Effects of planting quinoa on soil properties and microbial community in saline soil

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Abstract

Quinoa (Chenopodium quinoa), a herbaceous annual, has been widely cultivated in recent years because of its high nutritional value and strong tolerance to abiotic stresses. The study was conducted at two planting densities (LD, 10 plants/m2; HD, 65 plants/m2) on ameliorated coastal mudflats in Jiangsu Province, China (118° 46' E, 32deg 03' N). The results showed soil salinity and organic matter were higher in the HD than LD treatment, and salinity of the rhizosphere soil was higher than that of the non-rhizosphere soil. Quinoa grown in HD was taller, with thicker stalks and lower yields per plant, but higher yield per unit area. Amplicon sequencing showed that Proteobacteria, Bacteroidota and Acidobacteria were the dominant bacterial phyla. Regarding the rhizosphere soil, the Shannon index was higher in the HD than LD, and Proteobacteria and Bacteroidota were more abundant in the HD treatment. Fifty-one differential metabolites were identified by metabolomic assays, belonging to 14 annotated metabolic pathways. S-adenosylmethionine was the most abundant and up-regulated metabolite (fold change >1.67), and was more abundant in the roots from the LD than HD treatment. Docosahexaenoic acid was more abundant in the HD than LD treatment, and was down-regulated metabolite. In conclusion, planting density was an important factor affecting quinoa yield; compared with unplanted soil, planting quinoa at low density increased the content of the important metabolite S-adenosylmethionine in the root system of quinoa, and high density cultivation of quinoa increased soil salinity and microbial abundance and diversity.

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ABSTRACT

Quinoa (*Chenopodium quinoa*), a herbaceous annual, has been widely cultivated in recent years because of its high nutritional value and strong tolerance to abiotic stresses. The study was conducted at two planting densities (LD, 10 plants/m^2; HD, 65 plants/m^2) on ameliorated coastal mudflats in Jiangsu Province, China

(118° 46' E, 32deg 03' N). The results showed soil salinity and organic matter were higher in the HD than LD treatment, and salinity of the rhizosphere soil was higher than that of the non-rhizosphere soil. Quinoa grown in HD was taller, with thicker stalks and lower yields per plant, but higher yield per unit area. Amplicon sequencing showed that *Proteobacteria* , *Bacteroidota* and*Acidobacteria* were the dominant bacterial phyla. Regarding the rhizosphere soil, the Shannon index was higher in the HD than LD, and*Proteobacteria* and *Bacteroidota* were more abundant in the HD treatment. Fifty-one differential metabolites were identified by metabolomic assays, belonging to 14 annotated metabolic pathways. S-adenosylmethionine was the most abundant and up-regulated metabolite (fold change >1.67), and was more abundant in the roots from the LD than HD treatment. Docosahexaenoic acid was more abundant in the HD than LD treatment, and was down-regulated metabolite. In conclusion, planting density was an important factor affecting quinoa yield; compared with unplanted soil, planting quinoa at low density increased the content of the important metabolite S-adenosylmethionine in the root system of quinoa, and high density cultivation of quinoa increased soil salinity and microbial abundance and diversity.

Keywords

Quinoa, Planting density, High-throughput sequencing, Microorganisms, Root metabolome

1 INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd) is an annual herbaceous plant native to the Andes Mountains of South America. As the main traditional food of the Inca indigenous people, quinoa has been cultivated for more than 5000 years. The introduction of quinoa in China was relatively recent, with experimental research by the Tibetan Institute of Agriculture and Animal Husbandry and the Tibetan Academy of Agricultural Sciences in 1987, and small-scale trials in Tibet in 1992 and 1993. At present, small-scale plantings are done in Shanxi, Qinghai, Gansu, and Yunnan. Quinoa is a plant with strong environmental tolerance and can grow well under a variety of harsh conditions because it shows tolerance to cold, salinity and drought (Jacobsen et al., 2003). It also can grow on poorly fertile sandy and calcareous soils.

Quinoa grain is a rich source of a wide range of minerals, vitamins, fatty acids (e.g. linoleate and linolenate), natural antioxidants (Kozioł, 1992; Repo-Carrasco, 2003), and high-quality protein (with ample amounts of sulfur-containing amino acids) (Kozioł, 1992). Because of providing rich and balanced nutrition, quinoa has been ranked as one of the top 10 nutritious foods in the world (Wang et al., 2019). It is the only food considered by the Food and Agriculture Organization of the United Nations (FAO) to meet the basic nutritional needs of the human body from a single plant source (Ogungbenle, 2003).

The composition and activity of the soil microbial community largely determine biogeochemical cycles, organic matter turnover processes, and soil fertility and quality (Zelles, 1999). Rhizosphere microbiome influences plant growth and community succession (Herbert, 2009; Lambers et al., 2009). Plants interact with microorganisms, with plant residues and root secretions providing carbon and energy sources to soil microorganisms, and microorganisms decomposing organic compounds into inorganic nutrients for plant uptake and use (Hartmann et al., 2008; Marschner and Timonen, 2005). Therefore, understanding the composition and activity of soil microorganisms in interaction with plants can help us improve soil management and crop cultivation.

