounced in Salares than others. The faster primary root elongation allows this ecotype to produce lateral roots evenly distributed in the deeper soil from 50 to 75 cm, like the root density of plants grown under humid conditions. Instead, coastal ecotypes reach only 5 to 50 cm, with lower root densities (Alvarez-Flores et al., 2014).

### *2.4.2. Physiological and Biochemical Responses of Quinoa*

Studies have revealed that quinoa adapts different physiological and biochemical strategies to counter the negative consequences of drought (Nguyen et al., 2022; Forouzandeh et al., 2023). From these strategies, osmotic adjustments are crucial in maintaining turgor pressure during water limitations (Hinojosa et al., 2018; Gámez et al., 2019). The accumulation of osmolytes like glucose, trehalose, free amino acids, proline, and total soluble sugar significantly increases, directly stimulating antioxidant enzymes and decreasing lipid peroxidation and hydrogen peroxide content during drought stress (Sadak et al., 2019). Other mechanisms, such as the accumulation of calcium oxalate, enhanced protein stability, and thermostability of chlorophyll machinery (Killi and Haworth, 2017), also enhance drought tolerance in quinoa. Drought conditions cause a significant reduction in gaseous exchange traits, including stomatal conductance, transpiration rate, and photosynthesis rate, along with leaf water potential (Ain et al., 2023). A rapid closure of stomata is evident with a two-thirds reduction in transpiration and photosynthesis, and it becomes stable until water potential does not drop below −4 MPa and drought conditions remain. Importantly, unless severe drought conditions exist, quinoa does not respond to abscisic acid, and even if stomata close, they can photosynthesize at very low water levels for three days (Jacobsen et al., 2009) through oxalic acid conversion to carbon dioxide for photosynthesis. Stomatal closure in quinoa has been reported to occur at leaf water potential below −1.2 MPa (Jensen et al., 2000). The leaf water relation is characterized by low osmotic pressure and a low turgid weight to dry weight ratio, which sustains the potential gradient for water movement from soil to leaves to maintain turgor (Jensen et al., 2000; Akram et al., 2023). Another possible approach in quinoa for stomatal closure is the production of anti-transpirant compounds other than abscisic acid, particularly cytokinin, that act antagonistically to abscisic acid (Joshi et al., 2023). When its transport in the xylem is reduced, stomatal sensitivity to xylem abscisic acid increases accordingly (Jacobsen et al., 2009). It is reported that quinoa plants close their stomata to maintain leaf water potential and photosynthetic activity (Issa Ali

et al., 2019). Apart from this, the accumulation of soluble sugars, proline, and glycine betaine, also reported for quinoa under drought conditions, plays a role in osmotic adjustment (Sadak and Bakhoum, 2022). Furthermore, proline accumulation has a dual purpose, i.e., osmotic adjustment and osmoprotection simultaneously (Sachdev et al., 2023). It scavenges free radicals and prevents membrane protein denaturation because of osmotic stress caused by severe water limitations (Zulfiqar et al., 2020). Moreover, proline and sugar accumulation act as compatible osmolytes and maintain the cell turgor pressure required for cell expansion. Additionally, quinoa tolerates drought through growth plasticity and tissue elasticity (Al-Naggar et al., 2017). Recently, it has been reported that enhanced drought resistance in quinoa is also characterized by its ability to resume photosynthetic activity after eliminating drought conditions (Manaa et al., 2021).

### *2.4.3. Quinoa Anatomical Responses*

Quinoa exhibits massive leaf senescence and bladders or glands in the leaves and stems, the volume of which depends upon the severity of drought (Al-Naggar et al., 2017). It can rapidly recover from the consequences of drought by forming new leaves for leaf area expansion. Quinoa has smooth leaf surfaces without trichomes in mature leaves while containing a thicker cuticular epidermis (Prado et al., 2017). Moreover, the young leaves and stems are covered with bladders containing calcium oxalate and silicic anhydride that are hygroscopic in nature and help reduce transpiration (Riccardi et al., 2014). Additionally, epidermal bladder cells on the shoot, especially on younger parts of the plant (Otterbach et al., 2021), are used as additional reservoirs for moisture conservation that are released during water-limited conditions to maintain turgor pressure. Stomata are deeply sunken in the epidermis, aiding drought tolerance (Prado et al., 2017).

Al-Naggar et al. (2018) reported a significant decrease in leaf thickness and upper and lower epidermis under water stress. Alternatively, under severe water stress, the leaves exhibit a

substantial increase in palisade and spongy layer thickness, which helps in enhancing mesophyll conductance that ultimately aids in more $CO_2$ diffusion, whose role in increasing photosynthesis is crucial. Moreover, photosynthesis occurs in the palisade, so increasing its thickness increases the photosynthetic activity and the production of carbohydrates (Milla-Moreno et al., 2016).

## 2.5. BIOCHAR PRODUCTION AND USAGE FOR MANAGEMENT OF DROUGHT STRESS

Since 2009, the quantity and quality of research on biochar increased, just after the COP15 event organized by the European Commission in Copenhagen (Verheijen et al., 2014). Afterward, from 2014, a dramatic increase in articles on biochar was evident, about three times more than any other organic amendment (Aubertin et al., 2022). These articles characterize biochar's role in circular economy and role under different conditions of abiotic stress, including drought, salinity, heavy metals, and biotic stress (Figure 1). Biochar is a porous, high carbon-content product obtained through pyrolysis of different biomasses, such as plants, animals, and municipal wastes (forestry, agricultural and agro-industrial residues, manure, and sewage sludge), at elevated temperatures (250 to 900 °C) and under low- or no-oxygen conditions (Mašeket al., 2024). It has the ability to sequester atmospheric $CO_2$, thus helping in climate change mitigation (Murtaza et al., 2021). It significantly affects soil's physical (Kabir et al., 2023), hydraulic (Libutti et al., 2021), chemical (Hailegnaw et al., 2019), and biological properties (Arshad et al., 2024; Long et al., 2024). Biochar's presence neutralizes phytotoxic compounds in soil (Kumari et al., 2024). Plant growth stimulation is attributed to biochar's unique physicochemical properties, such as its highly porous structure, large inner surface area, greater negative surface charge, and charge density, which result in enhanced soil water retention capacity and mineral contents (Whalen et al., 2024), increased soil cation exchange capacity (Antonangelo et al., 2024), and decreased soil bulk density (Das and Ghosh, 2024). Recently, biochar has gained considerable attention because of its role in water retention and

soil remediation (Hou et al., 2024). Biochar is an essential soil amendment, as it binds all toxic elements and pollutants in the soil and increases crop production rates (Shoudho et al., 2024). It prevents water and nutrients from leaching, can retain moisture longer than non-amended soils (Maisyarah et al., 2023), and helps in reducing the adverse effects of drought. The multidimensional applications of biochar make it suitable for agricultural improvements and solid waste management (Shoudho et al., 2024). Presently, synthetic and other bio-based fertilizers dominate the agricultural sector. However, due to several initiatives, farmers are gradually spreading awareness to include biochar in farming activities (Kurniawan et al., 2023), thus creating huge avenues for market growth in the coming years.

Biochar's effects depend on different factors, including original feedstock, pyrolysis temperature, soil type, application rate, and plant species (Mishra et al., 2023). Biochar addition may positively affect the soil C sequestration and, thus, act as a sink and long-term storage of C due to its long residence time in the soil, ranging from 100 to 1000 years (Li and Tasnady et al., 2023). In particular, woody biochar, which has high carbon, is being recognized by scientists for its potential role (Khan et al., 2021). Although biochar contains higher C contents of up to 80%, it depends upon the pyrolysis conditions in which it was manufactured (Amalina et al., 2022). Significant variation exists among the characteristics of different biochars. For instance, biochar from pyrolysis conducted at a higher temperature (>350 °C) is characterized by higher pH, cation exchange capacity (CEC), and extractable $NO_3$. In contrast, biochar obtained at a lower temperature (<350 °C) has a higher concentration of extractable phenols, $NH_4$, and P (Khater et al., 2024). Furthermore, low pyrolysis temperature degrades the cellulose of the feedstock materials and may cause a considerable reduction in mass by volatilization, producing a hard, shapeless C matrix. Pyrolytic temperature is directly related to the concentration of aromatic C in biochar; i.e., an increase in temperature increases the proportion of aromatic C due to reduced volatile matter (Ippolito et al., 2020).

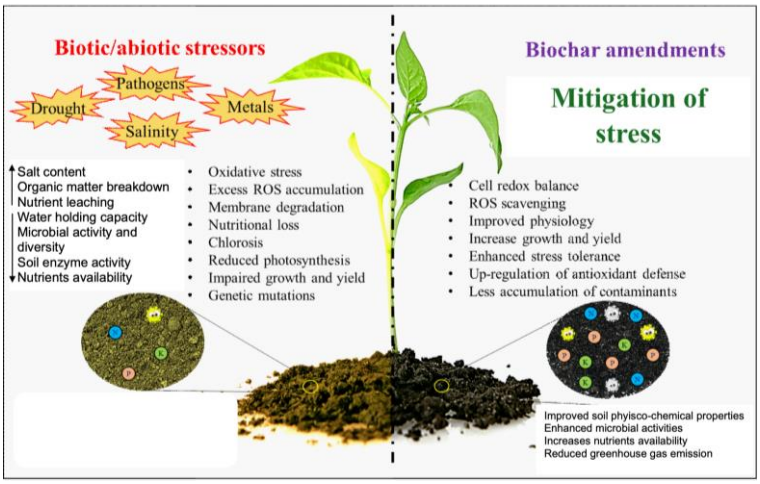


**Figure 1.** Role of biochar under biotic (de Medeiros et al., 2021; Jaiswal et al., 2024) and abiotic stress (Imran et al., 2022; Wu et al., 2023; Volpi et al., 2024). Upward arrows indicate increased levels of the indicated parameter, while downward arrows represent decreased levels.

Typically, biochar has two main morphological stages: crystalline graphene sheets in the form of layers and shapeless aromatic structures that are haphazardly arranged. Hydrogen (H), oxygen (O), nitrogen (N), phosphorus (P), and sulfur (S) are mainly present integrated within the aromatic rings as heteroatoms (Chen et al., 2017). The presence of heteroatoms imparts great heterogeneous surface chemistry and reactivity to biochar.

Furthermore, the biochar application rate to the soil exerts a significant impact on the soil's characteristics and plant growth. For instance, higher biochar doses than optimum may lock up ions, making them unavailable for plant uptake (Gale et al., 2017; Khan et al., 2021). A study revealed that larger biochar doses have a highly volatile matter that reduces plant nitrogen uptake and reduces plant growth (Khan et al., 2021). Similarly, these doses can reduce the population of beneficial mycorrhizal fungi due to changes in soil pH and the toxic effects of salts and heavy metal contents present in biochar (Mukherjee and Lal, 2014). Conversely, high application rates of biochar can offer environmental advantages, such as carbon storage and reduced nitrate pollution, but the absence of economic incentives may hinder its widespread adoption in the short term (Haider et al., 2017). However, these environmental benefits can

contribute to long-term sustainability (Idbella et al., 2024). Thus, any counterproductive effects should be comprehensively studied before adding biochar on a large scale.

During pyrolysis, formulation of several functional groups [e.g., hydroxyl (-OH), amino ($NH_2$), ketone (-OR), ester (-(C=O)OR), nitro (-$NO_2$), aldehyde (-(C=O)H), carboxyl (-(C=O)OH)] takes place mainly on the outside of the graphene layers and porous surfaces (Pustahija and Kern, 2023). Some of these groups behave as electron donors. Contrastingly, some act as electron acceptors determining the biochar state, either acidic/basic or hydrophilic/hydrophobic (Amonette and Joseph, 2012). Assimilation of H, N, O, P, and S in the aromatic rings is used to determine the electronegativity of the biochar, thereby improving CEC. The type of interaction between biochar and other soil components, such as soil particles, dissolved organic matter, gases, microorganisms, and water, is influenced by surface charge (Whalen et al., 2024). These biochar characteristics enhance the soil's physicochemical properties, increase soil fertility, and provide a large area for crop cultivation (Pandian et al., 2024). When the biochar particles remain in the soil for a long time, they increase the oxidation process and negatively charge at the soil surface, enhancing soil CEC (Whalen et al., 2024). Biochar particles, after oxidation, may attach to soil minerals by interacting with clay and silt-sized minerals, which might increase the sorption capacity of biochar to adhere to organic contaminants in the soil (Whalen et al., 2024). The small pore size of biochar particles increases their surface area and adsorption ability. Mesopores play a significant role in liquid-solid adsorption, while larger pores are necessary for air passage (Akhil et al., 2021). At the same time, macropores are essential for aeration, hydrology, movement of roots, and bulk soil structure (Faloye et al., 2024).

### 2.6. BIOCHAR-AMENDED SOILS UNDER DROUGHT STRESS

Biochar-amended soils have demonstrated potential for ameliorating the negative impacts of drought stress. By enhancing soil's physical properties, such as water retention and infiltration,

biochar contributes to improved soil water availability for plants (Li et al., 2021). Additionally, biochar can indirectly enhance drought resilience by stimulating beneficial microbial communities (Bolan et al., 2023), which can positively influence plant growth and development under water-limited conditions (Wu et al., 2023). As discussed in detail below, incorporating biochar plays a crucial role in mitigating the drastic effect of water limitations.

### *2.6.1. Effect of Biochar on Soil Properties under Drought Stress*

Many studies have reported that biochar addition improves the physicochemical properties of soil under limited water supply and drought conditions (Yavaş ET AL., 2022; Boudjabi et al., 2023). Biochar application to different soil types with different textures reduces the bulk density and improves the soil water contents (Wei et al., 2023). Guo et al. (2023) reported that a 5% biochar application rate substantially enhanced plant growth and soil hydraulic properties during drying. The decrease in soil bulk density due to adding biochar enhances the soil's water-holding capacity (WHC), significantly improving plant growth and yield under a limited water supply (Mao et al., 2019). Its addition to the soil significantly increases the WHC of the soil because of the formation of macroaggregates with a specific higher surface area and porosity that provide more binding sites for water molecules (Adhikari et al., 2022). Biochar's WHC is co-controlled by multiple properties of biochar working together, including its chemical makeup (elements and surface groups), carbon structure (graphitic structure and aliphatic structure), pore volume, and specific surface area (Adhikari et al., 2022). However, the quantitative contributions of the various properties of biochar to the WHC remain unknown, which hinders the understanding of the dominant mechanism controlling the WHC of biochar and the regulation of biochar-producing conditions to improve its WHC.

Ruan et al. (2024) reported that 100 t/ha application of maize straw biochar significantly decreased the root osmolality and malonaldehyde content. In contrast, root water potential, ascorbate peroxidase activity, and plant fresh weight were increased considerably under severe

drought conditions. Similarly, Gullap et al. (2024) revealed that applying hazelnut shell biochar to the sandy loam soil under drought conditions significantly enhances malondialdehyde, hydrogen peroxide, proline, sucrose, and antioxidant enzyme activities, including peroxidase, catalase, superoxide dismutase, and abscisic acid contents in soybean leaves. It has been demonstrated that the application of 15 t/ha from agricultural bio-waste (tomato plant residues) significantly improves barley and wheat growth by enhancing soil and plant nutrients N, P, and K, organic matter, and soil water retention capacity (Albalasmeh et al. 2023). Furthermore, under water limitations, the application of 10% (*w*/*w*) wheat straw biochar significantly eliminates the harmful impacts of drought by enhancing wheat growth and yield, as well as physiological plant attributes, including stomatal conductance, transpiration rate, water use efficiency, and chlorophyll content (Amin et al., 2023). The increase in the cumulative constancy of sandy-clay soil after biochar addition is influential in increasing soil water retention, specifically in drought stress conditions (Baiamonte et al., 2019). It is evident from these studies that soil biochar application effectively improves soil water-holding capacity. However, further details are needed to explain the role of biochar in enhancing soil water-holding capacity under different environmental conditions.

Biochar application and suitable microbial inoculants further improve plant growth and biomass under drought conditions (Noureen et al., 2024; Ullah et al., 2024). An indirect interaction of biochar and microbes to reduce drought stress has been reported, e.g., by altering soil properties such as pH (Ullah et al., 2021). Biochar can shelter microbes, which attach themselves to porous biochar surfaces. It also serves as a nutrient source for microbial growth and can positively modify soil physicochemical properties such as pH, water content, organic matter, etc. (Tsoils and Barouchas, 2023). Several reports indicate enhanced microbial communities with biochar addition under water-stressed conditions (Park et al., 2023). The uptake of N and P, growth, biomass, and nodulation in the lupin seedlings grown in a restricted water supply were enhanced

after adding biochar combined with *Bradyrhizobium* sp., compared to the inoculation of microbes only (Egamberdieva et al., 2020). The application of 2% woodchip biochar to sandy loam soil significantly reduced the bulk density and modified the soil pH and EC, which ultimately enhanced vegetative growth and quinoa biomass (Akram et al., 2024a). Therefore, it can be anticipated that applying biochar in combination with microbes may effectively decrease the water scarcity effect in plants and improve plant growth; however, it largely depends on microbial properties, soil conditions, and plant species.

### *2.6.2. Effect of Biochar-Amended Soils on Plant Growth under Drought Stress*

Biochar application improves the growth and biomass of plants growing under a limited water supply. The most common plant traits that were enhanced with biochar additions are morphology, biomass, pigment content, nutrient contents, water use efficiency, stomatal pore aperture, stomatal density, photosynthetic rate, relative water content, and membrane stability index. It has been shown that a 3% application rate of biochar from sesame residue to sandy soil enhances the morphological traits, leaf gas exchange, plant water status, yield, and water use efficiency while reducing proline levels in tomato plants in semi-arid and arid areas (Murtaza et al., 2024). Similarly, among two biochars tested (woodchip and vineyard pruning biochar), 2% woodchip biochar in sandy loam soil significantly enhanced the plant biomass by 23% while yielding contributing traits by 50% in quinoa under drought conditions (Akram et al., 2023). Similarly, the 2% woodchip biochar application under water shortage conditions enhanced Titicaca quinoa's morphological and yield attributes (Rivelli et al., 2024).

Different studies revealed that biochar helps plants cope with drought stress by improving the plant's physiological status through chlorophyll content, photosynthetic rate, stomatal conductance, water use efficiency, and relative water contents. However, Afshar et al. (2016) reported that the chlorophyll content, transpiration rate, plant biomass, stem height, and leaf area were not affected by increasing the biochar application rate from 1.0 to 2.0% in milk thistle

seedlings grown in fine sandy loam soil under moderate (60% of control) and severe (40% of control) drought stress, compared to the control (50% of field capacity: FC). Similarly, a study reported that the production of the vine was improved after the application of biochar (22 t $ha^{-1}$) to the field, specifically during the years of reduced rainfall, while the characteristic parameters of grapes, such as total soluble solids (°Brix), total acidity, and anthocyanins, were not altered (Genesio et al., 2015). Recently, Zhang et al. (2020b) reported an increase in grain yield and water use efficiency and a reduced negative effect of drought stress on *Glycine max* productivity under biochar amendments (10 g/kg).

Abideen et al. (2020) reported a one-fold increment of *Phragmites karka* biomass root/shoot ratio under the application of 0.75% of peanut husk biochar produced at high temperature (750 °C). Plant cells adjust osmotically under biochar application by accumulating organic solutes, which aids in water influx, reduces efflux, maintains plant water status, and inhibits the reactive oxygen species (ROS) harmful effects, protecting biomembranes and photosystem II efficiency (Abdou et al., 2024). Applying biochar to the soil improves essential nutrient availability and enzyme activity and enhances root traits under drought stress, especially diameter, surface area, volume, and density (Jabbrova et al., 2023). Its addition is also helpful for the rhizosphere environment because it stimulates the proportion of different bacterial communities, including Proteobacteria, which is responsible for symbiotic nitrogen fixation, and Acidobacteria, which aids in nutrient cycling remineralization (Babujia et al., 2016; Hou et al., 2021). Importantly, biochar could help delay root senescence, as reported by Han et al. (2023), who found that under biochar application, root length was maintained during grain filling stages, whereas a 21–34% reduction was observed in non-amended soils. Different studies highlighted that biochar could enhance the water absorption capacity of crops because of the stimulation of finer root growth and length under water-limited conditions (Zhang et al., 2023; Kartika et al., 2021).

Biochar addition to the soil not only improves the nutrient contents and structure in the soil but also enhances the absorption and utilization of nitrogen fertilizers that are important for grain quality, such as its appearance and its starch and protein contents (Xuan et al., 2020). The higher nitrogen availability under biochar application promotes the total amino acid content in the grain (Khan et al., 2020). As a soil conditioner, it significantly enhances fruit flowering, growth, quality, and yield. Sharma et al. (2022) reported that biochar addition in soil is associated with potential benefits for the growth and yield of fruit plants such as grapes and apples. Moreover, a significant reduction in the total acidity of Red Globe grapes and increased protein and firmness was observed under biochar application (Zhang et al., 2020a), while glucose, fructose, ascorbic acid, total acidity, and lycopene in tomatoes increased significantly (Almaroai and Eissa, 2020). The enhanced water availability under drought conditions because of biochar application is its vital benefit in sustaining gas exchange and the antioxidant defense system. The strategic usage of biochar during drought to sustain plant functionality and, ultimately, crop productivity is important in arid areas. Applying biochar in addition to Arbuscular mycorrhizal fungi positively regulates the fluorescein diacetate dehydrogenase and alkaline phosphatase activity, soil nutrients, growth, and physiological properties in turmeric plants (Jabborova et al., 2024). The studies reporting the biochar effect on growth and physiology of different plant species subjected to water deficit are summarized in Table 1.

**Table 1.** Application of biochar to alleviate drought stress on different crop species (selected studies were organized according to their bibliographic references, with the most recent ones listed first).

| Feedstock | Pyrolysis T. (°C) | Plant Species | Application Rate | Water Stress Application Method | Effects on Plants | Ref. |
|---|---|---|---|---|---|---|
| Hazelnut shells (*Corylus avellana* L.) | 500 | *Glycine max* L. | 3 and 6% *w*/*w* | 75% and 50% of FC | Boosted growth and chlorophyll content | Gullap et al., 2024 |
| Maize (*Zea mays* L.) | 400 | *Zea mays* L. | 100 t/ha | 40% and 20% of FC | Increased $K^+$ concentration, less $Ca^{2+}$ efflux, and increased apoplastic pH in roots. | Ruan et al., 2024 |
| Acai seeds (*Euterpe oleracea* Mart.) | 700 | *Glycine max* L. | 2.5 to 10% *w*/*w* | With-held watering (8 days) | Increased biomass, including leaf and root DM, PN, WUE, PN/Ci, and $g_s$. | Gonçalves et al., 2024 |
| Rice husk (*Oryza sativa* L.) | 500 | *Lolium perenne* L. | 5 and 10% *w*/*w* | 25% of FC | Enhanced root and shoot growth, leaf RWC and nutrient status, chlorophyll contents, and photosynthetic efficiency while reducing proline, $H_2O_2$, and MDA. | Safari et al., 2023 |
| Natural wood | - | *Solanum lycopersicum* L. | 20 g/kg | 75% and 45% of FC | Enhanced morphological parameters, such as PH, LA, NB, FW, DW, and productivity. | Afaf et al., 2023 |
| Apple wood (*Malus domestica* (Suckow) Borkh.) | 400 | *Onobrychis viciifolia* L. | 0.8 to 4% *w*/*w* | 80 to 40% of FC | Enhanced leaf RWC and reduced MDA and $H_20_2$ accumulation. | Roy et al., 2022 |
| Timber waste | 390 | *Triticum aestivum* L. | 2% *w*/*w* | 75 and 35% of FC | Increased flavonoids, anthocyanin, phenolics, proteins, GB, APX, POD and SOD. | Lalarukh et al., 2022 |

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| Date palm (*Phoenix dactylifera* L.) and pistachio (*Pistacia vera* L.) | 560 | *Solanum melongena* L. | 500 g/m$^2$ | 100 to 50% of FC | Improved growth, yield, and WUE. | Ebrahimi et al., 2021 |
| - | 700 | *Triticum aestivum* L. | 28 and 38 g/kg | Skip irrigation at tillering and grain formation | Higher mineral nutrient, Bray P, exchangeable K, soil C, N mineralization and respiration in the soil along with enhanced microbial activity. | Zaheer et al., 2021 |
| Oak wood (*Quercus robur* L.) | 400 | *Ehretia Asperula* L. | 5 to 20 t/ha | With-held watering (10 days) | Increased PH, NL, FW and DW of roots, shoots, and leaves, chlorophyll content, and leaf RWC. Reduced relative ion leakage | Hoang et al., 2021 |
| Wheat straw (*Triticum aestivum* L.) | 550 | *Glycine max* L. | 5 and 10 g/kg | 80 to 25% of FC | Increased GY and improved WUE while reducing negative impact on productivity. | Zhang et al., 2020b |
| Corn straw (*Zea mays* L.) | 500 | *Chenopodium quinoa* Willd. | 5% *w*/*w* | DI and ARD by restoring water to 100% FC when consumed 90% of water | Improved water relations and growth and ameliorated plant water status. Creating a balance between chemical signal (leaf ABA) and hydraulic signal (Ψ). | Yang et al., 2020 |
| Cattle manure | 600 | *Glycine max* L. | 1.25 to 5% *w*/*w* | 100 and 55% of FC | Improvement in plant growth and morphology increased $g_s$. Higher rates (2.5, 5%) adversely affected the plant growth and production due to excessive salinity. | Gavili et al., 2019 |
| Woody branches of button mangrove (*Conocarpus erectus* L.) | 450 | *Cicer arietinum* L. | 3% *w*/*w* | 60% throughout the experiment and half exposed to 50% of FC for 6 weeks | Increased plant growth, PH, LA, enhanced chlorophyll content and carotenoids, increased stomatal pore aperture, $g_s$, *Pn*, RWC, membrane stability index, and nutrient content. | Hashem et al., 2019 |

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| Eucalyptus bark (*Eucalyptus* sp.) | 350 | *Zea mays* L. | 5 to 60 g/kg | With-held watering (4 days) | Raised water retention, and micro/macropore ratio while reducing BD, additionally, increased PH, plant nutritional status, and growth. However, raised water retention due to biochar could not overcome the drought problems in soil | Tanure et al., 2019 |
| Rice straw (*Oryza sativa* L.) | 450 | *Oryza sativa* L. | 3 and 5% *w*/*w* | 50% and 35% of FC | Increased DM production, chlorophyll contents, *Pn*, $g_s$, WUE, *E*, reduction in $H_2O_2$ content, MDA and electrolyte leakage, enhanced enzymatic defense system. | Rizwan et al., 2018 |
| Rice straw (*Oryza sativa* L.) | 450–550 | *Triticum aestivum* L. | 3 and 5% *w*/*w* | 70%, and 35% of FC | Increased plant growth and yield, chlorophyll contents, *E*, *Pn*, $g_s$, WUE, increased antioxidant activity, decreased MDA, $H_20_2$ and electrolyte leakage. | Abbas et al., 2016 |
| Pinewood (*Pinus* L.), sewage sludge, paper sludge, and grapevine wood (*Vitis vinifera* L.) | 550–620 | *Helianthus annuus* L. | Two experiments I: 15 t/ha II: 1.5 t/ha | - | Increased LA, PH, wider inflorescences, enhanced vegetative growth, seed production, and reduced $g_s$ with greater WUE. | Paneque et al., 2016 |
| Woodchip | 500–600 | *Zea mays* L. | 1.5 and 3% *w*/*w* | 60% and 25% of FC | Increased biomass, WUE, leaf RWC, and Ψπ, N use efficiency, photosynthesis because of stimulated electron transport rate of PSII. | Haider et al., 2015 |
| Lantana stems (*Lantana camara* L.) | 450 | *Abelmoschus esculentus* L. | 1 and 3% *w*/*w* | 100 and 60% of FC | Improved WUE, $g_s$, *E*, *Pn*, WUE, plant DM. | Batool et al., 2015 |
| Cotton sticks (*Gossypium arboretum* L.) | 385 | *Vitis vinifera* L. | 1 and 2% *w*/*w* | 100% and 50% watering restoration | Increased PH, FW, DW, RL, chlorophyll, carotenoids and anthocyanin, and WHC. | Danish et al., 2014 |

T—temperature, FC—field capacity, DI—deficit irrigation, ARD—alternate root-zone drying, DM—dry matter, PH—plant height, LA—leaf area, NL—number of leaves, NB—number of branches, FW—fresh weight, DW—dry weight, GY—grain yield, RL—root length, WUE—water use efficiency, RWC—relative water content, Ψ—total water potential, Ψπ—osmotic potential, PN—net photosynthetic rate, $g_s$—stomatal conductance, *Pn*—photosynthesis rate, *E*—respiration, PN/Ci—carboxylation efficiency, MDA—malondialdehyde, GB—glycine betaine, APX—ascorbate peroxidase, POD—peroxidase, SOD—superoxide dismutase, BD—bulk density, WHC—water holding capacity.

## 2.7. ROLE OF SOILS AMENDED WITH BIOCHAR IN ALLEVIATING DROUGHT STRESS IN QUINOA

According to the literature, few studies have described biochar application's role in enhancing quinoa's drought tolerance. However, most experiments are concerned with soil amended with biochar under drought imposed during the reproductive growing cycle, especially during flowering (Kammann et al., 2011; Yang et al., 2020). The results revealed that biochar application under drought significantly enhances quinoa biomass (Kamman et al., 2011). They also reported that a higher dose of biochar could negatively affect quinoa growth because of excess negative charge on the soil surface. Its addition plays a significant role in greenhouse gas mitigation and C sequestration. They demonstrated that biochar addition stimulates taproot growth, increasing water uptake and reducing water loss by transpiration; however, lower content of proline and higher values of leaf osmotic potential increase drought tolerance. On the other hand, biochar-grown plants exhibit higher leaf areas than non-amended plants.

Similarly, Ramzani et al. (2017) introduced the deficit irrigation concept to cope with the negative consequences of less water application and sustain growth and productivity through biochar addition. They highlighted that the water deficit negatively affects the yield and overall plant growth. However, the application of 2% biochar effectively counters the adverse impact of drought. They also reported that a 2% biochar application could save up to 20% of the water without significantly impacting physiology, growth, and water use efficiency. Furthermore, a significant increment in yield-contributing traits and growth of quinoa was observed with woodchip biochar application under drought stress (Akram et al., 2023). Unlike the woodchip biochar, vineyard pruning biochar application negatively impacts quinoa growth, which aligns with the findings that biochar effectiveness depends upon the feedstock used (Ghorbani et al., 2024). Biochar addition to soil further regulates the soil's physicochemical properties, including pH and electrical conductivity, and reduces soil bulk density (Akram et al., 2024a; Kumari et

al., 2024), helping the extensive root system development for deep penetration in the soil for more water and nutrient uptake during severe water-limited conditions. The growth- and yield-contributing trait enhancement was evident in quinoa experiencing water shortage conditions, starting from the emergence of the 2% woodchip biochar application (Rivelli et al., 2024). The study found that a 2% woodchip biochar application was most effective in mitigating the adverse effects of water shortage, compared to a 4% dose, which showed no benefit or a negative effect. Under moderate water-limited conditions (60% of FC), biochar application to the soil significantly enhanced the bioavailability and uptake of the nutrients by quinoa roots, and significant effects were evident on plant biomass and yield enhancement (Tourajzadeh et al. 2024a; Tourajzadeh et al. 2024b).

Until now, most studies have focused on the role of biochar application under moderate water stress during quinoa's reproductive growing cycle, mainly at the flowering and grain-filling stages. However, severe water stress application during the vegetative stage can have a significant impact on plant growth, particularly roots, depending on the quinoa variety (Akram et al., 2024b). According to Geerts et al. (2006), the drought period during the vegetative growing cycle should be mitigated with a potential watering strategy, as water stress during this period can significantly reduce water use efficiency, plant growth, and grain yield. Applying biochar to the soil offers a promising strategy to combat quinoa growth problems caused by water stress and avoid drought's adverse effects. Therefore, future studies should investigate biochar's ability to enhance the vegetative growth of quinoa plants under severe drought conditions. A comprehensive overview of existing research investigating the effects of biochar application on quinoa cultivation is presented in Table 2.

**Table 2.** Effect of biochar under drought stress applied at different growth stages in quinoa (*Chenopodium quinoa* Willd.) (studies examining the effects of biochar-amended soils on quinoa compiled chronologically, from the earliest research to the most recent).

| Feedstock | Pyrolysis T. (°C) | Application Rate | Water Stress Application Stage | Water Stress Application Method | Effects on Plants | Ref. |
|---|---|---|---|---|---|---|
| Peanut hull residue (*Arachis hypogaea* L.) | 498 | 100 and 200 t/ha | After 27 days of sowing (F) | 60 and 20% of FC | Increased growth, LA, DT, leaf N, and WUE. | Kammann et al., 2011 |
| Maize cob (*Zea mays* L.) | 350 | 1% *w*/*w* | With-held watering every two weeks from the leaf development stage until maturity | 15–20% of FC | Improved plant growth, yield, physiological, chemical, and biochemical processes. Reduced anti-nutrients (phytate and polyphenols) and BD in soil, enhance bioavailability, and translocate essential nutrients from soil to plant. | Ramzani et al., 2017 |
| Corn straw (*Zea mays* L.) | 500 | 5% *w*/*w* | F | DI and ARD | ARD with biochar under salinity enhanced PH, SB, and grain. Balanced chemical signal (leaf ABA) and Ψ and increased iWUE. | Yang et al., 2020 |
| Pistachio tree (*Pistacia vera* L.) | - | 20 t/ha | From E | 70, 100 and 130 mm of pan evaporation | Applying biochar + vermicompost enhanced chlorophyll, LAI, PH, PL, 1000 SW, and GY while decreasing proline content. | Mohkami et al., 2022 |
| Rice straw (*Oryza sativa* L.) | 300 | 0.4 and 0.8% *w*/*w* | 4 leaf stage and F | 30% of FC | Increased concentration of macro and micronutrients in soil. | Alashti Rahimi et al., 2021 |
| Woodchip (Bw) and vineyard (*Vitis vinifera* L.) pruning (Bv) | - | 2% *w*/*w* | 12-leaf stage | 2 successive water stress cycles from FC to PWP | Bw increased plant growth, LA, FW and DW, main PL, NSP, and WUE. Reduced leaf Ψπ and TW:DW. Bv negatively affects the plant growth | Akram et al., 2023 |
| Woodchip | - | 2 and 4% *w*/*w* | From E to FI | Restoration 100% and 50% ET | Enhanced NL, LA, biomass, DM, PL, and NSP. | Rivelli et al., 2024 |

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| Woodchip (Bw) and vineyard (*Vitis vinifera* L.) pruning (Bv) | - | Two experiments: I: 2% *w*/*w* Bw and Bv II: 2 and 4% Bw | I: at 12-leaf stage II: from E to FI | I: two successive water stress cycles from FC to PWP. II: restoring 100% and 50% ET | I: Bw enhanced biomass, DM, PL, NSP. II: 2% Bw modified soil pH, and EC and reduced BD. | Akram et al., 2024a |
| Northern forest tree | 300 | 2 and 4% *w*/*w* | From 8-leaf stage | 100, 80, 60% of FC | Increased P, K, N pH and EC of soil, while reducing the actual specific gravity and the apparent specific gravity of soil. | Tourajzadeh et al., 2024a |
| Dried forest leaves | - | 2 and 4% *w*/*w* | After plant establishment | 100, 80, 60% of FC | Increased growth, PW, 1000 SW, and LAI. | Tourajzadeh et al., 2024b |
| Woodchip | - | 2% *w*/*w* | At 12 leaf stage | 2 successive water stress cycles from WHC to PWP | Increased root growth and development, plant growth and yield contributing traits | Akram et al., 2024b |

ET—evapotranspiration, DI—deficit irrigation, ARD—alternate root-zone drying, FC—field capacity, WHC—water holding capacity, PWP—permanent wilting point, E—emergence, FI—flowering initiation, LA—leaf area, PH—plant height, NL—number of leaves, SW—seed weight, SB—shoot biomass, LAI—leaf area index, PL—panicle length, NSP—number of sub panicles, PW—panicle weight, SW—grain weight, GY—grain yield, DT—drought tolerance, WUE—water use efficiency, iWUE—intrinsic water use efficiency, $\Psi\pi$—osmotic potential, TW:DW—turgid weight to dry weight ratio, EC—electrical conductivity, BD—bulk density, N—nitrogen, P—phosphorous, K—potassium, ABA—abscisic acid.

## 2.8. CONCLUSIONS AND FUTURE PROSPECTIVES

Although quinoa exhibits drought tolerance mechanisms, current climate change scenarios significantly impact both traditional and climate-proof crops, including quinoa. Different studies showed that quinoa positively responds to biochar addition in the soil regarding root development, gaseous exchange parameters, and alleviation of osmotic stress (production of reactive oxygen species) through enhanced antioxidant activity. It is evident from the above discussion that the characteristics of biochar can differ significantly because of feedstock, pyrolysis conditions, application rate, soil type, and plant species. Therefore, it is necessary to know biochar's properties and expected outcomes before its application to the soil. Additionally, most of the research work has been done under controlled conditions, with few studies conducted in field conditions. So, the focus must be diverted towards planning biochar usage under field conditions. Finally, future research should be focused on the following points:

I. Long-term field investigations are needed to elucidate the intricate interplay between biochar soil application and soil–plant systems under drought conditions.
II. Exploring synergistic interactions between biochar application and established agricultural practices could unveil novel avenues for achieving sustainable agricultural production.
III. Quinoa varietal response to biochar-amended soils under water stress conditions should be assessed through morphology, physiology and anatomical changes.

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# CHAPTER 3

# EVALUATION OF VEGETATIVE DEVELOPMENT OF QUINOA UNDER WATER STRESS BY APPLYING DIFFERENT ORGANIC AMENDMENTS

*This chapter is a state-of-the-art chapter, and its content has been already published:*

**Akram, M.Z.,** Libutti, A., & Rivelli, A.R. (2023). Evaluation of vegetative development of quinoa under water stress by applying different organic amendments. *Agronomy, 13(5), 1412.*

## 3.1. ABSTRACT

Prolonged drought periods, increasingly occurring worldwide due to global climate change, could affect the growth and productivity of both traditional and climate-resilient crops, including quinoa. Specifically, the vegetative growing cycle of this species is highly sensitive to drought conditions. In this context, using organic amendments could help plants cope with drought due to their ability to enhance soil water status. So, the current study aimed to investigate the effect of different organic amendments, i.e., two biochars (from woodchips and vineyard pruning) and a vermicompost (from cattle manure), applied to the soil alone and mixed at 2% rate (w/w), on the vegetative development of quinoa (cv. Titicaca), during which a period of water stress was imposed from the twelve-leaf stage to the bud stage. A set of growth-related parameters were measured both during and at the end of the experiment, along with a set of water-related parameters, at the end of the water-stress period and after soil re-watering. The results showed that woodchip biochar, both alone and mixed with vermicompost, significantly affected plant growth during the water-stress period, also allowing a quicker recovery once drought conditions ended. Indeed, the leaf number and area, SPAD index, leaf and stem fresh weight, and dry matter content in plants treated with woodchip biochar, alone and mixed with vermicompost, were higher than vineyard pruning biochar, alone and mixed with vermicompost and similar to the well-watered control plants. Similar results were observed considering the

yield contributing traits detected at the end of the experiment, including the main panicle length, number of sub-panicle, as well as fresh weight and dry matter content of both panicle and sub-panicles. Additionally, the water-related parameters, especially the low turgid weight to dry weight ratio of woodchip biochar treated plants, showed evidence of better growth than vineyard pruning biochar. At the end of the experiment, the WUE of plants treated with woodchip biochar and vermicompost, both alone and mixed, was higher than vineyard pruning biochar alone and mixed with vermicompost. Among the tested organic amendments, woodchip biochar alone and mixed with vermicompost positively affected the vegetative growth response of quinoa under water-stress conditions.

