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RECUPERACIÓN DE UN SUELO SALINO- SÓDICO CON ENMIENDAS COMBINADAS: IMPACTO SOBRE EL PERFORMANCE DE QUINOA Y CALIDAD BIOLÓGICA DEL SUELO

(RECLAMATION OF SALINE-SODIC SOIL WITH COMBINED AMENDMENTS: IMPACT ON QUINOA PERFORMANCE AND BIOLOGICAL SOIL QUALITY)

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HS = humic substances; B + G = biochar + gypsum; B + HS = biochar + humic substances; HS + G = humic substances + gypsum; B + HS + G = biochar + humic substances + gypsum. Source: Elaborated with own data.

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RECUPERACIÓN DE UN SUELO SALINO- SÓDICO CON ENMIENDAS COMBINADAS: IMPACTO SOBRE EL PERFORMANCE DE QUINOA Y CALIDAD BIOLÓGICA DEL SUELO.

RECLAMATION OF SALINE-SODIC SOIL WITH COMBINED AMENDMENTS: IMPACT ON QUINOA PERFORMANCE AND BIOLOGICAL SOIL QUALITY

RESUMEN

Evaluamos los efectos individuales y sinérgicos de la aplicación de Biocarbón (B), Sustancias Húmicas (SH), Yeso (Y) sobre las propiedades de un suelo salino-sódico, el crecimiento de plantas de quínoa y la calidad de la semilla (polifenoles, proteína y rendimiento). Los tratamientos incluyeron (B) 22 t ha^{-1}, (SH) 5 kg ha^{-1}, (Y) 47.7 t ha^{-1}. Dos genotipos de quínoa seleccionados en Zonas Áridas (AZ) fueron establecidos en ocho tratamientos. El tratamiento combinado B + SH + Y incrementó la biomasa radicular en los genotipos AZ-51 (206%) y AZ-103 (176%) comparado al control. Asimismo la conductancia estomática, índice de clorofila y rendimiento de las semillas incrementaron significativamente en todos los suelos tratados del genotipo AZ-103. El mismo parámetro en el genotipo AZ-51 incrementó en diferentes tratamientos. Además, la Conductividad Eléctrica (ECe); Razón de Adsorción de Sodio (RAS) y Porcentaje de Sodio Intercambiable (PSI) disminuyeron significativamente en todos los suelos tratados; el PSI en el tratamiento con yeso (decreció 11 veces), mientras que los tratamientos combinados B + Y; B + SH; B + SH + Y (decrecieron entre 9 – 13 veces) respecto al control. Asimismo, la biomasa microbiana del suelo incrementó 112 – 322% en el tratamiento B + SH + Y. La enmienda combinada (B + SH + Y) representa una alternativa para la recuperación de suelos degradados incluyendo suelos salinos-sódicos, en este estudio se reportan mejoras significativas respecto a las propiedades químicas y biológicas del suelo después de las aplicaciones de enmiendas químicas y orgánicas.

Palabras clave: biocarbón, sustancias húmicas, salinidad, sodicidad, PSI, biomasa microbiana.

ABSTRACT

We evaluated the individual and synergic effect application of Biochar (B), Humic Substances (HS) and Gypsum (G) on soil properties of saline-sodic soil over growth performance of quinoa plants and seed quality traits (polyphenols, protein and yield). Treatments included (B) 22 t ha^{-1}, (HS) 5 kg ha^{-1} and (G) 47.7 t ha^{-1}. Two quinoa genotypes selected in Arid Zones (AZ) were established in eight treatments. Combined treatment B + HS + G increased root biomass in AZ-51 and AZ-103 quinoa genotypes 206 and 176%, respectively. Likewise, stomatal conductance, SPAD index and seed yield increased significantly in AZ-103 genotype at all treated soil. The same parameters in AZ-51 genotype increased by different treatments. Furthermore, Electrical Conductivity (ECe), Sodium Adsorption Ratio (SAR) and Exchangeable Sodium Percentage (ESP) decreased significantly in all treated soils; ESP in G treatment decreased 11-fold, and B + G; B + HS; B + HS + G decreased 9 – 13-fold respect to control. Likewise, soil microbial biomass increased 112 – 322 % on B + HS + G treatment in AZ-51 and AZ-103 genotypes, respectively. Combined amendment (B + HS + G) represents an alternative for reclaiming degraded soils, including saline-sodic soils and significant improvements were determined regarding to soil chemical and biological properties after organic and inorganic amendment.

Key words: biochar; humic substances; salinity; sodicity; ESP; microbial biomass.

CAPÍTULO 1

INTRODUCCIÓN GENERAL

La degradación del suelo resultante de la salinidad y sodicidad es una importante limitación ambiental con graves efectos negativos sobre la fertilidad del suelo y productividad agrícola en regiones áridas y semiáridas del mundo (Young *et al*., 2015). Los suelos salinos-sódicos son degradados debido a los efectos simultáneos de la salinidad y sodicidad, que deteriora la estructura física del suelo debido al hinchamiento y dispersión de las arcillas debido a las altas concentraciones de Na^+ en la solución del suelo o en la fase de intercambio (Yu *et al*., 2010). Además de los efectos fisicoquímicos del suelo, las propiedades biológicas como la respiración y biomasa microbiana se deterioran (Wong *et al*., 2008). Por otro lado, la salinidad retarda el crecimiento de las plantas creando desequilibrios osmóticos y toxicidad iónica específica, causando limitaciones en los procesos morfológicos, histológicos, químicos, bioquímicos y metabólicos que reducen la apertura estomática y la tasa fotosintética, afectando negativamente el crecimiento de las plantas así como también el rendimiento y calidad (Parida y Das, 2005; Petropoulos *et al*., 2017). La adaptación de la agricultura a las condiciones climáticas cambiantes que implican mayor salinización del suelo, se basa en el uso de cultivos adecuados que muestran resistencia a los estreses bióticos y/o abióticos (Ruiz *et al*., 2016). La quínoa (*Chenopodium quinoa* Willd.) es un cultivo empleado en la diversificación de la agricultura debido a su extraordinaria adaptabilidad a estreses abióticos tales como salinidad del suelo, bajas temperaturas, sequía y suelos con deficiente estado nutricional (Razzaghi *et al*., 2015). En las últimas décadas, la quínoa atrajo la atención de investigadores y consumidores en todo el mundo por ser una de las únicas plantas de alimentos que son una fuente de aminoácidos esenciales, vitaminas, compuestos fenólicos y minerales (Nowak *et al*., 2010). Sin embargo, la alta salinidad puede influir negativamente sobre la longitud de la raíz y crecimiento de las plantas, la concentración de clorofila y el rendimiento del grano (Hariadi *et al*., 2010; Ruiz *et al*., 2016). De acuerdo con Ramzani *et al*. (2017), en suelos afectados por sal la calidad nutricional de la semilla de quínoa se reduce, el contenido de proteína disminuye mientras que el nivel de polifenoles se incrementa; por esta razón la recuperación de suelos salinos-sódicos es de gran importancia para la productividad agrícola (Yazdanpanah *et al*., 2013), así como para garantizar la seguridad alimentaria mundial (FAO, 2014). La remediación de un suelo salino-sódico requiere la eliminación de sodio de los sitios de intercambio en la matriz del suelo mediante cationes divalentes