Metabolomics aims to identify and quantify the range of primary and secondary metabolites (generally <1800 kDa) involved in biological processes (Llanesa et al., 2018). Current studies on the metabolomics of quinoa are mainly in the breeding of quinoa varieties (Song et al., 2020) and nutritional composition studies (Liu et al., 2020). Metabolomic studies related to the technical aspects of quinoa cultivation are virtually non-existent.

The research on quinoa is mainly on the nutritional value and physiological characteristics (Wright et al., 2002; Ferreira et al., 2015). In addition, the screening of quinoa germplasm resources (Zurita-Silva et al., 2014) and work on quinoa pests and diseases, and genetic diversity were carried out (Hinojosa et al., 2021). Regarding agronomy, the recommended cultivation density is 67,500 plants per hectare in high altitude

and cool regions, 97,500 plants per hectare in arid, semi-arid and irrigated regions, and 120,000 plants per hectare in medium altitude and arid regions (Iglesias-Puig et al., 2015). The recommended planting density varies among quinoa varieties, but studies on quinoa planting density as related to soil microbiome and root metabolome are rare or non-existent. In this study, we grew quinoa in the field at two planting densities followed by sequencing microorganisms in the rhizosphere and non-rhizosphere soils and determining quinoa root metabolome to provide a theoretical basis for the effects of planting density and soil microbiome on quinoa yield.

2 Materials and methods

2.1 Field trial

The experiment was conducted at the agricultural science and technology innovation and demonstration base (33° 24' N, 120° 09' E) of Xin Yang Agricultural Experiment Station in Yancheng City, Jiangsu Province. The base contains ameliorated coastal mudflat land with a subtropical monsoon climate (average annual temperature of 14 , total annual precipitation of 1100 mm, average annual sunshine of 2200 h, frost-free period of 210 d, and an altitude of less than 5 m). The soil texture of the test field was sandy loam.

2.2 Sample collection

The field experiment was set up in a randomized complete block design with three replications for each planting density. The fertilizer rates used in this trial were N 120 kg/ha, P 34.9 kg/ha and K 0. The area of the plot was 2 m^2 (1 m x 2 m) with a 0.5 m border row. The two quinoa transplanting densities were 40 x 25 cm (10 plants/m^2) and 20 x 7.5 cm (65 plants/m^2), which were denoted low-density (LD) and high-density (HD), respectively. The experimental variety tested was "Yan Suli NO.1", a quinoa material selected and bred at Xinyang Agricultural Experiment Station, Yancheng City, Jiangsu Province. Sowing of quinoa was done on September 15, 2020; on October 15, quinoa seedlings of similar height and number of leaves were selected for transplanting.

On November 25, 2020, during the quinoa fruit development (Sosa-Zuniga et al., 2017), soil samples were randomly taken at three points in an "S" mode at one depth (0-10 cm) using soil auger (6 cm diameter) in each of the three replicate plots in each treatment. We collected rhizosphere soil (soil attached to the plant root system) (Shao et al., 2018), non-rhizosphere soil (soil away from plant root system), and blank control soils (from the undisturbed and unplanted area adjacent to the experiment). The soil samples were mixed and placed in separate sterile plastic bags. At the same time, about 10 g of each soil sample was wrapped in tinfoil, labeled, snap-frozen in liquid nitrogen, and returned to the laboratory in an ultra-low temperature refrigerator at -80degC for subsequent analysis of soil microorganisms. The remaining soil samples were air-dried, passed through 0.15-mm (for biological analyses) or 0.05-mm sieves (for chemical analyses) and stored at room temperature.

On November 26, 2020, during the quinoa fruit development (Sosa-Zuniga et al., 2017), six quinoa plants were randomly taken from each plot, divided into four parts (roots, stem, leaves, and panicle), dried in an oven at 75degC, and weighed. A portion of the fresh root system was rinsed with deionized water, blotted with sterile filter paper, placed in plastic centrifuge tubes, labeled, snap-frozen in liquid nitrogen, and stored in an ultra-low temperature refrigerator at -80degC before being returned to the laboratory for subsequent metabolomic analysis.

At plant maturity on January 5, 2021, six randomly taken panicles from each plot were weighed to estimate total yield per plot.

2.3 Methods

2.3.1 Determination of soil pH and salt content

Salinity and conductivity and pH were measured in soil:water suspension 1:5. Organic matter content was determined by potassium dichromate oxidation colorimetry (Ru-kun, 1999).

2.3.2 Characterization of soil microorganisms

The treatments in this experiment were labeled as LDN (low-density non-rhizosphere soil), LDR (low-density rhizosphere soil), HDN (high-density non-rhizosphere soil), and HDR (high-density rhizosphere soil). Soil DNA was extracted from 0.3 g of sieved (1 mm) soil using a Power Soil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA). The extracted genomic DNA was assessed by 1% w/w agarose gel electrophoresis and stored at -80degC (Rodrigues et al., 2013).

PCR amplification of the V3-V4 region of bacterial 16S rDNA was conducted using the universal primers 341F (5'-CCTAYGGGRBGCASCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). All PCR reactions were carried out in 15 μL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs, Ipswich, MA, USA), 2 μM of each forward and reverse primers, and about 10 ng of template DNA. Thermal cycling consisted of initial denaturation at 98°C for 1 min, followed by 30 cycles of denaturation at 98°C for 10 s, annealing at 50°C for 30 s, and elongation at 72°C for 30 s. Final elongation was done at 72°C for 5 min. Each sample was measured in three technical repetitions.