*Keywords: Chenopodium quinoa, climate-resilient crop, vermicompost, vineyard pruning biochar, woodchip biochar, vegetative growing cycle, water stress, water-relations, plant growth*

## 3.2. INTRODUCTION

Recently, there is a growing interest in the use of climate-resilient crops to improve global food production and secure future food supply. These crops have the potential to adapt rapidly to changing climatic conditions, providing means to better cope with extreme events, such as drought, flooding, heat, chilling, freezing, and salinity (Ain et al., 2023). Among climate-resilient crops intended to maintain or increase crop yield under environmental stress conditions, there is quinoa (*Chenopodium quinoa* Willd.) (Akram et al., 2021). Quinoa is an annual herbaceous dicotyledonous species from the Andean region (Hafeez et al., 2022). Around 7000 years ago, quinoa was cultivated as a main staple food in the Andes region countries, with Bolivia and Peru being the countries that oversaw the most production. Later, from 1980 to 2014, it rapidly spread from 8 to 75 other countries and is now widely cultivated in Canada, the U.K., Denmark, China, India, Netherlands, Brazil, Cuba, Italy, and Pakistan (Akram et al., 2021; Hafeez et al., 2022; Bazile et al., 2015). The General Assembly of the

United Nations declared 2013 as the “International Year of Quinoa” due to its important role in food security as global attention focuses more on nutrition and poverty eradication (FAO, 2013). The plant forms a hollow erect stem with goosefoot shaped leaves and two types of inflorescences, i.e., glomerulate and amaranthiform, with the glomerulate ones (compact type) considered high yielding (Wu et al., 2019). Quinoa has a superior nutritional profile than other traditional crops because of the high content of proteins rich in all essential amino acids, vitamins (E, C and B complex), minerals (Ca, Cu, Mg, Mn, Fe, P, K, Na and Zn), and fiber, along with bioactive compounds, including ferulic and sinapinic acids, flavanols, kaempferol, and quercetin (Voronov et al., 2023). Its seeds are used to obtain multigrain flour, biscuits, and beer (Coţovanu et al., 2023). This species does not contain gluten and has a low glycaemic index (Bastidas et al., 2016).

Due to the diversity of quinoa ecotypes originating in contrasting agro-environmental conditions, this species has the ability to adapt to various levels of drought via both morphological and physiological adaptive strategies. The whole plant response to drought involves changes in root and leaf growth and, in some cases, a few ontogenic variations (Zurita Silva et al., 2015). Growth is much more sensitive to drought stress than photosynthesis. In stressed plants, growth reduction is not just from carbon starvation, i.e., reduced carbon concentrations that can amplify biotic attack because of low production of carbon-based defensive compounds such as resins (McDowell et al., 2008); there is a sharp and rapid decrease in leaf elongation rate in many plant species, including quinoa (Skirycz and Inzé, 2010; Jacobsen et al., 2009). In addition, root architecture traits may vary due to many interacting factors, such as growth conditions, drought duration, drought intensity, and plant phenology (Nicotra et al., 2002). Quinoa shows various drought resistance mechanisms, although not all in every genotype, such as tissue elasticity, thick-walled cells, and special epidermal cell bladders that can be used as external water reservoirs and vesicular glands (Al-Naggar et al.,

2017; Otterbach et al., 2021; Shabala et al., 2012). Many studies have revealed that quinoa evolved adaptive mechanisms to mitigate drought stress through high water-use efficiency and high shoot/root ratios. Garrido et al. (2013) showed a significant interaction between genotypes and the environment for harvest index and grain yield/$m^2$. Considerable variability was observed among stressed quinoa genotypes for grain yield and water-use efficiency. Geerts et al. (2008) also found a negative effect of water stress on grain yield and water-use efficiency. Drought stress at the grain filling stage can reduce quinoa plant yield sustainability by affecting plant leaf water potential (Zurita Silva et al., 2015). It also reduces the crop fresh and dry weights, as well as leaf area (Stikić et al., 2015). According to several authors (Beccari et al., 2021; Bilalis et al., 2019), the high resilience and tolerance of quinoa to adverse climatic conditions make it a good alternative to traditional Mediterranean crops.

Different agronomic strategies could be adopted to mitigate the adverse effects of drought on plant growth and productivity, including the application of organic amendments due to their role in improving soil water retention. Among the organic amendments, biochar and compost have gained even more attention over the last decades for their agronomic and environmental benefits (Das et al., 2020; Sayara et al., 2020). Biochar is a carbon-rich, porous, low-density material derived from the thermochemical decomposition of different biomasses (forest and agriculture residues, manure, sewage sludge) in the absence or limited supply of oxygen (pyrolysis or gasification) (Lee et al., 2019). Due to its long-term stability in soil, it provides a useful amendment to mitigate global climate change by sequestering atmospheric $CO_2$ in the soil and reducing greenhouse gas ($CO_2$, $CH_4$, $N_2O$) emissions (Laird et al., 2008). Moreover, because of its highly porous structure, large inner surface area, greater negative surface charge, and charge density, biochar may have a positive impact on the physical, chemical, and microbiological properties of soil. It is reported to improve soil's structure and porosity, water-holding capacity and hydraulic conductivity, cation and anion exchange capacity, nutrient

retention and availability (to decrease soil bulk density), and nutrient leaching to stimulate soil microbial biomass and activity (Lei et al., 2013; Manolikaki 2020; Murtaza et al., 2021; Subedi et al., 2017). In particular, the ability of biochar to increase soil water retention has been extensively reported in the literature, under both controlled (laboratory, lysimeters and pots) (Libutti et al., 2021) and open-field conditions (Libutti et al., 2019; Wang et al., 2019; Abd El-Mageed et al., 2020), although the effect is dependent on the characteristics of both soil (texture and structure) and biochar (feed-stock and thermochemical treatment). Compost is a humus-like product derived from the decomposition, stabilization, and sanitation of organic residues from plants and animals through the action of aerobic microorganisms under controlled conditions (Zhao et al., 2022). It is widely recognized as an organic amendment with beneficial agronomic advantages, with its effect depending on compost amount, type, degree of humification, and soil properties (Adugna et al., 2016; Azim et al., 2018). It is reported to efficiently enhance soil organic matter and nutrient content, including N, P, K, Ca, and Mg (Rivier et al., 2022), to improve soil structure, porosity (Abdelfattah et al., 2021), water retention, and hydraulic conductivity (Libutti and Rivelli, 2021). Compost also enhances soil fertility, plant growth, and productivity (Libutti and Rivelli, 2021; Libutti et al., 2020; Rivelli and Libutti, 2022; Cheng et al., 2023; Huang et al., 2023). Using organic amendments, such as biochar and compost, from eco-friendly technologies regarding the residue recycling of plant and animal organic waste (Lim et al., 2016; Ullah et al., 2021; Monlau et al., 2016; Zabaniotou et al., 2017; Zabaniotou et al., 2015) is now recognized as a sustainable strategy of soil management and agricultural productivity. This is also in line with most of the 17 Sustainable Development Goals (SDG) of the United Nations (2030 Agenda), including sustainable production and consumption and climate action, as well as with the targets for sustainable food production outlined by the “Farm to the Fork” (F2F) Strategy within the European Green Deal (e.g., reducing at least 20% of fertilizer application whilst retaining soil fertility).

Only a few studies are available in the literature regarding the cultivation of quinoa by using organic amendments. Kamman et al. (2011) found increased growth, drought tolerance, leaf N, and water-use efficiency despite larger leaf areas following the application of biochar from peanut hull residues to soil. Ramzani et al. (2017) reported that the application of acidified biochar is more valuable for improving the growth and yield of quinoa than acidified compost. They also observed an increase in the nutritional value of quinoa due to an increase in antioxidant levels, decrease in reactive oxygen species levels, and antinutrients (phytate and polyphenols). Moreover, the natural ability of quinoa to cope with water stress was mostly investigated when drought occurs during the reproductive growth stages (Jensen et al., 2000; Razzaghi et al., 2014; Riccardi et al., 2014) rather than the vegetative ones. However, the susceptibility of quinoa to drought is reported to be high during the vegetative stages of the growing cycle (Geerts et al., 2006). In light of this and also considering the capability of organic amendments in addressing drought stress among plants (Roy et al., 2022; Zhang et al., 2021), the current study aimed to investigate the effect of two biochar types and a vermicompost on a set of growth- and water-related parameters when quinoa is under water-stress conditions during vegetative development, i.e., from the twelve-leaf stage to the bud stage. We hypothesized that the application of organic amendments could improve the vegetative growth performance of quinoa (*cv.* Titicaca) when facing water-stress conditions.

## 3.3. MATERIAL AND METHODS

### *3.3.1. Experimental Layout and Characteristics of Soil and Organic Amendments*

The experiment was carried out during spring–summer in 2022 on quinoa (*Chenopodium quinoa*, Wild.), cultivar "Titicaca," at the University of Basilicata, Potenza (PZ, 40°38′N-15°48′ E, 819 m a.s.l.) in Southern Italy, under natural light in a temperature-controlled glasshouse maintained at 26°C during the day and 18°C during the night.

Plants were grown in pots on soil treated with different organic amendments, including two types of biochar, one derived from woodchips (B1) and the other from vineyard prunings (B2), and a vermicompost derived from cattle manure (V). Each organic amendment was applied to the soil alone (B1, B2, or V) at a rate of 2% of soil dry weight (dw) and mixed (B1+V or B2+V) with a B1:V and B2:V ratio equal to 1:1 (both biochar and vermicompost at 2% rate). An untreated soil was considered as the control and was replicated six times in order to have two set of control pots, indicated as Cw and Cs. While Cw was well-irrigated throughout the whole experimental trial, Cs was utilized in the water-stress period described in paragraph 2.2. Each experimental treatment was replicated three times. The resulting 21 experimental units were arranged according to a completely randomized design.

For the experiment, a soil collected from the 0–0.30 m upper soil layer within an agricultural farm located in the Potenza district was used. Based on analytical characterization performed before the experiment started and according to the official analytical methods reported in the Italian Official Gazette n. 248 (Decree, 1999), the resulting soil had a sandy-loam texture (USDA classification), with the physico-chemical characteristics reported in Table 1.

**Table 1.** Main physico-chemical properties of the soil used in the experiment.

| Property | Unit | Value |
|---|---|---|
| Sand | (%) | 66.1 |
| Silt | (%) | 11.5 |
| Clay | (%) | 22.4 |
| Field Capacity (FC) at −0.03 MPa | (% dw) | 22.8 |
| Permanent Wilting Point (PWP) at −1.5 MPa | (% dw) | 11.4 |
| pH | (-) | 7.6 |
| EC | (dS $m^{-1}$) | 0.6 |
| $C_{org}$ | (g $kg^{-1}$) | 5.9 |
| Organic Matter | (%) | 1.0 |
| $N_{total}$ | (‰) | 1.5 |
| C/N | (-) | 3.9 |

The biochar from woodchips was provided by a company located in northern Italy (Ivrea, Torino district), which specializes in producing commercial biochar in an industrial pyrolysis plant of one's own design, using wood wastes from the cleaning of green areas and woods

within a controlled supply chain. The biochar from vineyard pruning residues was self-produced at the STAR*Facility Centre of Foggia University (South Italy region). The residual biomasses from the pruning of a vineyard located in the agricultural area of the Foggia district were firstly dried (15% humidity), then chipped (approx. 50 mm), and finally pyrolyzed in a pilot-scale plant with a fixed-bed tubular reactor (30 L capacity) at a temperature of 750 °C (heating rate of 10 °C $min^{-1}$) for 8 h. Before the experiment, both biochars were crushed in finer fractions and sieved to ≤ 2 mm. The vermicompost was provided by a company located in southern Italy (Montescaglioso, Matera district), which specializes in producing high-quality organic fertilizers and amendments through an industrial composting process of organic residues from olive mills, crops, and livestock. More specifically, it was a commercial product derived from cattle manure bio-stabilization. Before use in the experiment, both biochars and the vermicompost were analyzed to determine the set of chemical properties shown in Table 2. The applied analytical procedures are detailed in Rivelli and Libutti (2022).

**Table 2.** Main chemical properties of the organic amendments used in the experiment.

| Property | Unit | Biochar | | Vermicompost |
|---|---|---|---|---|
| | | Woodchips | Vineyard Prunings | |
| pH | (-) | 8.9 ± 0.1 | 10.6 ± 0.1 | 7.6 ± 0.1 |
| EC | (mS $m^{-1}$) | 52.0 ± 0.0 | 249.0 ± 0.0 | 265.0 ± 0.0 |
| Moisture | (% dw) | 5.6 ± 0.1 | 15.3 ± 0.3 | 4.0 ± 0.2 |
| Volatile solids | (% dw) | 42.3 ± 0.4 | 15.3 ± 0.3 | 27.5 ± 0.6 |
| Ash | (% dw) | 4.4 ± 0.2 | 9.9 ± 0.0 | 72.2 ± 0.6 |
| Fixed carbon | (% dw) | 53.3 ± 0.2 | 74.8 ± 0.3 | 0.2 ± 0.0 |
| C | (% dw) | 68.3 ± 0.1 | 67.7 ± 0.9 | 11.3 ± 0.0 |
| H | (% dw) | 4.0 ± 0.0 | 2.1 ± 0.0 | 1.5 ± 0.1 |
| N | (% dw) | 1.0 ± 0.0 | 1.0 ± 0.0 | 1.5 ± 0.0 |
| S | (% dw) | 0.03 ± 0.0 | 0.2 ± 0.0 | 0.3 ± 0.0 |
| $C_{org}$ | (% dw) | 66.3 ± 0.1 | 67.0 ± 0.9 | 7.8 ± 0.1 |
| O | (% dw) | 22.3 ± 0.3 | 17.9 ± 1.5 | 5.2 ± 0.2 |
| $H/C_{org}$ | (-) | 0.7 ± 0.0 | 0.4 ± 0.0 | - |
| $O/C_{org}$ | (-) | 0.4 ± 0.0 | 0.2 ± 0.0 | - |
| C/N | (-) | 67.2 ± 2.0 | 66.2 ± 0.1 | 5.0 ± 0.2 |

Values are means ($n = 3$) ± s.e.

The two biochars were characterized by the typical alkaline pH value and high C content. They fully complied with the standards fixed by both the European Biochar Certificate (EBC) (EBC, 2012) and the International Biochar Initiative (IBI) (IBI, 2015), in terms of C (>50%) and $C_{org}$

(>60%) contents as well as $H/C_{org}$ (≤0.7) and $O/C_{org}$ (≤0.4) molar ratio values, both resulting in Class 1 biochar. The vermicompost was a stabilized organic amendment with good $C_{org}$ and N content and a slightly alkaline pH value. Further information about the chemical properties of the two biochars and the vermicompost are shown in Table 2.

### *3.3.2. Experimental Set-up and Conditions*

Plastic pots (25 cm height, 18 cm width and length) were filled with 5 kg of soil. The biochars and vermicompost were mixed into the soil before pot filling. After, the soil water content was brought to FC (previously determined at −0.03 MPa, along with the permanent wilting point (PWP) at −1.5 MPa) for each experimental treatment by using a pressure plate apparatus (Soil moisture Equipment Corp., California, USA). Then, 10 quinoa seeds were sown in each pot. After the complete seedlings emerged, the latter were thinned out to have one plant per pot. After thinning, soil surface was covered with a 3 cm layer of polythene beads in order to prevent water loss through evaporation.

All the treatments were well-watered until they reached the phenological twelve-leaf stage, i.e., at 26 days DAS. After that, they were subjected to a water-stress period that lasted up until the bud stage, i.e., at 35 DAS. An extra three control pots were maintained and well-watered throughout the whole experimental period (Cw). The well-watered conditions (for each experimental treatment) consisted of the application of an amount of water that brought the soil water content to FC at each watering by fixing the intervention limit at the depletion of 40% of the available water, and this was checked daily by weighing the pots at the same hour (8:00–9:00). For each experimental treatment, the water-stress period consisted of the application of two successive drought cycles by withholding watering until the soil water content reached the PWP. At the end of each drought cycle, re-watering increased soil water content to FC. After the second drought cycle, the well-watered conditions were maintained until the end of the experiment, when plants reached the phenological stage of flowering initiation, i.e., at 48 DAS.

At this time, plants were cut from the pots and divided into stems, leaves, panicles, and sub-panicles for the following measurements.

### *3.3.3. Plant Measurements*

From sowing until the end of the experiment, phenological stages were monitored, according to the scale defined by Jacobsen and Stolen (1993), by visiting the experimental area daily. The times of seedling emergence, leaf development stages (two-, six-, eight-, ten-, and twelve-leaf), bud and flowering initiation were recorded when more than 50% of plants in each treatment reached those stages.

During the experiment, a set of plant growth-related parameters was also measured, on average, twice a week. More specifically, the plant height (cm), stem diameter (mm), number of branches (n°), and leaves per plant (n°), as well as the length and width (cm) of each leaf per plant were recorded. Similarly, the SPAD index per plant was measured. For this, a hand-held SPAD-502 m (Konica-Minolta corporation, Ltd., Osaka, Japan) was used to obtain three SPAD readings on the youngest, fully expanded leaf per plant.

At the end of the experiment, the fresh weights (FW, g) of leaves, stems, main panicle, and sub-panicles per plant were measured. In addition, the length of main panicle (cm) and the number of sub-panicles per plant were recorded. The leaf area per plant (LA, cm2) was measured by using a LI-COR leaf area meter (Model 3100, Inc., Lincoln, NE, USA). After that, the leaves, stems, main panicle, and sub-panicles of each plant were dried in a ventilated oven at 70°C until they reached a steady weight to determine the corresponding dry weights (DW, g). Then, their dry matter content (DM, %) was calculated.

The LA per plant was also calculated by using the leaf length (L) and width (W) measurements obtained at the end of the experiment. For this, the LA of each leaf was first determined by applying Equation proposed for the “Titicaca” cultivar grown under glasshouse conditions by Talebnejad and Sepaskhah (2015): $LA = 0.64 (L \times W)$.

Then, the LA per plant was calculated by summing the LA of each leaf. A set of more than 600 leaves from all treatments was considered.

Successively, in order to verify the model validity within our experimental conditions, a correlation analysis was applied to measure and calculate LA data (Figure 1).

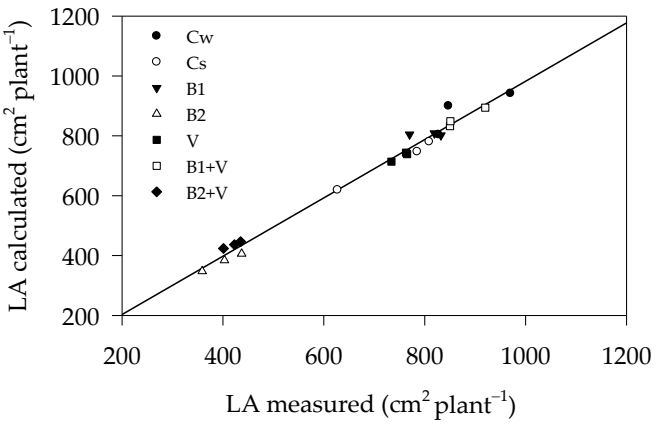


**Figure 1.** Relationship between leaf area measured and leaf area calculated by the model per plant. Fitted linear regression was derived from the following equation: $y = 0.974x + 8.233$ ($r^2 = 0.986$; $p \leq 0.0001$). Cw and Cs—well-watered and water-stressed control, respectively; B1—woodchip biochar; B2—vineyard pruning biochar; V—vermicompost.

A strong correlation ($r^2 = 0.98$; $p < 0.0001$) was observed between the two sets of data. Therefore, using the leaf length and width measurements obtained during the experiment, the model was applied to obtain the LA per plant over the whole growing cycle.

Finally, the Absolute Growth Rate (AGR, cm $d^{-1}$) per plant was calculated in the following three periods of the growing cycle: (1) from six- to twelve-leaf stage; (2) from twelve-leaf stage to bud stage (water-stress period); (3) from bud stage to flowering initiation stage. For this, the plant height measurements obtained at the start ($H_1$, cm) and end ($H_2$, cm) of each period, as well as the duration ($t_2$–$t_1$, days) of each period, were used to determine the $AGR_1$, $AGR_2$, and $AGR_3$ values, respectively.

A set of water-related parameters was measured both at the end of the water-stress period and after the re-watering that restored soil water content at FC. More specifically, the leaf relative water content (RWC, %) was determined by taking a segment from the youngest, fully

expanded leaf per plant and immediately weighing it for FW. After that, the leaf segment was kept in distilled water for 24 h at 4 °C to determine its turgid weight (TW, g). After drying in a ventilated oven at 70 °C until a steady weight, the DW was determined. The RWC was then calculated by Equation (2): RWC= (FW-DW/TW-DW) × 100 (2). The leaf turgid weight to dry weight ratio (TW/DW) per plant was also calculated.

In addition, the leaf water potential (Ψ, MPa), osmotic potential (Ψπ, MPa), and turgor (Ψp, MPa) were measured. In particular, the Ψ was measured at mid-day on the youngest, fully expanded leaf per plant by using the Scholander pressure chamber (PMS model 1000, Corvallis, OR, USA). For Ψπ measurement, leaf samples were frozen immediately in liquid nitrogen and stored at −20°C. The Ψπ was determined by extracting sap from leaf tissues, according to Smith and Lüttge (1985). Osmolarity of the sap was measured with a Micro-Digital Osmometer type 6, freezing point depression osmometer (Roebling Berlin, Germany), and then Ψπ was calculated from osmolarity by using the van't Hoff equation. Ψp was estimated to be the difference between Ψ and Ψπ.

At the end of the experiment, the total water consumption (TWC, L) over the growing cycle was determined by cumulating the amount of water supplied to the plants at each watering, along with the water-use efficiency (WUE, g $L^{-1}$), calculated as the ratio of total plant fresh weight (TFW, g) to TWC.

### *3.3.4. Statistical Analysis*

All the experimental data were first checked for normality and homogeneity of variance and then processed by one-way analysis of variance (ANOVA). When significant differences among means were detected, the latter were compared by Tukey's honest significance difference post hoc test at the 5% probability level.

Statistical analysis was carried out by using “Statistix” software version 8.1. Correlation analysis, applied in order to estimate the relationship between measured and calculated LA values, was performed using the same software.

## 3.4. RESULTS

### *3.4.1. Phenological Stages*

The time of the phenological stages recorded during the experiment, expressed as DAS, are reported in Table 3. Only the seedling emergence and the two-leaf stages were significantly influenced by soil treatment with organic amendments.

In particular, the time for seedling emergence was significantly higher ($p \leq 0.001$) in the soil amended with biochar from vineyard prunings, both mixed with vermicompost (B2+V) and alone (B2), than the controls (Cw and Cs). The contrary occurred in soil amended with biochar from woodchips and vermicompost, both applied alone (B1 and V, respectively) and mixed (B1+V), which resulted in similar seedling emergence times than Cw and Cs. The time of the two-leaf stage was higher in Cs, B1, and B2+V than Cw and V, with these latter not statistically different from B2 and B1+V.

Considering the other phenological stages, no statistical differences were found among the treatments. Irrespective of the latter, the plants showed the same phenological performance, taking a similar amount of time to get into the stages from six- to twelve-leaf development, bud initiation, and flowering initiation.

### *3.4.2. Growth-Related Parameters during the Experiment*

The time-trends of the growth-related parameters, i.e., plant height, stem diameter, number of branches, and number of leaves, as determined during the growing cycle, are reported in Figure 2. Plant height was significantly influenced by soil treatment with organic amendments. Initially, both at 19 and 21 DAS, plant height (Figure 2a) showed the highest ($p \leq 0.01$) value

in Cw than the other treatments, but successively, at 22 DAS, this parameter was higher ($p \leq 0.01$) in Cw than B1, B2, B1+V, and B2+V, without any statistical difference from Cs and V.

**Table 3.** Phenological stages during the vegetative growing cycle of quinoa treated with different organic amendments.

| Treatment | Phenological Stage (Days) | | | | | |
|---|---|---|---|---|---|---|
| | Seedling Emergence | Leaf Development Stages | | | Bud Initiation | Flowering Initiation |
| | | Two-Leaf | From Six-Leaf | To Twelve-Leaf | | |
| Cw | 4.3 C | 8.7 C | 19.7 | 26.3 | 28.7 | 41.3 |
| Cs | 4.3 C | 10.7 A | 21.0 | 25.3 | 28.6 | 42.7 |
| B1 | 4.7 BC | 11.0 A | 19.7 | 25.0 | 28.3 | 40.7 |
| B2 | 5.7 B | 9.3 BC | 21.3 | 26.3 | 29.0 | 43.0 |
| V | 4.7 BC | 8.3 C | 21.3 | 25.3 | 28.3 | 42.3 |
| B1+V | 3.7 C | 9.3 BC | 22.3 | 26.3 | 29.3 | 44.3 |
| B2+V | 7.0 A | 10.0 AB | 21.0 | 26.0 | 28.0 | 41.0 |
| Significance | *** | ** | ns | ns | ns | ns |

Values are means ($n = 3$). In each column, means followed by different letters are significantly different ($p \leq 0.05$; Tukey's test). ***—F test significant at $p \leq 0.001$; **—F test significant at $p \leq 0.01$; *ns*—not significant. Cw and Cs—well-watered and water-stressed control, respectively; B1—woodchip biochar; B2—vineyard pruning biochar; V—vermicompost.

At 23 and 24 DAS, as well as at the beginning of the water-stress period (at 26 DAS), plant height was also higher ($p \leq 0.01$) in B1, B2, and B1+V, with the lowest value in B2+V. Conversely, as the drought conditions proceeded, at 28 and 32 DAS, plant height was higher ($p \leq 0.001$) in Cw than Cs, B2, V, and B2+V, without any statistical differences from B1 and B1+V. At the end of the stress period, at 35 DAS, plant height reached the highest ($p \leq 0.001$) value again in Cw, with the lowest one in B2+V, and this trend was reflected in the subsequent period of the vegetative growth until the end of the experiment (from 35 to 48 DAS).

Additionally, with regards to the stem diameter (Figure 2b), the significant influence of the experimental treatments was observed. At the start of the drought period, at 26 DAS, both Cw and Cs, as well as B1, V, and B1+V, which did not differ from each other, showed a higher ($p \leq 0.001$) stem diameter than B2 and B2+V. As the water stress increased, at 32 and 35 DAS, the stem diameter of plants grown in Cw resulted in a higher ($p \leq 0.001$) value than Cs, B2, and B2+V, with no statistical differences from B1, V, and B1+V. These latter proved to minimize

the drought effects and support aspects of plant development, such as Cw, and this was also observed after the water-stress period, from 36 to 48 DAS.

The number of branches (Figure 2c) was shown to be significantly influenced by soil treatment with organic amendments by the fact that, at 28 DAS, Cw-, as well as B1 and B1+V-treated plants, exhibited a higher ($p \leq 0.001$) number of branches than Cs, B2, and B2+V. The same trend was observed during the water-stress period (32 and 35 DAS) and successively until the end of the experiment.

A very similar response was observed regarding the number of leaves (Figure 2d). The plants grown on B1, V, and B1+V always produced a significantly ($p \leq 0.001$) number of leaves (similar to Cw) than Cs, B2, and B2+V, starting from 24 to 48 DAS.

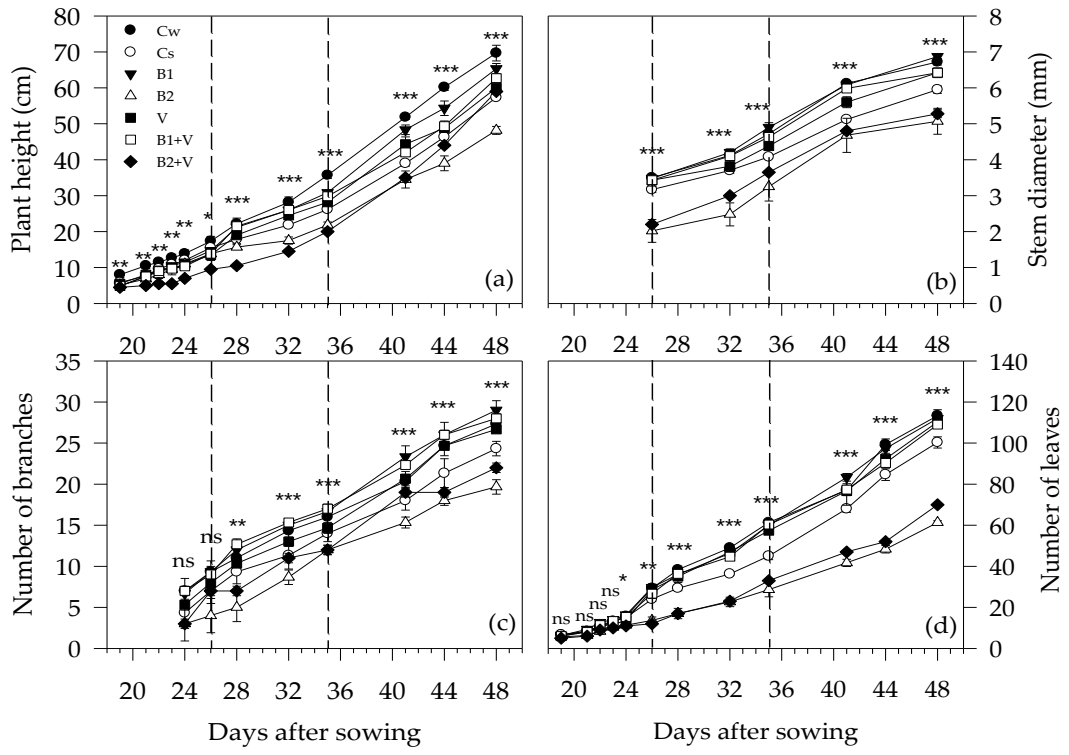


**Figure 2.** Time-trends during the experiment of plant height (a), stem diameter (b), number of branches (c), number of and leaves (d) of quinoa treated with different organic amendments. In each graph, dashed vertical lines indicate the water-stress period (from twelve-leaf stage, i.e., 26 DAS to bud stage, i.e., 35 DAS). Values are means (n = 3) ± s.e. The asterisks above the symbols indicate significant differences among treatments ($p \leq 0.05$; Tukey's test). *, **, ***—F test significant at $p \leq 0.05$, *0.01*, and *0.001*, respectively; *ns*—not significant. Cw and Cs—well-watered and water-stressed control, respectively; B1—woodchip biochar; B2—vineyard pruning biochar; V—vermicompost.

Additionally, the leaf area (LA) was significantly influenced by the experimental treatments, as reported in Figure 3a. When the water-stress period started (26 DAS), the two controls, Cw and Cs, as well as B1, V, and B1+V, which did not differ from each other, showed higher LA ($p \leq 0.001$) than B2 and B2+V. At 32 DAS, as drought conditions intensified, only Cw and B1 showed higher ($p \leq 0.001$) LA than the other treatments. At the end of the water-stress period (35 DAS), the highest ($p \leq 0.001$) LA was observed in Cw. Once removed from the water-stress conditions, from 41 DAS until the end of the experiment, B1 and B1+V treated plants increased their LA, with no statistical difference from Cw ($p \leq 0.001$).

The SPAD index (Figure 3b) proved to be influenced by soil treatment with organic amendments starting from 32 DAS until the end of the experiment. At 32 DAS, a higher ($p \leq 0.01$) SPAD index was recorded in Cw compared to Cs, B2, V, and B2+V. Moreover, this SPAD index was not statistically different from B1 and B1+V. At the end of the water-stress period (35 DAS), V also reached a higher SPAD index ($p \leq 0.001$) than Cs, B2, and B2+V, similar to Cw, B1, and B1+V. A similar trend of SPAD index was observed after the water-stress period until the end of the experiment.

Among the considered growth parameters, the Absolute Growth Rate (AGR) periodically determined throughout the vegetative cycle (Table 4) was shown to be influenced by the experimental treatments. Initially, the AGR1 calculated from the six-to twelve-leaf stage was similar in all the considered experimental treatments, except for B2+V, which, on the contrary, showed the lowest ($p \leq 0.05$) value. The AGR2, calculated successively, from the twelve-leaf to bud stage when water stress was applied, resulted in a higher ($p \leq 0.001$) value in Cw than Cs, B2, and B2+V, without any statistical difference from B1 and B1+V.

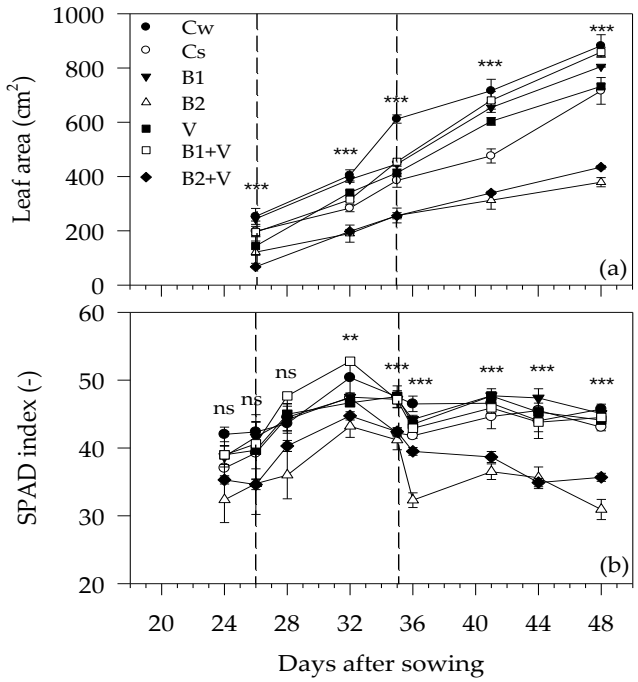


**Figure 3.** Time-trends during the experiment of leaf area (a) and SPAD index (b) of quinoa treated with different organic amendments. In each graph, dashed vertical lines indicate the period of water stress (from twelve-leaf stage, i.e., 26 DAS to bud stage, i.e., 35 DAS). Values are means (*n = 3*) ± s.e. The asterisks above the symbols indicate significant differences among treatments ($p \leq 0.05$, Tukey's test). **, ***—F test significant at $p \leq 0.01$ and *0.001*, respectively; *ns*—not significant. Cw and Cs—well-watered and water-stressed control, respectively; B1—woodchip biochar; B2—vineyard pruning biochar; V—vermicompost.

In contrast, in the final period of the vegetative cycle, from the bud to flowering initiation stage, the calculated AGR3 showed the highest ($p \leq 0.001$) value in B2+V than the other treatments. No statistical differences were found among the two controls (Cw and Cs), B1, V, and B1+V, with the lowest value in B2.

**Table 4.** Absolute Growth Rate (cm d$^{-1}$) of quinoa treated with different organic amendments in three periods of the growing cycle (AGR1, from the six-leaf to twelve-leaf stage; AGR2, from twelve-leaf stage to bud stage; AGR3, from bud to flowering initiation stage).

| Treatment | Absolute Growth Rate | | |
|---|---|---|---|
| | AGR1 | AGR2 | AGR3 |
| Cw | 1.33 a | 2.04 A | 2.62 B |
| Cs | 1.38 a | 1.19 C | 2.40 B |
| B1 | 1.41 a | 1.80 AB | 2.65 B |
| B2 | 1.24 a | 0.89 C | 2.03 C |
| V | 1.14 a | 1.63 B | 2.45 B |
| B1+V | 1.29 a | 1.78 AB | 2.53 B |
| B2+V | 0.72 b | 1.17 C | 2.98 A |
| Significance | * | *** | *** |

Values are means (n = 3). In each column, means followed by different letters are significantly different ($p \leq 0.05$; Tukey's test). *, ***—F test significant at $p \leq 0.05$ and $p \leq 0.001$, respectively. Cw and Cs—well-watered and water-stressed control, respectively; B1—woodchip biochar; B2—vineyard pruning biochar; V—vermicompost.

### *3.4.3. Growth-Related Parameters at the End of the Experiment*

At the end of the experiment, the significant effect of biochars and vermicompost on plant growth was clearly highlighted by all the considered parameters (Figure 4).

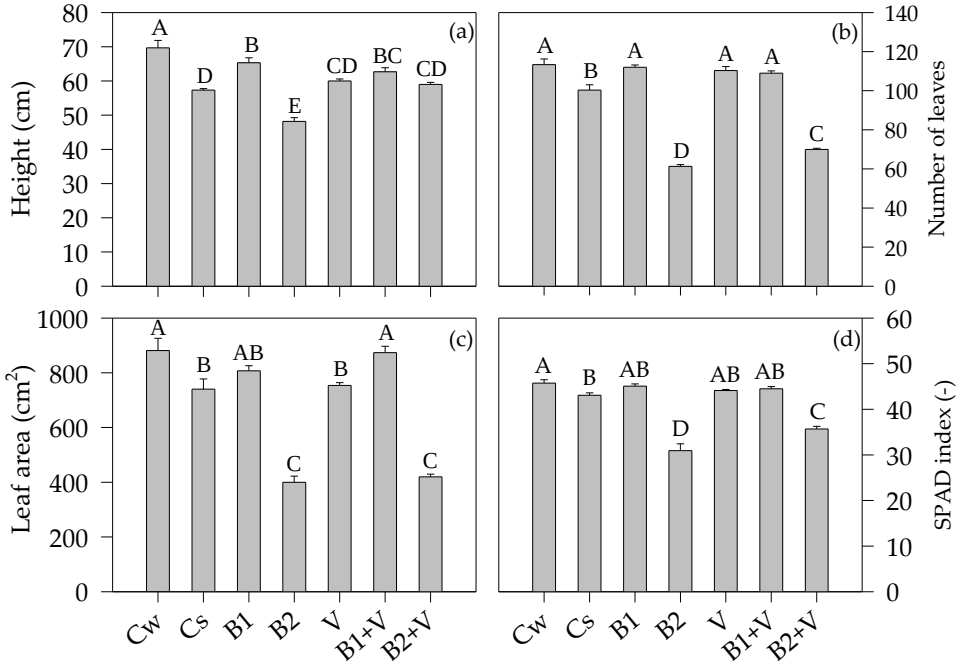


**Figure 4.** Plant height (a), number of leaves (b), leaf area (c), and SPAD index (d) of quinoa treated with different organic amendments, at the end of the experiment. Values are means ($n = 3$) ± s.e. In each graph, different letters above the bars indicate significant differences among treatments ($p \leq 0.05$, Tukey's test). F test significant at $p \leq 0.001$. Cw and Cs—well-watered and water-stressed control, respectively; B1—woodchip biochar; B2—vineyard pruning biochar; V—vermicompost.

More specifically, the plant height (Figure 4a), number of leaves (Figure 4b), leaf area (Figure 4c), and the SPAD index (Figure 4d) were always higher ($p \leq 0.001$) in the well-irrigated control (Cw) than the water-stressed one (Cs) and in B1, V, and B1+V treatments than B2 and B2+V.

A significant effect of the experimental treatments was observed also considering the fresh weights (FW) and dry matter (DM) contents of stem and leaves, as measured at the end of the experiment (Figure 5).