(preferiblemente Ca^{2+}) para promover la floculación del suelo. La enmienda típica usada en este tipo de suelo debe proporcionar calcio soluble al suelo, de esta forma el Na^{+} reemplazado se elimina por debajo de la zona de la raíz o fuera del perfil del suelo mediante la lixiviación del agua (Mahmoodabadi *et al*., 2013). El yeso es un producto comúnmente usado para la recuperación de suelos salinos-sódicos, puede mejorar las propiedades físicas y químicas de los suelos principalmente al mantener una concentración adecuada de electrólitos en la solución del suelo. Dado que el Na^{+} adsorbido en los sitios intercambiables de las partículas de arcilla se considera responsable de la dispersión del suelo, el yeso puede evitar esta dispersión manteniendo altas relaciones de Ca:Na y promoviendo así la floculación y estabilidad de la arcilla (Yaduvanshi y Sharma, 2008). Por otro lado, las enmiendas orgánicas pueden aumentar la disolución de minerales de calcita nativa ($CaCO_3$) mediante una mayor formación de ácido carbónico en el perfil del suelo; dicha solución podría liberar Ca^{2+} en la solución del suelo para facilitar la eliminación de Na^{+} en los sitios de intercambio catiónico (Yaduvanshi, 2017). Estas enmiendas representan una alternativa para recuperar una variedad de suelos degradados, incluyendo los suelos afectados por la sal, y se han informado mejoras significativas con respecto a las características físicas, químicas y biológicas del suelo después de la incorporación de materia orgánica (Tejada *et al*., 2006). Por ejemplo, en un suelo sódico las aplicaciones de yeso junto con enmiendas orgánicas mejoraron las propiedades del suelo, tales como: la disminución de la densidad aparente del suelo, Conductividad Eléctrica (CE), Porcentaje de Sodio Intercambiable (PSI), además incrementaron la actividad biológica del suelo (Gupta *et al*., 2016). Las sustancias húmicas son ampliamente reconocidas como componentes clave de la fertilidad del suelo porque controlan las propiedades fisicoquímicas y biológicas de la rizósfera; desempeñan diversas funciones en el suelo y regulan el crecimiento de las plantas. Sus efectos beneficiosos se deben principalmente a la mejora en las propiedades del suelo como la agregación, aireación y la capacidad de retención de agua (Clemente y Bernal, 2006; Ciarkowska, 2010). Además, Ciarkowska *et al.*, (2017), informaron que la aplicación al suelo de sustancias húmicas derivadas de lignito contribuyó sustancialmente a mejorar el estado de calidad del suelo, aumentó el carbono orgánico, actividad enzimática, además la biomasa radicular y rendimientos se vieron beneficiados positivamente. Otra enmienda orgánica es el biocarbón (Lehmann y Joseph, 2015), es más recalcitrante que las sustancias húmicas y, por lo tanto, es menos probable que influya directamente sobre el crecimiento microbiano. Sin embargo, se ha demostrado que el biocarbón puede promover significativamente la actividad biológica del suelo debido a la liberación de productos de pirolisis lábiles temporales (Lehmann *et al*.,

2011), pero es posible que este efecto sea transitorio (Chaganti y Crohn, 2015). No obstante, los suelos afectados por sales se benefician de la aplicación de biochar a través de: suministro de carbono orgánico y nutrientes K^+, Ca^{2+}, Mg^{2+}, Zn, Mn, aumento del área superficial, equilibrio en el contenido de agua y aire del suelo, retención de cationes polivalentes y el reemplazo de Na^+ de los sitios de intercambio al proporcionar Ca^{2+} en la solución del suelo (Zheng *et al*., 2018). En este contexto, la adición de enmiendas orgánicas junto con el yeso ha tenido éxito en la reducción de los efectos adversos sobre las propiedades del suelo asociados con la presencia de sodio. Sin embargo, se conoce muy poco sobre el efecto sinérgico entre el Biochar (B), las Sustancias Húmicas (SH) y el Yeso (Y) en la recuperación de suelos salinos-sódicos y su impacto sobre el crecimiento de quínoa.

HIPÓTESIS

La aplicación de enmiendas combinadas mejora las propiedades químicas y biológicas del suelo salino-sódico. Este efecto en las propiedades del suelo influye positivamente sobre la performance de plantas de quínoa.

OBJETIVO GENERAL

Estudiar los efectos de la aplicación combinada de yeso, biocarbón y sustancias húmicas sobre las propiedades químicas y biológicas de un suelo salino-sódico y el comportamiento de dos genotipos de quínoa (maduración temprana e intermedia).

OBJETIVOS ESPECÍFICOS

- Evaluar los cambios en las propiedades químicas y biológicas de un suelo salino-sódico después de la aplicación de las enmiendas (B), (SH), (Y) y sus combinaciones.
- Evaluar el efecto de estas enmiendas sobre la fisiología, productividad y calidad de semilla de dos genotipos de quínoa cultivados en un suelo salino-sódico.

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CAPÍTULO 2

RECLAMATION OF SALINE-SODIC SOIL WITH COMBINED AMENDMENTS: IMPACT ON QUINOA PERFORMANCE AND BIOLOGICAL SOIL QUALITY

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ABSTRACT

We evaluated the individual and synergic effect application of Biochar (B), Humic Substances (HS) and Gypsum (G) on soil properties of saline-sodic soil over growth performance of quinoa plants and seed quality traits (polyphenols, protein and yield). Treatments included (B) 22 t ha^{-1}, (HS) 5 kg ha^{-1} and (G) 47.7 t ha^{-1}. Two quinoa genotypes selected in Arid Zones (AZ) were established in eight treatments. Combined treatment B + HS + G increased root biomass in AZ-51 and AZ-103 quinoa genotypes 206 and 176%, respectively. Likewise, stomatal conductance, SPAD index and seed yield increased significantly in AZ-103 genotype at all treated soil. The same parameters in AZ-51 genotype increased by different treatments. Furthermore, Electrical Conductivity (ECe), Sodium Adsorption Ratio (SAR) and Exchangeable Sodium Percentage (ESP) decreased significantly in all treated soils; the ESP in G treatment decreased 11-fold, and B + G; B + HS; B + HS + G decreased 9 – 13-fold respect to control. Likewise, soil microbial biomass increased 112 – 322 % on B + HS + G treatment in AZ-51 and AZ-103 genotypes, respectively. Combined amendment (B + HS + G) represents an alternative for reclaiming degraded soils, including saline-sodic soils and significant improvements were determined regarding to soil chemical and biological properties after organic and inorganic amendment.

Key words: biochar, humic substances, salinity, sodicity, ESP, microbial biomass.

INTRODUCTION

Soil degradation resulting from salinity and sodicity is a major environmental constraint with severe negative effects on soil fertility and agricultural productivity in arid and semiarid regions of the world [1]. Saline-sodic soil are degraded due to their simultaneous effect of salinity and sodicity, which cause deteriorates soil physical structure by clay swelling and dispersion due to high concentrations of Na^+ in the soil solution or at the exchange phase, forming dispersed [2]. In addition to physicochemical effects, biological properties such as the microbial respiration and biomass are deteriorated [3]. On the other hand, salinity retards plant growth by creating osmotic imbalances and specific ion toxicities [4], caused limitations in morphological, histological, chemical, biochemical and metabolic processes which reduces stomatal opening and photosynthetic rate, leading towards low plant growth, crop yield and quality [4,5]. Adaptation of agriculture to changing climatic conditions, expected to entail increasing soil salinization, relies on the use of suitable crops displaying resistance to abiotic and biotic stresses [6]. Quinoa (*Chenopodium quinoa* Willd.) is a crop used for agricultural diversification due to its extraordinary adaptability to various environmental stresses such as soil salinity, low temperature, drought, and soil having poor nutritional status [7]. In the last decades, quinoa has attracted the attention of researchers and consumers worldwide for being one of the only food plants that are an excellent source of essential aminoacids, vitamins, phenolic compounds and minerals [8]. However, high salinity can negatively influence seedling root length, concentration chlorophyll, growth as well as seed yield [6, 9]. According to Ramzani et al. [10], in salt –affected soils the nutritional quality of quinoa seed is reduced; the protein content decreases, while that the level polyphenols increases. For this reason the successful reclamation of saline-sodic soils is of great importance for agricultural productivity [11], and ensure to global food security [12]. Reclamation of a saline–sodic soil requires the removal of sodium from the soil exchange sites into soil solution by divalent cations (Ca^{2+} preferably) to promote soil flocculation. The typical source of calcium can be an amendment which provides soluble calcium within the soil. The replaced Na^+ is removed either below the root zone or out of the soil profile by leaching water [13]. Gypsum is the most commonly used product for reclamation of saline -sodic soils, can improve the physical and chemical properties of soils primarily by maintaining a favourable soil solution electrolytes concentration. Since adsorbed Na^+ on exchangeable sites of clay particles are considered to be responsible for soil dispersion, gypsum can prevent it by maintaining high Ca: Na ratios and thus promoting clay flocculation and structure stability [14]. On the other hand, organic