The same volume of 1X loading buffer (containing SYB green) and PCR products were mixed and electrophoresed on 2% w/v agarose gel for detection. The mixed PCR products were purified using a Qiagen Gel Extraction Kit (Qiagen, Dusseldorf, Germany). Sequencing libraries were generated using a TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, San Diego, CA, USA) following manufacturer's recommendations, and index codes were added. The library quality was assessed on a Qubit@ 2.0 Fluorometer (Thermo Scientific, Waltham, MA, USA) and an Agilent Bioanalyzer 2100. The library was sequenced on an Illumina NovaSeq platform, and 250 bp paired-end reads were generated (Li et al., 2020; Shao et al., 2018).

Based on the 16S rDNA PCR amplification, a linear discriminant (LDA) effect size (LEfSe) analysis was conducted to identify taxa with significant differences in abundance among the treatments and to construct the relevant cladograms (Segata et al., 2011).

To estimate alpha diversity, the OTU table was rarified, and three metrics were calculated: Chao 1 index to estimate the abundance, the observed OTUs, and Shannon index to estimate diversity (Vishnivetskaya et al., 2011).

2.3.3 Metabolomic analyses

Quinoa roots stored at -80 °C were used for metabolomic analysis. Amount of 100 mg of ground root tissue was placed in an Eppendorf tube, 500 μL of 80% v/v methanol in water was added, vortexed and shaken, left to stand in an ice bath for 5 min, then centrifuged at 15,000 g at 4°C for 20 min. Supernatant was collected, diluted with mass spectrometry-grade water to reach 53% v/v methanol and analyzed by LC-MS (Want et al., 2013).

2.4 Statistics

Statistical analyses were performed using Microsoft Excel 2007, SPSS Statistics 23.0 (IBM, Armonk, New York, USA) and R package vegan (version 2.5-5). Adobe Illustrator CC 2020 and R package ggplot2 (version 3.2.0) were used to draw figures.

The average value of all parameters was taken from three replicates, and the standard error was calculated. One-way analysis of variance (ANOVA) was used to analyze the data (p[?]0.05). Correlation analysis used the Mantel test and Spearman's rank correlation coefficients. LDA Effect Size analysis (LEfSe) used the ANOVA and then the Wilcoxon rank sum test to analyze the differences among the treatments (threshold set to 0.05).

3 Results

3.1 Soil salinity, organic matter and biomass of quinoa

Soil salinity ranged from 1.21 to 3.83 g/kg (Fig. 1a). Compared with unplanted soil, the salt content was significantly higher in the rhizosphere soil in both density treatments as well as in the non-rhizosphere

soil of the high-density treatment. The soil organic matter content ranged from 10 to 13.4 (g/kg), being significantly lower in the unplanted soil compared with all other soil samples (Fig. 1b).

As the planting density increased, the stem diameter and total above-ground biomass and fresh panicle weight per plant decreased significantly, whereas total fresh panicle weight per unit area increased significantly (Table 1). There was a slight (non-significant) increase in plant height with an increase in planting density.

As shown in Fig. 2, the total above-ground biomass per plant showed a positive correlation with the fresh panicle weight and stem diameter. The fresh panicle weight was negatively correlated with the salt content in the rhizosphere soil.

3.2 Soil microorganisms

For the diversity of microbial communities detected in different samples at 97% consistency threshold, the following conclusions were drawn based on the analysis of Observed Taxonomic Units (OTUs) and Shannon and Chao1 indices (Table 2). The Shannon index showed the bacterial diversity was higher in high-density rhizosphere soils (HDR) than in low-density rhizosphere soils (LDR). However, there was no significant difference in bacterial diversity between rhizosphere and non-rhizosphere soils at the same planting density, and there was no variability in OTUs and Chao1 indices.

The bacterial OTU numbers in LDN, LDR, HDN, and HDR were 5018, 4789, 5168, and 5202, respectively (Fig. 3). The number of OTUs was higher in the high-density than low-density planting soil, but there was no obvious difference between the OTUs in the rhizosphere and non-rhizosphere soil.

Soil samples from 12 different treatments were annotated with the database Silva138. The top eight phyla in terms of relative abundance were *Proteobacteria* , *unidentified_Bacteria* ,*Bacteroidota* , *Acidobacteriota* , *Crenarchaeota* ,*Firmicutes* , *Actinobacteriota* , and *Gemmatimonadota* . Among them, *Proteobacteria* ,*unidentified_Bacteria* ,*Bacteroidota,*and *Acidobacteriota* had the highest relative abundance, accounting for more than 60% of all phyla. *Proteobacteria* was the most abundant phylum in soil; the relative abundance in LDN, LDR, HDN, and HDR was 26, 25, 27, and 26%, respectively. The relative abundance of *Bacteroidota* in LDN, LDR, HDN, and HDR was 9, 9, 10, and 13%, and that of *Acidobacteriota* was 8, 10, 9, and 7%, respectively (Fig. 4).