More specifically, 37% higher ($p \leq 0.001$) stem FW was observed in Cw than Cs (Figure 5a). The latter was not statistically different from the stem FW of B1, V, and B1+V (on average, 10

g). In addition, the stem FW of Cs, B1, V, and B1+V were, on average, 57% higher ($p \leq 0.001$) than B2 and B2+V, which did not differ from each other (on average, 6 g).

The stem DM (Figure 5b) was not statistically different in Cw and B1, showing 83% and 63% higher ($p \leq 0.001$) values than Cs, respectively. B1 did not differ from V and B1+V and were 39% higher, on average, than B2 and B2+V.

Regarding the leaf FW (Figure 5c), Cw was not statistically different from B1+V, which showed a 14% higher ($p \leq 0.001$) value than Cs (22 vs. 19 g). B2 and B2+V showed the lowest ($p \leq 0.001$) values (on average, 11 g).

The leaf DM (Figure 5d) reached the highest ($p \leq 0.001$) value in Cw (22%), with no significant differences among Cs, B1, V, and B1+V (on average, 18%), which, in turn, showed 29% higher leaf dry matter content than B2 and B2+V (on average, 14%).

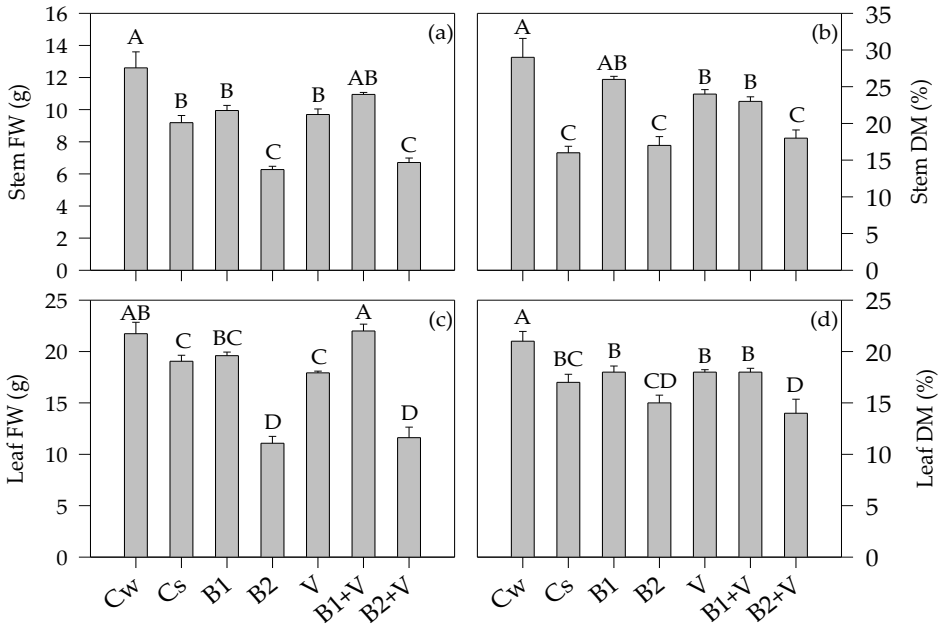


**Figure 5.** Stem fresh weight (a) and dry matter content (b) and leaf fresh weight (c) and dry matter content (d) of quinoa treated with different organic amendments, at the end of the experiment. Values are means ($n = 3$) ± s.e. In each graph, different letters above the bars indicate significant differences among treatments ($p \leq 0.05$, Tukey's test). F test significant at $p \leq 0.001$. Cw and Cs—well-watered and water-stressed control, respectively; B1—woodchip biochar; B2—vineyard pruning biochar; V—vermicompost.

The panicle length and number, as well as the panicle and sub-panicles FW and DM contents, were also affected by the experimental treatments (Figure 6).

In particular, 32% higher ($p \leq 0.001$) panicle length (Figure 6a) was detected in Cw compared to Cs. No statistical differences were found among Cw, B1, and B1+V (on average, 10 cm). The latter showed, in turn, a panicle length not statistically different from V and B2+V, while the lowest value of this parameter was observed in B2 (5 cm).

Additionally, the number of sub-panicles (Figure 6b) was higher ($p \leq 0.001$) in Cw than in Cs (18 vs. 12), with about a 50% increase. Cw was not statistically different from B1 and B1+V (on average, 16), B2+V resulted in a similar number of sub-panicles compared to Cs (on average, 11), while B2 had the fewest (6).

Regarding the panicle FW (Figure 6c), Cw showed the highest ($p \leq 0.001$) value (4 g). Cs was not statistically different from B1, V, and B1+V (on average, about 2 g), while B2 and B2+V showed the lowest value (on average, about 1 g). The sub-panicles FW (Figure 6c) did not differ between Cw and B1 (on average, 2 g), which showed values that were 115% higher ($p \leq 0.001$) than Cs. The lowest ($p \leq 0.01$) FW was shown in sub-panicles produced by B2 and B2+V (on average, 0.3 g).

Considering the panicle DM content (Figure 6d), V showed a value that was 33% higher ($p \leq 0.001$) than Cw, with no statistical difference from B1 and B1+V (on average 19%). The lowest value was observed in Cs (12%). The sub-panicle DM content (Figure 6d) did not differ among B1, V, and B1+V (on average, 19%), with the lowest ($p \leq 0.01$) values in both B2 and B2+V (on average, 13%).

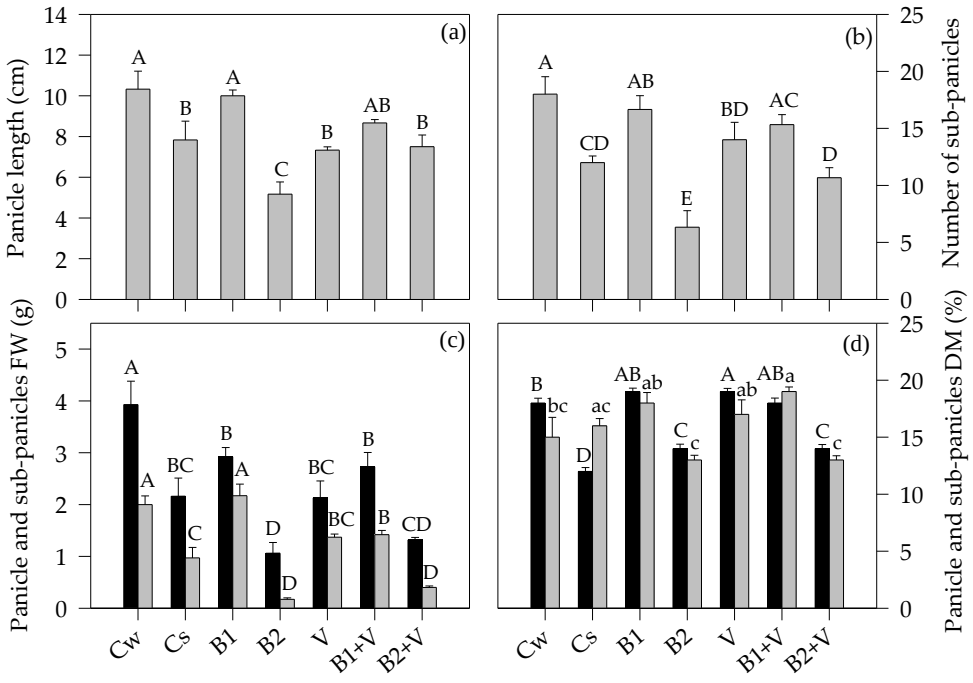


**Figure 6.** Panicle length (a), number of sub-panicles (b), panicle and sub-panicle fresh weight (c), and dry matter content (d) of quinoa treated with different organic amendments, at the end of the experiment. Values are means ($n = 3$) ± s.e. In each graph, different letters above the bars indicate significant differences among treatments ($p \leq 0.05$, Tukey's test). Lowercase and uppercase letters refer to F test significant at $p \leq 0.01$ and $0.001$, respectively. In graphs c and d, the black bars are panicle and the gray bars are sub-panicles. Cw and Cs—well- and water-stressed control, respectively; B1—woodchip biochar; B2—vineyard pruning biochar; V—vermicompost.

### *3.4.4. Water-Related Parameters*

At the end of the water-stress period, except for the leaf turgid weight to dry weight ratio (TW/DW), the water-related parameters were significantly affected by the application of biochars and vermicompost to the soil, as shown in Table 5. In particular, a higher ($p \leq 0.01$) value of leaf relative water content (RWC) was shown in Cw compared to Cs, which, in turn, did not differ from B1, V, and B1+V. B2, when combined with V, was not statistically different from Cw. Regarding the leaf water potential (Ψ), Cw and B2+V were not statistically different from each other and both significantly higher ($p \leq 0.01$) than the other treatments. A similar trend was observed with regard to the leaf osmotic potential (Ψπ). Except for Cw, the turgor (Ψp) was close to zero in all treatments (data not shown).

Additionally, after soil re-watering, except for Ψp, the experimental factors significantly influenced RWC, Ψ, Ψπ, and TW/DW (Table 5). More specifically, a higher ($p \leq 0.01$) RWC value was observed in Cw, followed by B2, B2+V, and B1; on the contrary, a lower RWC value was detected in Cs, which did not differ from V and B1+V. Regarding Ψ and Ψπ, the trends were very similar to those already observed at the end of water-stress period, with Cw, B2, and B2+V showing higher ($p \leq 0.05$) values than Cs, B1, and B1+V. On the contrary, Ψp did not result in any statistical difference among the experimental treatments. Finally, the TW/DW ratio was 21% higher ($p \leq 0.05$) in both B2 and B2+V than the other treatments.

**Table 5.** Relative water content (RWC, %), leaf water potential (Ψ, MPa), osmotic potential (Ψπ, MPa), turgor (Ψp, MPa), and turgid weight to dry weight ratio (TW/DW) of quinoa treated with different organic amendments, at the end of the water-stress period and after soil re-watering.

| Treatment | End of Water-Stress Period | | | | After Soil Re-Watering | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | RWC | Ψ | Ψπ | TW/DW | RWC | Ψ | Ψπ | Ψp | TW/DW |
| Cw | 75.4 A | −1.70 A | −1.81 A | 11.3 | 77.5 A | −1.37 A | −1.53 A | 0.17 | 10.5 C |
| Cs | 56.1 D | −2.50 B | −2.49 BC | 11.6 | 69.1 BC | −1.68 BC | −1.94 BC | 0.26 | 11.2 BC |
| B1 | 58.5 CD | −2.58 B | −2.51 BC | 10.2 | 70.7 AC | −1.71 BC | −1.88 BC | 0.17 | 10.8 C |
| B2 | 64.7 BC | −2.07 AB | −2.05 AB | 11.1 | 73.2 AB | −1.50 AB | −1.72 AB | 0.22 | 12.9 AB |
| V | 59.5 CD | −2.53 B | −2.54 C | 10.1 | 70.0 BC | −1.82 C | −1.91 BC | 0.09 | 11.1 BC |
| B1+V | 55.5 D | −2.56 B | −2.58 C | 8.1 | 66.5 C | −1.77 BC | −2.01 C | 0.25 | 10.2 C |
| B2+V | 69.6 AB | −1.73 A | −1.76 A | 10.3 | 72.2 AB | −1.52 AB | −1.68 AB | 0.17 | 13.1 A |
| Significance | ** | ** | ** | ns | * | * | * | ns | * |

Values are means ($n = 3$). In each column, means followed by different letters are significantly different ($p \leq 0.05$; Tukey's test). *, **—F test significant at $p \leq 0.05$ and $p \leq 0.01$, respectively; *ns*—not significant. Cw and Cs—well-watered and water-stressed control, respectively; B1—woodchip biochar; B2—vineyard pruning biochar; V—vermicompost.

The significant effect of soil treatment with biochars and vermicompost was also observed by considering the total fresh weight (TFW) and the total water consumption (TWC) per plant (Figure 7).

In particular, the TFW (Figure 7a) was significantly higher ($p \leq 0.001$) in the well-watered control (Cw) compared to the stressed one (Cs), accounting for an increase of 28%. Moreover, Cs did not differ from both B1 and V (on average, 32 g plant$^{-1}$). A drastic TFW reduction was observed in B2 and B2+V in comparison to both Cw (108%) and Cs (62%), while a TFW value that was not statistically different from Cw was observed in B1+V (on average, 39 g plant$^{-1}$).

The TWC (Figure 7b) was 38% higher ($p \leq 0.05$) in Cw than B2, V, and B2+V, with no statistically differences from Cs, B1, and B1+V (on average, 4 L plant$^{-1}$). The TWC reflected in the water-use efficiency (WUE) (Figure 7c) was higher ($p \leq 0.001$) in Cw than B2 and B2+V, with no statistical differences from Cs, B1, V, and B1+V.

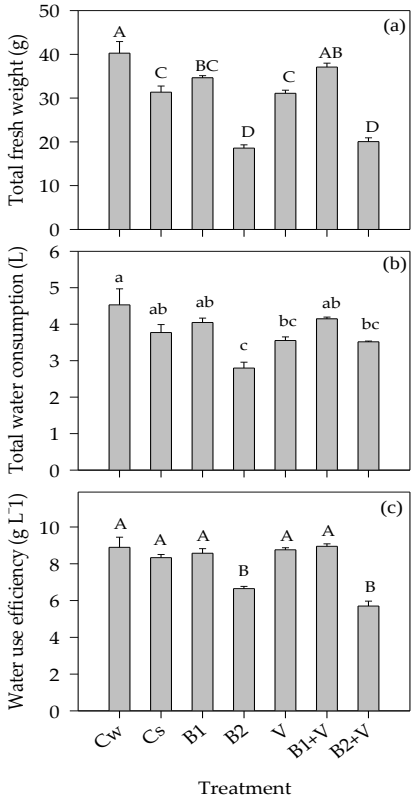


**Figure 7.** Total fresh weight per plant (a), total water consumption per plant (b), and water use efficiency (c) of quinoa treated with different organic amendments, at the end of the experiment. Values are means ($n = 3$) ± s.e. In each graph, different letters above the bars indicate significant differences among treatments ($p \leq 0.05$, Tukey's test). Lowercase and uppercase letters refer to F test significant at $p \leq 0.05$ and *0.001*, respectively. Cw and Cs—well-watered and water-stressed control, respectively; B1—woodchip biochar; B2—vineyard pruning biochar; V—vermicompost.

## 3.5. DISCUSSION

Due to increasing water scarcity and its negative impact on agricultural production, nowadays, there is an increasing interest in the use of organic amendments, especially compost and biochar, to improve soil-water relations, enhance plant-available water, and growth response (Ghorbani et al., 2023). Therefore, the current study was carried out to investigate the role of

two different biochar types and a vermicompost, both alone and mixed, under water-stress conditions occurring during the vegetative growing cycle of quinoa when the species is more susceptible to drought (Geerts et al., 2006).

Regarding the phenological growth stages of quinoa, the application of biochar from woodchips and vermicompost, alone and mixed, reduced the number of days between sowing and seedling emergence. This positive effect is also reflected in the successive two-leaf stage. On the contrary, biochar from vineyard prunings, particularly when combined with vermicompost, prolonged the two phenological stages. Therefore, the two biochars resulted in different phenological plant responses. According to Busch et al. (2012), the high ash content in biochar, particularly when produced at higher temperatures, can negatively influence seed germination due to saline stress. Moreover, seed germination can be also affected by high soil pH (Koger et al., 2004). This likely occurred in our experiment, considering both the higher ash content and electrical conductivity of vineyard pruning biochar, as well as the higher pH of the soil after its addition (data not shown) compared to woodchip biochar. In accordance with our results, the application of vineyard pruning biochar decreased the germination index in lettuce and watercress (Videgain-Marco et al., 2020); instead, woodchip biochar positively affected the seed germination rate of *Robinia pseudoacacia* L., which reached 100% 2 days before the control (Bu et al., 2020).

The application of biochar from woodchips and vermicompost, both alone and mixed, also positively affected plant growth performance during the water-stress period, as clearly shown by the higher values for plant height, stem diameter, number of branches, and number of leaves. Interestingly, this plant growth response was very similar to that observed in the well-watered control, showing that the two organic amendments, i.e., woodchip biochar and vermicompost, both when applied alone and in mixture, helped the plants to cope with the water-stress conditions they experienced from the twelve-leaf stage to the bud stage. Similar results were

observed during the water-stress period if considering the leaf area, which showed a marked reduction in plants treated with vineyard pruning biochar, alone and in mixture with vermicompost, and a very similar value to the well-watered control in plants treated with woodchip biochar. Consistent with the enhancement of growth was also the SPAD index following soil treatment with biochar from woodchips, both alone and mixed with vermicompost. On the contrary, vineyard pruning biochar, both alone and mixed with vermicompost, led to a drastically reduced SPAD index value. This prompted us to hypothesize a lower nitrogen availability for plant uptake in the soil treated with biochar from vineyard prunings, likely due to a higher porosity of biochar (Azuara et al., 2017; Pituello et al., 2015) that bound nutrients, especially N (Demiraj et al., 2018; Libutti et al., 2016). Ventura et al. (2013) applied different mixed fruit tree pruning biochars and highlighted that the changes in the SPAD index depend on the type and source of biochar. For instance, Wang et al. (2014) revealed an increased SPAD index with biochar from wood residues, while Akhtar et al. (2014) reported a decreased SPAD index after the application of biochar from mixed rice husk and shells of cotton seeds.

Except for the plants treated with vineyard pruning biochar, both alone and mixed with vermicompost, the limited plant-available water, progressively observed from 26 to 35 DAS, drastically reduced the water-related parameters in all the water-stressed plants, as shown by the lower RWC, $\Psi$, and $\Psi\pi$ values they reached at the end of the water-stress period—when soil was at the permanent wilting point. Moreover, these plants did not show turgor, suggesting a closure of stomata, probably also because the high temperatures registered at mid-day in the experimental site were concurrent with the end of the water-stress period. The negative effect of the high temperatures was confirmed by the well-watered plants showing a positive $\Psi p$, which although expected, was very low (0.11 MPa). The plants treated with biochar from vineyard prunings, alone and mixed with vermicompost, resulted in higher RWC, $\Psi$, and $\Psi\pi$

values (similar to well-watered control). Increases in water relations were observed following the application of biochar in wheat by Haider et al. (2020). In our study, the different effects of the two biochar types on plant water relations could be explained by considering that the Titicaca cultivar, usually characterized by fast growth and high leaf expansion (Sun et al., 2014), when grown on woodchip biochar-treated soil, showed far greater development (higher height, leaf number, and area), when more exposed to water-stress in comparison to vineyard pruning biochar-treated plants, which showed limited growth. Additionally, Jenson et al. (2000) found that quinoa maintained positive turgor down to zero turgor under severe water-stress imposition. Interestingly, only in plants treated with woodchip biochar, alone and mixed with vermicompost, the turgid weight to dry weight ratio showed a lower value. This result is in accordance with Andersen et al. (1996) who reported that, on a physiological level, drought tolerance can be expressed as low osmotic potential, low ratio of turgid weight to dry weight, less elasticity, and capacity to maintain positive turgor even at low leaf water potentials. After soil re-watering, plants treated with both the two biochars and vermicompost similarly increased their $\Psi$, $\Psi\pi$, and $\Psi p$ values, also showing a positive $\Psi p$. The turgid weight to dry weight ratio that was non-significant at the end of the water-stress cycle became significant, and still, the minimum value was observed in woodchip biochar alone and mixed with vermicompost, revealing more tolerance to drought than other treatments. Jensen et al. (2000) examined the effects of drying soil and concluded that the high net photosynthesis and specific leaf area in the early vegetative stage of quinoa resulted in early plant vigor by maintaining a low turgid/dry weight ratio. In this study, Titicaca cultivar showed a higher absolute growing rate and larger leaf area, but during drought stress, a significant reduction in leaf area, which was not recovered even after 7 days of re-irrigation, was observed, making this cultivar sensitive to drought (Sun et al., 2014); in our experiment, after 5 days of soil re-watering, Titicaca that

suffered from drought had a sudden increase in leaf expansion rate—more pronounced in plants treated with woodchip biochar alone and in mixture with vermicompost.

At the end of the vegetative growing cycle, plant response in terms of biomass, both total and partitioned into stems and leaves (fresh weight and dry matter content), as well as of yield contributing traits (main panicle and sub-panicles length, number, fresh weight, and dry matter content), confirmed the positive effect of woodchip biochar, both alone and mixed with vermicompost, in helping plants recover from water-stress conditions. Particularly, the higher main panicle and sub-panicle production in plants treated with this biochar type leads us to hypothesize a probable enhanced response in terms of grain yield. In this regard, our findings are in agreement with Kammann et al. (2015), who reported an increase in *Chenopodium quinoa* biomass of 305% following the application of different organic manures, along with 2% woodchip biochar (co-composted biochar) to a mixture of poor sandy soil. Using biochar boosted the nutritional availability and source–sink relationship, increasing panicle length and grain yield in rapeseed (Khan et al., 2021). Biochar application boosted spike number and length and grain yield in water-stressed wheat plants compared to no biochar application in droughted plants. A similar positive response of biochar has been described by Zhang et al. (2012) in rice, Foster et al. (2016) in maize, Mannan et al. (2016) in soybeans, and Agbna et al. (2017) in tomato plants.

A very interesting result also concerns the WUE of plants treated with biochar from woodchips, alone and mixed with vermicompost, and subjected to water stress, which was similar to that observed on well-watered control plants. Although the woodchip biochar-treated plants received 10% less water than the well-irrigated control, the water-use efficiency was not different, showing that quinoa had the ability to produce more with less water consumption. Additionally, Telahigue et al. (2017) and Aslam et al. (2020) reported a similar result in quinoa under drought conditions. It should be noted that the increase in biomass and yield in the plants

after biochar soil amendment is mainly due to the enhancement of the water content at the permanent wilting point (Tayyab et al., 2018), as well as an improvement in the uptake of essential mineral elements (de Melo Carvalho et al., 2014). On the other hand, in our study, woodchip biochar addition at a rate of 2% reduced the bulk density more effectively than vineyard pruning biochar (data not shown), which might also be a reason for the improved plant growth because less bulk density helps in better root penetration (Nawaz et al., 2013). Moreover, in our experiment, it can also be assumed that woodchip biochar has superior absorption and can better release water and nutrients compared to vineyard pruning biochar. However, further investigation is necessary to verify this hypothesis.

### 3.6. CONCLUSIONS

Due to current climate change conditions, regional droughts are occurring more intensively, threatening agricultural crop production. The keys to ensuring crop yield and food security are to identify the significant long-term impacts of climate change and the means for reducing its effect. In this context, increasing attention is now being paid to the use of organic amendments and the cultivation of climate-resilient crops.

The present study focused on quinoa, cv Titicaca, grown on soil treated with different organic amendments, such as two types of biochar from woodchips and vineyard prunings, respectively, along with a vermicompost derived from cattle manure, tested alone and in mixture, during the vegetative growing cycle under water-stress conditions. The results showed that, among the tested organic amendments, woodchip biochar, both alone and mixed with vermicompost, clearly improved plant vegetative development, also allowing a quickly recovery after the period of water stress, as well as an enhancement of WUE in comparison to vineyard pruning biochar. Furthermore, a similar positive effect was also observed on panicle and sub-panicle production, leading us to also hypothesize an enhancement of yield contributing traits.

In conclusion, the findings of the present study suggest that the use of soil treated with organic amendments, especially woodchip biochar and vermicompost, could be a useful approach to mitigate drought conditions during the vegetative growing cycle of quinoa, helping plants to develop. This could open new perspectives to boost the ability of quinoa to better cope with water stress and potentially broaden its spatial distribution across agricultural areas affected by drought and water scarcity.

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# CHAPTER 4

# WOODY BIOCHAR RATES AND WATER SHORTAGE IMPACT ON EARLY GROWTH STAGES OF *Chenopodium quinoa* Willd.

*This chapter is a state-of-the-art chapter, and its content has been already published:*

Rivelli, A.R., **Akram, M.Z.,** & Libutti, A. (2024). Woody biochar rate and water shortage impact on early growth stages of *Chenopodium quinoa* Willd. *Agronomy, 14(1), 53*.

## 4.1. ABSTRACT

The application of biochar to agricultural soils has been proven to have many advantages, including the improvement of soil water holding capacity and plant growth, particularly under limiting conditions of water supply. The response of quinoa (*Chenopodium quinoa* Willd.) to water shortage occurring during the vegetative growth stages is not well known. Therefore, the present study aimed to evaluate the combined effects of three wood chip biochar rates (0%, 2% and 4%) and two water regimes (100 and 50% evapotranspiration losses restitution) on the vegetative development and water status of quinoa (cultivar Titicaca). The results showed that the treatment with 2% wood chip biochar improved plant height, leaf and branch number and stem diameter during the vegetative growing cycle compared to the 0% (control) and 4% biochar treatments, which were not different from each other. At the end of the experiment, when the plants were at the flowering initiation stage, increases of 23% in leaf area, 22% in fresh biomass, 27% in main panicle length and 36% in sub-panicle number were observed. The application of woody biochar at a 4% rate, although improving the plant water status with increases of 10% in RWC and 18% in Ψ, did not enhance the vegetative development of the quinoa. The water shortage negatively affected both the growth performance and plant water status. The best growth response of quinoa was observed only when the plants were treated with a 2% biochar rate and were fully irrigated.

*Keywords: Woodchip biochar, water regime, water shortage, quinoa, vegetative growth, plant water status*

## 4.2. INTRODUCTION

It has been estimated that the persistence of anthropogenic $CO_2$ emissions at the current rate may lead to a potential rise in global temperatures of 1.5°C by 2052 (Masson-Delmotte et al., 2018). The escalating threat of climate change has emerged as a significant abiotic stressor for crop development, particularly in arid and semi-arid areas, resulting in frequent drought periods and water shortage conditions. In addition, the overutilization of agricultural chemicals, including fertilizers, herbicides and pesticides, has led to a negative impact on soil health, causing a decline in fertility over time. Water shortage and suboptimal soil conditions exert a substantial influence on the physiological processes in plants and plant growth (Heydari et al., 2023).

In this context, the up-to-date literature reports that the application of organic amendments to the soil can provide numerous benefits for agriculture and the environment. In particular, the use of biochar, a carbon-rich material derived from the thermochemical degradation of organic biomasses in an anaerobic or low-oxygen environment (Hamidzadeh et al., 2023), is a widely agreed agronomic strategy to enhance soil fertility and mitigate the negative effects of drought (Ajayi et al., 2016; Kapoor et al., 2022). Due to its highly porous structure and large surface area (Verheijen et al., 2012), biochar positively affects soil water and nutrient retention, soil water holding capacity and the water available to plants (Laird et al., 2010; Ajayi et al., 2016; Libutti et al., 2019; Libutti et al., 2021). Biochar has also gained attention as a potential solution for sequestering carbon in soils (Ding et al., 2023), binding contaminants (Aborisade et al., 2023) and mitigating climate change (Anand et al., 2022). The effects of biochar, when added to soil, can vary depending on the type and characteristics of the feedstock, the conditions of pyrolysis, the type of soil and the rate of application (Manikandan et al., 2023). Although adding

biochar to soil has been reported to be productive, before applying it on a large scale, any counterproductive effects should be comprehensively studied. An excessive biochar application rate may negatively affect (Lebrun et al., 2021; EL Moussaoui et al., 2023) plant response due to several effects, including altering the soil pH and immobilizing nutrients (Gale et al., 2017; Xiang et al., 2021). According to recent studies, application doses of up to 30 t $ha^{-1}$ enhanced rapeseed biomass, pods/plant and 1000-seed weight by 23%, 32% and 21%, respectively, and also increased yield, while higher doses adversely affected the growth and seed production of the rapeseed under drought (Khan et al., 2021). Among the different types of biochar, woody biochar has garnered attention for enhancing soil properties and fostering plant growth due to its composition, primarily comprising lignin, cellulose and hemicellulose (Lai et al., 2013; Laird et al., 2017). The specific ratios of lignin, cellulose and hemicellulose within woody biochar depend upon the wood feedstocks and are a factor that influences biochar's characteristics post-pyrolysis (Gholizadeh et al., 2019). Woody biochar stands out for its elevated porosity and augmented specific surface area, which are attributed to the robust thermal stability of lignin, which preserves the structural integrity of its pore architecture (Leng et al., 2021). Moreover, woody biochar exhibits a relatively higher carbon content (Li et al., 2023) compared to biochar derived from crop residues, thus affording it an amplified cation exchange capacity (Greco et al., 2019), accompanied by a diminished ash content (Mao et al., 2019). Woody biochar application at low rates improved the root growth of maize hybrids, significantly reducing their sensitivity to drought while enhancing their water use efficiency (Romdhane et al., 2019). Under drought conditions, the biomass of quinoa was increased up to 305% under the application of 2% co-composted wood chip biochar to the soil (Kammann et al., 2015).

Currently, the cultivation of alternative crops suited to adverse environmental conditions, including quinoa, is of growing interest in many countries (Akram et al., 2021). Quinoa,

originating from the South American highlands, has received much attention all over the world, also due to its unique nutritional profile and value, which make it suitable for specific food sectors (Angeli et al., 2020). It is recognized as a climate-resilient and drought-tolerant species (Jensen et al., 2000; Kumari et al., 2023). Its ability to adapt to different drought levels through physiological and morphological strategies depends on the genotype. Among the different genotypes, Titicaca has been shown to have a reduced stomatal conductance, plant height and seed yield while having an increased root length and density when subjected to water shortage (Razzaghi et al., 2020). The ability of Titicaca to reduce the leaf water potential (Ψ) contributes to its drought tolerance, as it can dynamically adjust its water status, exhibiting daily fluctuations in leaf Ψ with higher values at night and lower values during the day. This adaptation promotes groundwater extraction and survival under water shortage conditions (Fghire et al., 2017). According to Nanduri et al. (2019), the Titicaca genotype showed a high yield stability under different climate conditions. In a study by Issa Ali et al. (2019), the Danish variety Titicaca and the South American variety L119 exhibited varying physiological and morphological responses to water shortage; both were shown to be sensitive to drought in terms of leaf water potential, leaf relative water content, stomatal conductance and closure, while they differed in their drought resistance strategies depending on the period of the plant's cycle in which the water stress occurred. Quinoa presents diverse drought tolerance mechanisms, although not all are present in every genotype, emphasizing the need to screen the germplasm based on specific study objectives (Hafeez et al., 2022). A number of studies have addressed the impact of water shortage during quinoa's reproductive growth stages (Kammann et al., 2011; Walters et al., 2016; Ramzani et al., 2017), while those referring to the vegetative growth stages are relatively limited, despite the high vulnerability of quinoa in this developmental phase, as reported by Geerts et al. (2006). The present study follows our previous research (Akram et al., 2023) on quinoa Titicaca grown on soil treated with different types of organic

amendments, i.e., two biochars, one deriving from wood chips and the other from vineyard pruning, and a vermicompost, each applied alone or in combination at a 2% rate, and subjected to periods of water stress during the early vegetative growth stages. Among the tested amendments, the wood chip biochar resulted in enhanced plant growth. Therefore, the current experiment specifically focused on wood chip biochar with the aim of deeply investigating its effect by comparing different application rates under different water regimes throughout the vegetative development of quinoa Titicaca.

## 4.3. MATERIAL AND METHODS

### *4.3.1. Experimental Design and Setup*

A pot experimental trial was carried out in Potenza (PZ, 40°38′ N–15°48′ E, 819 m a.s.l.), southern Italy, at the greenhouse of the University of Basilicata, on quinoa plants (*Chenopodium quinoa* Willd., *cultivar* Titicaca) grown under conditions of natural light and with a controlled temperature, which was set at 26/18°C day/night.

The design of the experiment consisted of two factors: the biochar rate (B) and water regime (W). The biochar rates (w/w) were: 0% (B0, untreated soil or control), 2% (B2) and 4% (B4) of the dry soil weight (dw); the water regimes consisted of 100% (W100) and 50% (W50) restitution of evapotranspiration losses. Evapotranspiration was measured on a daily basis by weighing the pots at the same hour, and the intervention limit for watering was set at 50% depletion of the soil available water content, which was checked on the W100 treatments for each biochar rate. To evaluate the effect of each rate of biochar, each water regime and their combinations, a full-factorial experiment was set up. The six combinations (B0-W100, B0-W50, B2-W100, B2-W50, B4-W100 and B4-W50) were arranged according to a completely randomized block design and were replicated three times for a total of 18 experimental units.

For the trial, the soil was collected from the upper 0.30 m layer in an agricultural farm located in the Potenza district. Before the trial started, a soil physico-chemical characterization was

carried by following the analytical official methods reported in the Italian Official Gazette n. 248 (Italian Official Gazette, 1999). Accordingly, the soil resulted in a sandy-loam texture (USDA classification) with 66.1% sand, 11.5% silt and 22.4% clay; it had a field capacity (−0.03 MPa) of 22.8% dw and a wilting point (−1.5 MPa) of 11.4% dw; it had a pH of 7.6, an electrical conductivity (EC) of 0.6 dS $m^{-1}$, an organic carbon content of 5.9 g $Kg^{-1}$ and a total nitrogen content of 1.5%.

The biochar was acquired from the Nera Biochar Company located in Settimo Vittone (Turin district, Italy), whose quality was verified and attested by the University of Turin (Italy). The company is specialized in the production of biochar from wood chips that come from the cleaning of green areas and woods and from wood processing waste, and the biochar is produced in an industrial pyrolysis plant of its own design. According to the chemical characterization carried out before the application of the biochar to the soil, by following the procedures previously described (Rivelli and Libutti, 2022), the wood chip biochar was characterized by a pH of 8.9, an EC of 52 mS $m^{-1}$, C, H and N contents of 68.3%, 4% and 1% dw, respectively, a $C_{org}$ of 66.3% dw, a C/N value of 67.2, a $H/C_{org}$ value of 0.7, and an $O/C_{org}$ value of 0.4. Moreover, according to the European Biochar Certificate (EBC) (EBC, 2012) and the International Biochar Initiative (IBI) (IBI, 2012) standards that refer to the contents of C (>50%) and $C_{org}$ (>60%) and the molar ratio values of $H/C_{org}$ (≤0.7) and $O/C_{org}$ (≤0.4), the biochar resulted as a "Class 1 biochar". Plastic pots (25 cm height, 18 cm width and 18 cm length), with the bottom covered by a layer of expanded clay to facilitate the water drainage, were filled with 7 kg of air-dried soil, untreated or previously mixed with the wood chip biochar, according to the experimental treatments. The soil humidity was brought to field capacity on 6 May 2022, and ten seeds of Titicaca cultivar were sown in each pot. Once the emergence was completed, the plantlets were thinned in order to have one plant per pot, and the two water

regimes were applied until the experiment ended. The plants were cut at the beginning of flowering on 24 June 2022, 48 days after sowing (DAS).

### *4.3.2. Phenology, water relations and plant developmental measurements*

A daily visit to the experimental site was performed to monitor the seedling emergence, leaf development (two-, six-, eight-, ten- and twelve-leaf stage), budding and flowering initiation stages, based on the BBCH scale (Sosa-Zuniga et al., 2017). The criterion used to establish each stage was that more than 50% of the plants entered the given stage. A set of growth-related parameters were measured approximately twice a week throughout the experiment. In particular, the plant height (PH, cm), number of leaves (NL, n°) and branches (NB, n°) and stem diameter (SD, mm) were recorded. Furthermore, each leaf was recorded for its length (L) and width (W), which were further used to calculate the leaf area per plant (LA, $cm^2$) using equation presented by Talebnejad and Sepaskhah (2015) and verified by Akram et al. (2023) for the Titicaca cultivar: LA= 0.64 (L × W).

The leaf greenness or SPAD index was also measured on the youngest fully expanded leaves of each plant by taking three SPAD readings with a hand-held SPAD-502 m device (Konica-Minolta corporation, Ltd., Osaka, Japan). At the end of experiment, before plant cutting, a set of water-related parameters were measured. Specifically, the leaf relative water content (RWC, %) was determined by taking a small segment of the youngest fully expanded leaf per plant, immediately storing it in an ice bucket until taking its fresh weight (FW, g). After keeping the leaf segment in distilled water at 4°C for 24 h, its turgid weight (TW, g) was determined, and, after drying it in a ventilated oven at 70°C until reaching steady weight, the dry weight (DW, g) was measured. The RWC of the leaves was then calculated by equation (2): RWC= (FW-DW/TW-DW) × 100.

In addition, the turgid weight to dry weight ratio (TW:DW) was calculated. The total leaf water potential (Ψ, MPa) was measured at mid-day on the youngest fully expanded leaf per plant by

using a Scholander pressure chamber (PMS model1000, Corvallis, OR, USA). After plant cutting, the fresh weights (g) of the stem (SFW), leaves (LFW), panicle (PFW) and sub-panicles (SPFW) were measured, in addition to the above-mentioned growth-related parameters and SPAD index. The yield-contributing traits, i.e., the main panicle length (cm) and the number of sub-panicles (n°) per plant, were also recorded. Finally, the water use efficiency (WUE, g $L^{-1}$) was calculated by the ratio of the total plant fresh weight (TFW, g) to the total water consumption over the plant growing cycle (TWC, L).

#### *4.3.3. Statistical Analysis*

All the experimental data were first checked for normality and homogeneity of variance and then processed by two-way analysis of variance (ANOVA), following a factorial completely randomized design. When significant differences among the means were detected, the latter were compared by Tukey's honest significant difference post hoc test at the 0.05% level of significance. All the statistical analyses were carried out by using the "Statistix 8.1" software.

## 4.4. RESULTS

#### *4.4.1 Plant phenology and development*

Considering the phenological stages monitored during the experiment (data not reported), except for the seedling emergence and two-leaf development, the biochar rate (B) significantly affected the timing of the other stages. More specifically, in the plants treated with the lower biochar rate (B2), the leaf development (from the six- to twelve-leaf stages) and bud and flowering initiation occurred earlier in comparison to those with the higher rate (B4). The most evident differences concerned the flowering initiation that was reached 2.5 DAS ahead by the B2-treated plants in comparison to the B4-treated ones. A significant effect was also displayed by the water regime (W). Particularly, in the case of 50% evapotranspiration losses restitution (W50), the leaf development stage (from the six- to ten-leaf stages) was reached earlier than in

the case of 100% restitution (W100); on the contrary, the starting day of flowering initiation was delayed by 2 DAS in the W50-treated plants compared to the W100-treated plants.

Likewise, the biochar × water regime interaction (B × W) had a significant influence, with the B4-W100 treatment showing the highest number of days to reach the leaf development (from the six- to twelve-leaf stages) and bud initiation stages compared to the other treatments. In particular, the largest delay, equal to about 3 DAS, was registered on the twelve-leaf development stage. However, the B4-W100 treatment took a lower number of days, i.e., about 4 DAS fewer, to reach the flowering initiation stage than the B4-W50 treatment, without any difference compared to the other treatments. Considering the plant development throughout the experiment, in terms of the plant height and number of leaves, both B and W showed significant effects (Figure 1); on the contrary, their interaction never affected these two growth parameters. Specifically, a significant effect of B on the plant height (PH) (Figure 1a) was observed starting from 28 until 45 DAS when the B2-treated plants were clearly differentiated from the B0- and B4-treated ones, where they always showed a higher PH value. Instead, W affected the PH earlier (Figure 1b). Indeed, from 18 to 28 DAS, the W50-treated plants always showed a higher PH value than W100-treated ones. In particular, a 20% PH increase was observed at 23 DAS. This PH response to the water regimes reversed in the final part of growing cycle, with 23, 35 and 41% higher PH values obtained for the W100-treated plants compared to the W50-treated plants at 38, 41 and 45 DAS, respectively. As for the number of leaves (NL), a significant effect of B was observed in the second half of the growing cycle. From 32 DAS, the B2-treated plants started to show a higher NL value than the B0- and B4-treated ones, maintaining this growth advantage until the end of the experiment. Moreover, from 34 to 45 DAS, the NL was also significantly influenced by W (Figure 1d), always showing a higher value with the W100-treated plants than the W50-treated plants. In particular, at 45 DAS, the NL value observed for the W100-treated plants accounted for a 71% increase compared to the W50-treated ones.