amendments can increase the dissolution of native calcite ($CaCO_3$) minerals via increased formation of carbonic acid in the soil profile such dissolution could release Ca^{2+} in soil solution to facilitate the removal of Na^{+} from the cation exchange sites [15]. Represent an alternative for reclaiming a variety of degraded soils, including salt-affected soils and significant improvements have been reported with respect to soil physical, chemical and biological characteristics after organic matter incorporation [16]. For example Gupta et al.[17], found that the application of gypsum with amendments organic on sodic soil improved soil properties such as; decreasing of soil bulk density, Electrical Conductivity (EC) and Exchangeable Sodium Percentage (ESP), also increased soil biological activity. Humic substances are widely recognized as key components of soil fertility because they control physicochemical and biological properties of the rhizosphere and perform various functions in soil and regulate plant growth. Their beneficial effects are mainly through improvement of soil properties, such as aggregation, aeration and water holding capacity [18, 19]. Furthermore, Ciarkowska et al. [20] reported that a product containing lignite derived humic substances applied to soil contributed substantially to improving soil quality status, increased organic carbon, enzymatic activity, in addition root biomass and yield was positively benefited. Other organic amendment is the biochar [21], its more recalcitrant than humic substances and is therefore less likely to directly support microbial growth. However, it was shown that biochar can significantly promote soil biological activity after its addition due to the release of temporary labile pyrolysis products [22], but this effect could be very temporary [23]. Nevertheless the salt affected soils benefit from biochar application through increased content of soil organic carbon and nutrients (K^{+}, Ca^{2+}, Mg^{2+}, Zn, Mn), increased surface area, enhanced physical properties by balancing water content and air porosity, increased retention of polyvalent cations, and replacement of Na^{+} from exchange sites by providing Ca^{2+} in soil solution [24]. In this context the addition of organic amendments in conjunction with gypsum has been successful in reducing the adverse effects on soil properties associated with the presence of sodium. However, has very little is known above the synergistic effect between biochar (B), Humic Substances (HS) and Gypsum (G) on reclamation of saline- sodic soil and their impact on quinoa growth. The objectives of this study were: a) evaluate the changes in to chemical and biological characteristics of saline -sodic soil after the application combined amendments B, HS, G, b) evaluate the effect of these amendments to physiology, productivity, and seed quality of two quinoa genotypes. The hypothesis tested in this work was that the application combined amendments improve the chemical and biological

properties of the saline -sodic soil and this effect on soil properties may be significant on quinoa performance.

MATERIALS AND METHODS

Experimental Site

The experiment was conducted in pots (21 cm diameter, 30 cm height) that were filled with 1 kg of saline-sodic soil. All plants were grown under controlled greenhouse conditions (temperature between 20 ± 2 °C; day length 12 h; average humidity 65 %) at Soil Biology Laboratory of the Faculty of Agronomy, University of Concepción, Biobío Region, Chile. The lighting system was provided with lights 300 watts. The registered radiation range was 350 μmol m^{-2} s^{-1}.

Soil sampling

Saline–sodic soil samples (0-20 cm) were collected from Huasco commune (28°28'S; 71°10'W), Atacama Region, Chile. According to soil taxonomy [25], the soil under investigation was classified as a Typic Aquic Torriorthents. It was silty franc soil with bulk density of 1.10 g cm^{-3}, ECe of 218.5 dS m^{-1}, saturation paste 7.6 pH, 96.3% Sodium Adsorption Ratio (SAR), cation exchangeable capacity (CEC) of 189.4 cmol kg^{-1} soil, 2.96 % organic matter, 40.2 mg kg^{-1} P Olsen, 734.8 mg kg^{-1} available K. The climate at site of soil origin is arid, with an average temperature (15°C), relative humidity (70.2%) and annual precipitation (40 mm) [26].

Germination assay

To assess the germinability of quinoa seeds grown under natural conditions of soil salinity a germination test was performed. Twenty quinoa seeds were evaluated in 64 experimental units, placing in each pot per treatment and replicated four times. Germination (%) was evaluated 10 days after sowing [6]. This allowed measuring seed germination inhibition as a result of salt toxicity and cracking soil (germination average 6.7%; data not shown). However, all plants died few days after emergence. For this reason, to reduce cracking soil, vermiculite was added as 200 g pot^{-1} and mixed with soil thoroughly. Subsequently, soil leaching was carried out.

Leaching

To decrease the high salinity of the soil, leaching was applied before to sow seedlings in this study. The soil leaching consisted in adding partially tap water for thirty days, applying 15 l pot^{-1}. The soil electrical conductivity post leaching was 15 dS m^{-1}. Soil leaching is an agricultural practice commonly carried out in Huasco, in order to reduce soil salinity to levels that crop to be grown can tolerate. The average CEe value for Huasco soils is 17 dS m^{-1}[27]

Amendments

Pine woodchips biochar, humic substances were used as organic amendments and gypsum was used as an inorganic amendment. Pine woodchip biochar was producer under a slow pyrolysis process at 450°C for 30 minutes and cooling for 60 minutes. The processing of the carbonaceous material was carried at UDT (Technology Unit of Universidad de Concepción - Chile). Below are presented some properties of Biochar: Total carbon (C %) 46; Total nitrogen (N %) 0.5; C: N ratio 98.5; Total Ca – Mg (%): 0.8 – 0.12 [28]. Biosolve (Oiko, Chile), product containing 70% humic acids, 15% fulvic acids and 10% K_2O was used as source of humic substances derived from leonardite. Gypsum was obtained from the local market, SO_4 16-18%, CaO 23-24%.

The soil gypsum requirement was determined using the equation [29]:

$$GR = (ESPi - ESPf) \times CEC \times BD \times SD \times F\ 0.00086 \quad (1)$$

Where:

GR: is the net gypsum requirement of soil (t ha^{-1}), ESPi: is the initial exchangeable sodium percentage, ESPf: is final exchangeable sodium percentage, CEC: is cation exchangeable capacity (cmol kg^{-1}), SD: is soil depth (cm), BD: is bulk density (g cm^{-3}) and F: is Ca-Na exchange efficiency.

To reduce the initial soil ESP from 58% to 45%, 47.7 t ha^{-1} of GR was used.

Experimental Procedure

Quinoa selected genotypes in Arid Zones (AZ) AZ-103 and AZ-51 were obtained as dry seed batch from the Quinoa Breeding Program at INIA Intihuasi (Chile), and both corresponds to coastal/lowlands ecotype characterized by early and intermediate maturity habit: 150 - 170 days after sowing (DAS), respectively.

At two leaf stage were transplanted two plants per pot. Were applied N, in the order of 160 kg ha^{-1}, in 30% and 70% proportions, at sowing and branching, was omitted the

application of P_2O_5 and K_2O due to initial condition of the soil that provided sufficient amounts for normal development of the crop according to the results of soil analysis.

Plants were grown to the stage of harvest maturity: genotypes AZ-103 and AZ-51: 112 and 137 (DAS), respectively.

For the study amendments were applied at rate of: Biochar (B) 22 t ha^{-1} (1% w/w) [30], Humic Substances (HS) 5 kg ha^{-1} [31], Gypsum (G) 47.7 t ha^{-1}. In this way for each experimental unity with 1 kg soil treatments applied were: T1 = B (10 g pot^{-1}); T2 = G (22 g pot^{-1}); T3 = HS (2.3 mg pot^{-1}); T4 = B + G (10 + 22 g pot^{-1}); T5 = B + HS (10 g + 2.3 mg pot^{-1}); T6 = HS + G (2.3 mg + 22 g pot^{-1}); T7 = B + HS + G (10 g + 2.3 mg + 22 g pot^{-1}); T0 = Control (unamend soil). Eight treatments were evaluated under two quinoas genotypes, plants watered to 100% of field capacity. The moisture monitoring was done by means of the sensor (MORPHO, GS-1) and readings were recorded in the data logger (DECAGON-EM50 Series). And four replicates of each genotype were considered and two plants per treatment were evaluated in 64 experimental units.

Plant parameters measured

The shoot and root dry weight were determined by drying in the oven for 24 hours at 65° C and weighed on analytical balance repeating the procedure. Root length was processed using WinRhizo® Root Analysis System software (Régent Instrument Inc., Canada). Physiological variables of the plant were determined as Stomatal Conductance, during anthesis stage using a Porometer (DECAGON DEVICES model SC-1, USA). Chlorophyll index SPAD (soil plant analysis development) was determined by a portable meter 502 (Minolta Spectrum Technologics Inc., Plainfield, IL, USA), the measurements were taken on the fully expanded leaves from the upper part of the foliage.