Fig 5 showed the LEfSe analysis of soil prokaryotic microorganisms (bacterial domain) as influenced by different treatments. The cladograms showed the taxa (highlighted by small circles) that played an important role in the structure of the microbial community. The LDA scores distribution histograms showed significant differences in abundance among the treatment soils, with the length of the bars representing the magnitude of the microbial influence.

The LDA-based LEfSe identified four bacterial taxa with LDA thresholds greater than 3.5 in the LDR, namely: *Vicinamibacteraceae* (f) > *Vicinamibacteria* (c) >*Vicinamibacterales* (o) > *Latescibacterota*(p). Among them, *Vicinamibacteraceae* , *Vicinamibacteria* and*Vicinamibacterales* belong to the *Acidobacteriota* . The bacteria endemic in the LDN were *Bacillales* (o) >*Bacillus* (g) > *Bacillaceae* (f), whereas*Oceanospirillales* (o) were abundant in the HDR.

The soil properties explained a large proportion of variability in the relative abundance of *Bacteroidota* , *Actinobacteriota* ,*Acidobacteriota* , and *Firmicutes* . The relative abundance of*Bacteroidota* was correlated positively with salinity and pH, and negatively with organic matter. The relative abundance of*Actinobacteriota* was correlated positively with salinity, negatively with organic matter content, and was largely independent of soil pH. The relative abundance of *Acidobacteriota* was correlated positively with organic matter, and negatively with pH and salinity.*Firmicutes* were mostly independent of soil chemical properties (Fig. 6).

3.3 Root tissue metabolites

The Partial Least Squares Discrimination Analysis (PLS-DA) model separated the samples from the two planting treatments. PLS-DA is a supervised discriminant analysis statistical method, which uses partial least squares regression (Boulesteix et al., 2007) to establish the relationship model between metabolite

expression and sample category. PC1 and PC2 were the scores of the test samples in the first and second principal components, respectively. From Fig. 7, it was clear that the two planting density treatments had a certain degree of differentiation; hence, the subsequent data analysis was reliable.

The threshold values of Variable Importance in the Projection (VIP) >1.0, Fold Change (FC) >1.5 or <0.667 and P value< 0.05 were set as the screening criteria for significantly different metabolites. A total of 51 metabolites with significant differences were found. Plotting all metabolites of the low-density (L) vs high-density (H) treatments in the volcano map can help us quickly find the differences in expression of root tissue metabolites. Among the 51 different metabolites, 28 were significantly up-regulated and 23 were significantly down-regulated, with a relatively small difference (Fig. 7b). We selected the top 10 metabolites with the largest differences for the annotation study. These top 10 differential metabolites were: schisandrin C (up), s7p (down), pelargonidin chloride (down), N-feruloylspermidine (up), tyrosol (down), acetylharpagide (down), KMH (up), kanamycin (up), 3-ureidopropionic acid (up), and eicosapentaenoic acid (down) (Fig. 7c).

All the differentially expressed metabolites in the low-density vs high-density treatments were put into the KEGG database for annotations. The 14 annotated differential metabolites and the involved metabolic pathways are listed in Table 3. There were five up-regulated and nine down-regulated metabolites.

It can be seen from Fig. 7c that the metabolic pathways with the lowest P values was biosynthesis of unsaturated fatty acids, and the differential metabolites within the pathways were eicosapentaenoic acid and docosahexaenoic acid. Both eicosapentaenoic and docosahexaenoic acids were down-regulated.

The metabolite S-adenosylmethionine was the most enriched metabolite in all metabolic pathways, so we focused on it. The mean relative content of S-adenosylmethionine was 161 x 10^5 in the low-density treatment (LD) and 71 x 10^5 in the high-density treatment (HD), indicating the content of S-adenosylmethionine in the LD was twice that in the HD. The mean relative content of docosahexaenoic acids was 2971 x 10^5 in the LD treatment and 7183 x 10^5 in the HD treatment, i.e., the content of docosahexaenoic acids in the HD treatment was more than twice that in the LD treatment.

4 DISCUSSION

4.1 Effect of quinoa cultivation on soil physicochemical properties

Quinoa is an apomictic halophyte (Razzaghi et al., 2012) with salt glands or salt capsules, i.e. special epidermal cells that can isolate or exclude excess salt from metabolically active cells (Liphschitz and Waisel, 1982). In the present study, the soil salinity increased in the soil that had been planted with quinoa compared to the unplanted soil (CK), and the salt content of high-density rhizosphere soil (HDR) increased most significantly compared to CK. Nicole Goehring et al (Goehring et al., 2019) estimated quinoa biomass production and soil salinity with different irrigation managements and salinities, and the root zone salinity increased in all treatments, which was consistent with the results of this experiment. Zhang studied *Puccinellia ciliata* (a salt-tolerant species) through greenhouse experiments and found that salt accumulation also occurred between the roots of salt-tolerant species on saline soils (Zhang et al., 2005). It is suggested that quinoa continually effluxed salt from roots, contributing to increased salinity in the rhizosphere soil, especially in high-density planting (Fig. 1a). Therefore, cultivation of quinoa requires adequate water supply and drainage to remove salt from the soil in the high-density planting area and avoid inhibition of quinoa growth due to salt accumulation.