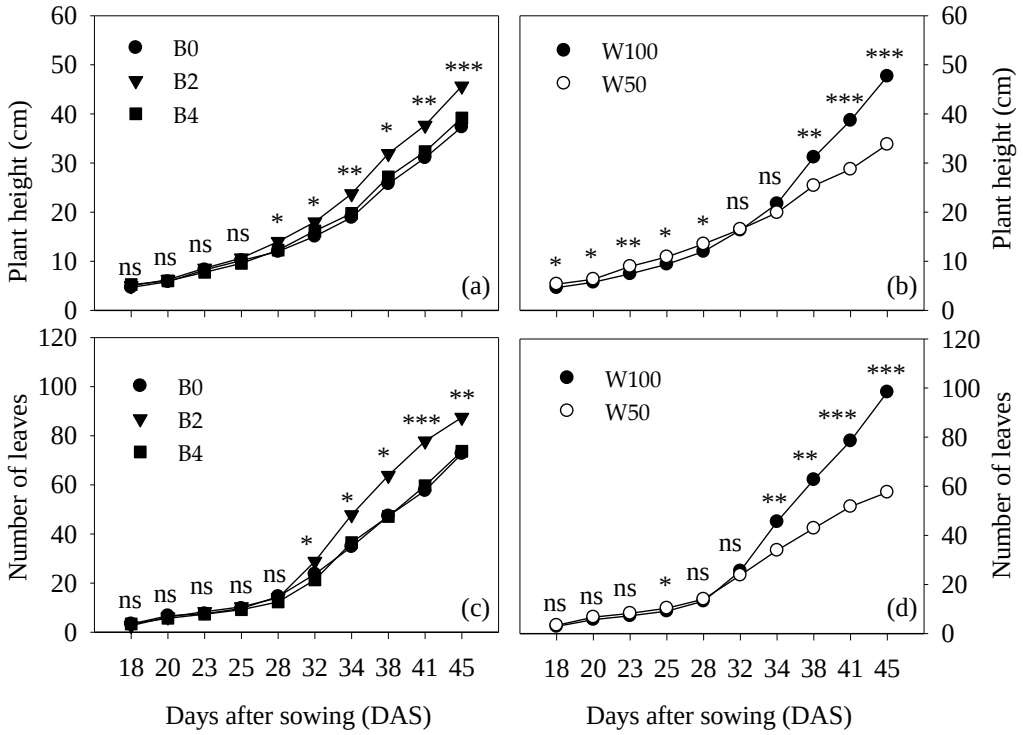


**Figure 1.** Plant height and number of leaves of quinoa as affected by biochar rate (a,c) and water regime (b,d) during the vegetative growing cycle. Values are means (n = 3). The asterisks above the symbols indicate significant differences among treatments ($p \leq 0.05$; Tukey's test). *, **, *** indicate F test significant at $p \leq 0.05$, *0.01*, *0.001*, respectively; ns, not significant; B0, B2 and B4 indicate 0%, 2% and 4% treatments of wood chip biochar, respectively; W100 and W50 indicate 100% and 50% treatments of evapotranspiration losses restitution, respectively.

As reported in Table 1, the leaf area (LA) was significantly affected by both the two factors, B and W, and their interaction. In particular, at 28 and 32 DAS, the B2-treated plants displayed the highest LA values. Successively, at 38 and 45 DAS, the B2-treated plants did not statistically differ from the B4-treated ones, both resulting in higher LA values than the B0-treated plants. Specifically, in terms of the average of the B2- and B4-treated plants, the higher LA values observed at 38 and 45 DAS accounted for 54 and 35% increases, respectively. On the other hand, by considering the two water regimes, the W100-treated plants always showed higher LA values than the W50-treated ones, accounting for the highest increase, equal to 113%, at 45 DAS. Relative to the effect of B × W, LA reached the highest value with the B2-W100 treatment at 28 DAS, a higher value with the B2-W100 treatment (not statistically different from B0-W100) compared to the B4-W100 treatment at 32 DAS and a value not

statistically different between the B2-W100 and B4-W100 treatments at 38 DAS. Finally, at 45 DAS, any statistically significant differences among the considered treatments disappeared, and the LA values were similar in all treatments. With regards to the SPAD index (Table 1), only starting from 32 DAS until 45 DAS, its values were significantly affected by both B and W, but not by their interaction. More specifically, the SPAD index always resulted in higher values with the B2-treated plants compared to the B4-treated plants, accounting for a 5% increase on average over the DAS, as well as with the W100-treated plants compared to the W50-treated ones, with the highest increase, equal to 12%, obtained at 45 DAS.

**Table 1.** Leaf area (LA, $cm^2$ $plant^{-1}$) and SPAD index (-) of quinoa grown under three biochar rates and two water regimes during the experiment.

| Experimental Factor | | 28 DAS | | 32 DAS | | 38 DAS | | 45 DAS | |
|---|---|---|---|---|---|---|---|---|---|
| | | LA | SPAD Index | LA | SPAD Index | LA | SPAD Index | LA | SPAD Index |
| Biochar (B) | | | | | | | | | |
| B0 | | 106 b | 44.3 | 201 b | 45.8 ab | 269 b | 43.3 ab | 446 b | 44.6 b |
| B2 | | 121 a | 46.2 | 271 a | 47.0 a | 419 a | 44.5 a | 585 a | 46.4 a |
| B4 | | 76 c | 46.7 | 177 b | 44.6 b | 409 a | 42.3 b | 618 a | 44.3 b |
| Water regime (W) | | | | | | | | | |
| W100 | | 106 a | 45.4 | 250 a | 46.9 a | 468 a | 44.3 a | 748 a | 47.7 a |
| W50 | | 96 b | 46.0 | 182 b | 44.7 b | 263 b | 42.4 b | 351 b | 42.5 b |
| Biochar × water regime | | | | | | | | | |
| B0 | W100 | 114 b | 44.0 | 260 ab | 47.3 | 350 b | 44.5 | 613 | 47.4 |
| | W50 | 99 bc | 44.5 | 142 d | 44.4 | 189 c | 42.0 | 279 | 41.7 |
| B2 | W100 | 135 a | 46.5 | 285 a | 47.6 | 517 a | 44.8 | 766 | 48.7 |
| | W50 | 106 b | 45.9 | 255 ab | 46.5 | 320 b | 44.2 | 404 | 44.1 |
| B4 | W100 | 70 d | 45.8 | 205 bc | 45.9 | 538 a | 43.5 | 865 | 47.0 |
| | W50 | 83 cd | 47.5 | 148 cd | 43.3 | 281 b | 41.1 | 372 | 41.7 |
| Significance | | | | | | | | | |
| B | | *** | ns | *** | * | *** | * | *** | ** |
| W | | ** | ns | *** | ** | *** | * | ** | *** |
| B × W | | *** | ns | * | ns | * | ns | ns | ns |

Values are means ($n = 3$). In each column, means followed by different letters are significantly different ($p \leq 0.05$; Tukey's test). *, **, *** indicate F test significant at $p \leq 0.05$, $0.01$, $0.001$, respectively; ns, not significant; B0, B2 and B4 indicate 0%, 2% and 4% treatment of wood chip biochar, respectively; W100 and W50 indicate 100% and 50% treatment of evapotranspiration losses restitution, respectively; DAS, days after sowing.

Concerning the stem diameter (SD) (Table 2), significant effects of both W and B × W interaction were observed at 32 DAS. The SD showed a 24% higher value with the W100-treated plants compared to the W50-treated plants, as well as with the B0-W100 treatment compared to the B0-W50 and B4-W50 treatments, accounting for 45 and 40% increases, respectively, without any statistically significant difference from the B2-W100, B2-W50 and

B4-W100 treatments. Successively, at 38 DAS, the SD was significantly influenced by the two factors B and W, displaying 18 and 39% higher values with the B2-treated plants compared to the B4-treated plants and with the W100-treated plants compared to the W50-treated ones, respectively. Finally, at 45 DAS, not only B and W but also their interaction affected the SD. The B2-treated plants showed 5 and 12% higher values than the B0- and B4-treated ones, respectively, and the W100-treated plants accounted for a 33% higher SD than the W50-treated ones. The highest SD value was recorded with the B2-W100 treatment, while the lowest one was recorded with the B4-W50 treatment, which showed a 36% decrease by comparison.

The number of branches (NB) was influenced by only W at 32 DAS (Table 2), showing a higher value with the W100-treated compared to the W50-treated plants. Successively, at 38 and 45 DAS, a significant effect was displayed by both the two factors B and W but not by their interaction. The SD was higher with the B2-treated plants than with the B0- and B4-treated plants, as well as with the W100-treated plants compared to the W50-treated ones.

**Table 2.** Stem diameter (SD, mm) and number of branches (NB) of quinoa grown under three biochar rates and two water regimes during the experiment.

| Experimental Factor | | 32 DAS | | 38 DAS | | 45 DAS | |
|---|---|---|---|---|---|---|---|
| | | SD | NB | SD | NB | SD | NB |
| Biochar (B) | | | | | | | |
| B0 | | 3.5 | 8.3 | 5.5 ab | 13.2 b | 5.9 b | 18.3 b |
| B2 | | 3.8 | 9.5 | 6.1 a | 15.3 a | 6.3 a | 20.3 a |
| B4 | | 3.4 | 8.3 | 5.2 b | 14.0 ab | 5.6 c | 18.5 b |
| Water regime (W) | | | | | | | |
| W100 | | 4.0 a | 9.2 a | 6.5 a | 16.0 a | 6.8 a | 20.9 a |
| W50 | | 3.2 b | 8.2 b | 4.7 b | 12.3 b | 5.1 b | 17.2 b |
| Biochar × Water regime | | | | | | | |
| B0 | W100 | 4.2 a | 9.0 | 6.5 | 15.0 | 6.6 b | 20.0 |
| | W50 | 2.9 c | 7.7 | 4.4 | 11.3 | 5.3 c | 16.7 |
| B2 | W100 | 3.9 ab | 9.7 | 6.8 | 17.3 | 7.1 a | 22.3 |
| | W50 | 3.8 ab | 9.3 | 5.5 | 13.3 | 5.4 c | 18.3 |
| B4 | W100 | 3.8 ab | 9.0 | 6.2 | 15.7 | 6.6 b | 20.3 |
| | W50 | 3.0 bc | 7.7 | 4.2 | 12.3 | 4.6 d | 16.7 |
| Significance | | | | | | | |
| B | | ns | ns | * | * | *** | ** |
| W | | *** | * | *** | *** | ** | *** |
| B × W | | * | ns | ns | ns | ** | ns |

Values are means ($n = 3$). In each column, means followed by different letters are significantly different ($p \leq 0.05$; Tukey's test). *, **, *** indicate F test significant at $p \leq 0.05$, $0.01$, $0.001$, respectively; ns, not significant; B0, B2 and B4 indicate 0%, 2% and 4% treatments of wood chip biochar, respectively; W100 and W50 indicate 100% and 50% treatments of evapotranspiration losses restitution, respectively; DAS, days after sowing.

### *4.4.2. Water- and growth-related parameters and yield-contributing traits at the end of the experiment*

At the end of the experiment, the considered plant water-related parameters, i.e., the leaf relative water content (RWC), turgid-weight-to-dry-weight ratio (TW:DW) and total leaf water potential (Ψ), were significantly influenced by both the two factors B and W (Table 3). In addition, the RWC was also affected by the interaction B × W. As for the RWC, about a 10% higher value was shown by the B4-treated plants compared to the B0- and B2-treated ones, which did not differ from each other. Similarly, the W100-treated plants accounted for a 14% higher RWC than the W50-treated ones. Moreover, higher values were observed with all the W100 treatments (B0-W100, B2-W100 and B4-W100) compared to the corresponding W50 ones (B0-W50, B2-W50 and B4-W50). A different response to the considered experimental factors was displayed by TW:DW, which accounted for a significant 9% lower value with the B2-treated plants compared to the B0-treated plants (both not statistically different from the B4-treated plants) and a 15% lower value with the W50-treated plants compared to the W100-treated ones. Finally, regarding the Ψ value, the B4-treated plants showed a 18% less negative value in comparison to the B0- and B2-treated plants, which were not statistically different from each other. A less negative Ψ value of about 25% was similarly observed with the W100-treated plants than W50-treated ones.

Also, the growth-related parameters and SPAD index measured at the end of the growing cycle (Table 4) were affected by the two factors B and W. Only SD and LA were also influenced by the interaction B × W. Specifically, higher PH, SD, NB, NL, LA and SPAD index values were always registered with the B2-treated plants compared to the B0- and B4-treated plants and with the W100-treated plants compared to the W50-treated ones. Moreover, the SD was higher with all the W100 treatments (B0-W100, B2-W100 and B4-W100; on average, 7.3 mm) than

with the corresponding W50 ones (B0-W50, B2-W50 and B4-W50; on average 5.6 mm), accounting for a 30% increase.

**Table 3.** Relative water content (RWC, %), turgid-to-dry weight ratio (TW:DW) and total leaf water potential (Ψ, MPa) of quinoa grown under three biochar rates and two water regimes at the end of the experiment.

| Experimental Factor | | RWC | TW:DW | Ψ |
|---|---|---|---|---|
| Biochar (B) | | | | |
| | B0 | 55.9 b | 10.1 a | −3.0 b |
| | B2 | 55.6 b | 9.1 b | −3.1 b |
| | B4 | 61.0 a | 9.5 ab | −2.6 a |
| Water regime (W) | | | | |
| | W100 | 61.4 a | 8.9 b | −2.6 a |
| | W50 | 53.6 b | 10.2 a | −3.2 b |
| Biochar × Water regime | | | | |
| B0 | W100 | 58.5 ab | 9.6 | −2.8 |
| | W50 | 53.3 bc | 10.5 | −3.2 |
| B2 | W100 | 62.2 a | 8.2 | −2.7 |
| | W50 | 49.1 c | 10.1 | −3.6 |
| B4 | W100 | 63.5 a | 8.9 | −2.3 |
| | W50 | 58.5 ab | 10.2 | −2.9 |
| Significance | | | | |
| | B | ** | * | *** |
| | W | *** | *** | *** |
| | B × W | * | ns | ns |

Values are means (n = 3). In each column, means followed by different letters are significantly different ($p \leq 0.05$; Tukey's test). *, **, *** indicate F test significant at $p \leq 0.05$, $0.01$, $0.001$, respectively; ns, not significant; B0, B2 and B4 indicate 0%, 2% and 4% treatments of wood chip biochar, respectively; W100 and W50 indicate 100% and 50% treatments of evapotranspiration losses restitution, respectively.

Similarly, the LA reached higher values with the W100 treatment than with the W50 treatment, with the highest value (1102 $cm^2$ $plant^{-1}$) showed by B2-W100, which accounted for a 25% increase compared to B0-W100 and B4-W100.

A significant effect of both the two experimental factors B and W was also observed when considering the fresh weights of the individual plant parts, such as stem, leaves, panicle and subpanicles, as well as the total plant weight (Table 5). More specifically, the B2-treated plants as well as the W100-treated ones always showed the highest fresh weights. With the B2-treated plants, the panicle FW showed the maximum increase, which was equal to 30% compared to the B0- and B4-treated plants. With the W100-treated plants, the maximum increase was observed with the stem FW, which was 172% higher than the W50-treated ones, followed by the panicle FW that accounted for an increase of 168%. In addition, both the leaf and the total FW were also affected by the interaction B × W, showing the highest value with the B2-W100

treatment (27.3 and 46.8 g plant$^{-1}$, respectively) and the lowest with the B0-W50 treatment (8.7 and 14.6 g plant$^{-1}$, respectively).

**Table 4**. Plant height (PH, cm), stem diameter (SD, mm), number of branches (NB, n.) and leaves (NL, n.), leaf area (LA, cm$^2$ plant$^{-1}$) and SPAD index (-) of quinoa grown under three biochar rates and two water regimes at the end of the experiment.

| Experimental Factor | | PH | SD | NB | NL | LA | SPAD Index |
|---|---|---|---|---|---|---|---|
| Biochar (B) | | | | | | | |
| B0 | | 47.7 b | 6.5 b | 20.3 b | 79.0 b | 621.1 b | 41.5 b |
| B2 | | 54.8 a | 6.8 a | 24.3 a | 105.0 a | 791.6 a | 44.6 a |
| B4 | | 50.0 b | 6.2 c | 21.2 b | 83.0 b | 674.6 b | 41.3 b |
| Water regime (W) | | | | | | | |
| W100 | | 60.1 a | 7.3 a | 23.8 a | 110.0 a | 955.4 a | 44.5 a |
| W50 | | 41.6 b | 5.7 b | 20.1 b | 68.0 b | 436.2 b | 40.4 b |
| Biochar × Water regime | | | | | | | |
| B0 | W100 | 57.0 | 7.1 a | 21.7 | 96.7 | 862.5 b | 44.2 |
| | W50 | 38.3 | 5.8 b | 19.0 | 61.3 | 379.7 c | 38.9 |
| B2 | W100 | 65.3 | 7.5 a | 26.7 | 128.7 | 1101.8 a | 46.4 |
| | W50 | 44.3 | 6.0 b | 22.0 | 81.3 | 481.4 c | 42.8 |
| B4 | W100 | 58.0 | 7.2 a | 23.0 | 104.7 | 901.8 b | 42.9 |
| | W50 | 42.0 | 5.1 c | 19.3 | 61.3 | 447.5 c | 39.6 |
| Significance | | | | | | | |
| B | | ** | *** | *** | *** | *** | ** |
| W | | *** | *** | *** | *** | *** | *** |
| B × W | | ns | ** | ns | ns | * | ns |

Values are means ($n = 3$). In each column, means followed by different letters are significantly different ($p \leq 0.05$; Tukey's test). *, **, *** indicate F test significant at $p \leq 0.05$, $0.01$, $0.001$, respectively; ns, not significant; B0, B2 and B4 indicate 0%, 2% and 4% treatments of wood chip biochar, respectively; W100 and W50 indicate 100% and 50% treatments of evapotranspiration losses restitution, respectively.

Also, the WUE was affected by the two factors B and W, as well as by their interaction, by showing 23 and 59% higher values with the B2-treated plants compared to the B4-treated plants and with the W100-treated plants compared to the W50-treated ones, respectively. Moreover, all the W100 treatments (B0-W100, B2-W100 and B4-W100) always showed higher WUE values than the corresponding W50 ones (B0-W50, B2-W50 and B4-W50). In particular, with the B0-W100 and B2-W100 treatments (not differing from each other), a 24% increase in WUE compared to the B4 W100 treatment was observed.

Finally, by considering the yield-contributing traits (Figure 2) detected on the plants at the end of the vegetative growing cycle, both the two factors B and W displayed a significant influence, while their interaction was not significant. Indeed, higher values of both the panicle length and number of sub panicle (Figure 2a and 2c, respectively), were observed with the B2-treated

plants compared to the B0- and B4-treated plants (not different from each other), accounting for 27 and 36% increases, respectively.

**Table 5.** Stem, leaf, panicle, subpanicle and total fresh weight (FW, g plant$^{-1}$) and water use efficiency (WUE, g L$^{-1}$) of quinoa grown under three biochar rates and two water regimes at the end of experiment.

| Experimental Factor | | FW | | | | | WUE |
|---|---|---|---|---|---|---|---|
| | | Stem | Leaves | Panicle | Subpanicles | Total | |
| Biochar (B) | | | | | | | |
| B0 | | 9.8 b | 16.2 b | 0.95 b | 0.71 b | 27.6 b | 6.5 ab |
| B2 | | 11.4 a | 19.5 a | 1.38 a | 0.85 a | 33.2 a | 7.2 a |
| B4 | | 9.0 b | 16.1 b | 0.99 b | 0.72 b | 26.8 b | 5.8 b |
| Water regime (W) | | | | | | | |
| W100 | | 14.8 a | 24.2 a | 1.47 a | 1.10 a | 41.5 a | 7.9 a |
| W50 | | 5.4 b | 10.3 b | 0.74 b | 0.41 b | 16.9 b | 5.0 b |
| Biochar × Water regime | | | | | | | |
| B0 | W100 | 14.7 | 23.6 b | 1.35 | 1.01 | 40.7 b | 8.3 a |
| | W50 | 4.9 | 8.7 c | 0.55 | 0.40 | 14.6 c | 4.6 c |
| B2 | W100 | 16.5 | 27.3 a | 1.81 | 1.22 | 46.8 a | 8.7 a |
| | W50 | 6.4 | 11.8 c | 0.96 | 0.48 | 19.7 c | 5.7 bc |
| B4 | W100 | 13.1 | 21.6 b | 1.27 | 1.06 | 37.1 b | 6.9 b |
| | W50 | 4.9 | 10.5 c | 0.71 | 0.37 | 16.5 c | 4.8 c |
| Significance | | | | | | | |
| B | | ** | *** | *** | *** | *** | ** |
| W | | *** | *** | *** | *** | *** | *** |
| B × W | | ns | * | ns | ns | * | * |

Values are means (n = 3). In each column, means followed by different letters are significantly different ($p \leq 0.05$; Tukey's test). *, **, *** indicate F test significant at $p \leq 0.05$, $0.01$, $0.001$, respectively; ns, not significant; B0, B2 and B4 indicate 0%, 2% and 4% treatments of wood chip biochar, respectively; W100 and W50 indicate 100% and 50% treatments of evapotranspiration losses restitution, respectively.

Similarly, 38 and 68% higher panicle length and number of subpanicles, respectively, were displayed by the W100-treated plants compared to the W50-treated plants (Figure 2b and 2d, respectively).

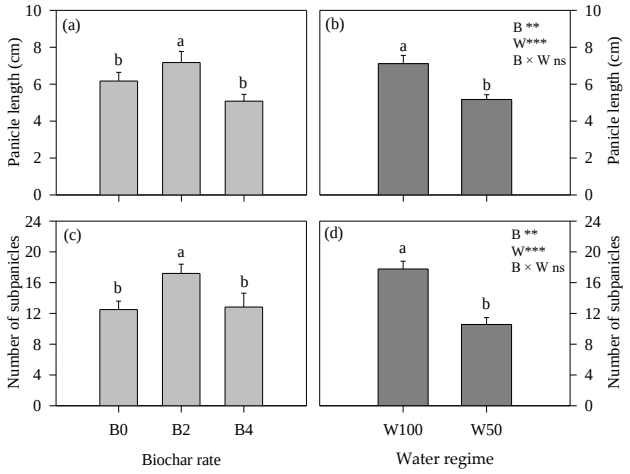


**Figure 2.** Yield-contributing traits, including panicle length and number of subpanicles of quinoa, as affected by biochar rate (a,c) and water regime (b,d) at the end of the experiment. Values are means (n = 3 ± se). In graph, different letters above the bars indicate significant differences among treatments ($p \leq 0.05$; Tukey's test). ** and *** indicate F test significant at $p \leq 0.01$ and $p \leq 0.001$; ns, not significant; B0, B2 and B4 indicate 0%, 2% and

4% treatments of wood chip biochar, respectively; W100 and W50 indicate 100% and 50% treatments of evapotranspiration losses restitution, respectively.

## 4.5. DISCUSSION

The impact of soil amended with biochar on plant growth and productive performance under water shortage is well documented for several crops (Paneque et al., 2015; Romdhane et al., 2019). The current study builds upon the valuable insights from our previous research (Akram et al., 2023) that prompted us to further explore the effects of soil amended with different wood chip biochar rates on the vegetative development of quinoa (cultivar Titicaca) grown under water-limiting conditions throughout the vegetative growing cycle. Previous results have consistently demonstrated that, among different organic amendments applied at a 2% rate, wood chip biochar alone and mixed with vermicompost showed a significant effect on quinoa plant growth during the water stress period that plants faced at the early growing stages, which also allowed a quicker recovery once the drought conditions ended. The leaf area was enhanced by up to 20% and biomass production was enhanced by 19% compared to untreated controls. This led us to state that the use of an organic amendment could play a pivotal role in enabling *Chenopodium quinoa* (cv Titicaca) to better deal with the negative consequences of an early period of severe water stress on plant development.

In the current experiment, Titicaca plants were grown on soil either not amended or amended with 2 or 4% treatments of wood chip biochar and were either well-watered or subjected to water shortage by 100% or 50% restitution of evapotranspiration losses, respectively. Referring to the plant phenology, all the considered stages, except for the emergence and the two-leaf stages, were significantly influenced by both the biochar rate and water regime. In particular, the flowering initiation was delayed, although by a few days, in the plants grown with 4% biochar and 50% evapotranspiration restitution. This might be due to the high phenological plasticity already reported for different genotypes of quinoa (Stanschewski et al., 2021). Maestro-Gaitán et al. (2022) highlighted the genotype effect associated with water limitations

in this crop. The delayed flowering used as a drought-adaptive mechanism, as already observed in the F15 (Maestro-Gaitán et al., 2022) and Santa Maria (Geerts et al., 2008) genotypes that showed an increased time to anthesis and physiological maturity under severe water deficiency (Geerts et al., 2008), might also occur in Titicaca. The delayed flowering phenomena was also reported in maize (Abrecht et al., 1993) and rice (Fukai et al., 1999) when water limitations were applied during the vegetative growing cycle.

Overall, our study highlighted that the application of 2% wood chip biochar, compared to the untreated control and 4% rate, was shown to be a better option in or-der to enhance quinoa growth in terms of plant height, leaf and branch numbers, stem diameter, leaf area and total biomass. These findings were consistent with previous studies indicating increased biomass production with similar biochar applications in quinoa (Kammann et al., 2015; Akram et al., 2023). It is important to note that biochar's impact can vary, with some cases showing non-significant or negative outcomes (Macdonald et al., 2014; Haider et al., 2015; Gavili et al., 2019; Ramlow et al., 2019). Therefore, careful consideration of biochar application rates is crucial for achieving the desired impact (Sandhu et al., 2017). Similarly, Gale and Thomas (2019) reported that the growth and ecophysiological responses of plants to biochar are highly dose-dependent and are mainly driven by nutrient availability, particularly N. In the present study, the 4% rate did not show any positive impact on plant growth, which was in agreement with other studies that have reported detrimental effects at higher biochar doses up to 4% due to reduced nutrient availability (Rajkovich et al., 2012; Lehman et al., 2012; Haider et al., 2015), increased negative charges leading to intensified electrostatic interactions with cations (Rees et al., 2014) on the soil surface and altered soil pH (Jeffery et al., 2011; Rees et al., 2015). A reduction in N availability likely occurred in the present experiment, as shown by the lower SPAD value under the 4% biochar rate, while an increased SPAD value was found under the 2% rate, as also reported by Hafez et al. (2020) in barley. This increase could be attributed to

the large specific surface area of biochar, facilitating enhanced adhesion and cohesion with water, increased soil acidity, improved nutrient availability in the root development zone and a subsequent heightened production of photosynthetic pigments (Hafez et al., 2020). Rees et al. (2015) reported a sharp decrease in nutrient availability at a high rate of woody biochar, which hindered plant growth. Moreover, the biochar liming effect may be beneficial at low rates, while at high rates, over-liming could reduce the availability of nutrients such as P, Mg and Fe (Gale and Thomas, 2019). Woody biochar at a low dose can retain and gradually release ions, specifically $Ca^{2+}$, $Mg^{2+}$ and $K^{+}$, while at high doses, it may "lock up" ions, making them unavailable (Gale et al., 2017). Considering the water regimes, a drastic reduction in growth-related parameters was observed under the 50% evapotranspiration losses restitution treatment throughout the vegetative growing cycle, in agreement with previous findings (Saddiq et al., 2021; Benaffari et al., 2022; Gholami et al., 2022). Moreover, under the water deficit conditions, a lowering of the leaf water potential (Ψ) and relative water content (RWC) were observed, as already reported by Jensen et al. (2000) and Issa Ali et al. (2019) on several quinoa genotypes, including Titicaca, under water stress.

The impact of the biochar rate and water regime on the response of quinoa Titicaca is further exemplified by the significant differences in the yield-contributing traits, such as the panicle length and sub-panicle number that, again, were higher following the 2% biochar rate application, outperforming both the non-treated soil and the 4% rate. These results were consistent with earlier research that demonstrated substantial biomass increases and enhanced grain yields with 2% co-composted woody biochar application (Kammann et al., 2015). Using biochar boosted the soil nutrient availability, increasing the panicle length and grain yield in rapeseed (Khan et al., 2021); however, a large biochar application significantly increased the soil pH and electrical conductivity, inhibiting the nutrient uptake (Gale et al., 2017) and crop growth (Yuan et al., 2011). Under deficit irrigation, a notable yield decrease has been observed

by several authors (Razzaghi et al., 2020; Maestro-Gaitán et al., 2022) for quinoa Titicaca. Telahigue et al. (2017) showed that re-establishing evapotranspiration by 60% and 30% significantly reduced the quinoa yield up to 27% and 74%, respectively. The inability to reach the full potential yield was attributed to sink limitations (Bertero, 2021).

Contrary to the growth response, the plant water status of Titicaca was enhanced by the 4% biochar rate, as shown by the higher Ψ and RWC values, although a lower TW:DW value observed with the 2% treatment, suggesting a better adaptation to water shortage conditions. Considering the growth habits defined by Rojas and Pinto for quinoa (Rojas and Pinto, 2013), Titicaca falls within habit 4, which presents more leaves and a larger leaf area, leading to a higher susceptibility to water losses due to the higher total leaf surface and higher sensitivity to water-limiting conditions. Therefore, the reduction in Ψ and RWC of the 2%-treated plants could be attributed to Titicaca's rapid growth and high leaf expansion rate (Sun et al., 2014), as also reported by Maestro-Gaitán et al. (2022). Regardless of the biochar rate, under the 50% restitution of evapotranspiration losses, the Ψ value decreased, reaching a value of −3.2 MPa compared to the 100% restitution conditions (−2.6 MPa), as previously reported by other authors for Titicaca (Razzaghi et al., 2020; Issa Ali et al., 2019).

In addition, the water use efficiency always exhibited higher values when the plants were fully irrigated, with the B2-treated plants displaying the highest WUE (8.7 g $L^{-1}$) and the B4-treated plants displaying the lowest one (6.9 g $L^{-1}$). Under the water shortage conditions, the rate of biochar did not have any effect on the WUE, with no differences being observed among the treatments. Similar results were found by Kammann et al. (2015), who reported an increase in water use efficiency only with a low biochar rate. Biochar characterized by a higher carbon content or derived from carbon-rich materials, such as wood, may have limited potential in enhancing the WUE when applied in excess, and in some instances, it may even yield negative effects (Gao et al., 2020; El Moussaou et al., 2023).

## 4.6. CONCLUSIONS

The present study investigated the effect of soil amendment with wood chip biochar at different rates on the vegetative development of quinoa cultivar Titicaca subjected to different water regimes. Within the context of the adopted experimental conditions, the findings, on the one hand, showed that the soil application with 2% wood chip biochar was the optimal choice for promoting the healthy growth of quinoa Titicaca; on the other hand, it evidenced the sensitivity of the tested cultivar to water limitation.

These outcomes suggest that further research could involve different quinoa cultivars in order to investigate the genotype-specific response to both the biochar type and rate under drought stress conditions. Such in-depth studies will help to advance our understanding about the relationship between biochar, water availability and the growth of quinoa with the perspective of the sustainable cultivation of the species.

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# CHAPTER 5

# ENHANCING VEGETATIVE GROWTH OF QUINOA AND SOIL PROPERTIES UNDER WATER SHORTAGE THROUGH TARGETED ORGANIC AMENDMENTS

*This chapter is a state-of-the-art chapter, and its content has been presented in an oral presentation of "The 2nd International Online Conference on Agriculture" conducted by MDPI, Switzerland and the proceeding article was published:*

**Akram, M.Z.,** Libutti, A., & Rivelli, A.R. (2024). Enhancing vegetative growth of quinoa and soil properties under water shortage through targeted organic amendments. In *Biology and Life Sciences Forum, 30(1): 4.*

## 5.1. ABSTRACT

The scarcity of water resources is considered a major threat and challenge for agriculture. Water limitations could strongly affect the growth and development of many crops including quinoa, a nutrition-rich, climate-resilient crop that is gaining attention globally. Organic amendment application has been reported as a suitable option to mitigate the detrimental impacts of water shortage on soil and plant growth. In this context, two experiments were conducted on *Chenopodium quinoa* "Titicaca"; in the first experiment, we investigated the effect of different organic amendments, namely woodchips biochar (Bw), vineyard pruning biochar (Bv), and vermicompost (V), applied (alone and mixed) at 2% soil dry weight, on soil properties and the plant biomass of quinoa subjected to a water stress period during vegetative development. Among organic amendments tested, Bw and Bw+V increased plant biomass on average by 15%, while Bv and Bv+V reduced the plant biomass by 62% compared to non-amended soil (C). A significant reduction in soil pH was observed with Bw (7.61), while BV increased pH (8.04) compared to C (7.76). The Bw and Bv also reduced soil bulk density (BD) (1.19 g/cm$^3$

and 1.13 g/cm$^3$, respectively) compared to C (1.28 g/cm$^3$). As Bw performed better in the first experiment, the second experiment assessed only Bw at different doses, i.e., 0%, 2%, and 4% under water shortage by restoring only 50% evapotranspiration losses, when soil water content reached the 50% of available water content. Considering the Bw rates, the plants treated with Bw2% showed 34% and 19% more biomass and 36% and 66% more panicles than Bw0% and Bw4%, respectively. The Bw2% decreased the soil pH (7.79 versus 7.85) and electrical conductivity (286 versus 307 µS/cm) compared to Bw0%, which was not different from Bw4%. No differences were observed in BD between Bw0% and Bw2% (on average 1.28 g/cm$^3$), while BD decreased in Bw4% (1.06 g/cm$^3$). The findings of both experiments highlighted that the appropriate type and dose of biochar could improve soil properties and help quinoa plants to grow better under water-limited conditions.

*Keywords: Chenopodium quinoa, Titicaca genotype, water-shortage, organic amendments, biochar rates, vegetative development, soil properties*

## 5.2. INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) is a resilient seed crop belonging to the Amaranthaceae family; native to the Andean region, nowadays it is cultivated in many parts of the world. Its exceptional nutritional qualities and tolerance against various abiotic stresses are well documented (Akram et al., 2021; Yadav et al., 2023). However, wide differences are reported among the germplasm collections of the species currently available (Hafeez et al., 2022). The Danish genotype Titicaca exhibited varying morphological and physiological responses, and notably revealed its sensitivity to drought (Issa Ali et al., 2019). As already known, drought significantly affects soil's physico-chemical properties, including soil fertility, soil aeration, decomposition of organic matter, and microbial activity (Quintana et al., 2023). Therefore, it is necessary to devise alternative methods for soil management that are environmentally friendly and sustainable, such as organic soil amendments (e.g., compost and biochar). Compost

contains a large amount of labile organic matter, which stimulates soil biota, promotes plant growth by enhancing microbial activity and many soil properties (Chen et al., 2023), mitigating the negative consequences of water limitations (Quintana et al., 2023); the biochar addition improves soil's structure, porosity and water-holding capacity, plant water status, and photosynthetic efficiency (Abideen et al., 2020); in quinoa, biochar enhances vegetative development when facing early periods of severe water stress (Akram et al., 2023). However, biochar's net benefits depend upon the feedstocks, pyrolysis conditions, dose, soil type, and crop (Manikandan et al., 2023). The present study aims to investigate the effects of different organic amendments on selected soil properties and the growth of quinoa plants (Titicaca genotype), followed by testing the best performing amendment at different application doses, when the species is subjected to water limitations.

## 5.3. MATERIAL AND METHODS

### *5.3.1. Description of Treatments and Experimental Designs*

Two experiments were carried out at the University of Basilicata, Potenza (PZ, 40°38′ N–15°48′ E, 819 m a.s.l.) in Southern Italy, during the spring–summer of 2022 on quinoa (*Chenopodium quinoa* Willd.), Titicaca genotype. Sandy loam textured soil (USDA classification) was used in the experiments. The biochar from woodchips (Bw) was purchased from Nera Biochar Company (Settimo Vittone, Italy), the biochar from vineyard prunings (Bv) was produced at the STAR*Facility Centre of Foggia University (South Italy), and the vermicompost from cattle manure (V) was purchased from a company located in Montescaglioso, Matera district (South Italy).

For experiment 1, pots were filled with 5 kg of soil or soil treated with different organic amendments, namely the two types of biochar, Bw and Bv, and vermicompost (V), alone or in mixed form (Bw+V and Bv+V) at a rate of 2% of soil dry weight (dw). Unamended soil was considered as a control (C). Then, soil was brought to field capacity (FC), previously

determined together with the permanent wilting point (PWP) at −0.03 and −1.5 MPa, respectively, for each treatment. The soil available water content (AWC, % dw) was calculated as the difference between FC and PWP. Following, 10 seeds were sown in each pot and thinned to one plant per pot once the emergence was completed. The six experimental treatments were replicated three times, and the 18 experimental units were arranged according to a completely randomized design. From the emergence until the twelve-leaf stage, the plants were kept well irrigated by restoring 100% of evapotranspiration losses at the depletion of 40% of the AWC; then a period of water stress was applied until the soil reached the PWP. At the end of this period, the pots were re-watered to FC and kept well-watered until the end of experiment (i.e., initiation of flowering when plants were cut and parameters were recorded).

Similarly, for experiment 2, the pots were filled with 6 kg of soil treated with three different rates (w/w) of woodchip biochar: 0% (Bw0% as control), 2% (Bw2%), and 4% (Bw4%) of dry soil weight (dw). Each experimental treatment was replicated three times, and the nine experimental units were arranged according to a completely randomized design. From seedling emergence to the beginning of flowering, plants were irrigated by restoring 50% of evapotranspiration losses at the depletion of 50% of the AWC.

At the end of both experiments, soil-related parameters (i.e., pH, electrical conductivity (EC), and bulk density (BD) were measured. Additionally, the total number of panicles (TNP, n°) and total plant fresh weight (TFW, g) were recorded. All the experimental data were processed by one-way analysis of variance (ANOVA) by using “Statistix 8.1” software. When significant differences among means were detected, the latter were compared by Tukey’s honest significance difference post hoc test at the 5% probability level.

## 5.4. RESULTS AND DISCUSSION

### *5.4.1. Soil and plant growth-related parameters of experiment 1*

The changes in soil-related parameters due to the application of different organic amendments at 2% rate are shown in Table 1. The pH variations ranged between 7.6 and 8.1. The woodchip biochar treatment (Bw) resulted in a significant decrease ($p < 0.001$) in soil pH (0.2 unit), while vineyard pruning biochar alone (Bv) and mixed with vermicompost (Bv+V) increased ($p < 0.001$) soil pH (0.3 unit) compared to the control (C). The electrical conductivity (EC) was not found to be significantly affected by treatments. Bulk density (BD) significantly ($p < 0.001$) declined in all treatments compared to C, with greater reduction in Bv+V (18%). The biochar produced from woody material has a larger surface area and lower ash content as compared to the biochar produced from crop residues or grasses (Li et al., 2023). In our case, the higher ash content in Bv (9.9%) is the driver for the higher pH than Bw (4.4%), which could reduce the availability of micronutrients, according to a previous study (Kishimoto et al., 1985). Moreover, the higher porosity of Bv (Azuara et al., 2017) than Bw reduced bulk density and likely increased nutrient retention (Azuara et al., 2017). The significant effect of the organic amendments tested on plant growth under a period of water stress is presented in Table 1. The Bw and Bw+V treated plants produced more ($p < 0.001$) panicles (on average 17) compared to Bv and Bv+V (on average 9.5).

