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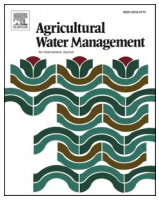

Assessing changes in growth, yield, and rhizosphere microbiome of red beetroot (*Beta vulgaris* subsp. *vulgaris* var. conditiva) cultivated under a saline integrated vegeculture-aquaculture system

Fahad Kimera [a], Muziri Mugwanya [a], Walaa Ahmed [a], Mahmoud A.O. Dawood [a,b], Hani Sewilam [a,c,*]

[a] Center for Applied Research on the Environment and Sustainability (CARES), School of Science and Engineering, The American University in Cairo, AUC Avenue, P.O. Box 74, New Cairo 11835, Egypt
[b] Department of Animal Production, Faculty of Agriculture, Kafrelsheikh University, Kafrelsheikh 33516, Egypt
[c] Managing Director, UNESCO Chair in Hydrological Changes and Water Resources Management, RWTH Aachen University, Germany

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ABSTRACT

In the past decade, red beetroot (*Beta vulgaris* L. ssp. *vulgaris*) has attracted the scientific community's attention as a functional food due to its essential biochemical properties for improving human health. Consequently, there has been an increase in the human and industrial demand for this crop, hence a growing interest in sustainable agricultural technologies to increase production. This study, therefore, aimed to investigate changes in the growth, yield, and rhizosphere microbiome of red beetroot cultivated in a saline integrated vegeculture-aquaculture system (IVAS). The study followed a completely randomized design of four salinity treatments: T1: 4.7 dSm^{-1}, T2: 7.5 dSm^{-1}, T3: 11.25 dSm^{-1}, and T4: control (fresh water, 0.63 dSm^{-1}) with four replicates. The study's results indicated that salinity negatively affected plant growth (plant height, leaf number, leaf length, leaf width, leaf shape index) and yield (leaf fresh and dry weights, root fresh weight, and root diameter), with the lowest values recorded in T3 and T2. Likewise, T3 and T2 recorded the lowest values for the relative water content at 15 days after transplanting compared to T1 and T4. Nutrient analysis indicated higher magnesium, zinc, and manganese content in red beetroot cultivated in T1, T2, and T3. On the other hand, results of the rhizosphere microbiome indicated that salinity influenced the abundance and diversity of bacteria and archaea species in the IVAS. *Proteobacteria* and *Euryarchaeota* were the dominant bacterial and archaeal phyla across all the salinity treatment groups. At the class level, *Gammaproteobacteria* were dominant in T1, T2, and T3, whereas T4 exhibited a variation in the dominance of different bacterial classes. For archaea, the dominant classes were *Thermoprotei* in T1 and T3, unclassified *Euryarchaeota* and unclassified archaea in T2, and unclassified archaea in T4. Overall, the microbial composition in the different treatments was weakly correlated with the sand's physical and chemical properties. In conclusion, therefore, red beetroot can be grown in an integrated vegeculture-aquaponics system in water salinities not exceeding 7.5 dSm^{-1}.

1. Introduction

Functional foods contain bioactive compounds such as proteins, vitamins, minerals, fibers, and phytochemicals (i.e. polyphenols) that benefit human health (Clifford et al., 2015; Nataraj et al., 2020). In the past decade, red beetroot (*Beta vulgaris* L. ssp. *vulgaris*) has attracted interest within the scientific community for not only its tolerance to salinity (i.e., salinity threshold of 4.0 dSm^{-1}) (Kurinc and Doganay, 2022), but also for its abundant source of several bioactive compounds, particularly betalains (betaxanthins or betacyanins), vitamins (ascorbic acid, pyridoxine, β-carotene, vitamin A, vitamin k, and tocopherol), mineral elements (zinc, potassium, selenium, iron, calcium, and magnesium), phenolics, carotenoids, flavonoids, and nitrates which are of a significant benefit for human health (Clifford et al., 2015; Fu et al., 2020; Hadipour et al., 2020). Moreover, red beetroot juice (BRJ), a popularly known drink among athletes, is consumed for its nitrate

* Corresponding author at: Managing Director, UNESCO Chair in Hydrological Changes and Water Resources Management, RWTH Aachen University, Germany.
E-mail addresses: FKim@aucegypt.edu (F. Kimera), muziri@aucegypt.edu (M. Mugwanya), walaa.ahmed@aucegypt.edu (W. Ahmed), mahmoud-dawood@aucegypt.edu (M.A.O. Dawood), sewilam@lfi.rwth-aachen.de (H. Sewilam).

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content, which has been linked to enhanced oxygen delivery to skeletal muscles, muscle efficiency, tolerance, and endurance, thus improving sports performance (Zamani et al., 2021). Besides red BRJ, beetroot is also consumed as a raw salad and, therefore, used in several cuisines and the food processing industry as a natural colorant (Akan et al., 2021; Chhikara et al., 2019). As such, the human and industrial demand for red beetroot is exponentially increasing, which warrants its increased and sustainable production.

Sandponics, a reasonably low-cost, low-technology, and sustainable soilless vegetable production technique, offers opportunities for the mass production of vegetables under greenhouse conditions (Makokha et al., 2020). This system, also called the integrated vegeculture-aquaculture system (IVAS) utilizes sand not only as a growth media for plant support but also as a physical and biological filter for wastewater purification (Kimera et al., 2023; Sewilam et al., 2022). Generally, sand has a low carbon-nitrogen ratio, which is not conducive to the proliferation of pathogenic microorganisms (El-Wahab et al., 2022). Furthermore, the particle size of the sand and its chemical properties determine its hydraulic properties (i.e. water retention and aeration), which impact the growth of plant roots. Previous studies have shown improved growth and yield of several vegetable and medicinal crops cultivated under sandponics systems. For instance, Sewilam et al. (2022) conducted a comparative study on different sand-based media for their suitability for the growth of Swiss chard (*Beta vulgaris* subsp. *cicia*) cultivated in an IVAS. The authors reported improved growth and yield of Swiss chard grown in sand-based media obtained from Fayoum compared to other locations (i.e. 6 October city and Beni Suef). Moreover, crops cultivated in sand-based media from Fayoum consumed less water. The chemical properties of this sand indicated lower concentrations of sodium, chloride, and potassium ions, which the authors anticipate could have contributed to better plant growth and water use efficiency of Swiss chard. In another study, Kimera et al. (2023) investigated the growth and yield response of kale (*Brassica oleracea*) and Nile tilapia (*Oreochromis niloticus*) under a saline IVAS, and the study results showed that integrating kale and Nile tilapia production in water salinities reaching up to 7.5 dSm^{-1} could be a sustainable alternative to increase food production in arid regions. Makokha et al. (2020) conducted a comparative study on multiplying sweet potato (*Ipomoea batatas* (L.) Lam) pre-basic seed in sandponics and conventional systems. They observed an increase in the vine multiplication rate under the sandponics system. El-Wahab et al. (2022) evaluated the growth, yield, and biochemical composition of two *Stevia rebaudiana* cultivars: Sugar High-A3 and Morita, cultivated in a sandponics system under greenhouse conditions. The study results indicated superior growth performance and biochemical composition (i.e. stevioside) of Morita under a sandponics system.