4.2 Growth indicators of quinoa at different planting densities

High salinity in the soil reduces plant growth and productivity (Pan et al., 2011). In the study presented here, negative correlation between the rhizosphere soil salinity and fresh panicle weight per plant (Fig. 2) suggested that the salt content of the rhizosphere soil contributed to decreased growth and yield of quinoa. As the planting density increased, the ventilation and light penetration through the canopy were limited, and stem diameter, total biomass, and fresh panicle weight per plant decreased with increasing density. In high-density planting, poor sunlight in the canopy induced plants to grow taller and thinner (Table 1).

4.3 Soil microbiome

The biomass of soil microorganisms is affected by a variety of factors such as temperature, soil moisture, plant growth and animal disturbance (Fierer and Jackson, 2006; Zhao et al., 2006). In the study presented here, the Shannon index was higher in high-density (HDR) than low-density rhizosphere soil (LDR), indicating a positive effect of high-density planting on the diversity of soil bacteria. This might have been related to the root exudation of quinoa providing nutrients for microbial growth and reproduction.

Proteobacteria were the overwhelmingly dominant phylum detected in this study. They are widespread in a variety of natural and artificial environments and represent one of the largest taxa of the entire bacterial kingdom (Ciccarelli et al., 2006).*Proteobacteria* are important in the global carbon, nitrogen and sulfur cycling (Dworkin et al., 2006) and can also serve as biological control agents in soil-plant ecosystems (Lueders et al., 2006). Thus,*Proteobacteria* have an important role in agriculture.

Burkholderiales (order in phylum *Proteobacteria*) were endemic to low-density rhizosphere soils in this study, known to inhabit mainly the roots and leaves of plants. Most of the major groups of*Burkholderiales* have the nitrogen fixation, phosphate solubilization and phytohormone production capacities that may promote plant growth and development (Peter et al., 2002; Castanheira et al., 2016; Zhang et al., 2000).

Both *Proteobacteria* and *Bacteroidota* were positively correlated with soil salinity, and the levels of these two bacterial phyla were relatively high in soils with high-density quinoa cultivation. Further study is needed to ascertain a potential role of these bacteria in promoting quinoa growth in saline soils.

4.4 Root tissue metabolites

A total of 14 annotated metabolic pathways were found in this study, and the most differentially expressed (upregulated) metabolite was S-adenosylmethionine (SAM) (in eight of the 14 metabolic pathways). SAM is second only to ATP in biological utilization and is involved in numerous intracellular metabolic reactions (Fontecave et al., 2004). Studies have shown that SAM has an important role in regulating plant adaptations to various abiotic stresses, such as iron deficiency (Lan et al., 2011), drought (Mayne et al., 1996) and salinity (Gong et al., 2016; Fujimoto et al., 2011) as well as biotic stresses (e.g. resistance to pathogenic bacteria) (Fujimoto et al., 2011) and has a complex time-dependent role in the senescence processes (Owiti et al., 2011). However, the effect of planting density on the abundance of SAM in roots has not been reported previously. In cucumber, soaking broken-root seedlings in SAM-containing solution significantly promoted root development, chlorophyll accumulation, enhanced photosynthetic rate, and improved plant uptake of N, P, and K (Liu et al., 2018). In the study presented here, the average abundance of SAM in quinoa roots in low-density planting was twice that in high-density planting. It is suggested that SAM can improve the adaptation of quinoa to coastal saline soils and may have some growth-promoting effects. Further research is needed to shed some light on the mechanism underpinning SAM-related promotion of quinoa growth.

5 Conclusion

In this study, we characterized soil chemical properties, microbial communities of rhizosphere and non-rhizosphere soils, and root tissue metabolites of quinoa planted at different densities. With increased planting density, soil salt content and microbial diversity increased. However, intense inter-plant competition in high-density planting resulted in taller and thinner plants with small panicles, but higher grain yield in gih-density than low-density planting. A high abundance of up-regulated metabolite S-adenosylmethionine was found in roots in low-density planting, which might have had a positive effect on plant growth and development. The mechanism by which S-adenosylmethionine promotes quinoa growth should be studied further.

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Tables and figures captions

Table 1 Quinoa growth indicators as influenced by different planting densities

low density (row spacing 40 × 25 cm); H-high density (row spacing 20 × 7.5 cm). The data are means ± standard errors (n=3). Different lowercase letters in a row denote significant differences.

Table 2 Comparison of the estimated operational taxonomic unit (OTU), Chao1 richness and Shannon diversity indices of the rhizosphere and non-rhizosphere soils in different quinoa planting density treatments.

LDN= Low-density treatment, non-rhizosphere soil; LDR= Low-density treatment, rhizosphere soil; HDN= High-density treatment, non-rhizosphere soil; HDR= High-density treatment, rhizosphere soil. Means (n = 3). Different letters in a column indicate significant (p [?]0.05) differences among the four treatments.

Table 3 KEGG enrichment results in the low-density vs high-density treatments

Fig. 1 Soil salinity (a) and organic matter (b) in the rhizosphere and non-rhizosphere soil as influenced by quinoa planting density.