**Table 1.** The pH, electrical conductivity (EC, µS/cm), and bulk density (BD, g/cm$^3$) of soil along with the total number of panicles (TNP) and total fresh weight (TFW, g) of quinoa plants grown under different organic amendments.

| Experimental | Soil-Related Parameters | | | Plant Growth | |
|---|---|---|---|---|---|
| Factors | pH | EC | BD | TNP | TFW |
| C | 7.8 B | 268 | 1.28 A | 13 B | 31.4 B |
| Bw | 7.6 C | 297 | 1.19 B | 18 A | 34.6 A |
| Bv | 8.0 A | 279 | 1.13 BC | 7 C | 18.6 C |
| V | 7.8 B | 291 | 1.20 AB | 15 AB | 31.1 B |
| Bw+V | 7.7 BC | 274 | 1.14 BC | 16 AB | 37.1 A |
| Bv+V | 8.1 A | 302 | 1.05 C | 12 BC | 20.1 C |
| Significance | *** | ns | *** | *** | *** |

Values are means ($n = 3$). In each column, means followed by different letters are significantly different ($p \leq 0.05$; Tukey's test). ***, F test significant at $p \leq 0.001$, respectively. C, control; Bw, woodchip biochar; Bv, vineyard pruning biochar; V, vermicompost.

The Bw and Bw+V treated plants produced more ($p < 0.001$) panicles (on average 17) compared to Bv and Bv+V (on average 9.5). Likewise, the Bw and Bw+V treatments yielded higher ($p < 0.001$) biomass (on average 35.9 g), while the Bv and Bv+V yielded lower biomass (on average 19.3 g). Using biochar boosted the nutritional availability and source-sink relationship under drought, increasing panicle length and grain yield also in rapeseed (Khan et al., 2021), and in quinoa, as reported by Kammann et al. (2015) under similar biochar application conditions. Probably, the higher porosity of Bv (Azuara et al., 2017) than Bw, although reducing the bulk density, increased nutrient retention, especially N (Rees et al., 2015), lowering their availability for plant growth.

### *5.4.2. Soil and plant growth-related parameters of experiment 2*

Applying different woodchip biochar rates significantly affected the soil properties under water shortage conditions (Table 2). More specifically, the addition of 2% biochar (Bw2%) decreased ($p < 0.001$) pH (7.79) more than the 4% rate (Bw4%) (7.82) as compared to the non-amended soil (Bw0%) (7.9). The EC was also reduced ($p < 0.05$) in Bw2% treated soil (286 µS/cm), while it increased in Bw4% (313 µS/cm) compared to Bw0% (307 µS/cm). Both Bw4% and Bw2% treatments reduced bulk density (BD) by 20% and 8%, respectively, compared to Bw0%. Several studies have previously reported changes (increase or decrease) in pH level resulting from the application of different biochar rates (Palansooriya et al., 2019). Consistent with our study, a decrease in soil pH after biochar addition was observed by Qiang et al. (2020). Soil pH variation after biochar application influences the microbial activity and enzyme production in addition to carbon and nutrient availability (Stark et al., 2014). The higher biochar application rate may increase soil salinity and EC, subsequently leading to undesirable impacts on plant growth (Tag et al., 2016). Moreover, biochar addition, by lowering soil bulk density, increases the total soil porosity, thereby positively impacting root development (Chang et al., 2021).

**Table 2.** The pH, electrical conductivity (EC, µS/cm), and bulk density (BD, g/cm$^3$) of soil along with total number of panicles (TNP) and total fresh weight (TFW, g) of quinoa plants grown under different rates of woodchips biochar.

| Experimental | Soil-Related Parameters | | | Plant Growth | |
|---|---|---|---|---|---|
| Factors | pH | EC | BD | TNP | TFW |
| Bw0% | 7.85 A | 307 A | 1.32 A | 11 B | 14.6 B |
| Bw2% | 7.79 B | 286 B | 1.23 A | 15 A | 19.7 A |
| Bw4% | 7.82 A | 313 A | 1.06 B | 9 B | 16.5 B |
| Significance | *** | * | ** | * | * |

Values are means (n = 3). In each column, means followed by different letters are significantly different ($p \leq 0.05$; Tukey's test). *, **, and ***, F test significant at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively. Bw0%, no biochar; Bw2%, 2% biochar; Bw4%, 4% biochar.

Significant variations of plant growth were observed among the different biochar rates under water shortage conditions (Table 2). In particular, the Bw2%-treated plants showed more ($p < 0.05$) panicles (15) compared to plants receiving Bw0% and Bw4% treatments (on average 10). Similarly, the Bw2% plants produced higher ($p < 0.05$) biomass (19.5 g) than Bw0% and Bw4% (on average 15.6 g). The significant enhancement of biomass and yield was reported in quinoa under water limited conditions by Kammann et al. (2015). However, some non-significant or negative impacts of biochar have also been reported (Haider et al., 2015; Ramlow et al., 2019), leading to the need to further consider appropriate biochar type and dose for desired responses. In the present study, Bw4% negatively affected plant growth, as reported by several studies due to detrimental effects including reduced soil nutrient availability under altered pH (Rees et al., 2015; Jeffery et al., 2011). The higher biochar rate increased negative charges on the soil surface, leading to intensified electrostatic interactions with cations (Rees et al., 2014), as also reported by Rees et al. (2015) under woody biochar application, resulting in detrimental effects on plant growth and yield due to reduced nutrient availability (Wang et al., 2016).

## 5.5. CONCLUSION

Among the organic amendments tested, woodchip biochar application, alone or mixed with vermicompost, was shown to be the better option to mitigate short-term water stress conditions arising during the vegetative growing cycle of quinoa, Titicaca genotype. Considering the different woodchip biochar rates, 2% application was determined to result in a greater growth

response of Titicaca under water limitations compared to 4% biochar, probably due to more favourable soil conditions. Both experiments pointed out that the proper type and dose of biochar could help quinoa plants to grow better and cope with water-limited conditions. Further investigations should be extended to the evaluation of different quinoa genotypes in order to better understand the specific response to biochar application in controlled and open field conditions.

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# CHAPTER 6

# BIOCHAR ADDITION PROMOTED GROWTH OF FIVE QUINOA VARIETIES UNDER WATER STRESS THROUGH IMPROVED PHYSIOLOGICAL ATTRIBUTES, PLANT WATER STATUS AND WATER USE EFFICIENCY

*Draft in preparation for Journal of Soil Science and Plant Nutrition but some of its contents was presented at the 53 National Congress of the Italian Society for Agronomy (Poster presentation)*

## 6.1. ABSTRACT

Among quinoa varieties cultivated specifically in Europe, Danish quinoa variety Titicaca is vulnerable to water scarcity during its early growth stages. However, adding 2% woodchip biochar has been found to improve plant water status and promote growth of Titicaca. Despite these findings, no comparative study has examined the responses of diverse quinoa varieties to biochar amendment under water-limited conditions. Thus, the current investigation aimed to study the interactive effects of biochar addition (i.e., B0: no biochar and B2: 2% woodchip biochar) under different watering conditions (well-watered: by restoring 100% ET and water stressed: with-held watering until soil in pots reaches PWP), applied during the vegetative growing cycle of five quinoa varieties from different origins. The results demonstrated that biochar significantly improved plant growth, water use efficiency (WUE), and yield-related traits across all varieties, particularly under water-stressed conditions. More specifically, biochar application enhanced plant water status, as indicated by lower pre-dawn water potential values (-1.52 vs -1.62 MPa) and increased relative water content (65 vs 62 %) and SPAD index (45 vs 41) than non-amended soil. Among different varieties tested, the Pakistani UAFQ7 showed superior growth with biochar, with a higher leaf number (179 vs 159) and area (1517 vs 1378 $cm^2$/plant). In contrast, the Danish variety Titicaca exhibited a greater sensitivity to

water stress, with a more pronounced reduction in leaf area (1268 vs 1386 cm²/plant), fresh (47 vs 55 g), and dry biomass (6.4 vs 7.4 g) compared to well-watered conditions. Notably, UAFQ7 didn't show a significant difference in leaf area between well-watered and water-stressed conditions (1598 vs 1562 $cm^2$/plant), indicating its ability to recover rapidly from water scarcity. The Italian variety Quipu produced longer panicles, further enhanced by biochar (17 vs 12 cm). A significant increase in WUE was observed in biochar-treated plants (16.4 g/L) compared to non-amended ones (14.1 g/L). The principal component analysis confirmed that biochar positively influenced plant water status and growth of the varieties tested. Correlation analysis further demonstrated a positive association between growth parameters and WUE. These findings suggest that biochar amendment can mitigate the adverse effects of water stress in quinoa, with varying responses among different varieties.

*Keywords: Chenopodium quinoa Willd.; morpho-physiological attributes; vegetative growing cycle; woodchip biochar rates*

## 6.2. 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 exhibited higher tolerance against abiotic stresses, 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, 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 under 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 in regions facing water scarcity. Although this specie exhibits drought resistance, its physiological processes can be compromised by water deficit, leading to significant reduction in 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). Recently, the European Union has 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 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). 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 improved soil structure (Morales-Salmerón et al. 2024).

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 stage, 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 early growth stage, 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 growing cycle on five quinoa varieties grown in biochar-amended soil by assessing morpho-physiological attributes, leaf nutrients and soil microbial communities.

## 6.3. MATERIALS AND METHODS

### *6.3.1. Experimental layout*

A pot experiment was conducted on different quinoa varieties during spring-summer (April-June 2023) at 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, while temperature maintained at 26/18°C day/night. Five quinoa varieties from diverse origins were selected for this study (Table 1). Among them, three varieties Titicaca, Quipu, and UAFQ7, are commercial varieties in Denmark, Italy, and Pakistan, respectively, while the other two, Regalona and Q126, are Chilean varieties and currently under trialed in Italy and Pakistan, respectively.

**Table 1:** Varieties used in the experiment

| Varieties | Codes |
|---|---|
| Titicaca | V1 |
| Quipu | V2 |
| Regalona | V3 |
| UAFQ7 | V4 |
| Q126 | V5 |

The five quinoa varieties (V1-V5) were cultivated without biochar served as a control (B0) and in soil amended with 2% (w/w) woodchip biochar (B2) rate based on the dry weight of the soil. The biochar was produced from woodchips and purchased from Nerabiochar Company (Ivrea, Torino, Italy). Based on biochar properties (C > 50%, Corg > 60%, H/Corg ≤ 0.7, O/Corg ≤ 0.4), that can be found in our previous study (Akram et al. 2023), it is certified as Class 1 biochar, according to the European Biochar Certificate (EBC) (EBC 2012) and the International Biochar Initiative (IBI) standards (IBI 2015).

Ten quinoa seeds were sown in 7.5 L plastic pots filled with 5 kg of either control or biochar-amended soil. At the 4-leaf stage, thinning was performed, and a single plant per pot was maintained. Then, a 3 cm layer of polythene beads was applied to the soil surface to minimize evaporation. Plants were maintained in well-watered (WW: by restoring 100% ET) conditions from emergence to the twelve-leaf stage ($t_1$). At this stage ($t_1$), half of the pots were kept under well-watered conditions, while others were subjected to two successive water stress cycles (WS: with-held watering until soil reaches the PWP) one after other. Different growth-related parameters were recorded at the start and end of each water stress cycle ($t_1$, $t_2$ and $t_3$, respectively). Following the water stress period, plants were rewatered to recover from water stress ($t_4$) and were well-watered until flowering initiation, when all treatments were cut and different parameters were recorded. The experiment was conducted under a factorial, completely randomized experimental design (three-way) with three replications for a total of 60 experimental units, considering variety (V), biochar rate (B), and watering conditions (W) as experimental factors.

### *6.3.2. Plant Measurements and analysis*

At $t_1$, $t_2$ and $t_3$, different growth-related parameters, including plant height (cm) and number of leaves ($n^0$) and branches ($n^0$), were recorded. Additionally, the length and width of each leaf were recorded and used for calculating leaf area ($cm^{2/}$plant) by using equation established by Talebnejad and Sepaskhah (2015) and validated by Akram et al. (2023): LA= 0.64 (L × W). Leaf greenness was simultaneously assessed by using a handheld SPAD-502 meter (Konica-Minolta Corporation, Ltd., Osaka, Japan), by recording three SPAD readings on the youngest fully expanded leaves per plant. At harvest, i.e., at flowering initiation stage, the aforementioned growth parameters were measured alongside the aboveground plant's fresh weight (TFW, g). Additionally, yield-contributing traits, including main panicle length (PL, cm) and the number of sub-panicles per plant ($n^0$) were recorded.

Total plant water consumption (TWC, L) throughout the experiment was determined by cumulating the amount of water applied at each watering. Water use efficiency (WUE, g $L^{-1}$) was then calculated as a ratio between the total dry biomass and the total water consumed by plant. Leaf relative water content (RWC, %) was assessed at $t_3$ and $t_4$. For that purpose, a small section of the youngest fully expanded leaf per plant was collected and immediately placed in an ice bucket and fresh weight (FW, g) was recorded. Then the leaf segment was submerged in distilled water to determine turgid weight (TW, g). The leaf tissue was then dried in a ventilated oven at 70°C until constant weight was achieved (DW, g). Then RWC was calculated by the equation RWC= (FW-DW/TW-DW) × 100

In addition, the ratio of turgid weight (TW) to dry weight (DW) was also calculated. Furthermore, the leaf's pre-dawn total water potential ($\Psi_{pd}$, MPa) was recorded at $t_3$ and $t_4$. For that purpose, plants were kept in the dark chamber for overnight, and measurements of leaf $\Psi_{pd}$ were made on the youngest fully expanded leaf per plant at 7:30 am using a Scholander pressure chamber (PMS model 1000, Corvallis, OR, USA).

The total bacteria count (TCB) in soil samples from control (B0) or biochar-treated pots (B2) was calculated as colony form units per milliliter (CFU/mL) at $t_1$, $t_2$ and $t_3$. For that purpose, the soil was collected, at $t_1$ from all pots and at $t_2$ and $t_3$ from water-stressed pots only, from both B0 and B2 treatments. The samples were processed as per the standard procedure (Olsen and Bakken 1987). The first phase concerned the preparation of the soil sample suspension. The utilized nutrient media Plate Counting Agar (PCA) was prepared. Regarding PCA media, the (g/L) tryptone 5, yeast extracts 2.5, glucose 1.0, and bacteriological agar 12.0 were mixed in distilled water at room temperature with the help of a magnetic mixer, and the pH was adjusted to 7.0±0.2. The prepared nutrient media was then autoclaved at 121°C/ 20 min.

One gram of each soil sample was homogenized, and 1 mL from each suspension was dissolved in 9 mL of distilled sterilized $H_2O$ (original suspension). The first suspension was vortexed for

30 seconds then three decimal dilutions of this solution were prepared by progressively pipetting 1 mL of the original suspension in 9 ml of water; four progressive decimal dilutions were prepared ($10^{-2}$, $10^{-3}$, $10^{-4}$ and $10^{-5}$). For TCB, 1 mL of the two highest decimal dilutions ($10^{-4}$ and $10^{-5}$) was pipetted into Petri dishes (Ø = 90 mm), and then PCA media was poured into petri dishes at 45°C. All plates of PCA were incubated at 30±2°C for 24-48 hours under aerobic conditions. After incubation, the colonies formed by bacteria were counted with the help of a stereomicroscope and expressed as colony-forming units (CFU) multiplied by the dilution factor to get the total number of colonies forming units per mL of each gram of soil.

At the end of the experiment, dried leaf samples were grounded to a homogenous 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) content was measured as a percentage of dry weight (% DW), according to the manufacturer's instructions (Flash 2000, Thermo Fisher Scientific Cambridge UK). It utilized dynamic flash combustion analysis, commonly called the modified Dumas method (Dumas, 1831).

### *6.3.3. Statistical Analysis*

All the experimental data were checked for normality and homogeneity of variance, then processed by two- and three-way analysis of variance (ANOVA), following 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 (PCA) analysis 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 RStudio software (Team 2013).

## 6.4. RESULTS

### *6.4.1. Water relation attributes*

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), were significantly influenced by experimental factors across the water stress period (Table 2). While initial differences among varieties were observed (data not shown) at $t_1$, the most pronounced effects emerged at $t_3$ and $t_4$. At $t_3$, Ψ and RWC were markedly affected by all experimental factors and interactions ($p \leq 0.001$). Importantly, biochar application improved plat water status, as evidenced by less negative Ψ and higher RWC values than control plants (Figure 1). The varieties V4 and V5 notably appeared to be most affected by water stress, with substantial reductions in RWC by 65% and 59%, respectively, compared to well-watered conditions. TW:DW was influenced by variety (V) ($p \leq 0.001$), biochar (B) ($p \leq 0.001$), and watering conditions (W) ($p \leq 0.001$), aligning with Ψ and RWC trends. The same varieties had significantly lower TW:DW ratio in biochar-amended soil than in non-treated soil.

**Table 2:** Water relations i.e., leaf pre-dawn water potential (Ψ), relative water content (RWC) and TW:DW ratio of different quinoa varieties grown without and with biochar under well-watered and water stress conditions.

| Experimental Factors | End of water stress period ($t_3$) | | | Recovery from 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 |
| **V × B** | | | | | | |
| 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 eg |
| 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 |
| **V × W** | | | | | | |
| 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 |
| **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 | *** |

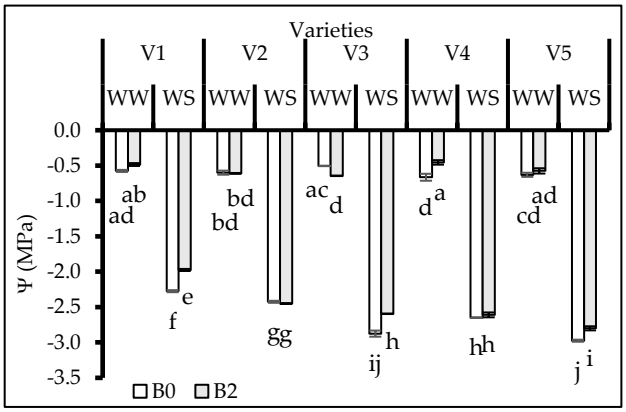


**Figure 1:** Leaf's pre-dawn water potential (Y) of different quinoa varieties grown without and with biochar under well-watered (WW) and water-stressed (WS) conditions recorded at end of water stress period ($t_3$). Values are means ($n = 3$) ± S.E. In each graph, different letters indicate significant 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.

### *6.4.2. Growth-related attributes during the experiment*

Plant growth attributes, including plant height (PH), leaf number (NL), branch number (NB), and leaf area (LA), were assessed at three time points, i.e., $t_1$, $t_2$, and $t_3$. Significant main effects were observed for variety (V) ($p \leq 0.001$) and biochar application (B) ($p \leq 0.001$) at all time points, while the watering regime (W) significantly influenced growth at $t_2$ and $t_3$ ($p \leq 0.001$). Additionally, non-significant interaction among all three factors V × B × W was observed (Table 3). V4 exhibited greater PH when grown with biochar at $t_1$, while V2 plants became taller with biochar and even more taller under well-watered conditions at $t_2$ and $t_3$.

Similarly, NL was significantly influenced by V ($p \leq 0.001$) and B ($p \leq 0.001$) at $t_1$, with no interactive effects detected. At $t_2$ and $t_3$, NL was affected by V, B, and W ($t_2$, $t_3$: $p \leq 0.001$), as well as the interaction of V with both B ($t_2$: $p \leq 0.001$, $t_3$: $p \leq 0.05$) and W ($t_2$: $p \leq 0.001$, $t_3$: $p \leq 0.001$) but not by V × B × W (Table 4). All varieties treated with biochar consistently exhibited higher NL, with an additional positive effect of watering. Consistent with the other growth parameters, NB and LA were significantly influenced simultaneously (Table 3 and 4).

For both parameters a significant variation was observed among V (NB: $t_1$, $t_2$, $t_3$: $p \leq 0.001$; LA: $t_1$, $t_2$, $t_3$: $p \leq 0.001$), B (NB: $t_1$, $t_2$, $t_3$: $p \leq 0.001$; LA $t_1$, $t_3$: $p \leq 0.001$, $t_2$: $p \leq 0.05$) and W (NB: $t_1$: $p \leq 0.05$, $t_2$, $t_3$: $p \leq 0.001$; LA $t_1$: $p \geq 0.05$, $t_2$,$t_3$: $p \leq 0.001$) as well as two-factor interaction V × B (NB: $t_1$, $t_2$, $t_3$: $p \leq 0.05$; LA $t_1$, $t_2$, $t_3$: $p \leq 0.001$) and V × W (NB: $t_1$, $t_3$: $p \leq 0.01$, $t_2$: $p \leq 0.05$; LA $t_1$ : $p \leq 0.01$, $t_2$, $t_3$: $p \leq 0.001$). In line with the other growth traits, V4 plants grown with biochar produced more branches and exhibited a bigger leaf area that similarly to plants grown under WW conditions.

**Table 3:** Plant height (cm) and leaf area ($cm^2$/plant) of different quinoa varieties grown without and with biochar during the experiment.

| Experimental Factors | start of water stress ($t_1$) | | end of first water stress cycle ($t_2$) | | end of second water stress cycle ($t_3$) | |
|---|---|---|---|---|---|---|
| | PH (cm) | LA ($cm^2$) | PH (cm) | LA ($cm^2$) | PH (cm) | LA ($cm^2$) |
| **Variety (V)** | | | | | | |
| V1 | 17.0 d | 430 d | 29.8 d | 689 d | 39.1 c | 993 c |
| V2 | 24.5 b | 623 b | 40.2 a | 968 b | 54.3 a | 1221 b |
| V3 | 22.2 c | 590 c | 35.9 c | 737 d | 48.3 b | 1177 b |
| V4 | 27.0 a | 684 a | 36.1 c | 1052 a | 49.0 b | 1305 a |
| V5 | 24.1 b | 588 c | 37.8 b | 870 c | 53.0 a | 1157 b |
| **Biochar (B)** | | | | | | |
| B0 | 22.0 b | 570 b | 34.3 b | 852 b | 46.4 b | 1058 b |
| B2 | 23.9 a | 595 a | 37.6 a | 875 a | 51.0 a | 1283 a |
| **Watering (W)** | | | | | | |
| WW | 22.9 | 585 | 39.6 a | 893 a | 52.7 a | 1208 a |
| WS | 23.0 | 581 | 32.3 b | 833 b | 44.7 b | 1133 b |
| **V × B** | | | | | | |
| V1-B0 | 17.7 d | 431 e | 28.0 f | 686 d | 35.8 g | 927 g |
| V1-B2 | 16.3 d | 429 e | 31.5 e | 692 d | 42.3 f | 1059 ef |
| V2-B0 | 24.5 bc | 617 bc | 39.8 ab | 1020 a | 52.5 ac | 1113 de |
| V2-B2 | 24.5 bc | 628 bc | 40.5 a | 916 b | 56.0 a | 1330 ab |
| V3-B0 | 21.5 c | 595 cd | 33.8 d | 709 d | 48.7 ce | 998 fg |
| V3-B2 | 22.8 bc | 584 cd | 38.0 b | 766 cd | 47.8 de | 1356 a |
| V4-B0 | 23.7 bc | 660 ab | 33.7 d | 1019 a | 44.7 ef | 1179 cd |
| V4-B2 | 30.3 a | 708 a | 38.5 b | 1085 a | 53.3 ab | 1430 a |
| V5-B0 | 22.7 bc | 549 d | 36.0 c | 825 c | 50.3 bd | 1074 df |
| V5-B2 | 25.5 b | 626 bc | 39.7 ab | 916 b | 55.7 a | 1241 bc |
| **V × W** | | | | | | |
| V1-WW | 16.7 | 428 e | 32.8 ef | 707 d | 42.8 e | 1006 e |
| V1-WS | 17.3 | 432 e | 26.7 g | 671 d | 35.3 f | 980 e |
| V2-WW | 24.3 | 636 bc | 43.8 a | 1049 ab | 58.3 a | 1361 a |
| V2-WS | 24.7 | 609 cd | 36.5 d | 887 c | 50.2 cd | 1082 de |
| V3-WW | 22.7 | 583 d | 40.3 bc | 737 d | 55.0 ab | 1124 cd |
| V3-WS | 21.7 | 596 cd | 31.5 f | 737 d | 41.5 e | 1230 bc |
| V4-WW | 26.3 | 706 a | 39.5 c | 1113 a | 52.2 bc | 1369 a |
| V4-WS | 27.7 | 662 ab | 32.7 ef | 991 b | 45.8 de | 1240 b |
| V5-WW | 24.3 | 571 d | 41.7 bc | 860 c | 57.8 a | 1182 bd |
| V5-WS | 23.8 | 605 cd | 34.0 e | 881 c | 48.2 cd | 1133 cd |
| **Level of Significance** | | | | | | |
| V | *** | *** | *** | *** | *** | *** |
| B | *** | *** | *** | * | *** | *** |
| W | ns | ns | *** | *** | *** | *** |
| V × B | *** | *** | *** | *** | *** | *** |
| V × W | ns | ** | * | *** | *** | *** |
| B × W | ns | ns | ns | * | ns | * |
| V × B × W | 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

Additionally, the SPAD index (Table 5) was also significantly influenced by V ($t_1$, $t_2$, $t_3$: p ≤ 0.001), B ($t_1$: $p \leq 0.05$, $t_2$, $t_3$: $p \leq 0.001$) and W ($t_2$, $t_3$: $p \leq 0.001$). Furthermore, the two-factor interaction V × B was found significant at $t_2$ and $t_3$ ($t_2$: $p \leq 0.001$, $t_3$: $p \leq 0.01$) and the three-way interaction V × B × W at $t_1$, $t_2$ and $t_3$ ($t_1$: $p \leq 0.05$, $t_2$: $p \leq 0.01$, $t_3$: $p \leq 0.001$).

**Table 4:** Leaf and branches number of different quinoa varieties grown without and with biochar during the experiment.

| Experimental Factors | start of water stress ($t_1$) | | end of first water stress cycle ($t_2$) | | end of second water stress cycle ($t_3$) | |
|---|---|---|---|---|---|---|
| | NL ($n^0$) | NB ($n^0$) | NL ($n^0$) | NB ($n^0$) | NL ($n^0$) | NB ($n^0$) |
| **Variety (V)** | | | | | | |
| V1 | 32 d | 9 d | 76 d | 14 d | 105 d | 19 d |
| V2 | 65 b | 14 b | 106 b | 19 ab | 132 c | 22 bc |
| V3 | 59 c | 13 bc | 76 d | 18 c | 107 d | 22 c |
| V4 | 76 a | 15 a | 122 a | 19 a | 156 a | 25 a |
| V5 | 58 c | 12 c | 103 c | 18 bc | 136 b | 23 b |
| **Biochar (B)** | | | | | | |
| B0 | 56 b | 12 b | 90 b | 16 b | 120 b | 21 b |
| B2 | 60 a | 13 a | 102 a | 20 a | 134 a | 24 a |
| **Watering (W)** | | | | | | |
| WW | 59 | 13 a | 101 a | 19 a | 135 a | 24 a |
| WS | 57 | 12 b | 92 b | 17 b | 119 b | 21 b |
| **V × B** | | | | | | |
| V1-B0 | 32 | 9 e | 70 f | 13 d | 96 g | 17 g |
| V1-B2 | 32 | 9 e | 81 e | 16 c | 113 e | 20 f |
| V2-B0 | 63 | 13 bd | 100 c | 17 c | 126 d | 21 f |
| V2-B2 | 68 | 15 ab | 112 b | 21 ab | 138 c | 24 bc |
| V3-B0 | 56 | 13 cd | 73 f | 16 c | 102 f | 21 f |
| V3-B2 | 62 | 13 cd | 80 e | 20 b | 111 e | 23 cd |
| V4-B0 | 74 | 13 bc | 115 b | 17 c | 147 b | 23 de |
| V4-B2 | 77 | 16 a | 128 a | 22 a | 164 a | 27 a |
| V5-B0 | 55 | 12 d | 94 d | 16 c | 129 d | 21 ef |
| V5-B2 | 60 | 13 cd | 112 b | 20 b | 143 bc | 25 b |
| **V × W** | | | | | | |
| V1-WW | 32 | 9 d | 80 e | 15 d | 112 d | 20 e |
| V1-WS | 32 | 9 d | 72 f | 13 e | 97 f | 17 f |
| V2-WW | 67 | 14 ab | 116 b | 19 ab | 148 b | 24 b |
| V2-WS | 64 | 14 bc | 96 d | 18 bc | 116 d | 21 de |
| V3-WW | 59 | 14 bc | 79 e | 18 bc | 110 d | 23 c |
| V3-WS | 58 | 13 bc | 73 f | 17 c | 103 e | 21 d |
| V4-WW | 78 | 15 a | 124 a | 21 a | 158 a | 26 a |
| V4-WS | 73 | 14 b | 119 ab | 18 c | 153 ab | 23 c |
| V5-WW | 57 | 12 c | 106 c | 19 ab | 147 b | 24 b |
| V5-WS | 58 | 13 bc | 99 d | 17 c | 125 c | 22 cd |
| **Level of Significance** | | | | | | |
| V | *** | *** | *** | *** | *** | *** |
| B | *** | *** | *** | *** | *** | *** |
| W | ns | * | *** | *** | *** | *** |
| V × B | ns | * | *** | * | * | * |
| V × W | ns | ** | *** | * | *** | ** |
| B × W | ns | ns | ns | ns | ns | ns |
| V × B × W | 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.

All varieties treated with biochar revealed higher values of the considered parameters at all time points, but a significant reduction was observed when WS conditions were applied at $t_2$ and $t_3$.

**Table 5:** SPAD Index (-) of different quinoa varieties grown without and with biochar during the experiment.

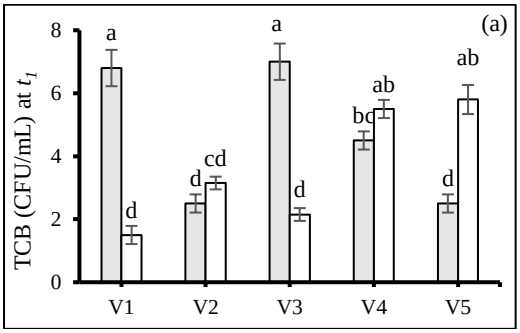
| Experimental Factors | start of | end of first water | end of second water |
|---|---|---|---|

| | water stress ($t_1$) | stress cycle ($t_2$) | stress cycle ($t_3$) |
|---|---|---|---|
| **Variety (V)** | | | |
| V1 | 42.2 b | 44.1 c | 45.3 a |
| V2 | 49.4 a | 45.2 ab | 44.9 ab |
| V3 | 42.1 b | 41.7 d | 41.8 c |
| V4 | 48.8 a | 44.9 bc | 43.8 b |
| V5 | 44.9 b | 45.8 a | 45.8 a |
| **Biochar (B)** | | | |
| B0 | 46.6 a | 43.8 b | 43.3 b |
| B2 | 44.3 b | 44.9 a | 45.3 a |
| **Watering (W)** | | | |
| WW | 45.1 | 45.0 a | 45.1 a |
| WS | 45.8 | 43.7 b | 43.5 b |
| **V × B** | | | |
| V1-B0 | 43.6 | 43.4 cd | 43.9 bc |
| V1-B2 | 40.8 | 44.8 bc | 46.6 a |
| V2-B0 | 52.0 | 44.0 c | 43.5 bc |
| V2-B2 | 46.8 | 46.4 a | 46.3 a |
| V3-B0 | 43.0 | 41.9 de | 40.7 d |
| V3-B2 | 41.1 | 41.5 e | 43.0 c |
| V4-B0 | 48.7 | 43.5 c | 42.6 c |
| V4-B2 | 48.9 | 46.3 a | 44.9 ab |
| V5-B0 | 45.6 | 46.1 ab | 45.8 a |
| V5-B2 | 44.2 | 45.5 ab | 45.9 a |
| **V × W** | | | |
| V1-WW | 42.5 bd | 44.5 bc | 46.5 a |
| V1-WS | 41.9 cd | 43.6 cd | 44.0 cd |
| V2-WW | 50.0 a | 46.8 a | 46.2 a |
| V2-WS | 48.8 ab | 43.7 cd | 43.5 d |
| V3-WW | 42.6 bd | 42.3 de | 43.5 d |
| V3-WS | 41.5 d | 41.1 e | 40.2 e |
| V4-WW | 49.1 a | 45.5 ab | 43.2 d |
| V4-WS | 48.4 ab | 44.4 bc | 44.4 bd |
| V5-WW | 41.6 d | 46.1 a | 45.9 ab |
| V5-WS | 48.2 ac | 45.5 ab | 45.7 ac |
| **Level of Significance** | | | |
| V | *** | *** | *** |
| B | * | *** | *** |
| W | ns | *** | *** |
| V × B | ns | *** | ** |
| V × W | * | ** | *** |
| B × W | ns | ** | ** |
| V × B × W | 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.*

### *6.4.3. Total Bacterial Community Count*

At $t_1$ (sampling all pots), $t_2$ and $t_3$ (sampling from water-stressed treatments), total bacterial community count (TBC) was affected by variety ($p \leq 0.001$), biochar ($p \leq 0.001$) and their interaction ($p \leq 0.001$) (Figure 3). At $t_1$, except for the variety V1 and V3, biochar-treated soils have more bacterial communities than non-amended soil. Contrary, when the same counting was done at $t_2$ and $t_3$ under water-stressed conditions, V4 and V5 grown in B2 treatment recorded a marked decrease of bacterial communities than non-treated soil (B0). Interestingly, at $t_2$, V1 grown on biochar-amended soil showed a remarkable increase in bacterial community count compared to B0 (220 vs 11.5) (the analysis was repeated three times for results confirmation).



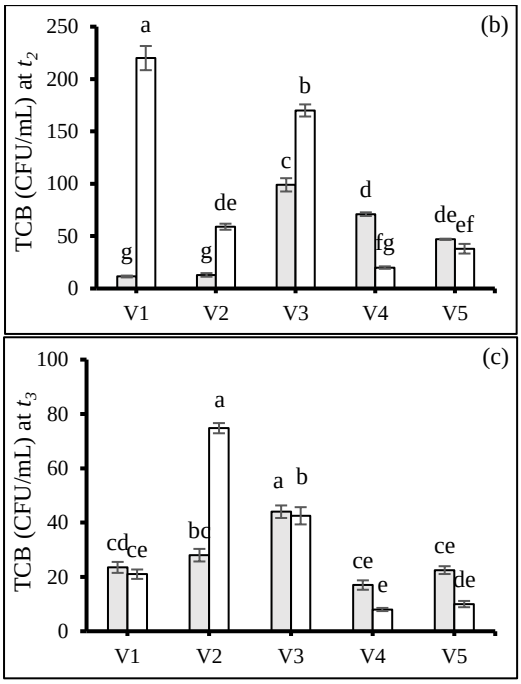



**Figure 2:** Total bacterial community count (TCB) at $t_1$ (**a**), $t_2$ (**b**) and $t_3$ (**c**) in soil without and with biochar. Values are means ($n = 3$) ± S.E. In each graph, different letters indicate significant differences among treatments ($p \leq 0.05$, Tukey's test). $t_1$— start of water 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.

### *6.4.4. Growth and yield related parameters at the end of the experiment*

Plant height was significantly affected by V, B and W, as well as by two-way interactions V × B and V × W ($p \leq 0.001$) (Table 6). The results displayed that V4 and V5 grown on biochar amended soil reached higher PH; while, all the considered varieties when subjected to well-watered conditions showed a significant PH enhancement. Similarly, the leaf and branches number showed the same trend of significance among experimental factors ($p \leq 0.001$) (Table 6). Interestingly, V4 generally showed the highest leaf and branches numbers and similarly when grown under WW. Furthermore, leaf area showed significant variation among V, B, and W ($p \leq 0.001$) and their two-factor interaction V × B ($p \leq 0.001$) and V × W ($p \leq 0.01$) (Table 6). Notably, V4 under both well-watered and water stressed conditions exhibited higher leaf area than the other varieties, showing higher recovery ability from water stressed conditions by more leaf expansion.

Total fresh weight (FW) and dry weight (DW) exhibited patterns similar to plant growth attributes, being influenced by V, B, W, and their interactions ($p \leq 0.001$) (Figure 3, Table 7). Biochar application enhanced both FW and DW, with V4 showing higher dry biomass accumulation, especially under well-watered conditions. Water use efficiency (WUE) affected by the main factors and their interactions (except for V × B × W), was generally higher in V4, in biochar-treated plants, and under water stress conditions. Except for V5, WUE was always higher in all water-stressed plants than well-watered ones (Table 7).

**Table 6:** Plant height (PH), leaf (NL) and branches (NB) number and leaf area (LA) of different quinoa varieties grown without and with biochar.

| Experimental Factors | PH (cm) | NL ($n^0$) | NB ($n^0$) | LA ($cm^2$) |
|---|---|---|---|---|
| **Variety (V)** | | | | |
| V1 | 64.9 b | 144 c | 25.9 c | 1327 d |
| V2 | 75.0 a | 174 b | 29.3 b | 1481 b |
| V3 | 74.7 a | 142 c | 26.5 c | 1453 b |
| V4 | 67.8 b | 214 a | 32.7 a | 1580 a |
| V5 | 68.0 b | 172 b | 28.3 b | 1397 c |
| **Biochar (B)** | | | | |
| B0 | 68.4 b | 159 b | 27.5 b | 1378 b |
| B2 | 71.7 a | 179 a | 29.5 a | 1517 a |
| **Watering (W)** | | | | |
| WW | 75.8 a | 177 a | 29.8 a | 1505 a |
| WS | 64.3 b | 162 b | 27.2 b | 1390 b |
| **V × B** | | | | |
| V1-B0 | 64.2 c | 137 f | 25.5 ef | 1243 e |
| V1-B2 | 65.7 bc | 152 e | 26.3 df | 1410 cd |
| V2-B0 | 74.8 a | 166 d | 27.3 ce | 1391 cd |
| V2-B2 | 75.2 a | 183 c | 31.2 ab | 1571 b |
| V3-B0 | 75.2 a | 133 f | 25.0 f | 1443 c |
| V3-B2 | 74.2 a | 152 e | 28.0 cd | 1463 c |
| V4-B0 | 62.3 c | 200 b | 32.5 a | 1469 c |
| V4-B2 | 73.2 a | 229 a | 32.8 a | 1691 a |
| V5-B0 | 65.7 bc | 162 d | 27.3 ce | 1345 d |
| V5-B2 | 70.3 ab | 182 c | 29.3 bc | 1450 c |
| **V × W** | | | | |
| V1-WW | 68.8 de | 148 ef | 26.5 d | 1386 ef |
| V1-WS | 61.0 f | 140 fg | 25.3 d | 1268 g |
| V2-WW | 85.0 a | 194 c | 31.7 b | 1561 ac |
| V2-WS | 65.0 ef | 155 e | 26.8 d | 1401 df |
| V3-WW | 78.7 b | 147 ef | 27.3 d | 1505 bc |
| V3-WS | 70.7 cd | 138 gj | 25.7 d | 1402 de |
| V4-WW | 74.3 bc | 223 a | 34.2 a | 1598 ac |
| V4-WS | 61.2 f | 206 b | 31.2 bc | 1562 ab |
| V5-WW | 72.3 cd | 174 d | 29.5 c | 1477 cd |
| V5-WS | 63.7 ef | 171 d | 27.2 d | 1317 fg |
| **Level of Significance** | | | | |
| V | *** | *** | *** | *** |
| B | *** | *** | *** | *** |
| W | *** | *** | *** | *** |
| V × B | *** | ** | ** | *** |
| V × W | *** | *** | ** | ** |
| B × W | ns | *** | ns | ns |
| V × B × W | 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.