The natural establishment of microorganisms in agricultural systems is of significant interest due to their role in nitrification, plant nutrient uptake, and tolerance against environmental stress (Eck et al., 2019, 2021; Mourouzidou et al., 2023). To the best of our knowledge, no study has been conducted to evaluate changes in the rhizosphere microbiome of red beetroot cultivated under a saline IVAS. The main objectives of this study, therefore, were to: (i) assess the influence of salinity on the growth performance and yield of beetroot cultivated in an IVAS, (ii) decipher changes in the rhizosphere microbiome in different salinity treatments of the IVAS, and (iii) elucidate the influence of the sand's physical and chemical properties on microbial composition.

2. Materials and methods

2.1. Experimental layout

A greenhouse experiment was conducted at the Center for Applied Research on the Environment and Sustainability, The American University in Cairo (30°01′11.7″N 31°29′59.8″E). The study was laid out in a completely randomized design of four salinity treatment groups, namely: T1: 4.7 dSm^{-1}, T2: 7.5 dSm^{-1}, T3: 11.25 dSm^{-1}, and T4: control (fresh water, 0.63 dSm^{-1}), with four replicates per treatment. These salinity levels were chosen based on the salinity tolerance level of 4.0 dSm^{-1} for red beetroot (Kurinc and Doganay, 2022) and 31.25 dSm^{-1} for *Oreochromis niloticus* (Dawood et al., 2022). Fig. 1 shows a schematic diagram of the experimental layout and the sandponics system. The chemical properties of the salt used in this study to prepare the different salinity treatments have been previously reported by Kimera et al. (2023) and are presented in Supplementary Table 1. Seeds of *Beta vulgaris* subsp. *vulgaris* var. conditiva were obtained from the local distributor and sown in a nursery bed for 14 days until transplanting. Uniformly grown seedlings were transplanted to the sandponics grow beds with an inter- and intra-row spacing of 30 cm and 50 cm, respectively. Salinity treatments were initiated 7 days after transplanting (DAT), and plants were drip irrigated daily at the rate of 8 $Lm^{-2}day^{-1}$ to maintain 100 % field capacity. Field capacity was measured 24 h after the last irrigation using a field-scout TDR 350 soil moisture meter (Spectrum Technologies, Illinois, USA).

2.2. Plant phenotypic parameters and relative water content

Plant phenotypic parameters such as plant height, leaf number, leaf length, and leaf width were measured at 15, 30, 45, and 60 days after transplanting (DAT). Plant heights were measured from the crown to the terminal apex of the plant using a meter rule. Leaf number per plant was determined by counting the number of fully expanded leaves, and averages were determined. Leaf length and width were measured using a ruler. The leaf shape index (LSI) was calculated as a ratio of leaf width to leaf length. At harvest, leaf fresh weight and root fresh weight were measured using a laboratory weighing balance. For dry weights, leaves were put in labeled paper bags and dried in the oven to constant weight at 70 °C for 72 h and then weighed using a laboratory weighing balance. Root diameter was measured using digital Vernier calipers.

The relative water content (RWC) was estimated according to the equation by Ahmed et al. (2019). Briefly, leaf discs were obtained from each salinity treatment group using a cork borer, and the samples were weighed to obtain the fresh weight. The samples were then submerged in distilled water for 24 h in the dark and weighed to obtain the turgid weight. Samples were then dried in an oven at 70 °C for 24 h and weighed to obtain the dry weights. The equation below was used to calculate the RWC.

RWC = [(fresh weight – dry weight) / (turgid weight – dry weight)] *100

2.3. Nutrient analysis

Freshly harvested beetroots were plunged in liquid nitrogen and immediately stored at −20 °C until further analysis. Samples were subjected to microwave digestion, and the resultant solution was injected into the atomic adsorption equipment to measure the concentration of macro and microelements. All samples were measured in triplicate, and averages were calculated.

2.4. Sand test

Soil samples from all the salinity treatment groups per replicate were collected and pooled to determine the sand's physical and chemical properties. The analysis was performed at the Soil, Water, and Environment Analysis lab at the Agricultural Research Center (ARC), Egypt. Briefly, electroconductivity (EC) values were obtained from the saturated soil paste extract. The soil pH was taken from the soil suspensions of 1:2.5 (Kimera et al., 2023). Soil nitrogen was determined using the micro-Kjeldahl technique. Atomic adsorption was used to determine the concentration of other macro and microelements. Organic matter and Organic carbon were determined as described by Miyazawa et al. (2000) and Meersmans et al. (2009), respectively.