Seed parameters measured

Seed yield (g $plant^{-1}$), the seeds of the plants of each plot were weighed on analytical balance. Seed protein (%) content was estimated by determining nitrogen by the Kjeldahl method. Then for the conversion of nitrogen to pure protein the factor was used (5.73).

Polyphenols were measured in seed samples by adopting Folin Ciocalteu Reagent method [32]. Were added 0.025 ml samples extract with 0.12 ml Folin Ciocalteau Reagent 1N and 1.6 ml H_2O. After 3 min 0.3 ml of 20% Na_2CO_3 were added. The mixture was incubated at laboratory temperature for 2 h and absorbance was measured at 760 nm against blank sample. Total polyphenol content was expressed as Gallic Acid Equivalents (GAE) in mg g^{-1}.

Chemical and biological soil analysis and foliar tissue analysis

Chemical analyses were performed measuring pH, ECe, and soluble cations (Ca^{2+}, Mg^{2+}, Na^{+}) of saturation paste following standard methods [33]. In addition, exchangeable cation concentrations were measured [33]. All analyses were conducted in the laboratories (ISO 17025 accredited) of the Department of Soils and Natural Resources of the University of Concepción.

The Exchangeable Sodium Percentage (ESP) was calculated by the following equation where the concentrations of exchangeable cations are expressed in cmol kg^{-1}:

$$ESP = Na^{+} / (Na^{+} + K^{+} + Ca^{2+} + Mg^{2+}) \times 100\% \quad (2)$$

The Sodium Adsorption Ratio (SAR) was calculated by the following equation where a chemical element symbol indicates a concentration in meq l^{-1}:

$$SAR = \frac{Na^{+}}{\sqrt{(Ca^{+2} + Mg^{+2})/2}}$$

Microbial soil biomass activity was determined by hydrolysis of fluorescein diacetate (FDA). For which, 0.5 g of wet soil was weighed in screw cap test tubes (samples were in triplicate and a blank was also included), then 4.95 mL of sodium phosphate buffer and 0.05 mL of FDA were added

with subsequent stirring and brought to the thermoregulated bath at 25 °C for 1 h, then samples were withdrawn and placed in an ice bath. 5 mL of acetone was added, shaken and filtered, and absorbance was read at 490 nm in a spectrophotometer against a reagent blank [34]. The soil respiration was determined by weighing 20 g of soil (in duplicate) was weighed per treatment, and introduced in a jar of incubation bottle. Added 7.5 mL of NaOH in centrifuge tube and placed in a bottle of incubation. Took bottles without soil (blank), all were hermetically closed and remained in an incubation chamber at 22 °C and humidity constant for 7 days. After the incubation time, was removed from the centrifuge tube 1 mL of NaOH was added 2 mL of $BaCl_2$, and titrated with HCl 0.1 M. The data were expressed as µg CO_2 g^{-1} - C soil oven dried (105 °C) [34].

Leaf analyses (Sodium) of the samples were performed following standard methods [35].

Statistical analysis

The study was conducted using a completely randomized design consisting of 2 factors (2x8), being the first factor the quinoa genotypes (AZ-51 and AZ-103) while the second factor corresponded to the amendments biochar, humic substances and gypsum (individually and combined). The data was analysed using one-way (ANOVA), where the normality test was done using the modified Shapiro-Wilks test, and mean separation by the Duncan test ($p < 0.05$). SAR, ESP, Na^+ values were transformed with the expression $(x+0.5)^{0.5}$ before entering data to the statistical software InfoStat version 2016e.

RESULTS

Amendment effects on growth and physiological parameters of quinoa plants

One major objective of this research was to evaluate effects of biochar, humic substances and gypsum applications on quinoa growth. The root biomass in AZ-51 quinoa genotype showed a significant increase of 413% in the B + HS + G treatment when compared to control. On the other hand, combined applications of B + G; B +HS and B + HS + G significantly increased the root biomass by 57; 73 and 93% compared to control in AZ-103 genotype, respectively (Table 1). In addition, HS + G; B; B + HS + G increased root length significantly by 51; 76; 106% respectively in AZ-51 genotype relative to control. Same parameter in AZ-103 genotype showed significant increases in B; B + G; B +HS treatments respect to control (Table 1).

We also evaluated some quinoa physiological variables such as chlorophyll index (SPAD) and stomatal conductance. SPAD values in AZ-51 genotype did not increased significantly after the amendment applications when compared to control soil (p value = 0.1028). Despite this, same parameter in AZ-103 genotype showed significant increases in all treatments compared to control. Highest SPAD values were obtained with (HS + G; G; B; B + G; B + HS). Percentage increases were 39; 33; 32; 30; 27% relative to the control, respectively (Table 2). Stomatal conductance of both quinoa genotypes increased significantly with amendments application. In AZ-51 genotype this parameter was significantly higher in B + HS + G; HS; B + G; G; B + HS treatments, whereas in AZ-103 genotype all treatments were significant higher than control (Table 2).

Table 1. Influence of chemical and organic amendments on root length and root dry weight of quinoa genotypes grown on saline-sodic soil.

Treatments	**Root dry weight** (g plant^{-1})		**Root length** (cm)	
	AZ-51	**AZ-103**	**AZ-51**	**AZ-103**
C	0.48±0.06 b	1.13±0.12 c	415±79.2 cd	329±41.8 d
B	0.83±0.23 b	1.49±0.06 bc	729±73.6 ab	480±31.5 abc
G	0.63±0.16 b	1.39±0.22 bc	357±40.1 d	369±48.3 bcd
HS	0.82±0.11 b	1.63±0.21 abc	533±77.1 bcd	365±23.4 cd
B + G	0.81±0.13 b	1.77±0.22 ab	569±61.6 bc	511±44.1 ab
B + HS	0.65±0.15 b	1.95±0.10 ab	388±51.2 cd	515±58.7 a
HS + G	0.60±0.05 b	1.51±0.24 bc	625±45.0 b	319±56.9 d
B+ HS + G	2.46±0.27 a	2.15±0.14 a	854±75.6 a	452±44.7 abcd
(p-value)	<0.0001	0.0124	0.0001	0.0148

Values are means of four replicates. Standard error: ±. Values followed by the same letter in the columns do not differ significantly ($p \leq 0.05$) by Duncan test. C = control; B = biochar; G = gypsum; HS = humic substances; B + G = biochar + gypsum; B + HS = biochar + humic substances; HS + G = humic substances + gypsum; B + HS + G = biochar + humic substances + gypsum. Source: Elaborated with own data.

Table 2. Influence of chemical and organic amendments on physiological parameters of quinoa genotypes grown on saline-sodic soil.

Treatments	Chlorophyll index SPAD		Stomatal conductance (mmol m $^{-2}$ s $^{-1}$)	
	AZ-51	AZ-103	AZ-51	AZ-103
C	47.49±1.20 a	46.85±1.57 d	106±10.64 c	100±4.93 d
B	53.40±2.17 a	61.70±1.24 ab	116±4.51 bc	136±7.04 bc
G	54.37±1.67 a	62.13±1.04 ab	137±6.98 ab	147±9.23 bc
HS	50.33±1.49 a	54.98±0.85 c	138±7.75 ab	133±7.74 c
B + G	55.80±2.43 a	61.03±0.66 b	137±8.63 ab	137±8.09 bc
B + HS	52.31±1.23 a	59.65±1.37 b	132±5.12 ab	127±8.66 c
HS + G	52.13±1.40 a	65.27±2.45 a	122±9.38 abc	160±5.92 ab
B+ HS + G	50.13±2.80 a	55.48±0.37 c	145±8.39 a	181±8.78 a
(p-value)	0.1028	<0.0001	0.0412	0.0001

Values are means of four replicates. Standard error: ±. Values followed by the same letter in the columns do not differ significantly ($p \leq 0.05$) by Duncan test. C = control; B = biochar; G = gypsum; HS = humic substances; B + G = biochar + gypsum; B + HS = biochar + humic substances; HS + G = humic substances + gypsum; B + HS + G = biochar + humic substances + gypsum. Source: Elaborated with own data.