**Table 7:** Fresh (FW) and dry biomass (DW) and water use efficiency (WUE) of different quinoa varieties grown without and with biochar.

| Experimental Factors | FW (g) | DW (g) | WUE (g/L) |
|---|---|---|---|
| **Variety (V)** | | | |
| V1 | 51.2 c | 6.9 d | 14.5 b |
| V2 | 61.9 a | 9.5 b | 16.1 a |
| V3 | 58.8 ab | 9.4 b | 15.0 ab |
| V4 | 61.2 a | 10.4 a | 15.8 ab |
| V5 | 55.3 b | 8.6 c | 14.9 ab |
| **Biochar (B)** | | | |
| B0 | 53.0 b | 8.3 b | 14.1 b |
| B2 | 62.3 a | 9.6 a | 16.4 a |
| **Watering (W)** | | | |
| WW | 61.9 a | 9.7 a | 13.6 b |
| WS | 53.4 b | 8.3 b | 16.9 a |
| **V × B** | | | |
| V1-B0 | 46.5 e | 6.2 g | 13.1 c |
| V1-B2 | 55.9 cd | 7.6 f | 15.8 ab |
| V2-B0 | 59.4 bc | 8.9 de | 15.6 ab |
| V2-B2 | 64.4 ab | 10.1 bc | 16.6 a |
| V3-B0 | 51.0 de | 8.4 e | 13.6 bc |
| V3-B2 | 66.5 a | 10.4 bc | 16.3 a |
| V4-B0 | 59.1 bc | 9.6 cd | 15.2 ac |
| V4-B2 | 63.3 ab | 11.2 a | 16.3 a |
| V5-B0 | 49.0 e | 8.4 e | 12.9 c |
| V5-B2 | 61.5 ac | 8.9 e | 16.9 a |
| **V × W** | | | |
| V1-WW | 55.4 ce | 7.4 e | 12.9 e |
| V1-WS | 47.0 f | 6.4 f | 16.1 ad |
| V2-WW | 69.4 a | 10.3 b | 14.9 ce |
| V2-WS | 54.3 de | 8.7 d | 17.4 ab |
| V3-WW | 61.1 bc | 10.4 b | 13.1 e |
| V3-WS | 56.4 be | 8.4 d | 16.9 ac |
| V4-WW | 63.0 ab | 11.4 a | 13.3 e |
| V4-WS | 59.4 bd | 9.4 c | 18.2 a |
| V5-WW | 60.5 bd | 8.8 cd | 14.0 de |
| V5-WS | 50.0 ef | 8.5 d | 15.8 bd |
| **Level of Significance** | | | |
| V | *** | *** | ** |
| B | *** | *** | *** |
| W | *** | *** | *** |
| V × B | *** | *** | * |
| V × W | ** | *** | * |
| B × W | *** | *** | *** |
| 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.

In line with the growth-related traits, yield contributing traits including panicle length and number of sub panicles were affected by V, B, W and their two-factor interaction V × B and V × W (PL, NSP: $p \leq 0.001$) (Figure 4, Table 8).

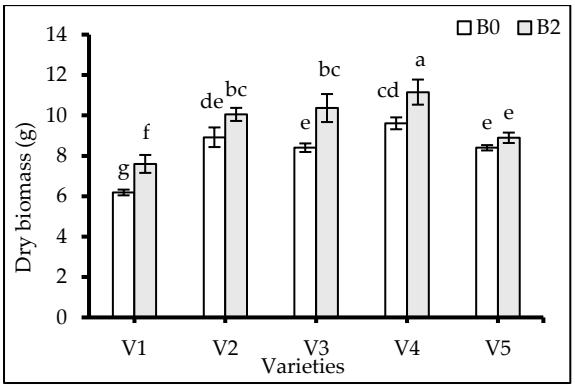



**Figure 3:** Dry biomass (DW) of different quinoa varieties grown without and with biochar under well-watered (WW) and water-stressed (WS) conditions. Values are means ($n = 3$) ± S.E. In each graph, different letters indicate significant differences among treatments ($p \leq 0.05$, Tukey's test). V1—Titicaca,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

The results revealed that the variety V2 produced the longest panicles that doubled both when grown with biochar and in WW conditions. Referring to the number of sub panicles, V4 showed the highest value than other varieties and similarly, results were observed when V4 was treated with biochar and was subjected to WW conditions.

**Table 8:** Panicle length (PL) and number of sub panicles (NSP) of different quinoa varieties grown without and with biochar.

| **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 |
| **V × W** | | |
| V1-WW | 11.2 c | 16.8 c |
| V1-WS | 9.0 d | 14.5 ef |
| V2-WW | 16.0 a | 19.2 b |
| V2-WS | 12.7 b | 15.0 df |
| V3-WW | 6.3 e | 16.5 cd |
| V3-WS | 5.2 e | 13.3 f |
| V4-WW | 9.0 d | 23.5 a |
| V4-WS | 7.8 d | 20.5 b |
| V5-WW | 3.2 f | 19.8 b |
| V5-WS | 3.7 f | 15.3 ce |
| **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.

The SPAD index, recorded at the end of the experiment, was significantly affected by all experimental factors ($p \leq 0.001$).

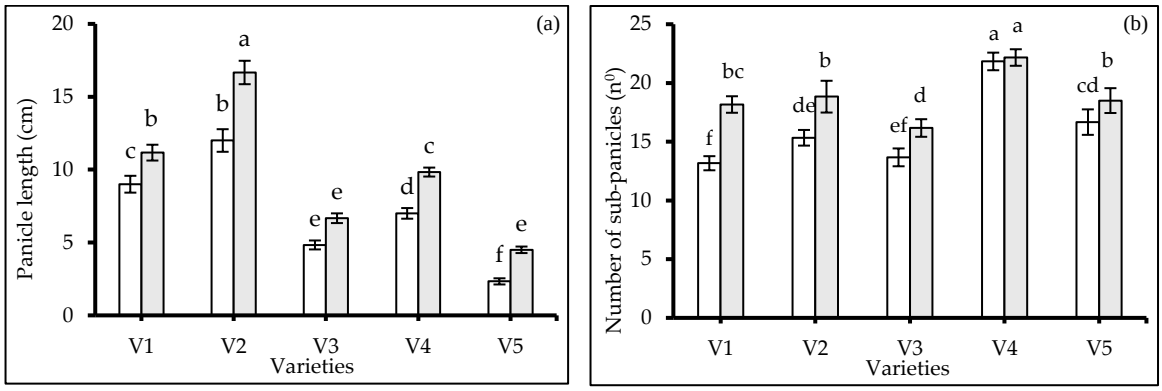



**Figure 4:** (**a**) Panicle length and (**b**) the number of sub-panicles of different quinoa varieties grown without and with biochar. Values are means ($n = 3$) ± S.E. In each graph, different letters indicate significant differences among treatments ($p \leq 0.05$, Tukey's test). V1—Titicaca, V2—Quipu, V3—Regalona, V4—UAFQ7, V5—Q126; white bars—no biochar, grey bars—2% woodchip biochar.

Biochar application enhanced SPAD index, with V1 showing the highest value when grown with biochar and under WW conditions, while V4 and V5 maintaining notable values regardless of water conditions. Nitrogen content was higher in V1 and V5 ($p \leq 0.001$), aligning with their higher SPAD values. Water-stressed plants exhibited increased leaf nitrogen content ($p \leq 0.001$). Consequently, V1 and V5 displayed lower C:N ratios ($p \leq 0.001$), indicating greater nitrogen uptake relative to carbon. Water-stressed plants also exhibited lower C:N ratios compared to well-watered conditions.

**Table 9:** SPAD index, Nitrogen (N%), Carbon (C%) and Carbon to Nitrogen ratio (C:N) in leaves of different quinoa varieties grown without and with biochar under well-watered and water stressed conditions.

| Experimental Factors | SPAD index | 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 |

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

### *6.4.5. PCA Correlation Analysis at the end of water stress period ($t_3$)*

A principal component analysis (PCA) was conducted to explore the relationships among the measured plant parameters (Figure 2). The two principal components (F1 and F2) explained 85.92% of the total variance. F1 was primarily associated with plant water status, as indicated by its strong correlations with RWC, $\Psi_{pd}$, and TW:DW ratio. In contrast, F2 was more closely

related to plant growth, with high loadings for NL, NB, PH, and LA. The analysis suggest that the treatments impacted both plant water status and growth, with distinct patterns emerging for different plant parameters.

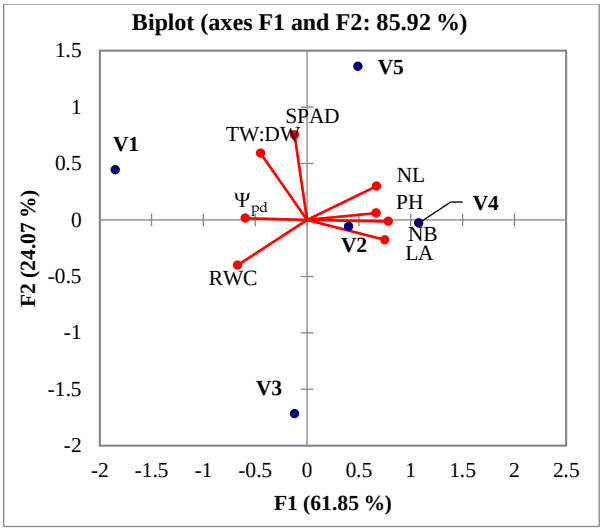



**Figure 2:** PCA correlation analysis at the end of water stress cycles. RWC: leaf relative water content, $\Psi_{pd}$: pre-dawn water potential, TW:DW: Turgid weight ratio dry weight, SPDA, SPAD index (leaf chlorophyll), NL: number of leaves, NB: number of branches, PH: plant height, LA: leaf area. V1, V2, V3, V4 and V5 showed the quinoa varieties Titicaca, Quipu, Regalona, UAFQ7 and Q126, respectively.

### *6.4.6. PCA Correlation Analysis at the end of the experiment*

In this case, the first principal component (PC1), explaining 59.2% of the total variance, was positively correlated with leaf-related traits, including nitrogen (N) and carbon (C) contents, and SPAD index (Figure 3). It showed that the varieties, V1 and V5 performed better in terms of these parameters. Conversely, the second principal component (PC2) demonstrated stronger associations with plant growth parameters, such as leaf area (LA), total dry weight (TDW), and water use efficiency (WUE), showed by V4. These findings suggest that PC1 primarily captures variations in leaf nutrient composition under biochar application rates and water regimes, while PC2 reflects treatment-induced changes in plant growth and water use strategies.

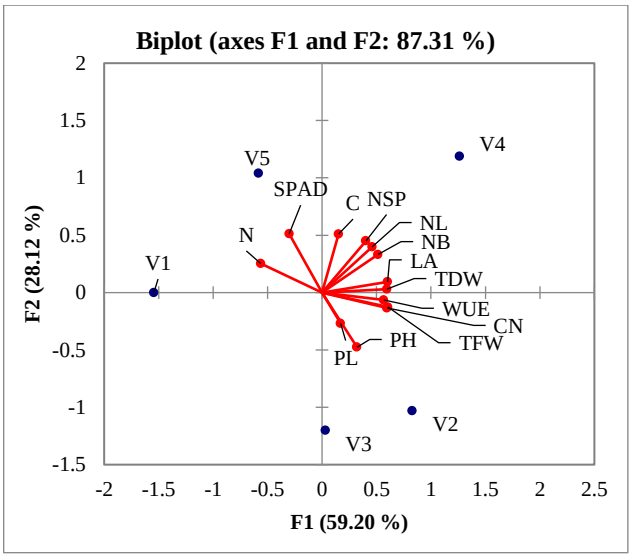



**Figure 3:** PCA correlation analysis at the end of the experiment. N: leaf nitrogen%, C: leaf carbon%, C:N: leaf carbon to nitrogen ratio, SPAD: SPAD index (leaf chlorophyll), NSP: number of sub panicles, NL: number of leaves, NB: number of branches, LA: leaf area, TFW: total fresh weight, WUE: water use efficiency, PL: panicle length, PH: plant height. V1, V2, V3, V4 and V5 indicated the varieties Titicaca, Quipu, Regalona, UAFQ7 and Q126, respectively.

### *6.4.7. Mental graph at the end of the experiment*

The correlation matrix (mental graph) revealed several important relationships between key plant traits (Figure 8). Strong positive correlations were observed between total fresh weight and leaf area, as well as between total fresh weight and plant height, 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, suggesting that higher leaf counts are associated with increased photosynthetic capacity. Conversely, leaf N% and C% displayed a weak negative correlation, implying that these traits are not closely linked. Mental's test identified significant correlations between plant height and carbon-nitrogen ratio and between panicle length and sub-panicle number, highlighting potential physiological and reproductive interactions. These results suggest that leaf area, plant height, and SPAD are vital in influencing plant growth and biomass accumulation.

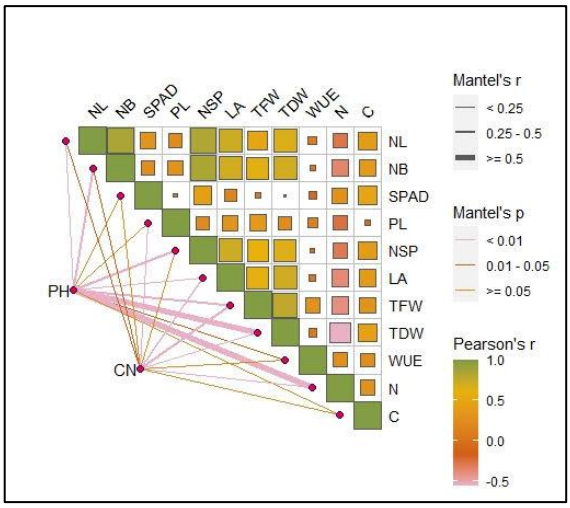



**Figure 4:** Mental graph of all growth-related traits measured at the end of the experiment. N: leaf nitrogen%, C: leaf carbon%, C:N: carbon to nitrogen ratio, SPAD: SPAD index (leaf chlorophyll), NSP: number of sub panicles, NL: number of leaves, NB: number of branches, LA: leaf area, TFW: total fresh weight, WUE: water use efficiency, PL: panicle length, PH: plant height.

## 6.5. DISCUSSION

Drought imposed during the vegetative phase significantly reduced the growth of 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 period (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, Akram et al. 2024b, Issa Ali et al. 2019).

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 3 and 4, Figure 4). These differences align with quinoa's diverse distribution, which has resulted from different quinoa ecotypes' behaviors (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 (Figures 6, 7 and 8) revealed that varieties with higher leaves and branching characteristics tend to develop more inflorescence (Table 5, Figure 5). Inflorescence length was also positively associated with plant height, indicating that varieties with greater plant height developed longer panicles, also reported in other study (Yang et al. 2016). Our study results, in line with 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 pre-dawn leaf water potential and RWC, were adversely affected by water stress. However, adding woody biochar to the soil improved plant water status, aiding in maintaining growth under water-limited conditions (Table 2, Figure 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 leaf count and branches. The improved plant water status might be due to biochar amendment that led to reduced $Na^+$ uptake and increased $K^+$ uptake, which are also supported by other study results (Yang et al. 2020). 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 other 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, Akram et al. 2024c). Further investigations into 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 presence can further enhanced the development of its roots under water-limited conditions (Xiao et al. 2016). The latter also showed a significant leaf expansion rate during post-water stress recovery, evident by no significant differences between well-watered and water-stressed conditions at the experiment's end (Table 4).

Water stress lowers the rate of photosynthesis by stomatal closure, which prevents leaves from absorbing $CO_2$, and decreases the photochemical efficiency of photosystem PSII by reducing electron transport and releasing reactive oxygen species (ROS) (Zahra et al. 2023), also evident in our experiment with low leaf carbon to nitrogen ratio that limit photosynthesis to conserve water, as also reported by other study (Zhao et al. 2020). In the current trial, the SPAD value of The Danish variety Titicaca (V1) was the most adversely affected by water stress (Figure 2). However, biochar addition mitigated these adverse effects, resulting in less growth reduction and SPAD 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 with biochar was attributed to its large surface area, facilitating 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 soil microbial communities (Elad et al. 2010, Joseph et al. 2021).

Variations in chlorosis among quinoa varieties, particularly in the Chilean Regalona, despite similar leaf nitrogen levels, highlight the potential role of the soil microbiome (Figure 3). The differential ability of quinoa varieties tested to harness microbial resources may explain the observed phenotypic differences. While total bacterial counts provide initial insights, a comprehensive microbial community composition and function analysis is necessary. Differences in rhizosphere microbiome structure and activity can significantly influence nutrient cycling, including nitrogen mineralization and availability (Bhowmik et al. 2017). This hypothesis necessitates further investigation into specific microbial taxa associated with each variety and their functional roles in nutrient acquisition and plant growth. Exploring the interactions between variety selection and agricultural management practices in rhizosphere microbial communities is essential.

## 6.6. CONCLUSION

The five quinoa varieties tested exhibited different growth and biomass production due to their varying growth rates and water-use efficiencies. The varieties under water stress conditions showed increased water-use efficiency, further improved in biochar-amended soil. The Pakistani variety UAFQ7 showed the highest biomass accumulation, further enhanced by soil biochar addition. A significant increment in the plant water status of all varieties grown in biochar-amended soil was observed under water stress conditions. Likewise, the Italian variety Quipu performed well under biochar-amended soil in yield-contributing traits by doubling the panicle length compared to non-treated ones. This research highlights the potential of biochar as a sustainable agronomic strategy to improve quinoa growth and productivity, as well as

adaptability to water limitations, emphasizing the importance of varietal selection for optimal plant performance.

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# CHAPTER 7

# MITIGATION OF DROUGHT STRESS FOR QUINOA (*Chenopodium quinoa* Willd.) VARIETIES USING WOODCHIP BIOCHAR-AMENDED SOIL

*This chapter is a state-of-the-art chapter, and its content has been already published:*

**Akram, M.Z.,** Rivelli, A.R., Libutti, A., Liu, F., & Andreasen, C. (2024). Mitigation of drought stress for Quinoa (*Chenopodium quinoa* Willd.) varieties using woodchip biochar-amended soil. *Plants, 13(16), 2279.*

## 7.1. ABSTRACT

Drought stress deteriorates agro-ecosystems and poses a significant threat to crop productivity and food security. Soil amended with biochar has been suggested to mitigate water stress, but there is limited knowledge about how biochar affects the physiology and vegetative growth of quinoa plants under soil water deficits. We grew three quinoa (*Chenopodium quinoa* Willd.) varieties, Titicaca (V1), Quipu (V2), and UAFQ7 (V3) in sandy loam soil without (B0) and with 2% woodchip biochar (B2) under drought conditions. The drought resulted in significant growth differences between the varieties. V3 performed vegetatively better, producing 46% more leaves, 28% more branches, and 25% more leaf area than the other two varieties. Conversely, V2 displayed significantly higher yield-contributing traits, with 16% increment in panicle length and 50% more subpanicles com-pared to the other varieties. Woodchip biochar application significantly enhanced the root development (i.e., root biomass, length, surface, and projected area) and plant growth (i.e., plant height, leaf area, and absolute growth rate). Biochar significantly enhanced root growth, especially fresh and dry weights, by 122% and 127%, respectively. However, biochar application may lead to a trade-off between vegetative growth and panicle development under drought stress as shown for V3 grown in soil with woodchip biochar. However, V3B2 produced longer roots and more biomass. Collectively, we suggest

exploring the effects of woodchip biochar addition to the soil on the varietal physiological responses such as stomatal regulations and mechanisms behind the increased quinoa yield under water stress conditions.

*Keywords: drought resistance; drought tolerance; physiological parameters; morphological attributes; root traits; soil amendments*

## 7.2. INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) belongs to the Amaranthaceae family (Rosentrater et al., 2018). It is an annual C3 crop originally from the South American highlands known for its tolerance to adverse abiotic stresses, particularly salinity and drought (Akram et al., 2021). It demonstrates remarkable adaptability to grow in poor soils and under extreme climatic conditions (El-Harty et al., 2023). Quinoa's drought tolerance is caused by its deep and extensive root system, white reflective and hygroscopic papillae on the leaf surface (Ain et al., 2023), leaf area reduction by shedding leaves to reduce water loss through transpiration, presence of special vesicular glands (i.e., small thick-walled cells used for water storage reservoirs maintaining turgor during water limitations) (Otterbach et al., 2021), and stomatal closure (Maestro-Gaitán et al., 2023). Jensen et al. (2000) revealed that the leaf water potential threshold for stomatal closure in quinoa varies between cultivars, occurring at −1.2 to −1.6 MPa. Quinoa's tolerance to drought is also attributed to its phenotypic plasticity, tissue elasticity, and inherently low osmotic potential (Jensen et al., 2000). This phenotypic plasticity, highlighted by Stanschewski et al. (2021), underscores the importance of exploring quinoa germplasm from diverse geographical locations. The vast genetic diversity within quinoa germplasm includes genes conferring resistance to both biotic and abiotic stresses (Murphy et al., 2016), suggesting its crucial role in quinoa's ability to adapt to various environmental challenges.

Inter-varietal variation in drought tolerance mechanisms necessitates targeted screening of quinoa germplasm for specific water deficit conditions (Hafeez et al., 2022). Earlier studies have shown that significant variations were evident between five quinoa ecotypes in drought tolerance mechanisms (Bazile et al., 2023). The ecotype Salares performed better under drought stress than other ecotypes through extensive root systems, low osmotic potential, epidermal bladder cells and stomatal control (Ain et al., 2023). Notable varieties from this ecotype are UAFQ7 (Akram et al., 2021) and Quipu (Casini et al., 2017), the first approved quinoa varieties in Pakistan and Italy, respectively. Akram et al. (2021) studied 13 superior genotypes screened from 117 imported quinoa lines from the United States Department of Agriculture (USDA) [10] and showed that UAFQ7 has longer roots and higher yield than other genotypes. Similarly, Valley and Altiplano ecotypes are considered short-duration and fast-growing varieties, like Titicaca, that showed drought escape mechanisms by completing their life cycle earlier to avoid the drought effects at later stages. However, it was more susceptible to water deficits under prolonged drought conditions (Taaime et al., 2022).

Application of organic amendments, including compost, manure, and biochar, can significantly mitigate the adverse effects of water stress on plant growth (Ullah et al., 2021). They improve soil structure, water retention, and nutrient availability (Aytenew et al., 2020). Among these, biochar is particularly advantageous due to its exceptional water-holding capacity, nutrient adsorption, and ability to stimulate beneficial soil microbial communities, and thereby enhance plant growth under water stress conditions (Niu et al., 2024). Biochar, a carbon-rich byproduct of pyrolysis (Blenis et al., 2023), offers a promising approach to mitigating climate change and enhancing agricultural productivity under drought stress. Several studies have documented its positive effects on plant growth (Kammann et al., 2011; Rivelli et al., 2024), yield (Han et al., 2023), nutrient uptake (Wan et al., 2023), and plant physiological properties (Usman et al., 2023) under drought. Specifically for quinoa, biochar application has been shown to enhance

both vegetative (Akram et al., 2023) and reproductive growth (Kammann et al., 2011) under drought stress through changes in soil physicochemical and biological properties (Bogati et al., 2022). Studies have documented significant improvement in soil structure, water-holding capacity, and other physicochemical properties under drought conditions after biochar application (Wei et al., 2023). The improved soil environment enhances plant biomass and nutrient uptake (Kammann et al., 2011). Furthermore, biochar application positively influences plant physiological attributes under drought stress, such as increased photosynthetic rate, stomatal conductance, and plant water status in quinoa (Ramzani et al., 2017). Biochar's porous structure retains water and may enhance root proliferation and growth. Thus, an improved root system could maintain leaf turgor pressure, stomatal conductance and photosynthesis during drought stress by facilitating increased water and nutrient uptake (Khan et al., 2021). In biochar-amended soil, Kammann et al. (2011) observed better quinoa growth and water-use efficiency under drought conditions.

Apart from its advantages, biochar can adversely affect plant growth by immobilizing essential nutrients through altering soil pH (Brtnicky et al., 2021). High application rates can create physical barriers, hindering root development due to the highly porous nature of the biochar which accelerates the loss of water and nutrients from the soil (Xu et al., 2016). The influence of biochar application on quinoa's physiological response to drought stress throughout vegetative development remains understudied (Geerts et al., 2006). While previous work has shown that biochar can enhance the growth of the drought-sensitive quinoa cultivar Titicaca (Akram et al., 2023), the effect under prolonged water deficits remains unknown (Rivelli et al., 2024).

With the significant phenotypic diversity within quinoa germplasm in mind, this study aimed to contribute substantially to our understanding of drought tolerance mechanisms of quinoa varieties under biochar application. We studied the impact of woodchip biochar application at

a rate of 2% in the soil to enhance drought tolerance among different quinoa varieties grown under water stress conditions. We utilized morphological and physiological analyses to evaluate the impact of successive water stress cycles on quinoa growth and physiology to elucidate the interaction between drought stress and biochar application, mainly focusing on their combined effects during the vegetative growth stage. We hypothesized that application of woodchip biochar to the soil helps the quinoa varieties to avoid the negative impact of water stress conditions occurring during the vegetative growing cycle of the plant.

## 7.3. MATERIALS AND METHODS

### *7.3.1. Plant Material and Growing Conditions*

Two identical experiments were conducted under controlled greenhouse conditions at the Department of Plant and Environmental Sciences, University of Copenhagen, Taastrup, Denmark (55.66 N-12.30 E, 27 m a.s.l.). The first experiment (Exp. 1) was conducted from October 2023 to January 2024, and the other (Exp. 2) from January to March 2024. A completely randomized factorial design with nine replicates was employed, with the three varieties Titicaca (V1), Quipu (V2), and UAFQ7 (V3) (Table 1) grown in soil without (B0) and with biochar (B1) resulting in a total of six treatments, i.e., V1B0, V2B0, V3B0, V1B2, V2B2, V3B2. In each experiment, nine plastic pots (diameter: 26 cm; height: 20 cm; volume: 7.5 L) were filled with sandy loam soil collected from the Taastrup campus fields and nine were filled with sandy loam soil mixed with woodchip biochar manufactured by Nerabiochar (Nerabiochar, Ivrea, Torino, Italy).

**Table 1.** Varieties used in the experiments.

| Varieties | Code | Origin | Collection Site/Company |
|---|---|---|---|
| Titicaca | V1 | Denmark | University of Copenhagen, Taastrup, Denmark |
| Quipu | V2 | Italy | Tuttoquinoa di Vannuzzi Dario, Manciano, Italy |
| UAFQ7 | V3 | Pakistan | Muhammad Nawaz Shareef University of Agriculture (MNSUAM), Multan, Pakistan |

The properties of the biochar are shown in Table 2. The biochar was incorporated into the soil (Table 3) at the rate of 2% based on the dry weight of the soil. Soil moisture was estimated

(Equation (1)) (Soil Water Content (%)= (FW-DW/DW) × 100) after weighing the soil before (*FW*) and after drying in a drying chamber for two days at 105 °C (*DW*). The pots were placed in a greenhouse with 16/8 h light/darkness, at a temperature of 20 ± 2 °C, and a relative humidity of 70% ± 7%.

**Table 2.** Woodchip biochar amendment properties used in the experiment (from Rivelli et al., 2024)

| Parameters | Biochar |
|---|---|
| pH | 8.9 ± 0.13 |
| EC | 0.52 ± 0.04 dS $m^{-1}$ |
| Moisture | 5.6 ± 0.11% dw |
| Volatile solids | 42.3 ± 0.44% dw |
| Ash | 4.4 ± 0.21% dw |
| Fixed carbon | 53.3 ± 0.24% dw |
| C | 68.3 ± 0.11% dw |
| H | 4 ± 0.04% dw |
| N | 1 ± 0.03% dw |
| Corg | 66.3 ± 0.06% dw |
| C/N | 67.2 ± 1.96 |
| S | 0.03 ± 0.01% dw |
| O | 22.3 ± 0.29% dw |
| H/Corg ratio | 0.06 ± 0.01 |
| O/Corg ratio | 0.4 ± 0.01 |

Values are means (n = 3) ± s.e. dw = dry weight

Similarly, the soil properties used in the experiment are listed in Table 3.

**Table 3.** Soil properties used in the experiment.

| Parameters | Units | Soil |
|---|---|---|
| Phosphorus | (mg/100 g) | 4.7 |
| Potassium | (mg/100 g) | 19 |
| Magnesium | (mg/100 g) | 5.9 |
| Sodium | (mg/100 g) | 2.6 |
| Manganese | (mg/kg) | 30.2 |
| Copper | (mg/kg) | 1.5 |
| Zinc total | (mg/kg) | 2.4 |
| Boron | (mg/10 kg) | 3.4 |
| Molybdenum | (mg/10 kg) | 0.92 |
| Organic matter | (%) | 2.14 |
| Clay (<0.002 mm) | (%) | 9.1 |
| Silt (0.002 mm) | (%) | 7.0 |
| Fine sand (0.02–0.2 mm) | (%) | 43.5 |
| Coarse sand (0.2–2.0 mm) | (%) | 38.4 |

Ten seeds per pot were sown. When plants had 2−4 leaves, the number of plants was reduced to three uniform plants. At the 6-leaf stage, the plants were thinned to one plant per pot. From emergence to the 12-leaf stage, the plants were kept well-watered. At the 12-leaf stage, two successive drought cycles were imposed.

### *7.3.2. Water Stress Treatment*

From emergence to the 12-leaf stage, pots were regularly watered to 100% pot water holding capacity (WHC) when soil water content reached 70% of WHC, determined by daily weighing the pots between 8:00−9:00 AM. The 100% pot WHC was defined as pot weight when drainage had stopped after saturation of the soil. At the 12-leaf stage, two progressive drought cycles were applied, one after the other. In both experiments, the stress period started after the water content in all pots was brought to 100% pot WHC at 35 Days After Sowing (DAS) (time = $t_1$). At $t_1$, three replications were harvested at the soil surface, and plant height, number of leaves and branches, leaf area, and fresh and dry biomass of the above-ground plants were recorded. The watering of the remaining pots was retained until all available water was used at 45 DAS (time = $t_2$). Then, plants were rewatered to field capacity, and the second drought period began. It finished at 56 DAS (time = $t_3$). At $t_3$, three replications were harvested, and their yield components were recorded. The remaining three replications kept growing under well-watered conditions until flowering initiation happened 80 DAS (time = $t_4$). Then, plants were harvested, and their yield components were measured.

### *7.3.3. Measurements*

Plant height (cm), number of leaves ($n^0$), and branches ($n^0$) were recorded at times $t_1$, $t_3$, and $t_4$ by sampling three plants. The fresh weight (g) of above-ground biomass (*TFW*) was measured at each time. Afterwards, the plants were dried in an oven at 70 °C until constant weight and the dry weights were recorded. Yield components such as main panicle length (cm) and the number of sub-panicles plant$^{-1}$ were determined at $t_4$. At each harvest point ($t_1$, $t_3$, $t_4$), the leaves were collected, and the leaf area ($LA_1$, $LA_3$, $LA_4$) was determined by a leaf area measuring instrument (model 3100; LI-COR, Inc., Lincoln, NE, USA). Finally, the Absolute Growth Rate (*AGR*) of the plants was calculated for the drought period (DP, between $t_1$ to $t_3$) by recording

the plant dry weight at the start ($DW_1$) and end of the drought treatment ($DW_2$): AGR (g/d)= ($DW_2$-$DW_1$/$t_3$-$t_1$)

Likewise, *AGR* was calculated for the recovery period (RP, between $t_3$ to $t_4$) using $DW_3$ and $DW_2$.

Root fresh and dry weight (g), and root shoot ratio (R:S) were estimated at $t_1$ and $t_4$ from 3 plants. Additionally, at the same time, roots were scanned in a root scanner by using Epson scan 2 software (https://epson.com/Support/Scanners/Expression-Series/Epson-Expression-12000XL---Photo/s/SPT_12000XL-PH?review-filter=Windows+11), and root length (cm), projected area ($cm^2$), surface area ($cm^2$), volume ($cm^3$), and average diameter (mm) were measured via WinRhizo software 7.6.5.

At the end of the drought periods ($t_3$) and the trials ($t_4$), the total water consumption (*TWC*) was calculated by cumulating the amount of water consumed by the plants. Water consumption was measured by weighing the pots daily. Then, the whole plant water use efficiency (*WUEwp*) during the drought period was calculated according to equation (Akhtar et al., 2020): $WUEwp= (DW_2-DW_1)/TWC$.

where *TWC* is the total water consumed during the drought period. Likewise, *WUEwp* was calculated for the recovery period using $DW_2$, $DW_3$ and *TWC*. Midday leaf water potential (Ψ) was measured in a pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA) on three fully expanded upper canopy leaves between 12:00 and 13:00 h at $t_2$, $t_3$ and $t_4$. For that purpose, the young fully expanded leaf was detached from the plant along with its petiole. The leaf was then placed in the chamber with the petiole protruding outside. Afterwards, the pressure was increased gradually in the chamber and the reading was recorded immediately when a droplet of water came out from the protruding petiole surface.

Relative leaf chlorophyll content was assessed using a chlorophyll meter (SPAD-502; Konica Minolta Sensing, Inc., Osaka, Japan). Five measurements were recorded on five fully expanded top leaves of each plant, and the values were subsequently averaged.

Three dried leaf samples were collected at $t_4$ and finely ground. The total leaf N, C, and H content (% *DW*) were estimated with a CHNS/O elemental analyzer (Flash 2000, Thermo Fisher Scientific, Cambridge, UK) using the dynamic flash combustion technique known as the modified Dumas method (Dumas, 1831).

### *7.3.4. Statistical analysis*

A two-way analysis of variance was computed using the statistical software RStudio 4.2.0 (R Core Team, 2013), considering varieties and biochar levels as the main factors. Mean comparisons among treatment groups were performed using Tukey's Honestly Significant Difference (HSD) test at a significance level of $p \leq 0.05$. Similarly, correlation analysis between SPAD values and N% in the leaves and principal component analysis (PCA) was done by using the same software.

## 7.4. RESULTS

### *7.4.1. Plant Growth and Biomass during the Experiment*

At all three harvests ($t_1$, $t_3$ and $t_4$) in both experiments, there were significant differences between the varieties' response to biochar (Exp. 1: $t_1$: $p \leq 0.0008$; $t_3$: $p \leq 0.0000$; $t_4$: $p \leq 0.0066$; Exp. 2: $t_1$: $p \leq 0.0042$; $t_3$: $p \leq 0.0024$; $t_4$: $p \leq 0.0320$) in height (PH). Plants grown in biochar-amended soil were taller (Exp. 1: $t_1$: $p \leq 0.0055$; $t_3$, $t_4$: $p \leq 0.0000$; Exp. 2: $t_1$, $t_3$: $p \leq 0.0000$; $t_4$: $p \leq 0.0329$), but there was no significant interaction between varieties and soil type. In both experiments, V1 was the tallest at $t_1$, while V2 was the tallest at the final harvest ($t_4$) (Figure 1a). Contrarily, in Exp. 2, an interaction was observed for plant height at $t_1$ and $t_3$ ($t_1$, $p \leq 0.0227$; $t_3$, $p \leq 0.0109$). In particular, V1 grown with biochar attained the greatest height at both harvests. Plants grown in soil with biochar achieved higher numbers of leaves (NL), branches (NB), and

larger leaf areas (LA). Similarly, an interaction was recorded for the number of leaves and branches at $t_3$ and $t_4$ for Exp. 2 ($t_3$, $t_4$: $p \leq 0.0000$). At both times, V3 showed a positive response to biochar possessing a higher number of leaves and branches.

In both experiments, at all three harvest times, V3 produced the highest number of leaves (Exps. 1 and 2: $t_1$, $t_3$, $t_4$: $p \leq 0.0000$), branches (Exp. 1: $t_1$: $p \leq 0.0008$; $t_3$, $t_4$: $p \leq 0.0000$; Exp. 2: $t_1$, $t_4$: $p \leq 0.0000$; $t_3$: $p \leq 0.0013$), and leaf areas (Exps. 1 and 2: $t_1$, $t_3$, $t_4$: $p \leq 0.0000$). All varieties grown in soil with biochar had significantly higher numbers of leaves, branches, and leaf areas at all harvests (Figure 1b–d). A significant two-factor interaction was observed in Exp. 2 at $t_1$, $t_3$ and $t_4$ ($t_1$, $t_3$, $t_4$: $p \leq 0.0000$). Consistent with the number of leaves and branches, V3 showed the positive effect of biochar addition by always producing bigger leaf areas. Importantly, at $t_4$ biochar treatment enhances the number of leaves by 18% and 40% in Exp. 1 and 2, respectively. Similarly, plants grown with biochar produced bigger leaf areas than non-amended ones by 20% and 60% in Exp 1. and 2, respectively.

In both experiments, the fresh biomass (FB) varied between varieties (Exp. 1: $t_1$, $t_3$: $p \leq 0.0000$, $t_4$: $p \leq 0.0008$; Exp. 2: $t_1$, $t_3$, $t_4$: $p \leq 0.0000$) and increased for all varieties at all harvests when biochar was added (Exps. 1 and 2: $t_1$, $t_3$, $t_4$: $p \leq 0.0000$). In Exp. 1, at $t_1$, there was interaction between the varieties and soils ($p \leq 0.0017$). In Exp. 2, an interaction was observed at $t_1$, $t_3$, and $t_4$ ($t_1$, $t_3$, $t_4$: $p \leq 0.0000$). In both experiments at all sampling times, consistent with the other growth parameters, V3 produced higher fresh biomass, especially when grown with biochar amendment (Figure 2a). Particularly at $t_4$, plants grown with biochar produced more fresh biomass than controlled soil by 20% and 32% in Exp. 1 and 2, respectively. Dry biomass showed the same trend (Exp. 1: $t_1$, $t_3$, $t_4$: $p \leq 0.0000$; Exp. 2: $t_1$, $t_4$: $p \leq 0.0000$, $t_3$, $p \leq 0.0425$). In both experiments, plants grown in soil with biochar produced more biomass at $t_3$ (Exps. 1 and 2: $p \leq 0.0000$) and $t_4$ (Exps. 1 and 2: $p \leq 0.0000$). In Exp. 1, there was an interaction between varieties and soils at the $t_3$ ($p \leq 0.0000$) and $t_4$ ($p \leq 0.0000$).