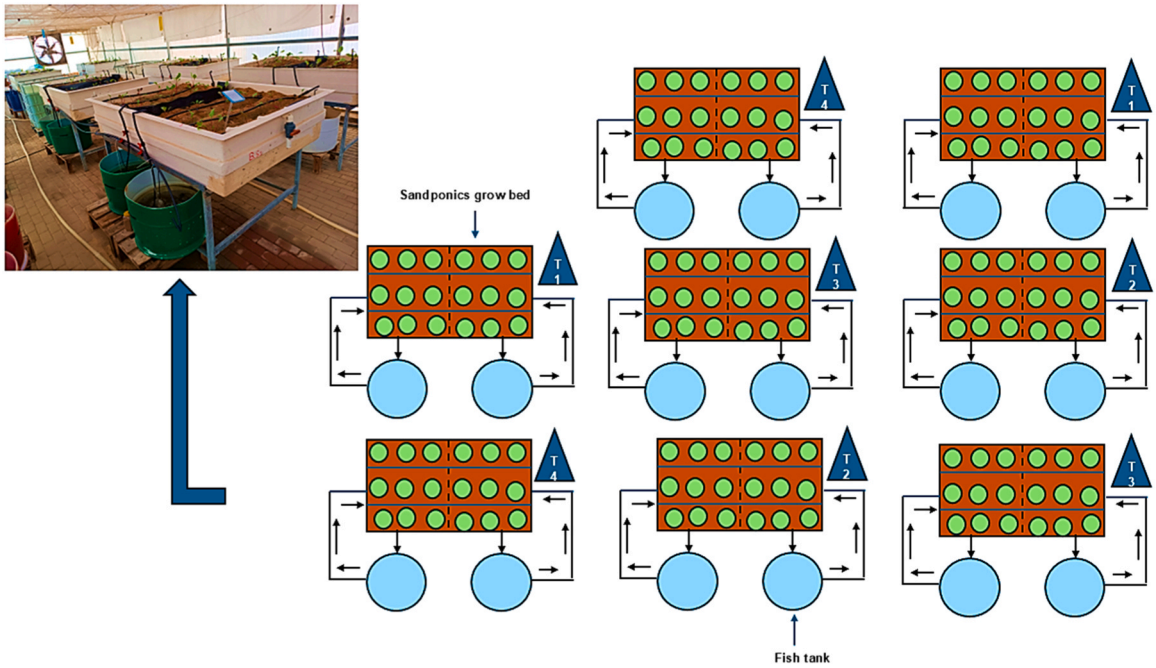

Fig. 1. Schematic diagram of the experimental layout and the sandponics system. Treatments; T1: 4.7 dSm^{-1}, T2: 7.5 dSm^{-1}, T3: 11.25 dSm^{-1}, and T4: control (fresh water, 0.63 dSm^{-1}).

2.5. Rhizosphere DNA extraction and high-throughput sequencing

Soil samples from the rhizosphere per salinity treatment group per replicate were obtained and pooled for DNA extraction. The pooled samples were immediately plunged into liquid nitrogen and stored at −20 °C until further analysis. The total genomic DNA of the soil rhizosphere samples was extracted using innuSPEED Soil DNA Kit 2.0 (LOT # 004–23) following the manufacturer's instructions. DNA concentration was determined by NanoDrop. Purity and completeness were evaluated using 1 % agarose gel electrophoresis. The 16S rRNA genes were amplified using the specific primers for V3 and V4 hypervariable regions: 341 F (CCTAYGGGRBGCASCAG) and 806 R (GGACTCANNGGGTATCTAAT) (Wu et al., 2024). PCR was performed in a 30 μL reaction with 15 μL Master Mix (New England Biolabs), 0.2 μM each of forward and reverse primers and 10 ng of template DNA. The thermocycling conditions were as follows: predegeneration at 98℃ for 1 min, 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, and extension at 72℃ for 30 s, followed by a final extension at 72℃ for 5 min. Electrophoretic detection was performed on a 2 % agarose gel. Samples with a bright main band between 400 and 450 bp were selected for subsequent experiments. PCR products were purified using a Kit (Tiangen Biotech). The purified products were used to prepare the library. Sequencing libraries were generated using TIANSeq Fast DNA Library Prep Kit (Tiangen Biotech). Library quality was assessed on a Qubit 2.0 fluorometer (Thermo Scientific) and an Agilent 2100 Bioanalyzer. Finally, the libraries were sequenced on the Illumina platform using a 2 × 250 bp paired-end protocol.

2.6. Amplicon data processing

Bioinformatics analysis of high-throughput sequencing data was performed using Mothur (version1.42.6) following the tutorial on their website (www.mothur.org/wiki/MiSeq_SOP) with slight modifications Kozich et al. (2013). Briefly, raw sequences were quality-filtered, denoised, merged, and chimera-removed using several commands in Mothur. This was followed by species annotation. Alpha diversity analysis was used to reflect the complexity of the species diversity for the sample by observed species, ACE, and Simpson indices. All indices were calculated and visualized using the R statistical programming language (version 4.3.2). The Kruskal-Wallis test was used to detect significant differences in the Alpha-diversity indices. Beta diversity analysis was used to assess sample differences in species complexity. Beta diversity was calculated using the Non-metric multidimensional scaling (NMDS) with the Bray-Curtis index distance method. PERMANOVA was computed to statistically determine the association of microbial composition with covariates of interest. Constrained ordination analysis (CCA) was used to understand the difference in microbial community structure of the four salinity treatment groups and identify the significant soil properties that influence their community structure. The Mantel test based on Pearson's product-moment correlation was computed to elucidate the relationship between the soil properties and different classes of microbial species. Raw sequence reads have been deposited in the National Center for Biotechnology Information (NCBI) under a BioProject accession identification number PRJNA1150080.

2.7. Fish effluent quality parameters and fish growth performance

Fish effluent quality parameters such as pH, dissolved oxygen (DO), and temperature were monitored by automated digital Nilebot technologies sensors by Conative Labs, Egypt. Reagent kits H193715–01, H193728–0, and H193707–01 from HANNA instruments were used to measure the concentrations of ammonia, nitrate, and nitrite, respectively, throughout the experimental period. Absorbance was measured using a photometer (HANNA instruments) and results were displayed as ammonia, ammonium, Nitrate-Nitrogen, and Nitrite-Nitrogen.

2.8. Statistical analysis

Data analysis and visualization were performed using the R statistical programming language (version 4.3.2). Before the analysis of variances (ANOVA) was computed, all datasets were subjected to normality and equality of variances tests using QQ plots and Levene's test, respectively. One-way ANOVA was conducted to determine significant differences in the measured parameters at $p < 0.05$. The Duncan Multiple Range test (DMRT) was used as a posthoc test to detect differences in treatment means.