Seed yield presented significantly increases in AZ-51 genotype by 116 and 85% in G and HS + G treatments compared to control, respectively (Duncan test $p > 0.05$). However, in AZ-103 genotype seed yield significantly increased in all treated soils compared to control (Figure 1).

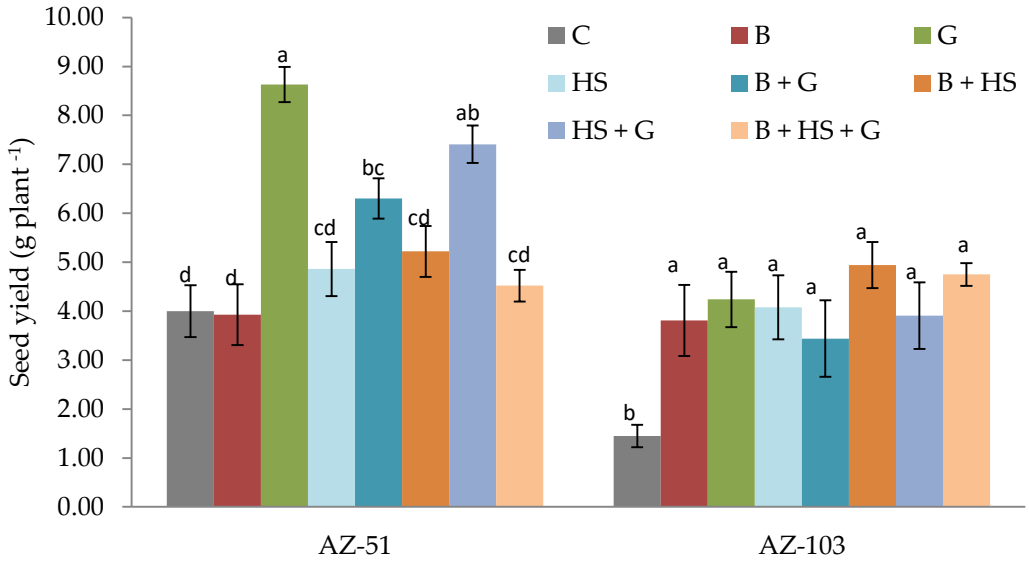

Figure 1. Seed yield (g plant $^{-1}$) of quinoa genotypes grown in saline-sodic soil in response to chemical and organic amendments. Values are means of four replicates. Error bars represent the standard error. Values with the same letter are not significantly different according to ($p \leq 0.05$) by Duncan test. C = control; B = biochar; G = gypsum; HS = humic substances; B + G = biochar + gypsum; B + HS = biochar + humic substances; HS + G = humic substances + gypsum; B + HS + G = biochar + humic substances + gypsum. Source: Elaborated with own data.

Polyphenols and Protein Content in quinoa seeds

Polyphenols content GAE did not revealed significant differences (p value = 0.2239) in AZ-51 genotype treatments compared to control. Nevertheless, we detected significant differences in AZ-103 genotype that resulted in a 28% significant decrease of GAE concentration in B + HS + G treatment compared to control (Table 3).

Protein content under HS; HS + G; B + HS; B + HS + G treated soils were significantly increased in AZ-51 genotype by 45; 34; 29; 26% respectively, relative to control soils. In AZ-103 genotype, protein content in quinoa seed significantly increased (23%) in biochar treatment, when compared to control (Table 3).

Table 3. Contents of polyphenols (Gallic Acid Equivalent) and Protein in quinoa seed

Treatments	Polyphenols (GAE mg g^{-1})		Protein (%)	
	AZ-51	AZ-103	AZ-51	AZ-103
C	1.02±0.08 a	1.2±0.07 ab	9.3±0.72 d	11.4±0.27 b
B	0.75±0.06 a	1.05±0.09 bc	11.1±0.61 bcd	14.0±0.53 a
G	0.91±0.06 a	1.22±0.14 ab	10.4±0.82 cd	10.8±0.19 b
HS	0.71±0.03 a	1.02±0.09 bc	13.5±0.26 a	10.3±0.48 b
B + G	0.81±0.16 a	1.38±0.11 a	10.7±0.76 bcd	11.9±0.37 b
B + HS	0.87±0.12 a	1.21±0.11 ab	12.0±0.10 abc	11.3±0.70 b
HS + G	0.78±0.15 a	1.12±0.09 abc	12.5±0.44 ab	11.8±0.92 b
B+ HS + G	0.73±0.05 a	0.87±0.08 c	11.8±0.50 abc	11.3±0.37 b
(p-value)	0.2239	0.0137	0.0018	0.0029

Values are means of four replicates. Standard error: ±. Values followed by the same letter in the columns do not differ significantly ($p \leq 0.05$) by Duncan test. C = control; B = biochar; G = gypsum; HS = humic substances; B + G = biochar + gypsum; B + HS = biochar + humic substances; HS + G = humic substances + gypsum; B + HS + G = biochar + humic substances + gypsum. Source: Elaborated with own data.

Amendment effects on chemical and biological properties of saline - sodic soils

Electrical conductivity of the saturation paste extract decreased significantly for most of soil amendments compared to control soils. Salinity (ECe) after amendment applications was significantly reduced ranging from 33 to 46% (in AZ-51 genotype), except in biochar treatment, where it was parallel to control soil, while ECe was significantly reduced in AZ-103 genotype in all treatments ranging from 52 to 68% when compared to control soils, (Table 4).

Sodium Adsorption Ratio showed significantly decrease in all amended soils compared to control soils. Maximum decrease in SAR for individual applications was obtained with gypsum treatment (in average decreased 10.9 – fold) compared to control, though for combined applications the highest decrease in SAR was found in B + G (in average decreased 15.2 – fold) respect to control (Table 4).

All amendment treated soils had significantly lower soil ESP as compared to control soils. The largest reduction in ESP for individual applications was observed with G, which exhibited an eleven-fold decline in soil ESP relative to control; whilst for combined

applications the maximum decrease in ESP was registered in B + G; B + HS; B + HS + G (nine- and thirteen-fold decrease in soil ESP respect to control; Table 4).

Table 4. Chemical soil properties measured after the application of amendments**.**

Treatments	ECe (dS m^{-1})		SAR (meq $l^{-1})^{1/2}$		ESP (%)	
	AZ-51	AZ-103	AZ-51	AZ-103	AZ-51	AZ-103
C	9.1±0.3 a	13.2±0.4 a	9.7±0.7 a	10.0±0.3a	15.0±1.3 a	21.5±1.2 a
B	8.1±0.7 a	5.5±0.8 bc	1.4±0.2 b	2.2±0.4 b	2.2±0.4 bc	4.5±0.5b
G	5.7±0.4 b	6.1±0.7 b	0.9±0.0 cd	0.9±0.1de	1.3±0.1 d	2.1±0.2 def
HS	5.3±0.4 b	4.5±0.3bc	1.3±0.1 bc	1.4±0.2cde	2.2±0.1 b	3.7±0.4 bc
B + G	6.1±0.8 b	6.3±0.6 b	0.6±0.0 d	0.7±0.1e	1.2±0.1 d	1.7±0.2 f
B + HS	6.1±0.8 b	4.2±0.4 c	0.8±0.1 d	1.2±0.1 de	1.4±0.2 cd	2.0±0.3 ef
HS + G	4.9±0.2 b	4.7±0.5 bc	1.2±0.2 bc	1.9±0.2 bc	2.1±0.1 b	4.0±0.5 bcd
B+ HS + G	5.3±0.4 b	4.6±0.6 bc	0.7±0.0 d	1.36±0.3cd	1.2±0.0 bcd	2.4±0.5 cde
(p-value)	0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Values are means of four replicates. Standard error: ±. Values followed by the same letter in the columns do not differ significantly ($p \leq 0.05$) by Duncan test. C = control; B = biochar; G = gypsum; HS = humic substances; B + G = biochar + gypsum; B + HS = biochar + humic substances; HS + G = humic substances + gypsum; B + HS + G = biochar + humic substances + gypsum. Source: Elaborated with own data.