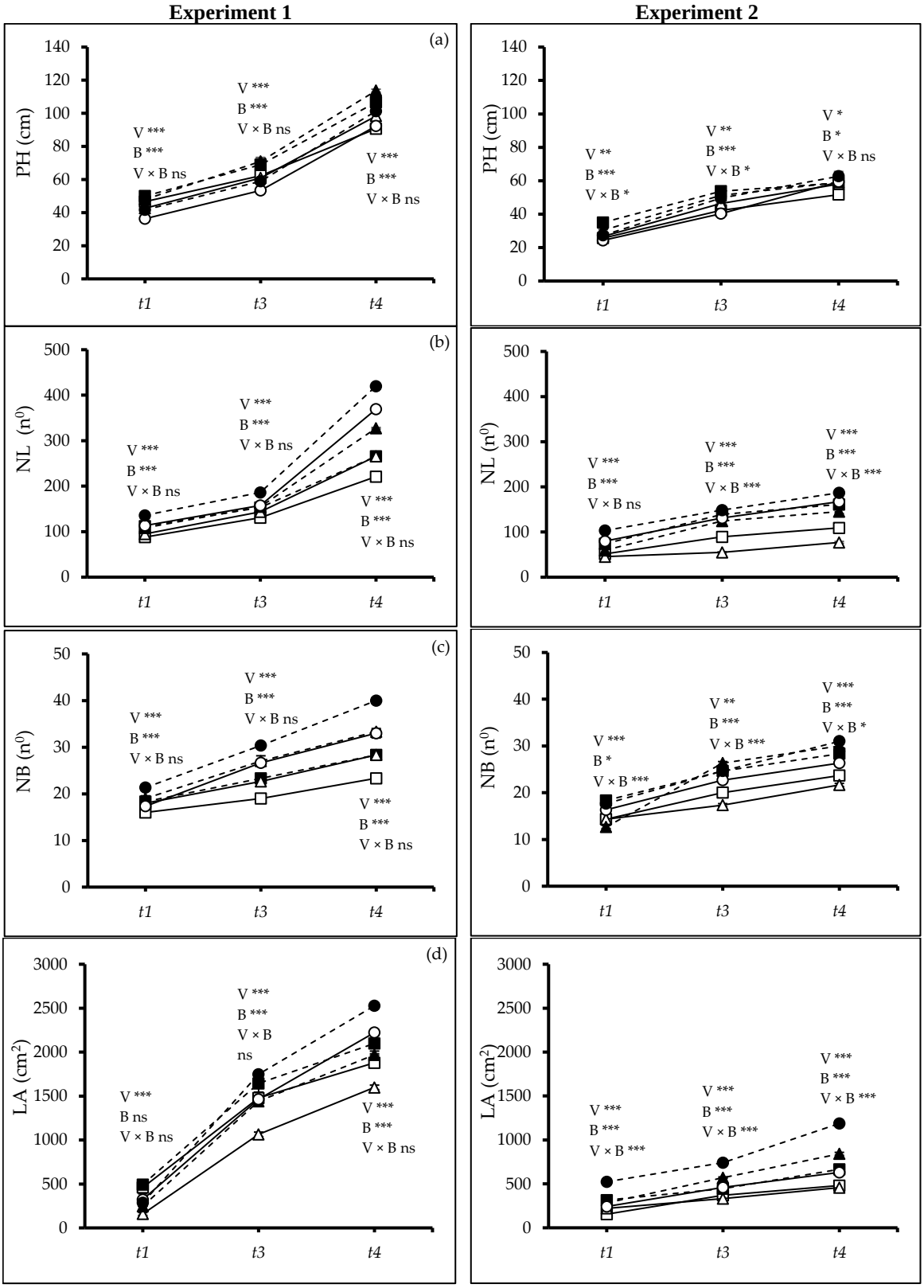



**Figure 1.** (**a**) Plant height (PH), (**b**) number of leaves (NL), (**c**) number of branches (NB), and (**d**) leaf area (LA) of quinoa (*Chenopodium quinoa* Willd.) varieties treated without and with woodchip biochar. Values are means ($n$ = 3) ± S.E. In each graph, different stars above and below the lines indicate significant differences among treatments ($p \leq 0.05$, Tukey's test). F-test significant at *—($p \leq 0.05$), **—($p \leq 0.01$), ***—($p \leq 0.001$). $t_1$—start of drought, $t_3$—end of 2nd drought cycle and $t_4$—end of the experiment. White squares: quinoa variety 1 (V1) without biochar. Black squares: quinoa variety 1 (V1) with biochar; white triangles: quinoa variety 2 (V2) without

biochar; black triangles: quinoa variety 2 (V2) with biochar; white circles: ; quinoa variety 3 (V3) without biochar; black circles: quinoa variety 3 (V3) with biochar; solid lines—(B0) no biochar, dash lines—(B2) 2% biochar.

In Exp. 2, fresh biomass and dry biomass accumulation were affected by a two-factor interaction at $t_1$ and $t_4$ ($t_1$: $p \leq 0.0101$, $t_4$: $p \leq 0.0000$). In both experiments, V3 consistently had the highest dry matter production, especially in soil amended with biochar. Specifically, biochar-treated plants increased dry biomass in both experiments by 50% at $t_4$ compared to non-amended soils (Figure 2b), indicating a potentially beneficial interaction under drought stress.

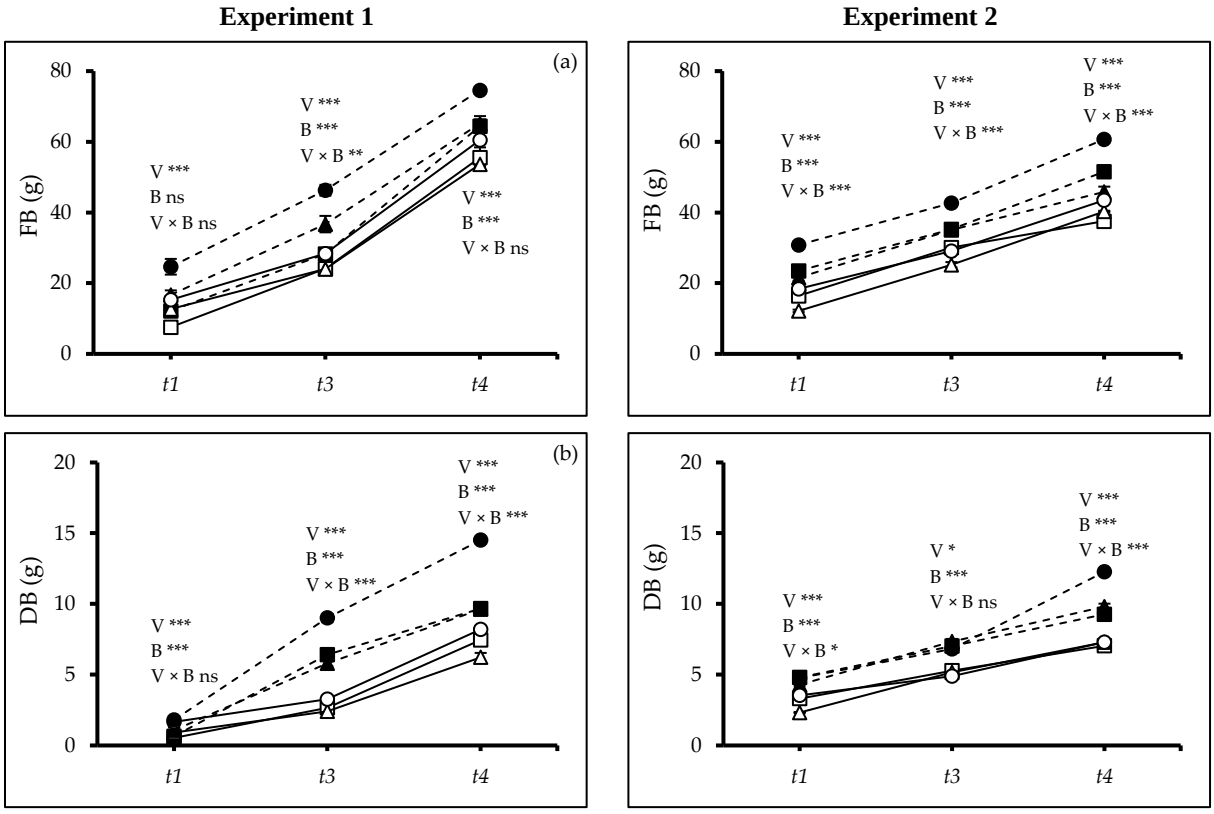
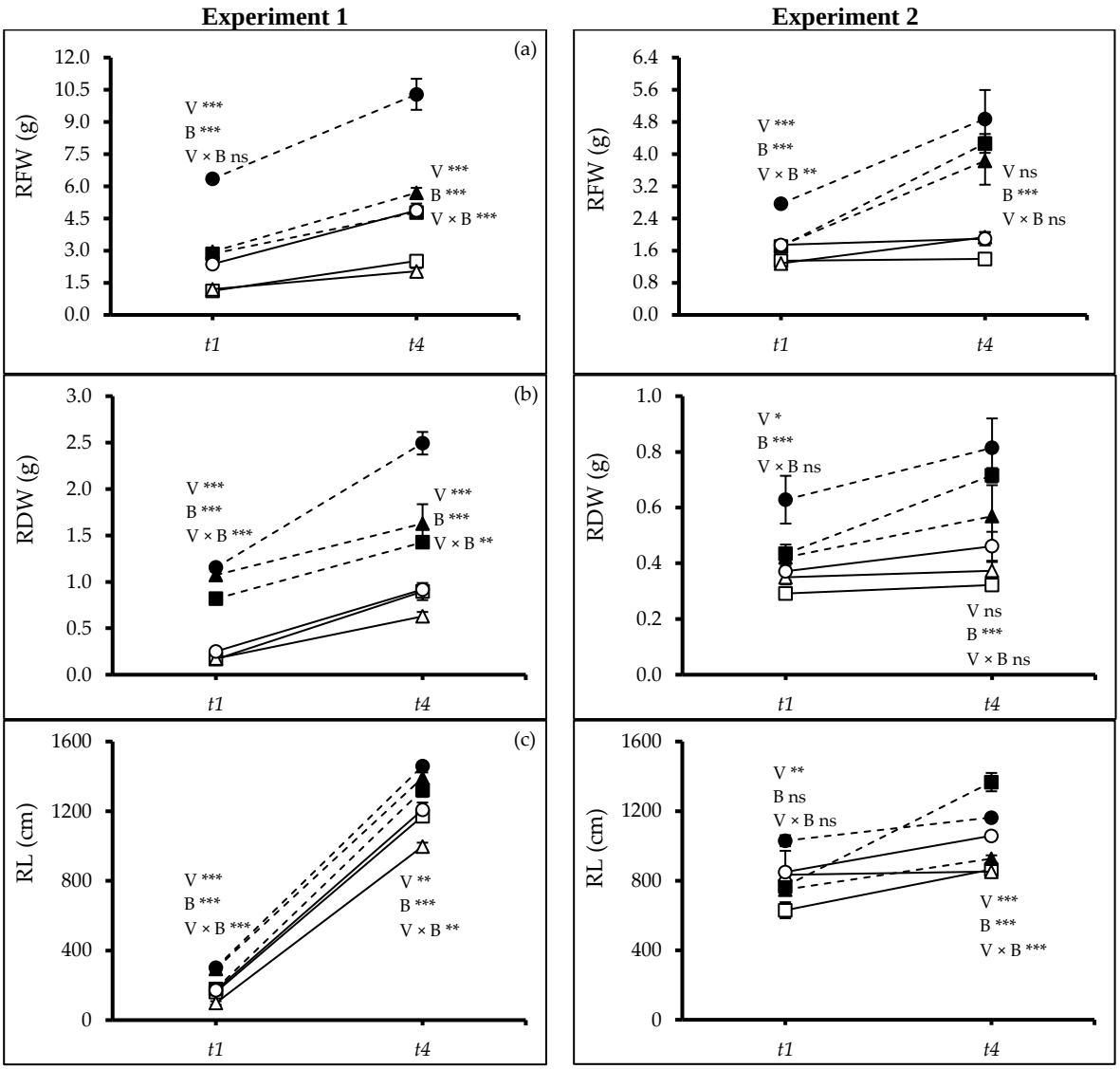



**Figure 2.** (a) Fresh biomass (FB), and (b) dry biomass (DB) of quinoa (*Chenopodium quinoa* Willd.) varieties treated without and with woodchip biochar. Values are means ($n = 3$) ± S.E. In each graph, different stars above and below the lines indicate significant differences among treatments ($p \leq 0.05$, Tukey's test). F-test significant at *—($p \leq 0.05$), **—($p \leq 0.01$), ***—($p \leq 0.001$). $t_1$—start of drought, $t_3$—end of 2nd drought cycle and $t_4$—end of the experiment. White squares: quinoa variety 1 (V1) without biochar. Black squares: quinoa variety 1 (V1) with biochar; white triangles: quinoa variety 2 (V2) without biochar; black triangles: quinoa variety 2 (V2) with biochar; white circles: quinoa variety 3 (V3) without biochar; black circles: quinoa variety 3 (V3) with biochar; solid lines—(B0) no biochar, dash lines—(B2) 2% biochar.

### *7.4.2. Root Traits during the Experiments*

The root traits varied significantly in both experiments between varieties and growth media, and there was also an interaction. At $t_1$ and $t_4$, significant differences were observed between varieties, biochar amendment, and interaction for root fresh (RFW) and dry weight (RDW) and

root length (RL). Interestingly, plants grown with biochar-amended soils exhibited 130% and 110% more fresh and dry root biomass, respectively, at $t_4$ in both experiments. V3 produced the largest root weight, especially in soil amended with biochar (Exp. 1: $t_1$: $p \leq 0.0000$, $t_4$: $p \leq 0.0096$; Exp. 2: $t_1$, $t_4$: $p \leq 0.0000$) (Figure 3 a–c). In Exp. 1, root shoot ratio (R:S) at $t_1$ and $t_4$ was affected by biochar ($t_1$, $t_4$: $p \leq 0.0001$) but not by varieties. In Exp. 2, R:S varied between varieties at $t_1$ ($p \leq 0.0071$) and biochar at $t_4$ ($p \leq 0.0342$). In both experiments, biochar amendment resulted in a higher R:S, probably by enhancing nutrient availability (Figure 3d).



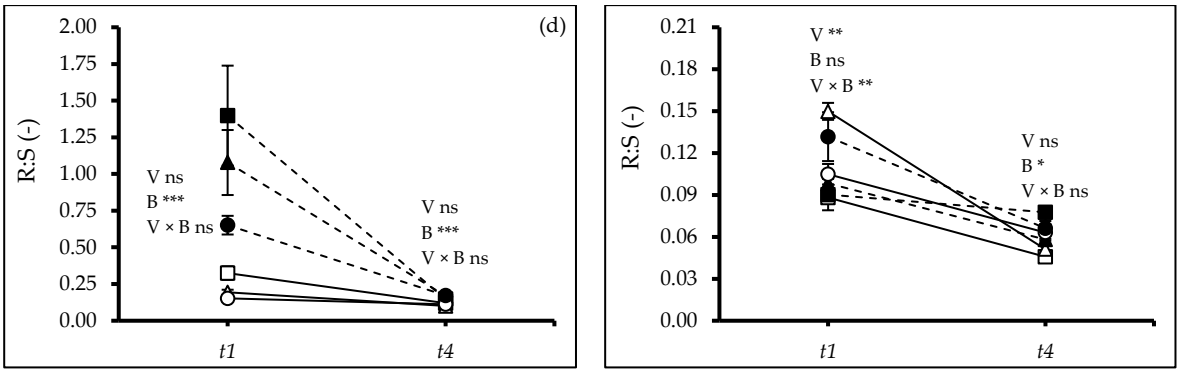



**Figure 3.** (a) Root fresh weight (RFW), (b) root dry weight (RDW), (c) root length (RL), and (d) root shoot ratio (R:S) of quinoa (*Chenopodium quinoa* Willd.) varieties treated without and with woodchip biochar. Values are means ($n = 3$) ± S.E. In each graph, different stars above and below the lines indicate significant differences among treatments ($p \leq 0.05$, Tukey's test). F-test significant at *—($p \leq 0.05$), **—($p \leq 0.01$), ***—($p \leq 0.001$). $t_1$—start of drought, and $t_4$—end of the experiment. White squares: quinoa variety 1 (V1) without biochar. Black squares: quinoa variety 1 (V1) with biochar; white triangles: quinoa variety 2 (V2) without biochar; black triangles: quinoa variety 2 (V2) with biochar; white circles: quinoa variety 3 (V3) without biochar; black circles: quinoa variety 3 (V3) with biochar; solid lines—(B0) no biochar, dash lines—(B2) 2% biochar.

Similarly, root surface (SA) and projected areas (PA) varied in both experiments between varieties (Exps. 1 and 2: $t_1$, $t_4$: $p \leq 0.0000$) and the two soils (Exps. 1 and 2: $t_1$, $t_4$: $p \leq 0.0000$) both at $t_1$ and $t_4$ (Figure 4 a, b). In both experiments, V3 grown in soil amended with biochar had the highest surface and projected root area at $t_1$ and $t_4$. Root volume and average diameter were different between varieties in Exp. 1 ($t_1$: $p \leq 0.0004$, $t_4$: $p \leq 0.0000$), soils ($t_1$, $t_4$: $p \leq 0.0000$), and there was an interaction between the two factors ($t_1$, $t_4$: $p \leq 0.0000$). However, in Exp. 2, root volume (Vol) varied between the varieties at $t_4$ ($t_4$: $p \leq 0.0026$) but not at $t_1$. Biochar had a positive effect at both times, i.e., $t_1$ and $t_4$ ($t_1$: $p \leq 0.0091$, $t_4$: $p \leq 0.0000$). An interaction was also found at $t_4$ ($p \leq 0.0292$). In Exp. 2, the average diameter (AvgD) varied between soil types at $t_4$ ($p \leq 0.0000$). In soil with biochar, V3 consistently showed the highest values (Figure 4c,d).

**Experiment 1** **Experiment 2**

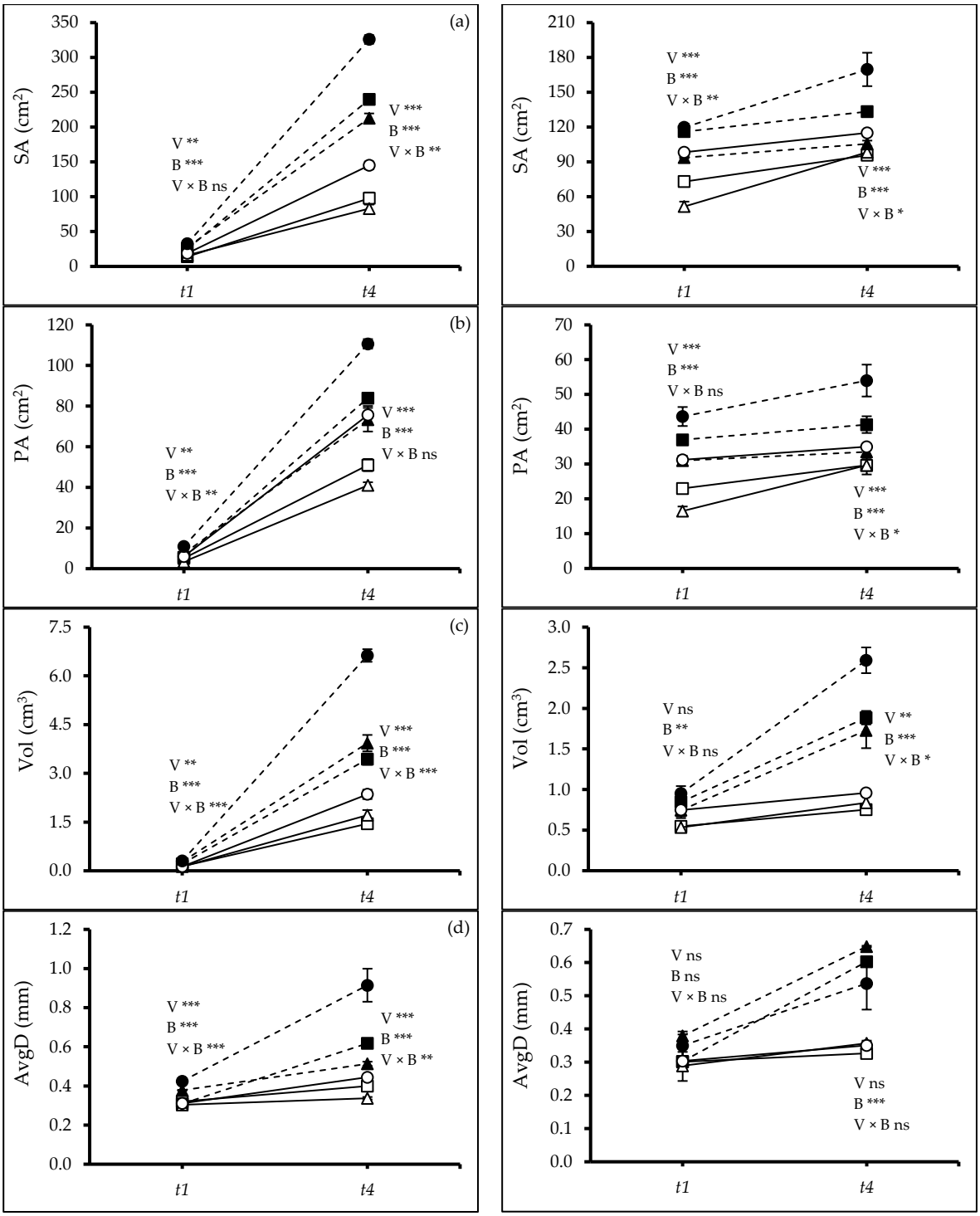
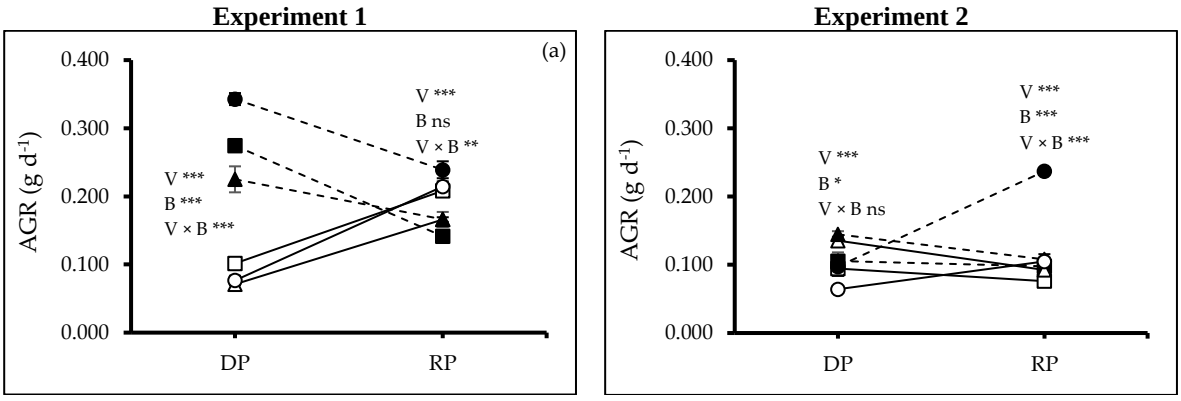



**Figure 4.** (a) Root surface area (SA), (b) root projected area (PA), (c) root volume (Vol), and (d) root average diameter (AvgD) of quinoa (*Chenopodium quinoa* Willd.) varieties treated without and with woodchip biochar. Values are means ($n = 3$) ± S.E. In each graph, different stars above and below the lines indicate significant differences among treatments ($p \leq 0.05$, Tukey's test). F-test significant at *—($p \leq 0.05$), **—($p \leq 0.01$), ***—($p \leq 0.001$). $t_1$—start of drought, and $t_4$—end of the experiment. White squares: quinoa variety 1 (V1) without biochar. Black squares: quinoa variety 1 (V1) with biochar; white triangles: quinoa variety 2 (V2) without biochar; black triangles: quinoa variety 2 (V2) with biochar; white circles: quinoa variety 3 (V3) without biochar; black circles: quinoa variety 3 (V3) with biochar; solid lines—(B0) no biochar, dash lines—(B2) 2% biochar.

### *7.4.3. Growth- and Yield-Contributing Traits*

During the drought (DP, between $t_1$ to $t_3$) and recovery period (RP, between $t_3$ to $t_4$), the varieties performed differently (Exps. 1 and 2: DP, RP, $p \leq 0.0000$). In Exp. 1, soil amended with biochar affected the absolute growth rate positively during DP (DP, $p \leq 0.0000$), but not during RP, while in Exp. 2, biochar had a positive effect during both periods (DP, $p \leq 0.0224$; RP, $p \leq 0.0000$). In Exp. 2, there was also interaction between the two factors (DP, $p \leq 0.0000$, RP, $p \leq 0.0046$). V3, grown in soil amended with biochar, had the highest growth rate under drought stress (Figure 5a). The varieties also showed different panicle lengths (Exp. 1: $p \leq 0.0000$; Exp. 2: $p \leq 0.0003$), which was also positively affected by biochar amendment (Exp. 1: $p \leq 0.0002$; Exp. 2: $p \leq 0.0000$). The plants grown with biochar had 57% and 66% longer panicles than controlled soil in both experiments, respectively. V2 had the longest panicles, which became even longer when the plants were grown in soil amended with biochar (Figure 5b). Similarly, during both experiments, the three varieties produced different numbers of sub-panicles (Exp. 1 and 2: $p \leq 0.0000$), and biochar amendment had a positive effect on the number produced (Exp. 1 and 2: $p \leq 0.0000$). In Exp. 1, there was also an interaction between the variety and soil ($p \leq 0.0000$). V2 grown in soil amended with biochar produced the most sub-panicles (Figure 5c).



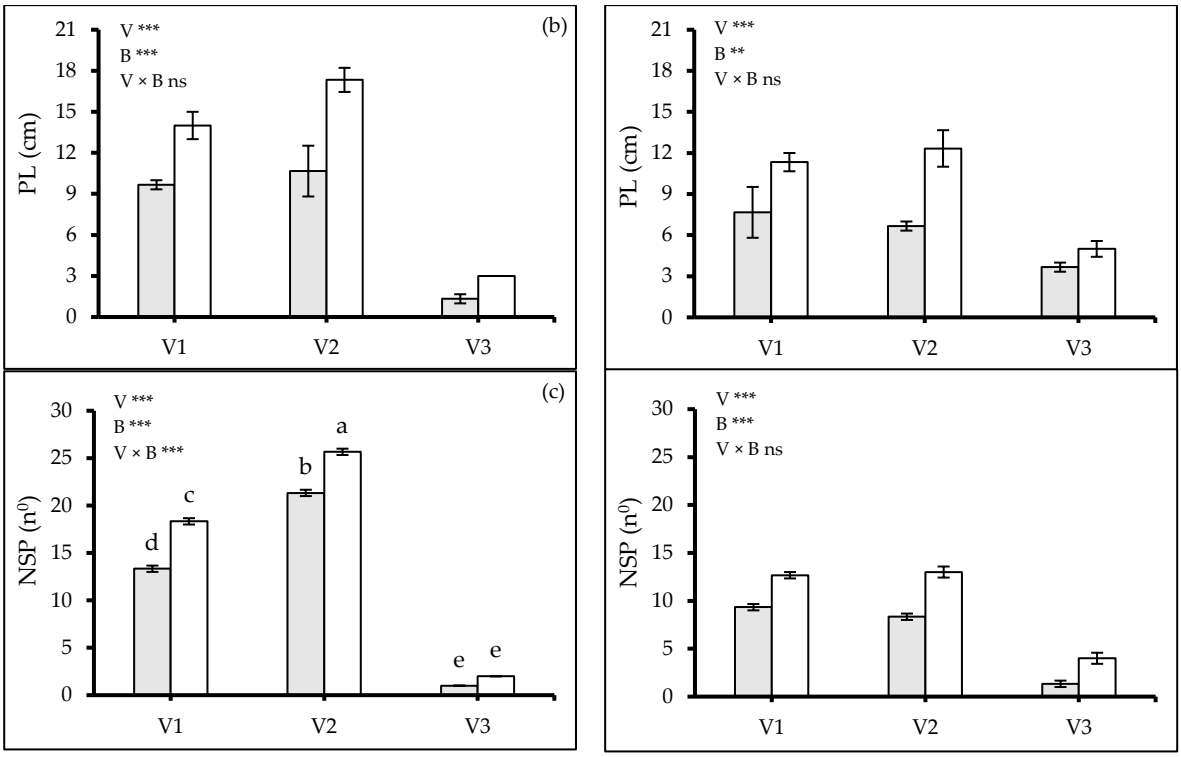



**Figure 5.** (a) Absolute growth rate (AGR), (b) panicle length (PL) and (c) number of subpanicles (NSP) of quinoa (*Chenopodium quinoa* Willd.) varieties treated without and with woodchip biochar. Values are means ($n = 3$) ± S.E. In each graph, different stars above and below the lines indicate significant differences among treatments ($p \leq 0.05$, Tukey's test). F-test significant at *—($p \leq 0.05$), **—($p \leq 0.01$), ***—($p \leq 0.001$). DP—drought period, RP—recovery period. White squares: quinoa variety 1 (V1) without biochar. Black squares: quinoa variety 1 (V1) with biochar; white triangles: quinoa variety 2 (V2) without biochar; black triangles: quinoa variety 2 (V2) with biochar; white circles: quinoa variety 3 (V3) without biochar; black circles: quinoa variety 3 (V3) with biochar; solid lines and grey filled bars—(B0) no biochar, dash lines and unfilled bars—(B2) 2% biochar.

### *7.4.4. Physiological and Water-Related Parameters*

In both experiments, the midday leaf water potential (Ψ) was recorded at three different times: (1) at the end of the 1st drought period ($t_2$), (2) at the end of the 2nd drought period ($t_3$) and (3) after the recovery period ($t_4$). At all times, a difference was recorded between varieties (Exp. 1: $t_2$, $t_3$, $t_4$: $p \leq 0.0000$; Exp. 2: $t_2$: $p \leq 0.0153$, $t_3$: $p \leq 0.0011$) and soil types (Exp. 1: $t_2$: $p \leq 0.0009$, $t_3$: $p \leq 0.0000$, $t_4$: $p \leq 0.0228$; Exp. 2: $t_2$, $t_3$, $t_4$: $p \leq 0.0000$), while there were also interactions at $t_3$ (Exp. 1: $p \leq 0.0092$; Exp. 2: $p \leq 0.0141$) and $t_4$ (Exp. 1: $p \leq 0.0007$; Exp. 2: $p \leq 0.0383$) in both experiments. At $t_2$, V1 and V3 had lower Ψ values than V2. Biochar amendment significantly improved plant water status. This positive effect of biochar was sustained at $t_3$, where V1 and V3 in soil without biochar amendment exhibited the most negative values in both experiments (Figure 6a). Following rehydration at $t_4$, V3 and V1 plants under biochar

application recovered leaf water potential more rapidly, suggesting enhanced recovery potential from drought stress.

The total water consumption by plants in Exp. 1 was not significantly different among all experimental factors, while for Exp. 2 the water consumption significantly varied among soil treatment ($p \leq 0.0000$). Importantly, water consumption by plants treated with biochar was 25% larger than without biochar. Based on this water consumption, *WUEwp* was estimated for two periods: DP (between $t_1$ to $t_3$) and RP (between $t_3$ to $t_4$). For DP, in Exp. 1, *WUEwp* varied between varieties ($p \leq 0.0000$), soil treatments ($p \leq 0.0000$), and there was also an interaction ($p \leq 0.0000$). During Exp. 2, the differences were observed among varieties ($p \leq 0.0000$) but not when they were grown in soil with biochar. More specifically, V3 grown in soil amended with biochar displayed the highest *WUEwp* in both experiments (Figure 6b). For RP in both experiments, a positive effect was observed among varieties (Exp. 1: $p \leq 0.0011$; Exp. 2: $p \leq 0.0000$) and also an interaction (Exp. 1: $p \leq 0.0139$; Exp. 2: $p \leq 0.0000$). There was only an effect of biochar in Exp. 2 ($p \leq 0.0026$). V3 grown in soil with biochar maintained the highest *WUEwp* in both experiments (Figure 6b).

The SPAD value (leaf chlorophyll content index) was measured at $t_1$, $t_3$, and $t_4$ in both experiments (Figure 6c). At $t_1$, $t_3$, and $t_4$, SPAD values differed between varieties (Exp. 1: $t_1$: $p \leq 0.0146$, $t_3$: $p \leq 0.0375$, $t_4$: $p \leq 0.0146$; Exp. 2: $p \leq 0.0218$, $t_3$: $p \leq 0.0077$, $t_4$: $p \leq 0.0026$), however, there was no effect of biochar amendment at $t_1$ but it varied at $t_3$ and $t_4$, ($t_3$: $p \leq 0.0000$, $t_4$: $p \leq 0.0146$) in Exp. 1, while in Exp. 2, it was different at all times ($t_1$, $t_3$, $t_4$: $p \leq 0.0000$). In Exp. 2, a significant interaction was observed at $t_4$ ($p \leq 0.0138$). However, in both experiments, V3 had the highest SPAD values, followed by V1. More importantly, control treatment values were significantly reduced, while a sharp increment was observed for biochar-treated plants in both experiments. The same was observed at $t_4$ in Exp. 1 with a difference between varieties ($p$

≤ *0.0025*) and soil treatments ($p \leq 0.0076$), while for Exp. 2, V3 increased SPAD values but it became more pronounced when grown with biochar.

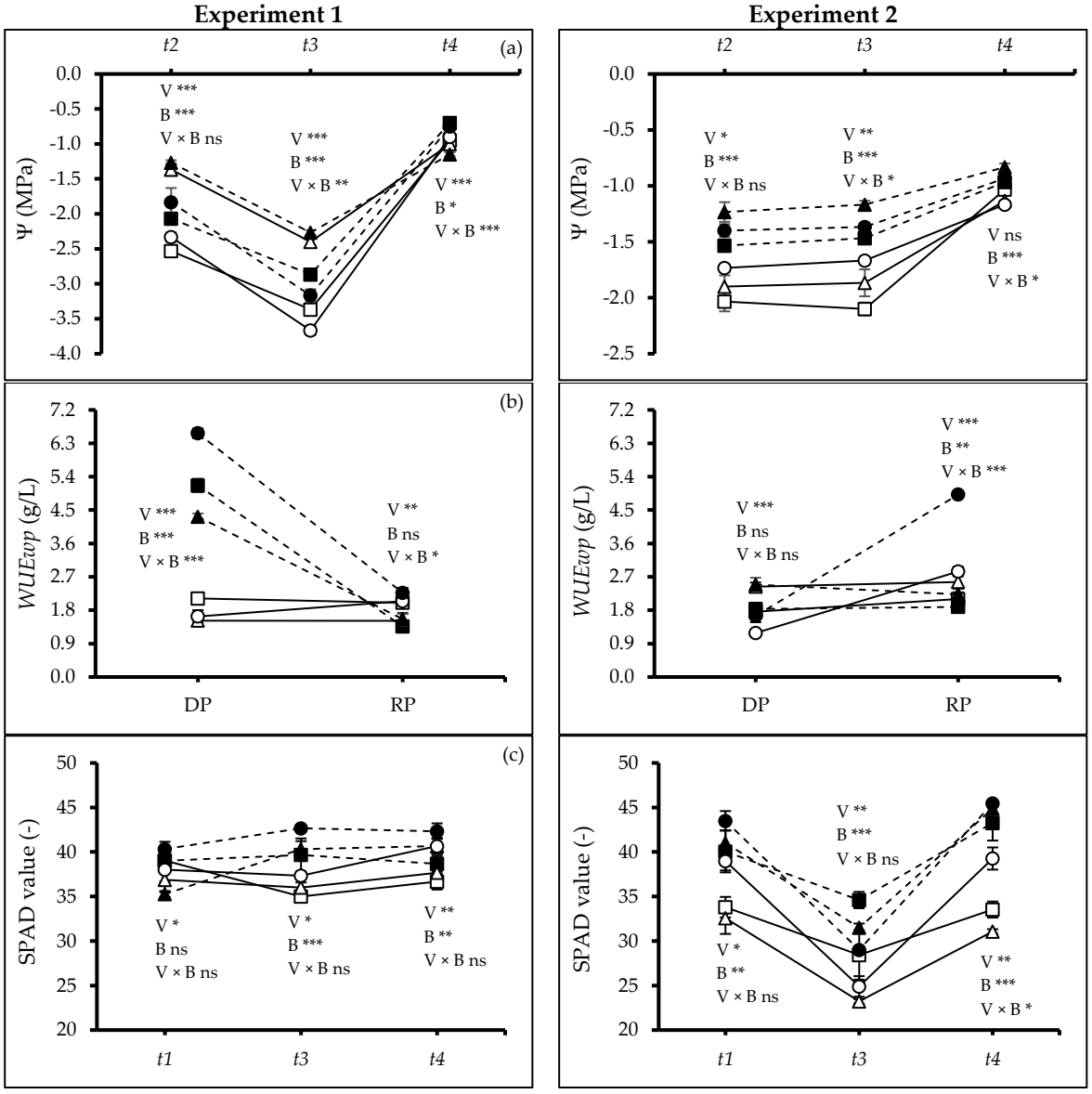



**Figure 6.** (a) Total water potential (Ψ), (b) whole plant water use efficiency (WUEwp) and (c) SPAD index of quinoa (*Chenopodium quinoa* Willd.) varieties treated without and with woodchip biochar. Values are means ($n = 3$) ± S.E. In each graph, different stars above and below the lines indicate significant differences among treatments ($p \leq 0.05$, Tukey's test). F-test significant at *—($p \leq 0.05$), **—($p \leq 0.01$), ***—($p \leq 0.001$). $t_1$—the start of drought, $t_2$—end of 1st drought cycle, $t_3$—end of 2nd drought cycle, and $t_4$—end of the experiment. DP—drought period, RP—recovery period. White squares: quinoa variety 1 (V1) without biochar. Black squares: quinoa variety 1 (V1) with biochar; white triangles: quinoa variety 2 (V2) without biochar; black triangles: quinoa variety 2 (V2) with biochar; white circles: quinoa variety 3 (V3) without biochar; black circles: quinoa variety 3 (V3) with biochar; solid lines—(B0) no biochar, dash lines—(B2) 2% biochar.

### *7.4.5. Carbon and Nitrogen Contents*

At $t_4$, variations were observed in carbon% (C, %) between varieties in Exp. 1 ($p \leq 0.0024$) but not in Exp. 2. However, biochar did not have any effect in Exp. 1 but showed variations in Exp.

2 ($p \leq 0.0335$). The highest carbon% was found in V2 followed by V1 in Exp. 1, while for Exp. 2 plants grown with biochar exhibited higher carbon% (Figure 7a). Biochar had a negative effect on the nitrogen% (N, %) during both experiments (Exp. 1: $p \leq 0.0014$, Exp. 2: $p \leq 0.0018$). It did not vary between varieties in Exp. 1 but a significant variation was recorded in Exp. 2 ($p \leq 0.0100$) (Figure 7b). The results demonstrated that the plants treated with biochar had reduced N in leaves by 21% in both experiments. Plants grown in soil amended with biochar had a greater carbon–nitrogen ratio (C:N) (Exp. 1: $p \leq 0.0026$, Exp. 2: $p \leq 0.0032$) (Figure 7c).