3. Results

3.1. Plant phenotypic parameters and relative water content

Plant phenotypic parameters recorded throughout the study period are shown in Table 1. At 15 days after transplanting (DAT), T1 and T2 significantly ($p < 0.05$) recorded higher values for the average plant height compared to other treatments. Furthermore, T1 significantly ($p < 0.05$) recorded the highest values for the number of leaves, leaf length, and leaf width compared to other salinity treatment groups. No significant differences in the leaf shape index (LSI) were noted across all the salinity treatment groups. At 30 and 45 DAT, the results of this study showed that T1 and T2 significantly ($p < 0.05$) recorded higher values for the average plant height and number of leaves, with T3 significantly recording the lowest values for leaf length and leaf width. At 60 DAT, T4 significantly ($p < 0.05$) recorded the highest values for the average plant height, leaf length, and leaf width compared to other treatments. No significant differences in the LSI were noted across all the salinity treatment groups.

Fig. 2 summarizes the results of plant phenotypic parameters recorded at harvest. The average fresh leaf weight ranged between 85.35 g/plant to 160 g/plant with T1 and T4 significantly ($p < 0.05$) recording higher values compared to other treatments (Fig. 2**a**). Likewise, results of the average leaf dry weight (Fig. 2**b**) indicated that T1 and T4 significantly ($p < 0.05$) recorded higher values compared to T3. The root fresh weight ranged from 318.5 g to 596.33 g, with T4 significantly ($p < 0.05$) recording the highest values compared to other treatments (Fig. 2**c**). Similarly, T4 significantly ($p < 0.05$) recorded the highest values for the root diameter compared to other treatments (Fig. 2**d**).

The relative water content (RWC) of plant leaves was recorded at 15 and 30 DAT, and the results are shown in Fig. 3. At 15 DAT, T1 and T4 significantly ($p < 0.05$) recorded higher values for the RWC of leaves, followed by T2 and T3, respectively (Fig. 3**a**). However, no significant differences in the RWC of leaves were recorded across all treatments at 30 DAT (Fig. 3**b**).

Table 1
Plant growth parameters of beetroot cultivated under different salinity treatments.

Treatments	Plant height (cm)	Leaf Number plant^{-1}	Leaf length (cm)	Leaf width (cm)	Leaf shape index
15 DAT					
T1	21.58^{a} ± 2.14	5.45^{a} ± 0.99	21.45^{a} ± 2.35	5.28^{a} ± 0.57	4.11^{a} ± 0.58
T2	20.15^{a} ± 2.76	4.75^{b} ± 0.79	19.40^{b} ± 3.18	4.75^{b} ± 0.70	4.11^{a} ± 0.61
T3	16.45^{b} ± 3.12	4.10^{c} ± 1.02	15.78^{c} ± 3.06	3.70^{c} ± 0.55	4.30^{a} ± 0.82
T4	17.33^{b} ± 2.75	4.27^{bc} ± 0.59	16.30^{c} ± 2.17	3.77^{c} ± 0.73	4.45^{a} ± 0.66
30 DAT					
T1	28.60^{a} ± 6.85	7.55^{a} ± 0.89	28.00^{a} ± 6.61	9.15^{a} ± 1.33	3.07^{b} ± 0.62
T2	27.78^{a} ± 5.32	7.65^{a} ± 0.88	26.90^{ab} ± 4.93	8.30^{ab} ± 1.88	3.29^{b} ± 0.35
T3	20.90^{b} ± 3.90	6.75^{b} ± 1.25	20.15^{c} ± 3.74	5.63^{c} ± 1.35	3.66^{a} ± 0.52
T4	25.23^{a} ± 2.78	6.60^{b} ± 1.12	24.40^{b} ± 3.03	7.60^{b} ± 1.61	3.28^{b} ± 0.47
45 DAT					
T1	40.55^{a} ± 4.89	9.65^{a} ± 1.09	38.80^{a} ± 4.46	12.37^{a} ± 2.25	3.20^{a} ± 0.52
T2	37.40^{a} ± 6.40	9.25^{a} ± 1.52	35.30^{b} ± 6.38	10.65^{b} ± 2.98	3.46^{a} ± 0.61
T3	31.15^{b} ± 5.54	7.75^{b} ± 1.65	30.55^{c} ± 5.07	9.13^{c} ± 1.76	3.38^{a} ± 0.40
T4	38.07^{a} ± 3.53	8.33^{b} ± 1.18	37.07^{ab} ± 3.53	11.93^{ab} ± 1.43	3.16^{a} ± 0.57
60 DAT					
T1	41.30^{b} ± 5.76	12.50^{a} ± 1.57	39.15^{b} ± 4.98	12.73^{b} ± 1.31	3.08^{a} ± 0.27
T2	39.35^{b} ± 5.53	12.05^{ab} ± 2.04	37.75^{b} ± 5.00	11.50^{b} ± 2.17	3.35^{a} ± 0.52
T3	33.30^{c} ± 7.28	10.90^{b} ± 2.31	32.15^{c} ± 7.51	9.50^{c} ± 2.14	3.49^{a} ± 1.24
T4	45.60^{a} ± 4.67	11.80^{ab} ± 1.66	43.80^{a} ± 5.23	14.07^{a} ± 2.12	3.17^{a} ± 0.56

Data is expressed as mean ± SD (Standard deviation), n = 20. Treatments within the same column having different letters are significantly different ($p < 0.05$). Treatments; T1: 4.7 dSm^{-1}, T2: 7.5 dSm^{-1}, T3: 11.25 dSm^{-1}, and T4: control (fresh water, 0.63 dSm^{-1}).

3.2. Nutrient analysis

The results of the nutrient analysis are shown in Table 2. The macro and micronutrients in red beetroot are summarized in Table 2. The study showed that the potassium (K) content ranged from 99.12 to 101.65 mg/L. However, no significant differences were noted among the treatments. For magnesium (Mg) content, the values ranged from 68.56 to 82.53 mg/L, with T4 significantly ($p < 0.05$) recording the lowest values compared to other treatments. Likewise, T4 and T3 significantly ($p < 0.05$) recorded lower values for the Zinc (Zn) content compared to other treatments. Data on the iron (Fe) content indicated no significant differences in concentration between T1 and T3. The copper (Cu) content ranged from 0.07 to 0.17 mg/L, with T1 significantly ($p < 0.05$) the highest value compared to other treatments. No significant differences in the manganese (Mn) content were recorded across all the treatments.