Exchangeable Ca^{2+} concentrations were 40; 27; and 23% significantly higher in G; B + HS + G and HS + G treated soils than in control soils, respectively in AZ-51 genotype. Same treatments showed similar trend in AZ-103 genotype, adding to this group B + HS; B + G and B treatments that significantly increased exchangeable Ca^{2+} concentrations from 54 to 14 % relative to untreated soil, respectively (Table 5).

Soil Exchangeable Mg^{2+} was significantly lower in both quinoa genotypes (AZ-51 and AZ-103) in B + G treated soils (20 and 24 %, respectively) than in control soils (Table 5).

Soil Exchangeable Na^{+} concentrations were significantly reduced in all treated soils than control soils (Table 5). In AZ-51genotype, exchangeable Na^{+} concentrations decreased ranging from 85% to 93% when compared to control soil (in combined applications); whereas exchangeable Na^{+} concentrations reduced ranging from 87% to 91% when compared to control soil (in individual applications). Similar fashion was observed in AZ-103 genotype,

where exchangeable Na^+ concentrations reduced ranging from 82 to 92% and 81 to 89% relative to control soil (in combined and individual applications, respectively).

Table 5. Soil exchangeable Ca^{2+}, Mg^{2+} and Na^+ concentrations after the application of amendments.

Treatments	Ca^{2+} (cmol kg^{-1})		Mg^{2+} (cmol kg^{-1})		Na^+ (cmol kg^{-1})	
	AZ-51	AZ-103	AZ-51	AZ-103	AZ-51	AZ-103
C	52±0.9 d	50±0.9 d	8.4±0.8 ab	11.1±0.7 ab	10.9±1.2 a	16.8±1.6 a
B	57±4.2 bcd	57±1.7 c	7.7±0.2 bc	10.5±0.6 ab	1.40±0.2 bc	3.20±0.4 b
G	73±2.3 a	76±1.1 a	8.0±0.3 ab	9.5±0.4 bc	1.03±0.0 bc	1.87±0.1 cde
HS	54±2.1 cd	54±0.6 cd	8.4±0.2 ab	11.1±0.7 ab	1.38±0.1 bc	2.50±0.3 bcd
B + G	59±1.9 bcd	67±2.3 b	6.7±0.2 c	8.4±0.5 c	0.80±0.1 c	1.29±0.2 e
B + HS	57±2.9 bcd	77±0.6 a	8.4±0.2 ab	10.2±0.3 ab	0.95±0.1 c	1.77±0.3 de
HS + G	64±1.8 bc	70±1.2 b	8.9±0.2 a	11.7±0.6 a	1.59±0.1 b	3.07±0.5 bc
B+ HS + G	66±5.7 ab	65±0.3 b	8.2±0.5 ab	10.9±0.7 ab	0.89±0.1 c	1.94±0.5 cde
(p-value)	0.0011	<0.0001	0.0192	0.0127	<0.0001	<0.0001

Values are means of four replicates. Standard error: ±. Values followed by the same letter in the columns do not differ significantly ($p \leq 0.05$) by Duncan test. C = control; B = biochar; G = gypsum; HS = humic substances; B + G = biochar + gypsum; B + HS = biochar + humic substances; HS + G = humic substances + gypsum; B + HS + G = biochar + humic substances + gypsum. Source: Elaborated with own data.

Regarding biological soil analysis, soil and respiration microbial biomass presented significantly increments in most of treatments compared to control. All amended soils displayed significantly increments in soil microbial biomass compared to control soils (Figure 2). Thus, in both genotypes the biggest values of microbial biomass were obtained with following treatments: B + HS + G; HS + G; B; B + HS (ranging from 12.2 to 14.1 µg FDA g^{-1} soil).

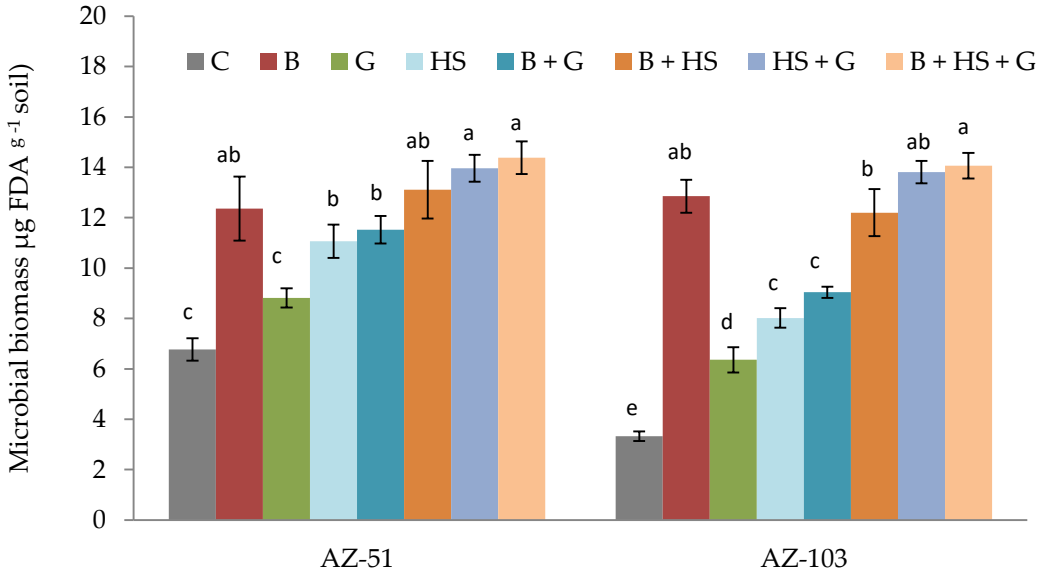

Figure 2. Microbial biomass (Fluorescein diacetate hydrolyzing activity µg FDA g^{-1} soil in saline-sodic soil after application of chemical and organic amendments. Values are means of four replicates. Error bars represent the standard error. Values with the same letter are not significantly different according to ($p \leq 0.05$) by Duncan test. C = control; B = biochar; G = gypsum; HS = humic substances; B + G = biochar + gypsum; B + HS = biochar + humic substances; HS + G = humic substances + gypsum; B + HS + G = biochar + humic substances + gypsum. Source: Elaborated with own data.

Soil microbial respiration was also significant increased in most treated soils as compared to control soils (Figure 3). In AZ-51genotype the higher values of soil microbial respiration were observed in B + HS + G; B + G; B (130; 88; 85% higher than that in control, respectively). In AZ-103 genotype all treated soils increased significantly values of soil microbial respiration respect to untreated soils. In turn, the highest values of soil microbial respiration were determined in B + HS + G; B; HS + G (285; 227; 211% higher than that in control, respectively) treatments.

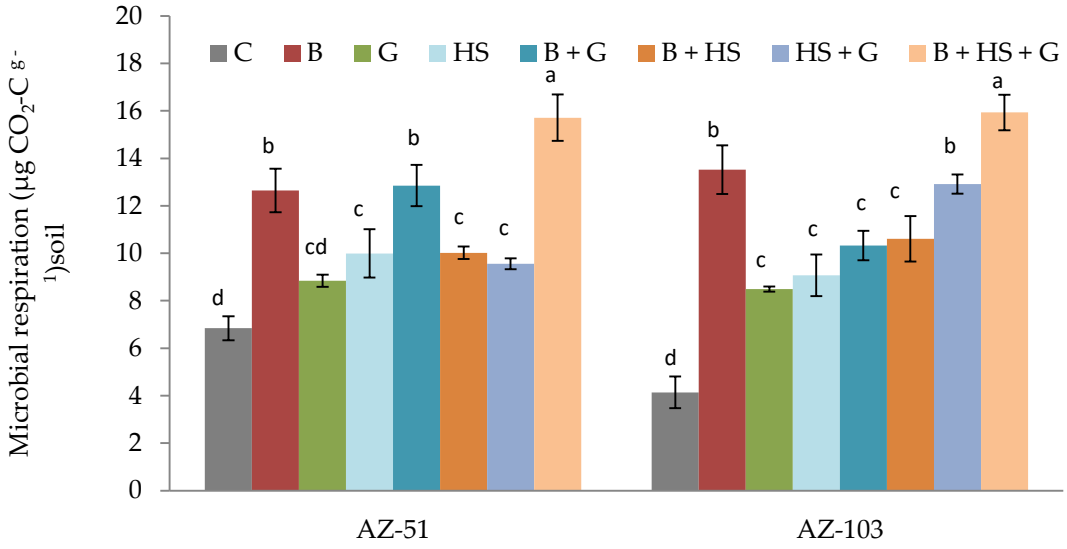

Figure 3. Microbial respiration (µg CO_2-C g^{-1}) in saline-sodic soil after application of chemical and organic amendments. Values are means of four replicates. Error bars represent the standard error. Values with the same letter are not significantly different according to ($p \leq 0.05$) by Duncan test. C = control; B = biochar; G = gypsum; HS = humic substances; B + G = biochar + gypsum; B + HS = biochar + humic substances; HS + G = humic substances + gypsum; B + HS + G = biochar + humic substances + gypsum. Source: Elaborated with own data.