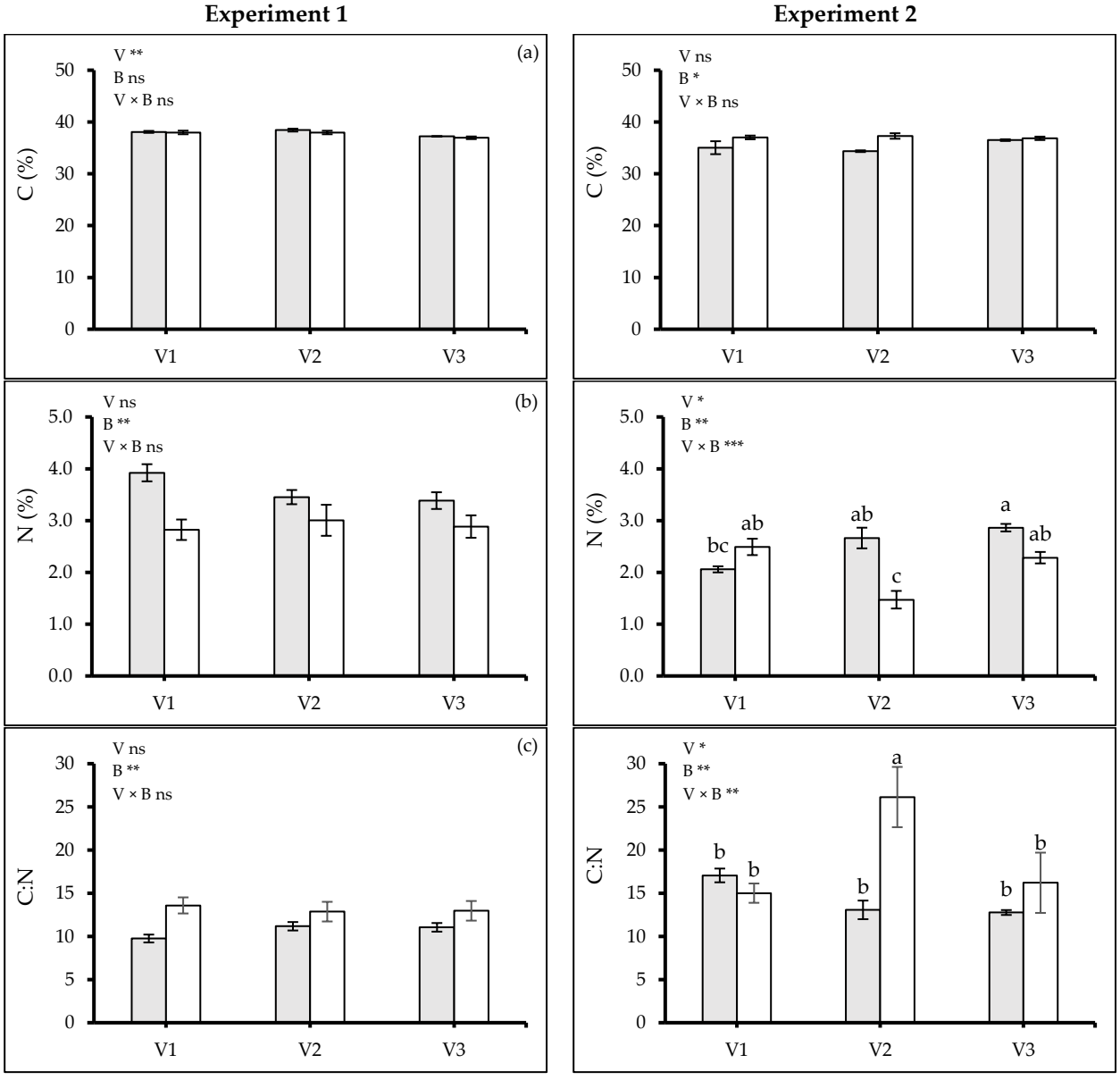



**Figure 7.** (a) Total Carbon% (C, %), (b) total Nitrogen% (N, %), (c) Carbon–Nitrogen ratio (C:N) of quinoa (*Chenopodium quinoa* Willd.) varieties treated without and with woodchip biochar. Values are means ($n = 3$) ± S.E. In each graph, different stars indicate significant differences among treatments ($p \leq 0.05$, Tukey's test). F-test

significant at *—($p \leq 0.05$), **—($p \leq 0.01$), ***—($p \leq 0.001$). V1, V2 and V3—quinoa varieties; grey bars—(B0) no biochar, white bars—(B2) 2% biochar.

### *7.4.6. Correlation Analysis between SPAD Value and Nitrogen Percentages*

The observed relationship between SPAD and nitrogen content was weak, as indicated by low R2 values of 0.252 and 0.1557, for Exp. 1 and 2, respectively (Figure 8). These findings suggest that while SPAD may be a contributing factor to nitrogen variation, it does not fully explain the observed patterns and highlight the need for further investigation into other factors influencing nitrogen dynamics.

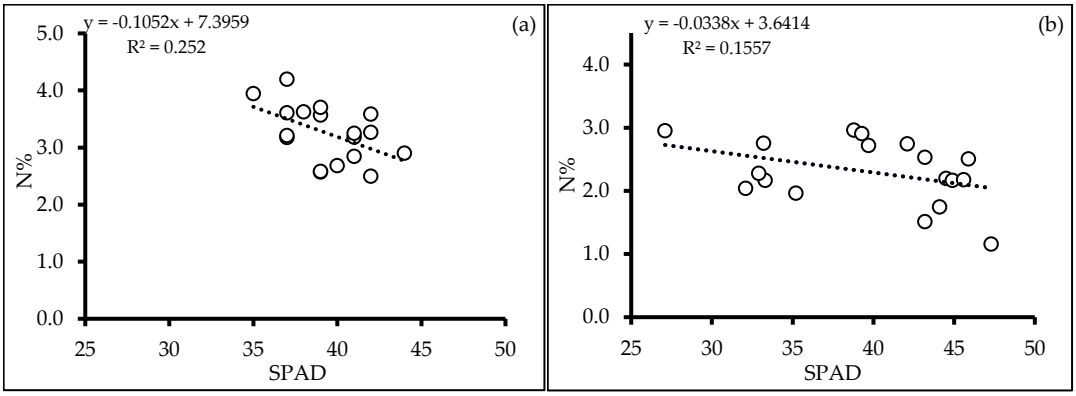



**Figure 8**. Linear correlation analysis between SPAD values and N content (%) in leaves from experiment 1 (a) and experiment 2 (b) of quinoa (*Chenopodium quinoa* Willd.) varieties treated without and with woodchip biochar. White circles are measurements.

### *7.4.7. PCA Correlation Analysis at $t_4$*

A scatter plot was generated to visualize the relationships among plant traits (Figure 9). The PCA suggested potential positive correlations between plant height (PH) and number of leaves (NL), number of sub-panicles (NSP) and panicle length (PL), carbon percentage (C%) and nitrogen percentage (N%), SPAD, and various root parameters (volume, fresh and dry weight, surface and projected area, and length). Conversely, a potential negative correlation was observed between root to shoot ratio (R:S) and both fresh weight (FW) and dry weight (DW).

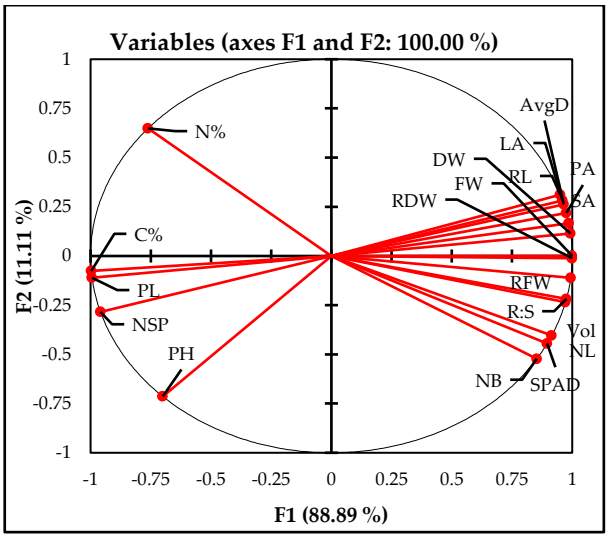



**Figure 9.** PCA correlation analysis of growth-related attributes at $t_4$ (similar observations were recorded for both experiments). Red arrows indicate the variables included in the PCA, with arrow length representing the contribution of each variable to the principal components. PH—plant height, NSP—number of sub-panicles, PL—panicle length, C%—carbon% in the leaves, N%—nitrogen% in leaves, NB—number of branches, SPAD—SPAD index, NL—number of leaves, Vol—root volume, R:S—root to shoot ratio, RFW—root fresh weight, RDW—root dry weight, FW—fresh weight, DW—dry weight, SA—root surface area, PA—root projected area, AvgD—average density and RL—root length.

## 7.5. DISCUSSION

### *7.5.1. Influence of Biochar Application on Growth under Drought Conditions*

The woodchip biochar application significantly enhanced growth under water stress during the vegetative phase for all varieties but especially for the drought-resistant variety UAFQ7 (V3) (Figures 1 and 2). The drought-sensitive variety Titicaca was more negatively affected by prolonged water shortage than the others even when it grew in soil amended with biochar (Rivelli et al., 2024). The experimental results showed significant differences between varieties in terms of plant height, number of leaves and branches, and leaf area when grown with and without biochar amendment (Figures 1 and 2), which is in line with previous studies (Akram et al., 2023; Rivelli et al., 2024; Akram et al., 2024). The drought-sensitive variety Titicaca produced more biomass when it grew in the biochar-amended soil as previously observed (Akram et al., 2023; Rivelli et al., 2024). Panicle numbers and length are early determinants of grain yield (Raza et al., 2023), and water stress affected these parameters negatively (Saddiq et al., 2021). Our results confirmed that the application of biochar as soil amendment offers a

promising mitigation strategy, as shown before (Ali et al., 2017). García-Parra et al. (2020) reported that quinoa's developmental stages influenced the panicle formation and other yield components (Noulas et al., 2017). The faster vegetative growth of UAFQ7 results in increasing production of leaves and branches, and a larger biomass. However, the enhanced vegetative growth came at the expense of panicle formation (Manjarres-Hernández et al., 2021). Some UAFQ7 plants even exhibit shorter panicles or lack sub-panicles entirely (Figure 5b,c). This suggests a potential trade-off between vegetative growth and panicle development under biochar application during water limitations.

### *7.5.2. Influence of Biochar Application on Physiology and Water-Related Traits under Drought Conditions*

Our study revealed that biochar addition to the soil significantly improved the plants' water status by making the midday leaf water potential less negative than plants grown in unamended soil (Figure 6a). Some studies found that quinoa initiates stomatal closure when leaf water potential falls below −1.2 to −1.6 MPa (Jacobsen et al., 2009; Sun et al., 2014). Accordingly, the stomata might have been closed at the end of drought stress in the present study as the leaf water potential had dropped to below −2.0 MPa (Experiment 1) and −1.5 MPa (Experiment 2), particularly for those grown in soil without biochar amendment. The lower water potential could be due to the intrinsic lower osmotic potential of the plants (Jensen et al., 2000). The osmolyte accumulation contributes to lowering the water potential and enhancing root growth in quinoa (Jensen et al., 2000; Saddiq et al., 2021). Our findings support the hypothesis that biochar application helps plants to maintain water status under drought. Gaskin et al. (2010) reported an increased accumulation of osmotically active substances like potassium ($K^+$) in plant tissues when biochar was added to the soil. The high cation content in biochar may facilitate improved plant water uptake and higher water use efficiency. Besides, drought tolerance in quinoa has also been linked to dense root elongation supported by our study

(Figures 3 and 4) and the presence of hygroscopic calcium oxalate crystals in leaf cuticles, which can further minimize water loss (Riccardi et al., 2014). Issa Ali et al. (2019) observed the same for Titicaca and L119 varieties. Biochar application improved SPAD values in quinoa varieties under water stress (Figure 6c). The increase in SPAD could be caused by the large surface area of biochar, facilitating enhanced adhesion and cohesion with water and increased soil acidity and subsequent heightened production of photosynthetic pigments. Anee et al. (2022) also found an increased SPAD index of wheat when plants were grown in soil amended with biochar. However, the increased SPAD value was seemingly not associated with the changes in leaf nitrogen content, as the latter was negatively affected by biochar amendment.

### *7.5.3. Influence of Biochar Application on Root Morphology and Leaf Nutrient Concentrations under Drought Stress*

Biochar application stimulated the root growth resulting in a deeper root system (Figures 3 and 4), which could further enhance water acquisition through the finer pores within the biochar itself, reducing the negative leaf water potential and subsequently improving plant water use efficiency (Figure 6a,b). Jabborova et al. (2021) reported significant improvement in the root morphology of okra under woody biochar applications likely caused by an enhanced nutrient acquisition (Elad et al., 2010) and potentially improving the soil microbial community (Joseph et al., 2021). Biochar amendments significantly enhanced the leaf's relative water content, osmotic potential, photosynthetic rate, and WUE reflected in higher SPAD values (Figure 6c). Similar results were found for maize cob biochar (Ramzani et al., 2017) and woody biochar (Kammann et al., 2011) on quinoa and crops like rice (Zhang et al., 2012), wheat (Haider et al., 2020), maize (Foster et al., 2016), soybean (Mannan et al., 2017), and tomato (Agbna et al., 2017). Our findings on increased root-to-shoot ratio and absolute growth rate under woodchip biochar application (Figures 3d and 5a) align with previous studies (Noguera et al., 2010; Liao et al., 2019). The woodchip biochar has probably increased the nutrient absorption capacity of

the quinoa roots. Additionally, biochar application may contribute to better plant health by accelerating systemic defenses and reducing the soil pathogen load (Elad et al., 2010). Collectively, these mechanisms likely contribute to the observed improvement in plant growth efficiency under biochar application. Furthermore, woodchip biochar addition can increase soil organic carbon, potentially leading to a higher carbon-nitrogen ratio (Figure 7), as reported by Zhao et al. (2020). This can enhance soil nitrification and improve nitrogen bioavailability, ultimately promoting plant biomass accumulation (Figure 2).

## 7.6. CONCLUSION

Biochar application improved plant water status, growth, and root development. The positive effect of woodchip biochar is likely caused by influencing several mechanisms like improved water uptake, soil features, and enhanced nutrient availability. However, biochar application may lead to a trade-off between vegetative growth and panicle development under water stress in some quinoa varieties. Overall, this study suggests that a 2% application of woodchip biochar is a promising strategy for enhancing drought tolerance and growth of quinoa, although the positive effect varies between varieties (Titicaca < Quipu < UAFQ7). As these varieties responded differently to water stress conditions, so, further investigation is needed to elucidate the underlying mechanisms by which biochar influences stomatal behavior and drought response among different quinoa varieties.

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# CHAPTER 8

# QUINOA VARIETAL RESPONSES TO BIOCHAR AND WATER STRESS: A COMPARATIVE STUDY OF STOMATAL MORPHOLOGY AND GASEOUS EXCHANGE

*Draft in preparation for Journal of Soil Science and Plant Nutrition*

## 8.1. ABSTRACT

Quinoa (*Chenopodium quinoa* Willd.), known for its exceptional adaptability to adverse climate change conditions, particularly drought, relies significantly on stomatal regulation to manage water loss and gas exchange. Previous studies have shown the role of biochar in stomatal regulation under drought stress. For that purpose, the present study explores the impact of biochar, a carbon-rich soil amendment, on the stomatal morphology and drought response of three quinoa varieties, Titicaca, Quipu, and UAFQ7. The plants were grown in soil without (B0) and with (B2) 2% of woodchip biochar (w/w) amendment. The water stress was applied at the 12-leaf stage by withheld watering until the soil in the pots reached the PWP. Biochar application was found to enhance plant water status and mitigate the adverse effects of water stress on quinoa. Specifically, biochar improved stomatal conductance and transpiration rates, indicating better water use efficiency under drought conditions. Among the varieties tested, Quipu showed the highest water potential, while UAFQ7 exhibited more recovery post-stress, increased stomatal density and larger guard cell dimensions. These findings highlight biochar's potential to enhance quinoa's drought resilience by positively influencing stomatal behavior, thereby contributing to more effective water resource management in water-limited environments.

*Keywords: Stomatal regulations; stomatal aperture dimensions; transpiration; water relation*

## 8.2. INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.), a resilient Andean grain, has gained significant global attention due to its exceptional nutritional qualities and adaptability to adverse environmental conditions, particularly drought (Nazeer et al., 2022). Its ability to thrive under water-limited conditions is attributed to a range of drought resistance mechanisms, including drought escape, tolerance, and avoidance strategies (Ain et al., 2023). These mechanisms are evidenced by faster vegetative growth, early maturation, tissue elasticity, low osmotic potential, and a well-developed root system capable of deep water extraction (Hinojosa et al., 2018).

Stomatal behaviour, as the primary regulator of gas exchange and water loss, plays a crucial role in quinoa's drought tolerance. Previous studies have highlighted the close relationship between stomatal conductance, photosynthetic efficiency and drought response in quinoa, with variations observed across different varieties (Killi et al., 2017; Manaa et al., 2021). Abscisic acid (ABA), a plant hormone, has been implicated in regulating stomatal closure under drought conditions, although the precise mechanisms remain a subject of ongoing research (Joshi et al., 2024). Stomatal morphology, including stomatal density, size, and pore aperture, also influences water use efficiency (WUE) and overall plant performance.

Recent advances in agricultural practices have explored the use of biochar, a carbon-rich material derived from the pyrolysis of organic materials (Mašek et al., 2024), as a soil amendment to enhance plant resilience under water stress (Afshar et al., 2016; Murtaza et al., 2024). Biochar has been shown to improve soil properties, including water retention, nutrient availability, and microbial activity (Li et al., 2021). Moreover, soil biochar application has been reported to influence stomatal morphology and behaviour, potentially contributing to improved WUE and drought tolerance in crops (Hou et al., 2023). Under severe water stress, quinoa leaves exhibit a substantial increase in palisade and spongy layer thickness, which helps in enhancing mesophyll conductance that ultimately aids in more $CO_2$ diffusion, whose role in increasing photosynthesis is crucial (Al-Naggar et al., 2018).

The current study is the extension of our previous studies (Akram et al., 2023; Rivelli et al., 2024; Akram et al., 2024a; Akram et al., 2024b) that demonstrated that the application of woodchip biochar to the soil helps the plants to cope the adverse effects of water stress applied during the vegetative growing cycle. They displayed that biochar application significantly enhances the above and below ground biomass and substantially increased the yield contributing traits by improving plant water status. So, the current study investigates the responses of three quinoa varieties, Titicaca, Quipu and UAFQ7, to woodchip biochar application specifically examining changes in stomatal morphology, as well as key physiological parameters, such as total water potential, stomatal conductance, and transpiration. By applying a period of water stress at early growth stages, we aim to elucidate the comparative effects of biochar on quinoa's drought response, with a focus on stomatal regulation and gas exchange. The insights gained from this study will contribute to the development of more drought-resistant quinoa varieties and inform sustainable agricultural practices under changing climatic conditions.

## 8.3. MATERIAL AND METHODS

A greenhouse pot experiment was conducted at the Department of Plant and Environmental Sciences, University of Copenhagen, Taastrup, Denmark. Three quinoa varieties (V1, V2 and V3) and two biochar rates (0, 2% DW) were used in the experiment. The experiment was conducted by following factorial completely randomized design by considering variety and biochar as two main factors. The varieties used in the experiment were described in Table 1. Ten seeds were sown in plastic pots filled with 5 kg of either sandy soil collected from Tasstrup Garden, or a mixture of sandy soil and 2% woodchip biochar collected from Nerabiochar Company (Ivrea, Torino, Italy). Based on its properties already described in Akram et al., (2023), the biochar is classified as Class I biochar.

Both the soil and the soil-biochar mixture had undergone a previous crop cycle, so, the biochar had been sitting for more than four months prior to the experiment. From emergence to the 12-leaf stage, all plants were kept under well-watered conditions. At 12 leaf stage, the plants undergo two successive water stress cycles (one after another) by raising the soil water content up to 100% by with-held watering until all available water was consumed.

**Table 1:** Code, origins and collection site of varieties used in the experiment

| Variety | Code | Origin | Collection site |
|---|---|---|---|
| Titicaca | V1 | Denmark | University of Copenhagen, Denmark |
| Quipu | V2 | Italy | Tuttoquinoa di Vannuzzi Dario, Manciano, Italy |
| UAFQ7 | V3 | Pakistan | Muhammad Nawaz Shareef University of Agriculture (MNSUAM), Multan, Pakistan |

Then, one other cycle was repeated with same approach. After the two successive water stress cycles, the plants went through a recovery period from water stress and then at flowering initiation they were cut and a set of parameters were recorded.

### *8.3.1. Parameters observed*

Total leaf water potential (Ψ) was measured by using pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA) on five fully expanded upper canopy leaves between, 12:00 and 13:00 h at the end of each drought period and after the plants had recovered from the drought. For that purpose, the young fully expanded leaf was separated from the plant along with its petiole. The leaf was then placed in the chamber with the petiole protruding outside. Afterwards, the pressure was increased gradually in the chamber and the reading was recorded immediately when a droplet of water came out from the protruding petiole surface.

During the drought period, *in situ* stomatal conductance (*gs*) and respiration rate (*E*) were measured daily using a steady-state diffusion porometer (model SC-1, Deca-gon Devices, Inc., Pullman, WA, USA). The readings were recorded at the start of stress, at the end of each water stress cycle and after recovery from stress conditions. The porometer was calibrated daily prior

to measurements. Measurements of *gs* and *E* were recorded on the adaxial surface of the uppermost, fully expanded leaf exposed to direct sunlight.

For stomatal morphology, leaf epidermal imprints were acquired from the abaxial leaf surface. A thin layer of crystal-clear nail polish was applied to the cleaned leaf and allowed to solidify for 10-15 minutes. Then, the hardened nail polish film was carefully removed using adhesive tape and placed on a microscope slide. Stomatal density (SD) was determined from digital images captured using a Leica DMRD light microscope (Leica Mikroskopie & Systeme GmbH, Wetzlar, Germany) equipped with a camera (Leica DFC 420). A 1 $mm^2$ grid overlay facilitated stomatal counting. Stomatal size parameters, including guard cell length (Ls), guard cell width (Ws), stomatal pore aperture length (La), and stomatal pore aperture width (Wa), were measured using ImageJ software v. 1.43u. Stomatal aperture (SA) was calculated according to Doheny-Adams et al. (2012) using the equation: $a_{max} = (\pi \times Wa \times La)/4$.

### *8.3.2. Statistical Analysis*

A two-way analysis of variance was applied using the statistical software RStudio version 4.2.0, considering variety and biochar level as the main factors. Mean comparisons among treatment groups were performed using Tukey's Honestly Significant Difference (HSD) test at a significance level of $p \leq 0.05$.

## 8.4. RESULTS

### *8.4.1. Total water potential*

Total water potential (Ψ) was significantly affected by both variety and biochar treatment. The V2 consistently demonstrated superior water status compared to other varieties, as indicated by less negative Ψ values throughout the experiment. Biochar application mitigated the decline of Ψ, particularly during the second drought cycle. While all varieties exhibited recovery during the post-stress period, with V2 demonstrating the most pronounced recovery.

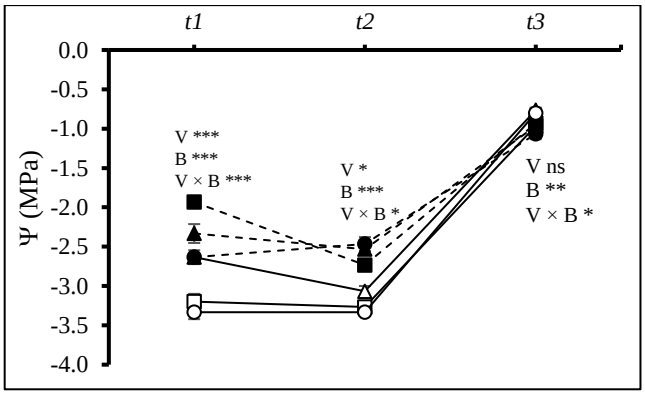


**Figure:** Total water potential of different quinoa varieties grown without and with biochar. Values are means (n =3) ± S.E. In each graph, different stars above and below the lines indicate significant differences among treatments ($p \leq 0.05$, Tukey's test). F-test significant at *—($p \leq 0.05$), **—($p \leq 0.01$), ***—($p \leq 0.001$). $t_1$—end of 1st drought cycle, $t_2$—end of 2nd drought cycle, $t_3$—recovery from stress treatment. White squares: quinoa variety 1 (V1) without biochar. Black squares: quinoa variety 1 (V1) with biochar; white triangles: quinoa variety 2 (V2) without biochar; black triangles: quinoa variety 2 (V2) with biochar; white circles: quinoa variety 3 (V3) without biochar; black circles: quinoa variety 3 (V3) with biochar; solid lines—(B0) no biochar, dash lines—(B2) 2% biochar.

### *8.4.2. Gas-exchange attributes*

Stomatal conductance (*gs*) and transpiration rate (*E*) exhibited significant reductions in response to progressive water stress conditions across all quinoa varieties tested (V1, V2, V3) and under both control (B0) and biochar-amended soil (B2). Biochar application resulted in a significant enhancement of both physiological parameters compared to the non-treated soil. Furthermore, quinoa variety significantly influenced stomatal conductance and transpiration rate, with V2 consistently demonstrating superior performance relative to V1 and V3. As per the results, V3 regulated its stomatal activity more efficiently as it sensed the soil drying conditions by reducing its stomatal conductance and transpiration compared to other varieties. The interactive effects of biochar and variety were also significant, indicating differential responses among varieties to soil biochar application.

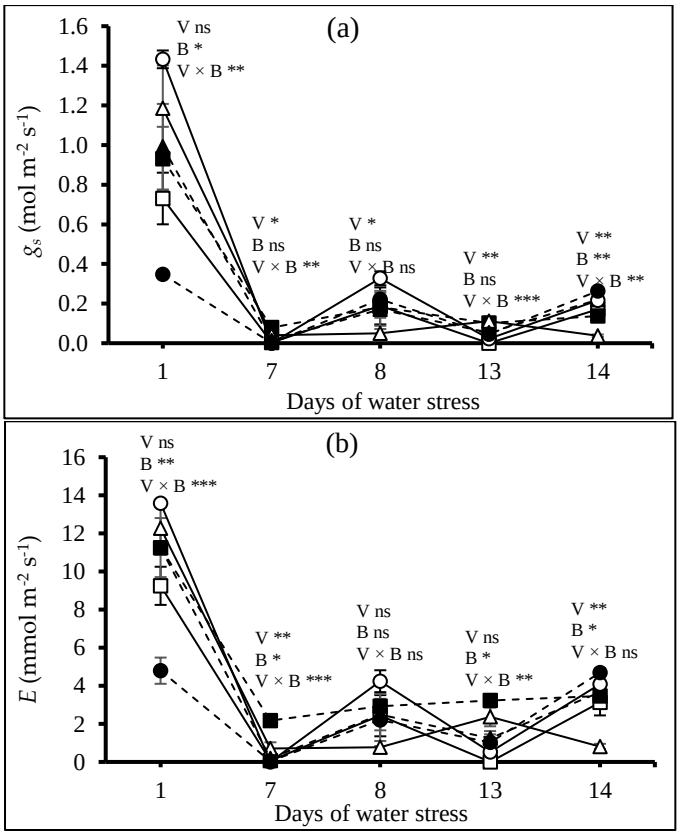


**Figure:** (a) Stomatal conductance (*gs*) and (b) transpiration rate (*E*) of different quinoa varieties grown without and with woodchip biochar. Values are means (n =3) ± S.E. In each graph, different stars above the lines indicate significant differences among treatments ($p \leq 0.05$, Tukey's test). F-test significant at *—($p \leq 0.05$), **—($p \leq 0.01$), ***—($p \leq 0.001$). 1—start of stress, 7—end of 1st stress cycle, 8—recovery from 1st stress cycle, 13—end of 2nd stress cycle and 14—recovery from 2nd stress cycle. White squares: quinoa variety 1 (V1) without biochar. Black squares: quinoa variety 1 (V1) with biochar; white triangles: quinoa variety 2 (V2) without biochar; black triangles: quinoa variety 2 (V2) with biochar; white circles: quinoa variety 3 (V3) without biochar; black circles: quinoa variety 3 (V3) with biochar; solid lines—(B0) no biochar, dash lines—(B2) 2% biochar.

### *8.4.3. Stomatal morphology*

Stomatal morphology, as assessed by guard cell length (Ls) and width (Ws), stomatal aperture length (La), width (Wa), density (SD), and maximum aperture ($a_{max}$), varied significantly among variety and biochar treatment. The Pakistani (V3) consistently exhibited larger stomatal dimensions (Ls, Ws, La, Wa) and higher stomatal density (SD) compared to V1 and V2. These differences were statistically significant ($p \leq 0.05$). Biochar application resulted in a significant increase in stomatal density (SD) and stomatal aperture ($a_{max}$) ($p \leq 0.05$). These morphological variations suggest potential differences in gas exchange capacity and water use efficiency among quinoa varieties and their response to biochar.

**Table :** Stomatal morphology of different quinoa varieties grown without and with biochar recorded at the end of water stress cycles.

| Experimental Factors | Ls (mm) | Ws (mm) | La (mm) | Wa (mm) | SD ($mm^{-2}$) | $a_{(max)}$ ($\mu m^2$) |
|---|---|---|---|---|---|---|
| Variety (V) | | | | | | |
| V1 | 50.5 b | 29.4 c | 38.8 a | 11.5 c | 82.0 b | 414.4 a |
| V2 | 49.3 b | 32.2 b | 32.0 b | 12.7 b | 75.5 c | 414.3 a |
| V3 | 54.4 a | 35.0 a | 31.9 b | 13.9 a | 96.8 a | 306.8 b |
| Biochar (B) | | | | | | |
| B0 | 48.4 b | 30.4 b | 30.8 b | 11.7 b | 72.3 b | 327.7 b |
| B2 | 54.5 a | 34.0 a | 37.7 a | 13.7 a | 97.2 a | 429.3 a |
| Level of Significance | | | | | | |
| V | ** | *** | *** | *** | *** | *** |
| B | ** | *** | *** | *** | *** | *** |
| V × B | ns | ns | ns | ns | *** | ns |

Ls: guard cell length, Ws**:** guard cell width, La**:** stomatal aperture length, Wa: stomatal aperture width, SD: stomatal density, A(max): maximum stomatal aperture

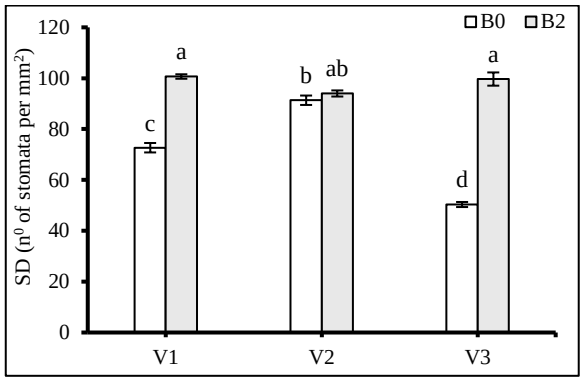


**Figure:** Stomatal density (SD) of different quinoa varieties grown without and with biochar recorded at the end of water stress cycles. Values are means (n =3) ± S.E. In each graph, different letters indicate significant differences among treatments ($p \leq 0.05$, Tukey's test). F-test significant at *—($p \leq 0.05$), **—($p \leq 0.01$), ***—($p \leq 0.001$).

## 8.5. DISCUSSION

Under water stress conditions, the decrease of water plant availability affects the physiological performance. It is commonly noted that plants actively lower their osmotic potential as a means of maintaining turgor in environments characterized by water scarcity (Mohammadi et al., 2023). In the current trial, it was noted that the leaf water potential decreased in response to the reduction of water content in the soil, thereby, the osmotic potential also decreased under such conditions, ultimately affecting the growth of plants (Sajid et al., 2023). However, the soil biochar application effectively decreased the negative effects of water stress on the water potential of plants (Zhang et al., 2023; Obadi et al., 2023). Biochar nutrients' ability to sustain

high turgor potential and water content in cells, even under conditions of increasing osmotic potential caused by solute accumulation, is the mechanism responsible for improved water relations (Afaf et al., 2023). Moreover, the higher water potential was observed in V2 followed by V1 and V3. The higher water potential might be due to more accumulation of solutes under water stress to maintain cell's turgor potential already reported by Razzaghi et al. (2014).

In the present study, stomatal conductance (*gs*) and transpiration rate (*E*) of all quinoa varieties were decreased under water stress; however, the less effect in *gs* and *E* was observed in V2 followed by V1 than other varieties. According to Lazar (2002), plants typically close their stomata when stressful situations arise in order to conserve water. This lowers photosynthesis, indicating that a decrease in *gs* is the primary cause of the arrest of *E* and is correlated with a decrease in chlorophyll levels. The secondary effects resulting from stomatal closure and inhibition of photosynthetic enzymes may be the consequence of water stress (Zahra et al., 2023). The current study findings are corroborated with the outcomes of Rehman et al. (2024), Ishfaq et al. (2024), and Asargew et al. (2024). However, the biochar amendment enhances stomatal conductance and transpiration under water stress that might be due to their signaling behaviors to avert the stress-induced damage. These findings suggest that biochar may play a role in mitigating the negative effects of water stress on stomatal function, potentially through hormonal signaling pathways that influence guard cell behavior. The increase in *gs* and *E* was observed by the biochar soil amendment. The current study findings are with the outcomes of Murtaza et al. (2024), Işik and Ortaş (2024), and Jia et al. (2024).

Research has indicated that when drought severity increases, stomatal density varies depending on the species, either increasing or decreasing, or increasing and then decreasing (Xu et al., 2008; Li et al., 2017; Wang et al., 2019). During water stress, stomatal width and aperture dramatically shrank and underwent the following processes: guard cells went from completely dry to opening, and surrounding leaf cells shrank from smooth to severe (Hu et al., 2023). In

our trial, the maximum guard cell length (Ls), width (Ws), stomatal aperture length (La), width (Wa), density (SD) was observed in V3 followed by V1 and V2 varieties. Smaller and fewer stomatal openings help preserve water by minimizing the amount of water lost through transpiration. This alteration aids plants in withstanding difficult environmental conditions. The observed changes in stomatal morphology and function highlight the adaptive strategies employed by quinoa to cope with water stress. Understanding the genetic basis of these traits could facilitate the development of drought-tolerant varieties. The genetic mechanisms governing stomatal attributes and regulation must be understood in order to improve stress tolerance, regulate development rate, and maintain water balance (Hasanuzzaman et al., 2023).

## 8.6. CONCLUSIONS

This study highlights the role of biochar in improving drought resilience in quinoa through its effects on stomatal morphology and gas exchange. Among the varieties studied, Quipu (V2) showed better water retention and recovery under water stress, while UAFQ7 (V3) demonstrated effective stomatal regulation with larger stomatal dimensions and higher density. Biochar application enhanced these features, leading to better water use efficiency across all varieties. These findings suggest that combining biochar with specific quinoa varieties, like Quipu and UAFQ7, could be a valuable strategy for improving drought tolerance in the cultivation of the specie. Further research is needed to elucidate the mechanisms through which biochar enhances drought tolerance in quinoa, including its effects on nutrient uptake, and plant hormone signaling. This investigation will contribute to the development of sustainable agricultural practices for quinoa production under water-limited conditions and identification of more drought-tolerant varieties can be used in the future breeding quinoa programs.

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# CHAPTER 9

# GENERAL CONCLUSION

Biochar, a carbon-rich material produced from the pyrolysis of organic matter, has emerged as a promising strategy for enhancing agricultural productivity, particularly in regions facing water scarcity. Among the different climate-resilient crops, quinoa, a pseudocereal with high nutritional value, has been extensively studied during this investigation to evaluate its response to biochar-amended soils under drought-stressed conditions.

The current study has consistently demonstrated the positive influence of woodchip biochar compared to other organic amendments, on quinoa growth, water use efficiency, and overall adaptability under water limitations. We found that woodchip biochar significantly promoted quinoa's growth, while biochar from vineyard pruning had a negative impact under water stress conditions applied during vegetative growing cycle, highlighting the importance of the type of amendments (feedstock and pyrolysis conditions etc.) to identify for a specific soil and crop. Further experimentation revealed that a 2% application rate of woodchip biochar under water shortage conditions optimized quinoa growth and yield-contributing traits. The rate more than 2% did not yield significant benefits or may even had negative consequences. This also suggested that biochar efficiency depends upon plant type and application rate also evident in other studies. The 2% rate of biochar positively influenced soil physico-chemical properties, such as bulk density, soil pH and electrical conductivity that could increase nutrient availability. However, excessive biochar application can lead to negative soil charges that bind nutrients, reducing their availability to plants and hindering quinoa growth.

The study was further elaborated to investigate the responses of different quinoa varieties to the optimized dose under water stress conditions. The results highlighted that responses can vary depending on the cultivated quinoa variety. For instance, the Pakistani UAFQ7 and the Italian Quipu exhibited more pronounced responses to biochar-amended soils than others by enhanced

growth and yield-contributing traits under water stress limitations. Therefore, adapting biochar application strategies to the specific needs of the crop and the existing environmental conditions is paramount. The notable response of these varieties was observed in under-ground traits i.e., roots suggesting that although varieties from Salares ecotypes (UAFQ7 and Quipu) have longer root system, but it substantially enhanced due to biochar presence in the soil that significantly contributed to the overall adaptability under water stress conditions. Additionally, biochar positively influenced stomatal morphology and gas exchange, enabling above mentioned varieties to recover more effectively from water stress through efficient stomatal regulation, characterized by larger stomatal dimensions and higher density. Overall, biochar emerges as a valuable tool for enhancing quinoa adaptability to drought and promoting sustainable agricultural practices.

The research conducted has provided valuable insights into the benefits of biochar for quinoa, but further studies are recommended to fully understand its long-term impacts and optimize its application in various agricultural scenarios. For instance, long-term field experiments are essential to assess the sustainability of biochar-amended soils and the potential for nutrient leaching or other unintended consequences. Additionally, exploring the interactions between biochar and other soil amendments or agricultural practices can help identify optimal combinations for enhancing quinoa productivity and tolerance based on genotypes.

Furthermore, it's essential to consider the economic and environmental implications of biochar use in agriculture. While biochar can provide significant benefits, its production and application can involve energy and resource inputs. Therefore, it's essential to evaluate biochar use's overall cost-benefit ratio and identify strategies for minimizing its environmental footprint.

# LIST OF PUBLICATIONS

Results of this PhD thesis have been published in international peer-reviewed journal or are currently under drafting.

The list of all peer-reviewed papers submitted manuscripts and drafts, oral and poster presentations is given below.

***Peer-reviewed papers***

- Akram, M.Z., Libutti, A., & Rivelli, A.R. (2024). Drought stress in quinoa: effects, responsive mechanisms, and management through biochar amended soil: A Review. *Agriculture*, 14(8), 1418.
- Akram, M.Z., Libutti, A., & Rivelli, A.R. (2023). Evaluation of vegetative development of quinoa under water stress by applying different organic amendments. *Agronomy*, 13(5), 1412.
- Rivelli, A.R., Akram, M.Z., & Libutti, A. (2024). Woody biochar rate and water shortage impact on early growth stages of *Chenopodium quinoa* Willd. *Agronomy*, 14(1), 53.
- Akram, M.Z., Rivelli, A.R., Libutti, A., Liu, F., & Andreasen, C. (2024). Mitigation of drought stress for Quinoa (*Chenopodium quinoa* Willd.) varieties using woodchip biochar-amended soil. *Plants*, 13(16), 2279.

***Submitted manuscripts and drafts***

- Akram M.Z, Libutti A., Andreasen C., Liu F. & Rivelli A.R. (2024). Biochar addition promoted growth of five quinoa varieties under water stress through improved physiological attributes, plant water status and water use efficiency (*Drafted*).
- Akram M.Z, Libutti A., Andreasen C., Liu F. & Rivelli A.R. (2024). Quinoa varietal responses to biochar and water stress: a comparative study of stomatal morphology and gaseous exchange (*Drafted*).

***Oral and poster presentations***

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