3.3. Physical and chemical properties of the soil

Table 3 summarizes the results of the soil's physical and chemical properties recorded in the different salinity treatment groups. T3 recorded the highest values for the electrical conductivity (EC), sodium ions (Na^{+}) concentration, chloride ions (Cl^{-}) concentration, nitrogen (N), phosphorus (P), and potassium (K) concentrations compared to the other salinity treatment groups. However, T2 recorded higher values for manganese (Mn), zinc (Zn), iron (Fe), and copper (Cu) concentrations compared to the other salinity treatment groups. T1 recorded the highest values for organic matter (OM) and organic carbon (OC) percentages compared to the other salinity treatment groups.

3.4. Taxonomic profiles of the rhizosphere bacterial and archaeal communities

The differences in the rhizosphere bacterial and archaeal communities were evident across all the salinity treatment groups. For bacteria, our study results showed that Proteobacteria were the most abundant phylum across all the salinity treatment groups (Fig. 4**a**). Other dominant phyla across all the salinity treatment groups were *Bacteroidetes*, *Firmicutes*, *Actinobacteria,* as well as unclassified bacteria. However, *Cyanobacteria* were dominant in T1 and T2, whereas *Chloroflexi* and *Acidobacteria* were dominant in T4. On the other hand, the dominant bacterial classes observed in T1, T2, and T3 were *Gammaproteobacteria* and *Alphaproteobacteria*. The dominant bacterial classes in T4 were *Alphaproteobacteria*, unclassified bacteria, and *Gammaproteobacteria*. *Sphingobacteria* were detected in T1, T3, and T4, whereas *Negativicutes* were detected in T2 (Fig. 4**b**). Our study results also revealed *Euryarchaeota*, *Crenarchaeota*, and unclassified archaea as the dominant archaeal phyla across treatments T1, T3, and T4. However, the dominant archaeal phyla in T2 were *Euryarchaeota* and the unclassified archaea (Fig. 4**c**). The dominant archaeal classes observed in T1, T3 and T4 were *Thermoprotei*, *Halobacteria*, unclassified *Euryarchaeota*, and unclassified archaea, except for *Methanomicrobia,* which were detected in only T3 (Fig. 4**d**). The dominant archaeal classes in T2 were unclassified *Euryarchaeota* and unclassified archaea (Fig. 4**d**). PERMANOVA test

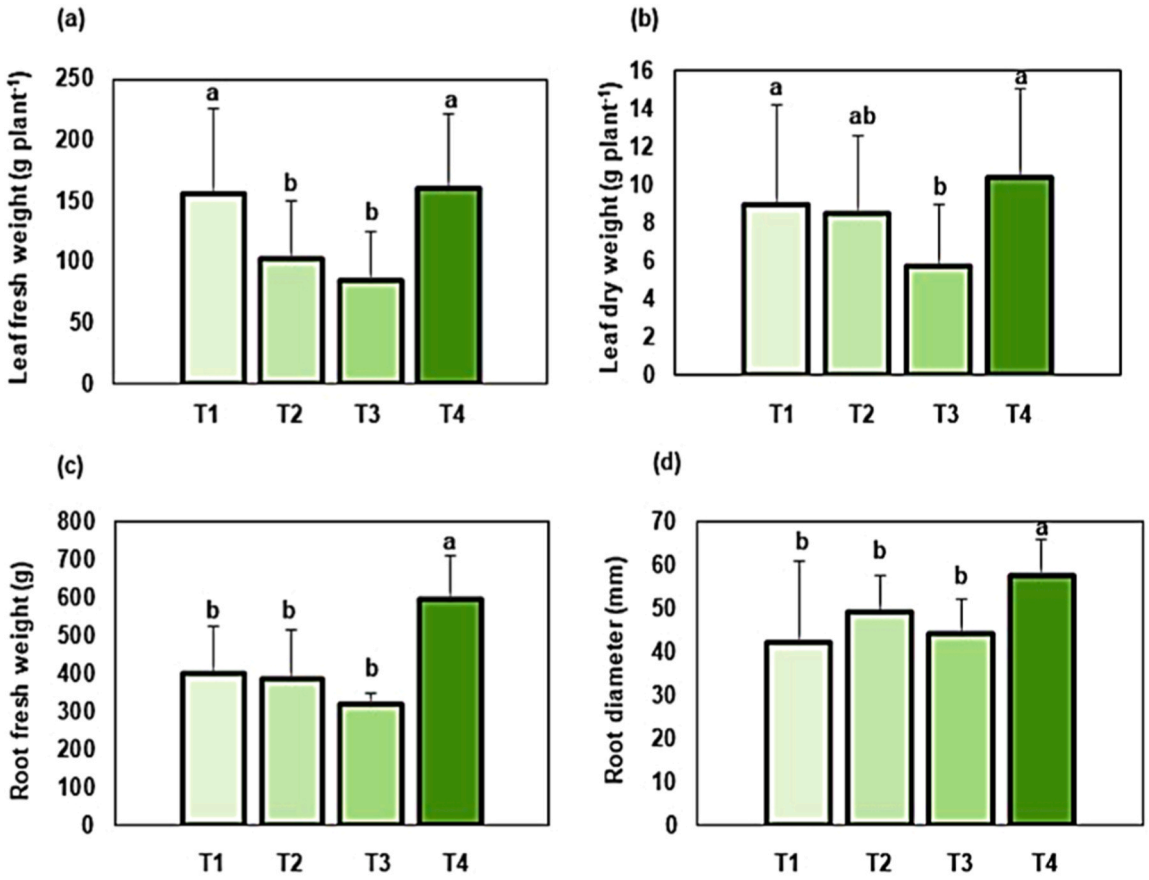

Fig. 2. Plant phenotypic parameters (a): Leaf fresh weight, (b): Leaf dry weight, (c): Root fresh weight, and (d): Root diameter. Data is expressed as mean ± SD. Errors bars represent the standard deviation. Bar columns having different letters are significantly different at $p < 0.05$. Treatments; T1: 4.7 dSm^{-1}, T2: 7.5 dSm^{-1}, T3: 11.25 dSm^{-1}, and T4: control (fresh water, 0.63 dSm^{-1}).