Different lower-case letters represent significant differences (p[?] 0.05). Means +- SE (n=3). LDN= Low-density treatment, non-rhizosphere soil; LDR= Low-density treatment, rhizosphere soil; HDN= High-density treatment, non-rhizosphere soil; HDR= High-density treatment, rhizosphere soil.

Fig. 2 Correlation between growth indicators of quinoa and the rhizosphere soil organic matter and salinity.

The color gradient denotes Pearson's correlation coefficients.

Fig. 3 Venn diagram of the bacterial OTUs

LDN= Low-density treatment, non-rhizosphere soil; LDR= Low-density treatment, rhizosphere soil; HDN= High-density treatment, non-rhizosphere soil; HDR= High-density treatment, rhizosphere soil.

Fig. 4 Comparison of relative abundance of bacterial phyla

LDN= Low-density treatment, non-rhizosphere soil; LDR= Low-density treatment, rhizosphere soil; HDN= High-density treatment, non-rhizosphere soil; HDR= High-density treatment, rhizosphere soil.

Fig. 5 LDA Effect Size (LEfSe)

The LDA scores distribution histograms (a) showing taxa with significantly different abundance. The differences are mapped to cladograms (taxonomic trees) (b). LDN= Low-density treatment, non-rhizosphere soil; LDR= Low-density treatment, rhizosphere soil; HDR= High-density treatment, rhizosphere soil. In the

cladograms, the taxa associated with small circles and the shading in the color of a specific soil played an important part in the structure of the microbial community in that soil (significantly different from other soils). The diameter of the small circle represents relative abundance of the taxa. The taxa without a significant difference are colored yellow.

Fig. 6 Redundancy analysis of soil chemical properties and relative abundance of bacterial taxa. The blue arrows represent environmental factors (soil properties), and the red arrows represent the top ten bacterial phyla in terms of relative abundance.

LDN= Low-density treatment, non-rhizosphere soil; LDR= Low-density treatment, rhizosphere soil; HDN= High-density treatment, non-rhizosphere soil; HDR= High-density treatment, rhizosphere soil.

Fig. 7 PLS-DA score plot (a), Volcano map of differential metabolites (b) and KEGG Enrichment scatterplot (c). L = low-density treatment, H = high-density treatment.

Table 1 Quinoa growth indicators as influenced by different planting densities

Growth index	Density L	Density H
Stem diameter (cm)	0.7±0.05a	0.5±0.05b
Plant height (cm)	73±4a	76±4a
Total above-ground biomass (g/plant)	287±81a	121±3b
Fresh panicle weight (g/plant)	9.8±1.4a	3.9±1.1b
Fresh panicle weight (g/m^2)	98±14b	254±73a

L-low density (row spacing 40 × 25 cm); H-high density (row spacing 20 × 7.5 cm). The data are means ± standard errors (n=3). Different lowercase letters in a row denote significant differences.

Table 2 Comparison of the estimated operational taxonomic unit (OTU), Chao1 richness and Shannon diversity indices of the rhizosphere and non-rhizosphere soils in different quinoa planting density treatments.

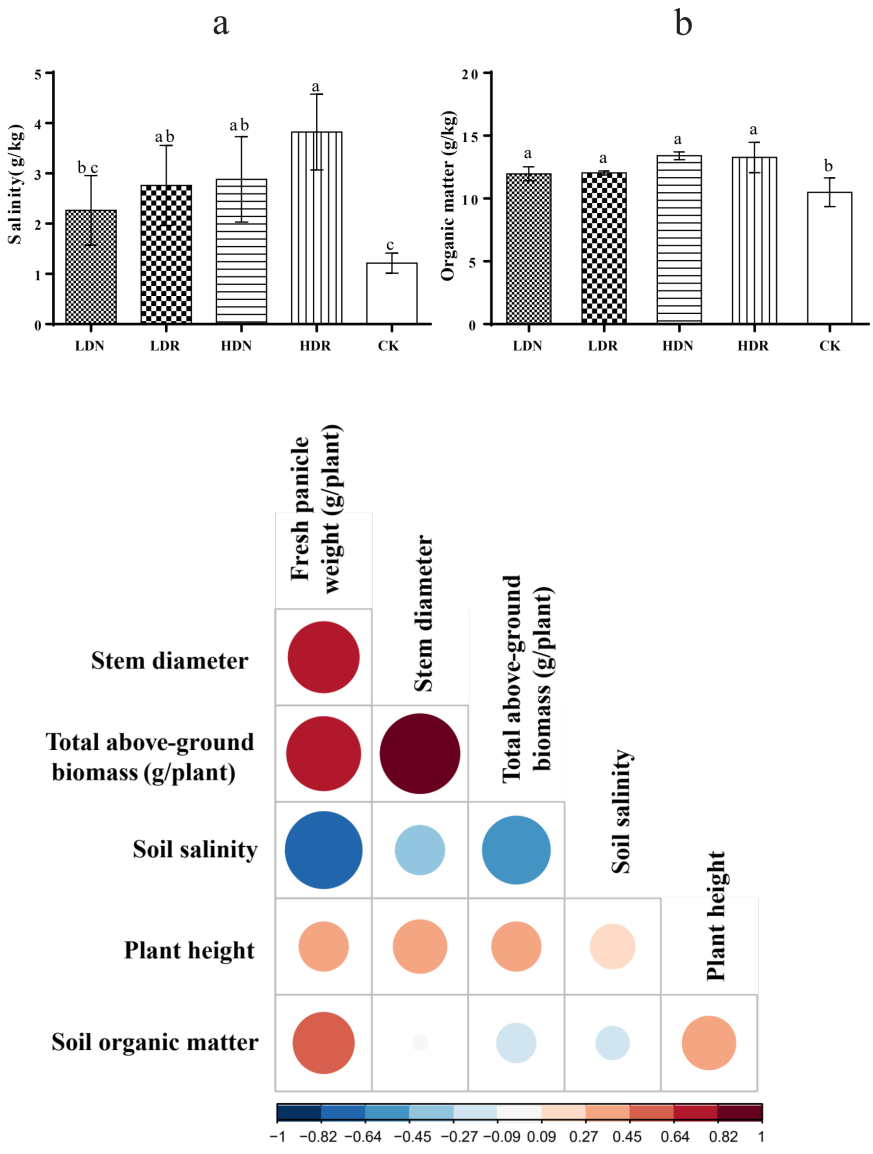
Sample name	OTUs	Shannon	Chao1
LDN	3135^{a}	9.89^{ab}	3477^{a}
LDR	2949^{a}	9.73^{b}	3319^{a}
HDN	3258^{a}	9.94^{ab}	3671^{a}
HDR	3244^{a}	10.01^{a}	3675^{a}