DISCUSSION

Soil salinity greatly reduces plant performance by osmotic stress, hormonal imbalance, nutrient deficiencies and specific ion toxicity [36]. Our results indicated that application of biochar, humic substances and gypsum could mitigate part of these negative effects on performance of quinoa plants.

We found that quinoa root biomass (AZ-51 genotype) was significantly improved by combined amendments B + HS + G. Similar treatment in AZ-103 genotype showed the highest increases of root biomass in comparison to control (Table 1).This may be explained because combination of HS and B induces exudation of organic compounds from microbiota that positively affects root development [37, 38]; additionally, B can exclude salts from root system [39]. Organic amendments and G in combination significantly improve soil properties, which in turn, supported prolific root growth of plants [40]. Similar results were also determined by Kammann et al. [37], who observed enhanced root growth (fine root mass) of quinoa plants after B applications. According to Akhtar et al. [39] B influences positively plant root growth under saline conditions, due to its high adsorption capacity, which might led to reduce Na^+ uptake or enhanced Na^+ exclusion or both from roots. Our results are in line with this observation, showing significantly decreased of leaf Na^+ concentrations in amended soils (data not shown). HS improve soil microbial activity, because have a high hormonal

activity auxin-like substance production that stimulate root morphology [38]. We observed that SPAD chlorophyll index significantly increased in all treated soils in AZ-103 genotype (Table 2). Under saline conditions, chlorophyll content generally decreases in salt-sensitive plants, whereas in salt-tolerant ones' increases [41]. This information is consistent with our results, where non-amended treatment showed significantly lower SPAD index value, evidencing a positive effect of amendments that increased chlorophyll content in plants under salinity stress. In line with similar studies, Ramzani et al. [10] confirmed that chlorophyll content in quinoa plants grown under saline conditions was positively affected by B and compost applications over control. Despite this, AZ-51 genotype showed no significantly response on SPAD index (Table 2). Indeed, this might be related to genetic diversity among genotypes, where it's possible that leaf internal mechanisms in AZ-51 plants, which allowed to better adapt to salinity conditions maintaining an adequate photosynthetic activity. For this reason, responses were not so evident to amendment applications. Some genotypes harbour salt adapting mechanisms with physiological responses that result in phenotypic leaf plasticity to abiotic stress, in this fashion leaf morphological traits such as associated photosynthetic function vary depending of the environment and its genetic plasticity [42].

Overall, in our experiment most of amendment applications (individual and combined) showed statistically significant increments in stomatal conductance respect to untreated soils (Table 2). Our results are in line with Akhtar el al. [43], where B incorporation increased both stomatal aperture and density, implying reduced stress in wheat plants. This can be also attributed to B that can enhance soil water-holding capacity, which should also help to mitigate salt-induced osmotic stress and ion toxicity to plants due to dilution effect. Moreover, HS increased photosynthesis and respiration rates in plants, due to presence of phytohormones such as auxin and gibberellins that are directly involved in various biochemical mechanisms inside plant cells and for enzymatic activities involved in carbohydrate metabolism [44]. Conversely, we found that stomatal conductance was decreased in non-amended soils due to stomatal closure to endure osmotic constraint. In this condition, plants are more restrictive to water loss via transpiration by a sensitive stomatal closure mechanism, which leads to a restricted availability of CO_2 for carboxylation reaction favouring formation of Reactive Oxygen Species (ROS) [45].

Salt stress has also been identified as major factor constraining crop productivity in saline-sodic soils [36]. Here we present results showing that quinoa seed yield significantly increased in G; HS + G (in AZ-51 genotype); in the same mode, all treated soils in AZ-103 genotype improved significantly seed yield relative to control (Figure 1). Similar results have

been reported by Lashari et al. [46], who evaluated wheat growth in salt stressed soils and found increments of grain yields by B and compost applications. This could be attributed to multiple effects by amendment applications on quinoa plants grown in salt affected soil such as root development, hormonal and metabolic activity leading to higher seed yield. Indeed, HS increased root growth due to providing indole acetic acid (IAA), resulting in enhanced root surface area, thus enabling plant access to nutrients and to boosts in yield [47]. In addition, according to Turan et al. [48], increments in seed yield may be explained because HS increased the production of adenosine triphosphate (ATP) within plant cells and thus increasing permeability, resulting in rises of nutrient transport to sites of metabolic demand. Also, B application supplied nutrients to plants, triggered changes in soil redox conditions, enhanced microbial biomass and rise water holding capacity as it might increase plant tolerance to saline stress but also increase in plant dry yield [22]. Moreover, Spokas et al. [49], suggested that B amendments being incorporated to the soil are an ethylene source that stimulated plant growth and yield. Furthermore, in the study of Agegnehu et al. [50], who evaluated effects of B on soil quality and maize growth, B increased growth and grain yield due to greater pore spaces leading to a higher nutrient supply to plants. Likewise, Mahmood et al. [51], reported significant improvement on wheat plant growth and yield (25% to 43%) in saline-sodic soils reclaimed with G, which has been attributed to increases in Ca^{+2} build-up, elimination of Na^{+} toxicity and nutritional balance in plants. Although G and HS improved productivity of AZ-51 genotype, B did not significantly increased genotype yield, probably because contribution of C labile or leachable and some nutrient fractions were very transient [22], considering that this genotype exhibited a late maturation respect to AZ-103 genotype (25 days after). The B and HS rate is another factor that could have restricted effect in this variable [52], since this genotype displayed a higher average yield of 45% (greater export of nutrients) than genotype with early maturation.

Polyphenols content were evaluated as a functional quality indicator of quinoa seeds whose role is to reduce the damages caused by ROS (Table 3). Most of the treatments did not significantly affect the GAE content. Both genotypes presented a certain degree of tolerance to salinity level of studied soil that could have a negative influence on the induction to protective mechanisms such as polyphenol synthesis. Quinoa plants might use enzymatic antioxidant and non-enzymatic antioxidant compounds as polyphenols, which have a strong ability to scavenge ROS [53]. This was supported by Ruiz et al. [6], who reported that seeds harvested from R49 and VR quinoa genotypes, grown on saline soils showed higher GAE content, relative to controls.

We found the highest significant protein content in quinoa grains grown in HS and B treatments (in AZ-51 and AZ-103 genotypes, respectively) compared to control (Table 3). Our findings agreed with results of previous experiments where organic amendment applications into a saline soil improved nutritional quality in quinoa seed [10]. Similar response was observed in wheat grain grown on calcareous soils [54]. The mechanism held responsible for this enhancement in protein content of quinoa seed could be due to the role of organic amendments for overall improvement in soil health and delivery of essential nutrients to plants [55]. In addition, reduction in salt toxicity to plants through the adsorption of Na^+ on large B surface areas in the soil might be another possible mechanism for enhancement in protein content of quinoa seeds [22, 56].

Soil salinity also generates osmotic effects in plants and often causes physiological drought if it exceeds a critical threshold in crops [57]. Amendment applications improved chemical properties of soils (decreases soil salinity), influenced by effective salt leaching from soil profile. In our experiment, most of amended soils (except B treatment in AZ-51 genotype), showed significantly decreases of soil ECe compared to control soils (Table 4), suggesting that these amendments have a strong efficiency in reducing soil ECe. Similar results were also reported by Tejada et al. [16] and Gupta et al. [17] where soil EC was significantly lower when organic amendments and G were applied to reclaim salt affected soils compared to control.