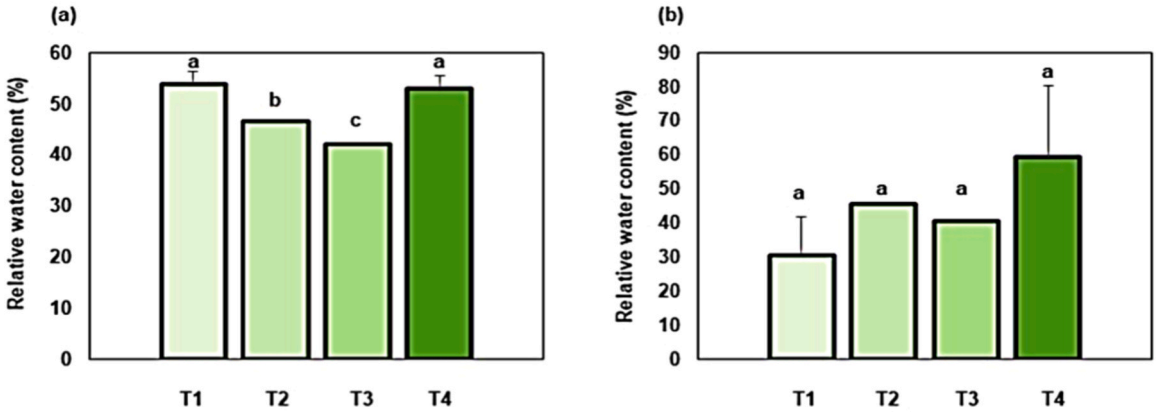

Fig. 3. Relative water content (a): 15 days after transplanting and (b): 30 days after transplanting. Data is expressed as mean ± SD. Errors bars represent the standard deviation. Bar columns having different letters are significantly different at $p < 0.05$. Treatments; T1: 4.7 dSm^{-1}, T2: 7.5 dSm^{-1}, T3: 11.25 dSm^{-1}, and T4: control (fresh water, 0.63 dSm^{-1}).

indicated no significant differences in bacterial and archaeal composition ($p > 0.05$) across all the salinity treatment groups.

3.5. *Unique and shared operational taxonomic units (OTUs) in different salinity treatment groups*

Fig. 5**a** summarizes the number of unique and shared bacterial OTUs in different salinity treatment groups. The number of unique OTUs in T1, T2, T3, and T4 was 1393, 1384, 1817, and 5333, respectively. OTUs shared between T1 and T2 were 238, T1 and T3 were 428, T1 and T4 were 574, T2 and T3 were 299, T2 and T4 were 233, T3 and T4 were 534, T1, T2, and T3 were 160, T1, T3, and T4 were 254, T2, T3, and T4 were 147, T1, TT2, and T4 were 136, T1, T2, T3, and T4 were 107. Similarly, Fig. 5**b** summarizes the number of unique and shared archaeal OTUs in different salinity treatment groups. The number of unique OTUs in T1, T2, T3, and T4 was 22, 11, 35, and 105, respectively. OTUs shared between T1 and T2 were 3, T1 and T3 were 11, T1 and T4 were 13, T2 and T3 were 6, T2 and T4 were 2, T3 and T4 were 13, T1, T2, and T3

Table 2
Macro and micronutrients in red beetroot at harvest.

Treatments	K (mg/L)	Mg (mg/L)	Zn (mg/L)	Fe (mg/L)	Cu (mg/L)	Mn (mg/L)
T1	99.12 ± 2.43^{a}	82.53 ± 0.30^{a}	0.77 ± 0.18^{a}	2.94 ± 0.23^{a}	0.17 ± 0.02^{a}	0.35 ± 0.02^{a}
T2	101.13 ± 4.74^{a}	79.49 ± 2.57^{ab}	0.52 ± 0.09^{b}	1.51 ± 0.05^{c}	0.07 ± 0.02^{b}	0.39 ± 0.13^{a}
T3	101.65 ± 1.47^{a}	77.26 ± 1.81^{b}	0.33 ± 0.01^{c}	2.86 ± 0.32^{a}	0.08 ± 0.01^{b}	0.32 ± 0.06^{a}
T4	100.53 ± 1.15^{a}	68.56 ± 1.80^{c}	0.32 ± 0.03^{c}	2.24 ± 0.11^{b}	0.07 ± 0.01^{b}	0.20 ± 0.01^{a}

Data is expressed as mean ± SD (Standard deviation), n = 20. Treatments within the same column having different letters are significantly different ($p < 0.05$). Treatments; T1: 4.7 dSm^{-1}, T2: 7.5 dSm^{-1}, T3: 11.25 dSm^{-1}, and T4: control (fresh water, 0.63 dSm^{-1}). Macro and micronutrients; K: potassium, Mg: magnesium, Zn: zinc, Fe: iron, Cu: copper, and Mn: manganese.

were 3, T1, T3, and T4 were 6, T2, T3, and T4 were 2, T1, T2, and T4 were 2, T1, T2, T3, and T4 were 2.

3.6. Alpha and beta diversity indices

The alpha diversity measure indices for bacteria and archaea are shown in Fig. 6a and Fig. 6**b**, respectively. Values for the observed and ACE bacterial species richness were higher in T4, followed by T1, T2, and T3, respectively. Likewise, the values for Simpson's index for bacterial evenness were higher in T4, followed by T1, T2, and T3, respectively. For archaea, values for the observed and ACE species richness were higher in T4, followed by T1, T3, and T2, respectively. However, the Simpson index values for archaeal evenness were higher in T3, followed by T1, T4, and T2, respectively. Overall, results of the Kruskal-Wallis test indicated no significant differences ($p = 0.392$) in the alpha diversity measure indices (i.e. Observed, ACE, and Simpson) of bacteria and archaea across all the salinity treatment groups.

Non-metric multidimensional scaling (NMDS) was conducted with the Bray-Curtis index distance method for beta diversity for both

Table 3
Soil physical and chemical properties.