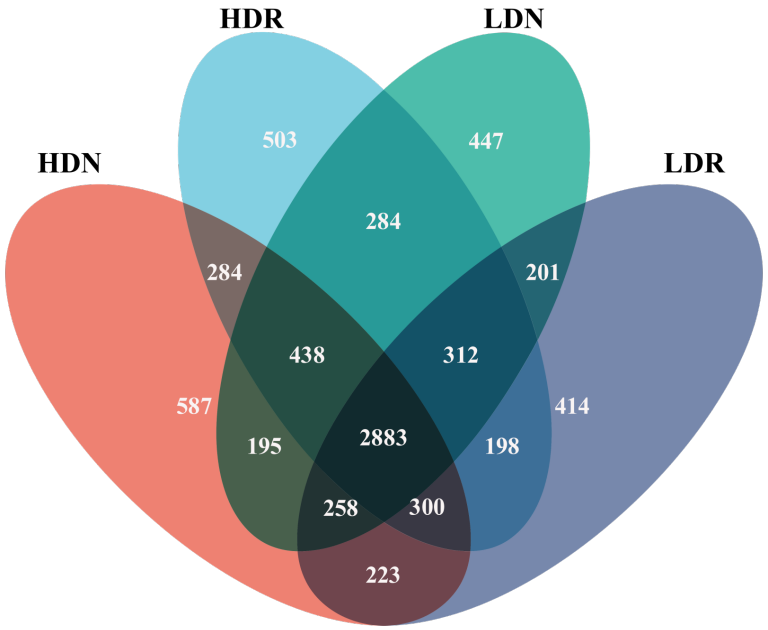
LDN= Low-density treatment, non-rhizosphere soil; LDR= Low-density treatment, rhizosphere soil; HDN= High-density treatment, non-rhizosphere soil; HDR= High-density treatment, rhizosphere soil. Means (n = 3). Different letters in a column indicate significant (p [?]0.05) differences among the four treatments.

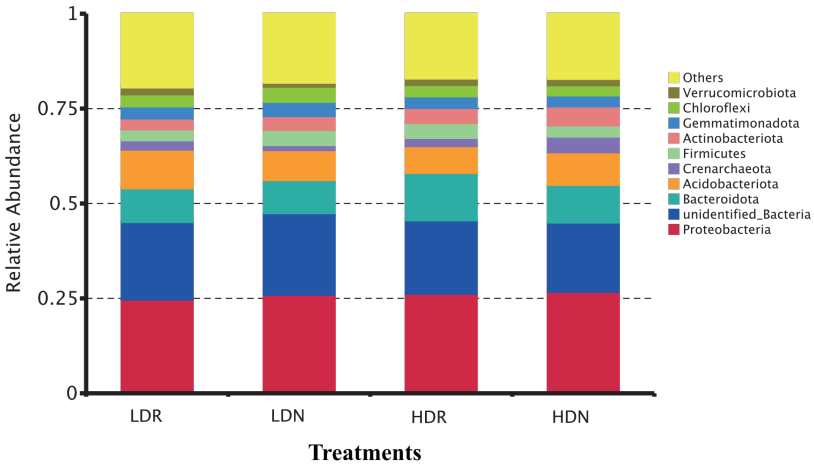
Table 3 KEGG enrichment results in the low-density vs high-density treatments