Application of chemical and organic amendments increases the concentration of Ca^{2+} and promotes the displacement of adsorbed Na^+. We found that SAR and ESP values were reduced significantly in all amended soils related to control soils. These results are consistent with the study of Chaganti et al. [58], who found that organic amendments (biochar, biosolids, green waste compost) improved soil hydraulic conductivity, thus facilitating salt leaching of saline-sodic soil, dropped significantly soil ESP and SAR. Moreover, Mahmoodabadi et al. [13], reported that organic amendments decreased soil SAR due to a reduction in Na^+ solution concentrations and enhanced Ca^{2+} supply. Relative changes in soil exchangeable Na^+ are dependent on chemical reactions in soil matrix. Chemical reactions take place between soil solution and exchange phases due to changes in chemical concentrations of monovalent and divalent cations [23]. In this regard, Nan et al. [57] applied G and HS in a sodic soil and observed improved soils structural stability and reduced of ESP from soil colloids, in this fashion salts could be leached down to deeper layers. Moreover, Vijayasatya et al. [23] reported that B incorporation to saline-sodic soil reduced ESP by 83% when compared to control after leaching. Likewise, Aktar et al. [39, 43], ascribed reduction of soil salinity with

B to (1) adsorption/ retention of salts such as Na^+ on biochar surfaces, or salt physical entrapment in B fine pores, and (2) biochar-induced reduction in upward movement of saline water.

We found significant higher concentrations of Soil Exchangeable Cation (Ca^{2+}) after application of combined amendments and G treatments (Table 5). These results suggest that HS, B and G have a synergistic effect to carry more Ca^{2+} into soil solution. HS, B and G increased Ca^{2+} content in saline-sodic soil, which improved exchange of sodium from soil exchange sites [14, 15, 57, 58]. Nevertheless, in our study individual application (B) and (HS) did not significantly increased soil exchangeable cation (Ca^{2+}) or this increase was very low (e.g. HS in the AZ-103 genotype; Table 5). This could be probably ascribed to chemical composition of Biochar (0.8% Ca).

Some differences in the final concentrations of bivalent cations (e.g. Ca, Mg) arise from chemical composition of applied organic amendments [13]. In our study Soil Exchangeable Mg^{2+} did not significantly increased after amendment applications (Table 5). Our results are consistent with findings of Chaganti et al. [23], who applied composts and B in a saline-sodic soil and noticed that exchangeable Mg^{2+} concentrations did not statistically differed respect to untreated soil.

It is widely acknowledged that divalent cation Ca^{2+} can replace adsorbed Na^+ in soil colloids, causing flocculation of colloids and improving soil structure [59]. This is fundamental of successful reclamation of saline-sodic soil. We found soil exchangeable Na^+ concentrations significantly decreased in all treated soils respect to untreated soils (Table 5). These results are in agreement with previously reported findings [13, 17, 57, 58]. Nevertheless, individual applications of B and HS did not increase Ca^{2+} concentrations in soil but did significantly decrease Na^+ concentrations; these effect might be explained due to HS and B applications to saline soils results in reduction of sodium salt concentrations, which is not correlated with salt leaching, yet it may be correlated with improving root growth and accumulation of Na^+ in the root/ surface area (biochar) [22, 60].

Several processes and materials able to alter soil C content can affect biomass and activity of microbial communities. B and HS can improve microorganisms' growth in salt-affected soils in many ways, including: increasing water retention and supply to microbes, releasing soil nutrients for microbes, stimulating root exudation of dissolved organic carbon and nitrogen that are needed for microbial metabolism. We found that soil microbial biomass (FDA) values were significantly increased by the most of treatments of both quinoa genotypes under study, except at G treatment of AZ-51 genotype, which was similar to control (Figure 2). These

results are consistent with our hypothesis that B and HS enhanced soil microbial activity. Zheng et al. [24] noticed that B (peanut Shell 350°C) induced a microbial response in the rhizosphere. Moreover, Bhaduri et al. [61] also reported that B application (peanut Shell 300°C, in 5% dose) on saline soil influenced microbial growth and C use efficiency led to increments in soil microbial biomass. According to Pukalchik et al. [62], addition of HS stimulated heterotrophic microorganisms in soil and consequently restored soil microbial respiration in a multi-contaminated soils. Our results indicated a synergistic effect between (B + HS + G) that leads to enhancements in soil microbial biomass and respiration (Figure 1, 3). The positive effect of B on microbial biomass can be explained because internal pore system of B particles may protect microorganisms. Microbial sorption to B may occur via flocculation, adsorption on surfaces and entrapment in a matrix [22]. According to Whitman et al. [63], microbial responses to B addition could be attributed to modified substrate availability (e.g. C and N), due to labile C input from biochar and soil chemical properties such as salt stress (e.g. ECe, ESP, Table 3). On the other hand, microorganisms can be stimulated by a HS addition through enriched surfactant-like interaction. Lipczynska-Kochani et al. [64] suggested that HS have direct effects on enzymes and oxidative stress defence. In addition, HS should be considered a significant carbon source for soil bacteria [64]. Control soils displayed values of microbial biomass FDA (enzymatic activities) significantly lower than those observed in other treated soils (Figure 3), which indicates that biochemical quality is negatively affected in saline-sodic soils. This may be due to a salting-out effect, which involves a decrease in enzyme solubility through dehydration (osmotic effect), thus altering enzyme catalytic sites [16, 65].

Consequently, combined amendments may be considered as promising technique to enhanced saline- sodic soil biology quality. However, role of biochar in soil biological processes represents a frontier in soil science research, with many unexplained phenomena awaiting for exploration [22].

CONCLUSIONS

The combination of biochar, humic substances and gypsum (B + HS + G) promoted the highest increments of root biomass in both quinoa genotypes. Stomatal conductance, SPAD index and seed yield in quinoa increased significantly in all treated soil as compared to control in AZ-103 genotype; however, these parameters in AZ-51 genotype increased by different treatments. AZ-51 genotype reached to double its seed productivity after applications of (G; HS + G); whereas in AZ-103 genotype similar increments were obtained

in all amended soils. Seed quality (protein content) was affected positively by B and HS amendment applications. These results suggest that each genotype responded differentially to amendment applications. On the other hand, soil sodicity and salinity (ESP; CEe) were significantly reduced with all individual and combined amendments. The most effective treatments in reducing sodicity levels through increments of soil exchangeable Ca^{2+} concentrations were: G; B + G; B + HS; B + HS + G. Moreover, soil microbial biomass was mostly stimulated with combined treatment (B + HS + G). In this fashion, combined amendment improved chemical and biological soil properties, reducing negative effects of saline- sodic soil in quinoa genotypes' performance. This paper depicts first results of AZ-51 and AZ-103 quinoa genotypes behaviour grown on saline sodic- soil and their responses to different amendments. It is also compelling to fully understand best treatments pointed out in this experiment (B + HS + G; B + G; B + HS) with thorough studies under field conditions.

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CAPÍTULO 3

CONCLUSIONES GENERALES

- La aplicación individual o combinada de biocarbón y sustancias húmicas mejoró las propiedades químicas y biológicas de un suelo salino sódico. Estos cambios en el suelo se reflejaron en el óptimo desarrollo y rendimiento de las plantas de quínoa (AZ-51, AZ-103).

- El tratamiento combinado (B + SH + Y) promovió un mayor incremento de biomasa radicular en ambos genotipos de quínoa, dotando a las plantas de tolerancia al estrés salino mediante un robusto sistema radicular.

- Los tratamientos más efectivos para reducir los niveles de sodicidad mediante incrementos de las concentraciones de Ca^{+} intercambiables en el suelo fueron: Y; B + Y; B + SH; B + SH + Y.

- La calidad biológica del suelo (biomasa microbiana y respiración microbiana), fue mayoritariamente estimulada en los suelos tratados con B + SH + Y.

- El tratamiento combinado (B + SH + Y) mejoró las propiedades químicas y biológicas del suelo salino sódico, por lo que representa una alternativa para la recuperación de suelos degradados tales como los salinos sódicos evaluados, reduciendo los efectos negativos de la salinidad y sodicidad sobre el performance de las plantas de quínoa.