Treatments	pH 1:2:5	EC dS/m	Na^{+} (meq/L)	Cl- (meq/L)	N (mg/kg)	P (mg/kg)	K (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	Fe (mg/kg)	Cu (mg/kg)	OM (%)	OC (%)
T1	7.81	1.55	8.10	9.5	83	14.43	53	0.90	2.63	3.13	0.43	2.16	1.58
T2	7.65	1.46	6.15	8.5	48	28.75	51	2.81	3.26	5.26	0.53	0.60	1.51
T3	7.51	2.91	13.60	18.5	120	30.02	70	1.95	2.41	4.16	0.49	0.24	1.43
T4	7.73	1.66	8.15	9.6	30	25.05	65	1.37	2.10	3.91	0.40	0.24	1.50

EC: electroconductivity, Na^{+}: sodium ions, Cl^{-}: chloride ions, N: nitrogen, P: phosphorus, K: potassium, Mn: manganese, Zn: zinc, and Cu: copper, O.M: organic matter, O.C: organic carbon, Treatments T1: 4.7 dSm^{-1}, T2: 7.5 dSm^{-1}, T3: 11.25 dSm^{-1}, and T4: control (fresh water, 0.63 dSm^{-1}).

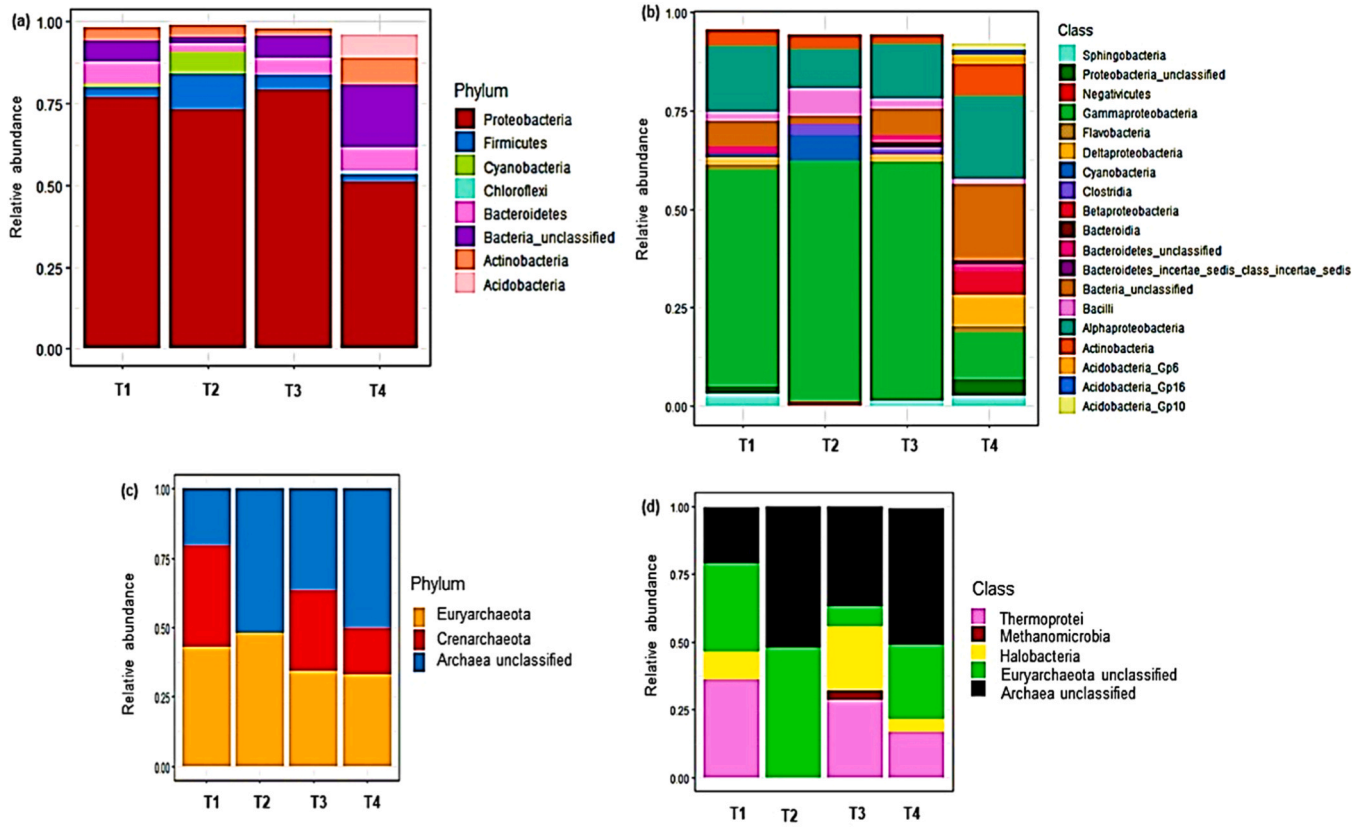

Fig. 4. Relative abundance of (a): dominant bacterial phyla, (b) dominant bacterial classes, (c): dominant archaeal phyla, and (d): dominant archaeal classes identified through the 16 s rDNA V4 hypervariable region annotated through the SILVA database. Treatments; T1: 4.7 dSm^{-1}, T2: 7.5 dSm^{-1}, T3: 11.25 dSm^{-1}, and T4: control (fresh water, 0.63 dSm^{-1}).