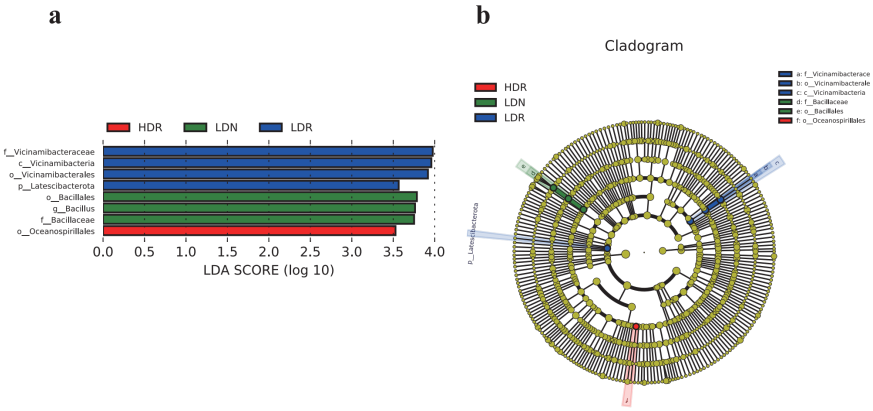
Metabolite	Regulated	KEGG pathway annotation
Eicosapentaenoic acid	down	Biosynthesis of unsaturated fatty acids (map01040)
Docosahexaenoic acid	down	Biosynthesis of unsaturated fatty acids (map01040)
Glutaric acid	up	Fatty acid degradation (map00071); Lysine degradation (map00310)
trans-cinnamic acid	up	Ubiquinone and other terpenoid-quinone biosynthesis (map 00130); Phenylalanin
Loganic acid	down	Monoterpenoid biosynthesis (map00902); Biosynthesis of secondary metabolites (
Pelargonidin chloride	down	Anthocyanin biosynthesis (map00942); Flavonoid biosynthesis (map00941); Biosy
Sepiapterin	down	Folate biosynthesis (map00790)
S-adenosylmethionine	up	Zeatin biosynthesis (map00908); Sulfur relay system (map04122); Monobactam b
Kaempferide	down	Flavone and flavonol biosynthesis (map00944)

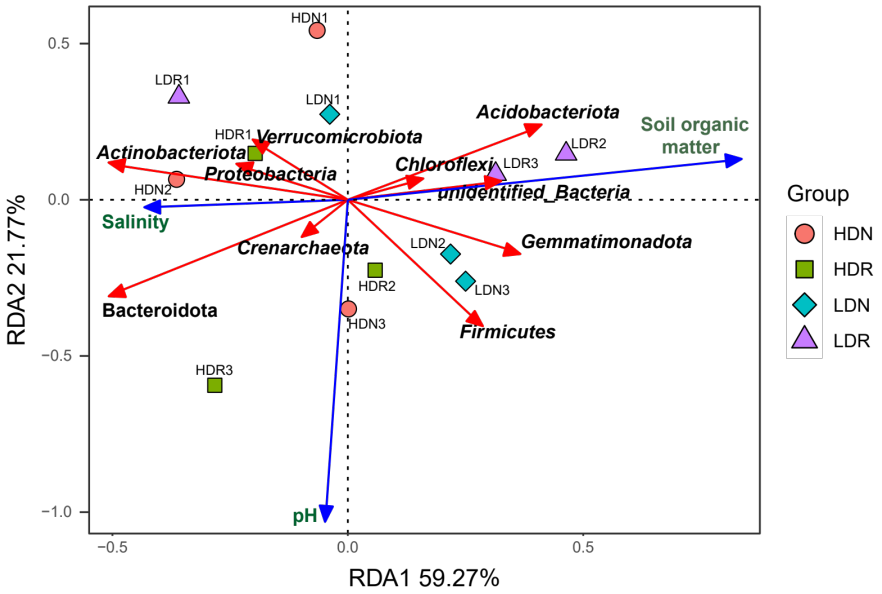
Tyrosol	down	Tyrosine metabolism (map00350)
Hypoxanthine	down	Purine metabolism (map002300); Metabolic pathways (map01100)
Protocatechuic acid	up	Phenylalanine, tyrosine and tryptophan biosynthesis (map00400); Biosynthesis of
Cytidine-5'-monophosphate	up	Pyrimidine metabolism (map002400); Metabolic pathways (map011000)
Lupinine	down	Tropane, piperidine and pyridine alkaloid biosynthesis (map00960); Biosynthesis











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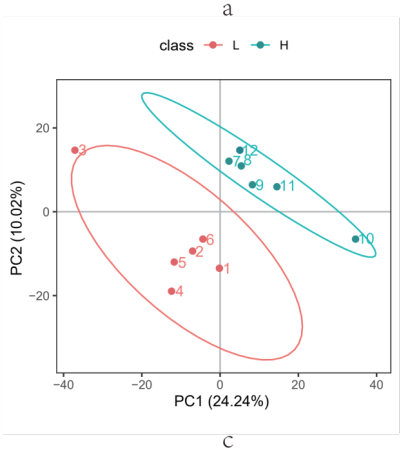

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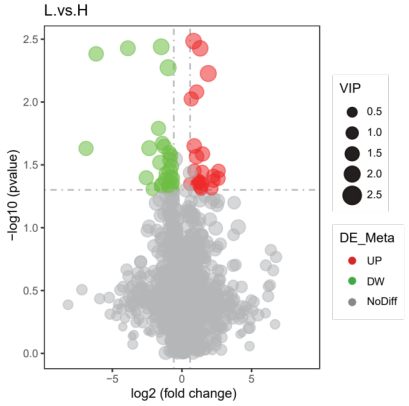

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