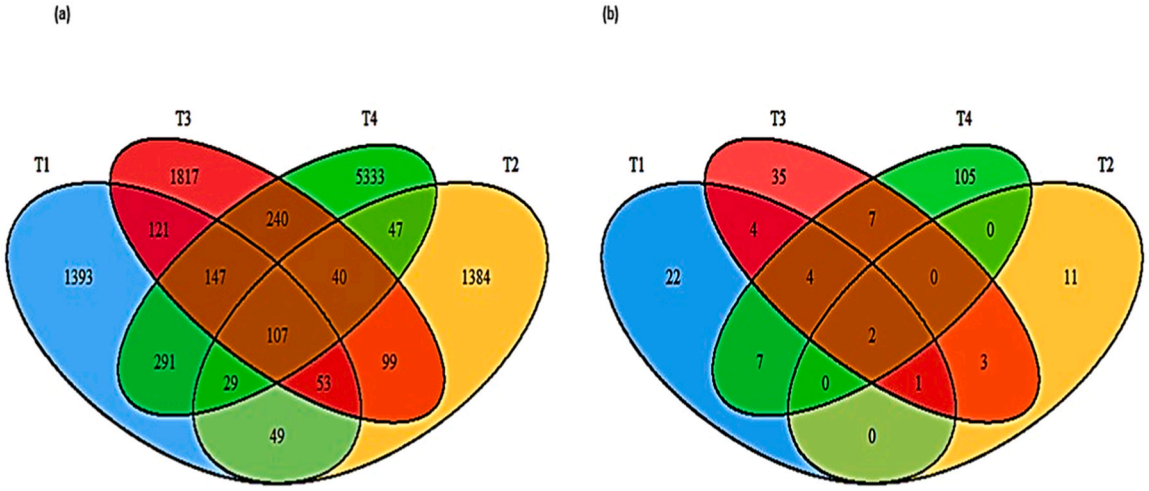

Fig. 5. Venn diagrams of unique and shared Operational Taxonomic Units (OTUs) in (a): bacteria and (b): archaea. The numbers in the overlapping part represent the number of OTUs common to the four groups, whereas the numbers in the non-overlapping part represent the number of OTUs specific to the corresponding group. Treatments; T1: 4.7 dSm^{-1}, T2: 7.5 dSm^{-1}, T3: 11.25 dSm^{-1}, and T4: control (fresh water, 0.63 dSm^{-1}).

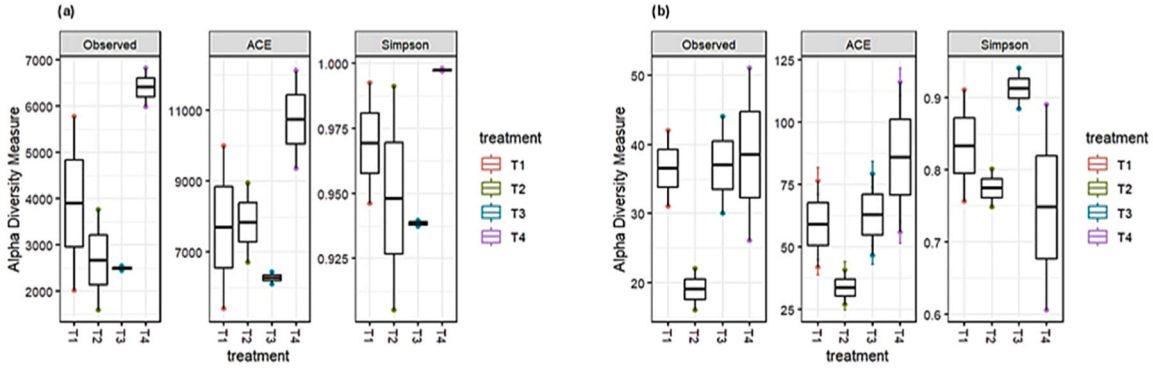

Fig. 6. Alpha diversity measures of total (a): bacterial and (b): archaeal community based on Operational Taxonomic Units (OTUs) as calculated using the SILVA database. Treatments; T1: 4.7 dSm^{-1}, T2: 7.5 dSm^{-1}, T3: 11.25 dSm^{-1}, and T4: control (fresh water, 0.63 dSm^{-1}).

bacteria (Fig. 7**a**) and archaea (Fig. 7**b**). Each salinity treatment group represented the combined abundance of all OTUs with T1 and T3 clustering **(**Fig. 7**a**), thus indicating a similarity in composition of certain bacterial species between these treatments. Likewise, T1 and T3 clustered (Fig. 7b) for the archaeal species, unlike other salinity treatment groups, thus indicating a similarity in archaeal composition between these treatments.

Constrained ordination analysis was used to understand the difference in bacterial and archaeal community structure of the four salinity treatment groups and identify the significant soil properties that influence their community structure (Fig. 8). The constrained ordination plots demonstrated that the bacterial communities in the four salinity treatment groups were substantially distinct (Fig. 8**a**). Organic matter (OM), organic carbon (OC), and nitrogen (N) were identified as the most critical soil properties influencing the composition of bacterial communities. Likewise, our results also showed that the archaeal communities in the four salinity treatment groups were substantially distinct, with OM, OC, and N being the most critical soil properties influencing the composition of archaeal communities (Fig. 8**b**). The Mantel test based on Pearson's product-moment correlation was computed to elucidate the relationship between the soil properties and different bacterial and archaeal taxa (class). The results of this test showed that there was a weak and positive correlation between the soil properties and bacterial taxa (class) ($r = 0.085$, $p = 0.028$). Likewise, there was a low positive correlation between the soil properties and archaeal taxa (class) ($r = 0.350$, $p = 0.001$)

3.7. Fish effluent quality parameters and fish growth performance

The results of fish effluent quality parameters recorded throughout the study are shown in Fig. 9. The concentration of ammonium ranged from 0.93 mg/L to 2.11 mg/L. No significant differences in the concentration of ammonium were noted across all the salinity treatment groups (Fig. 9**a**). Data on ammonia-nitrogen indicated that the concentration ranged from 0.72 mg/L to 1.64 mg/L; however, no significant differences were noted across all the salinity treatment groups (Fig. 9**b**). The concentration of nitrate-nitrogen ranged from 22.16 mg/L to 27.18 mg/L, with T4 significantly ($p < 0.05$) the lowest values compared to the other salinity treatment groups (Fig. 9**c**). For nitrite-nitrogen, the concentration ranged from 8.50 mg/L to 10.89 mg/L; however, no significant differences were noted across all the salinity treatment groups (Fig. 9**d**).

Table 4 summarizes the results of fish growth performance throughout the study period. The average body weight gain ranged from 383 g to 482 g, with T1 and T4 recording the highest and lowest values, respectively. However, all the salinity treatment groups recorded no significant differences in average weight gain. Likewise, all the salinity treatment groups noted no significant differences in specific growth rate (SGR) and feed conversion ratio (FCR). The survival rate ranged from 83.25 % to 87.50 %. No significant differences were noted across all the salinity treatment groups.

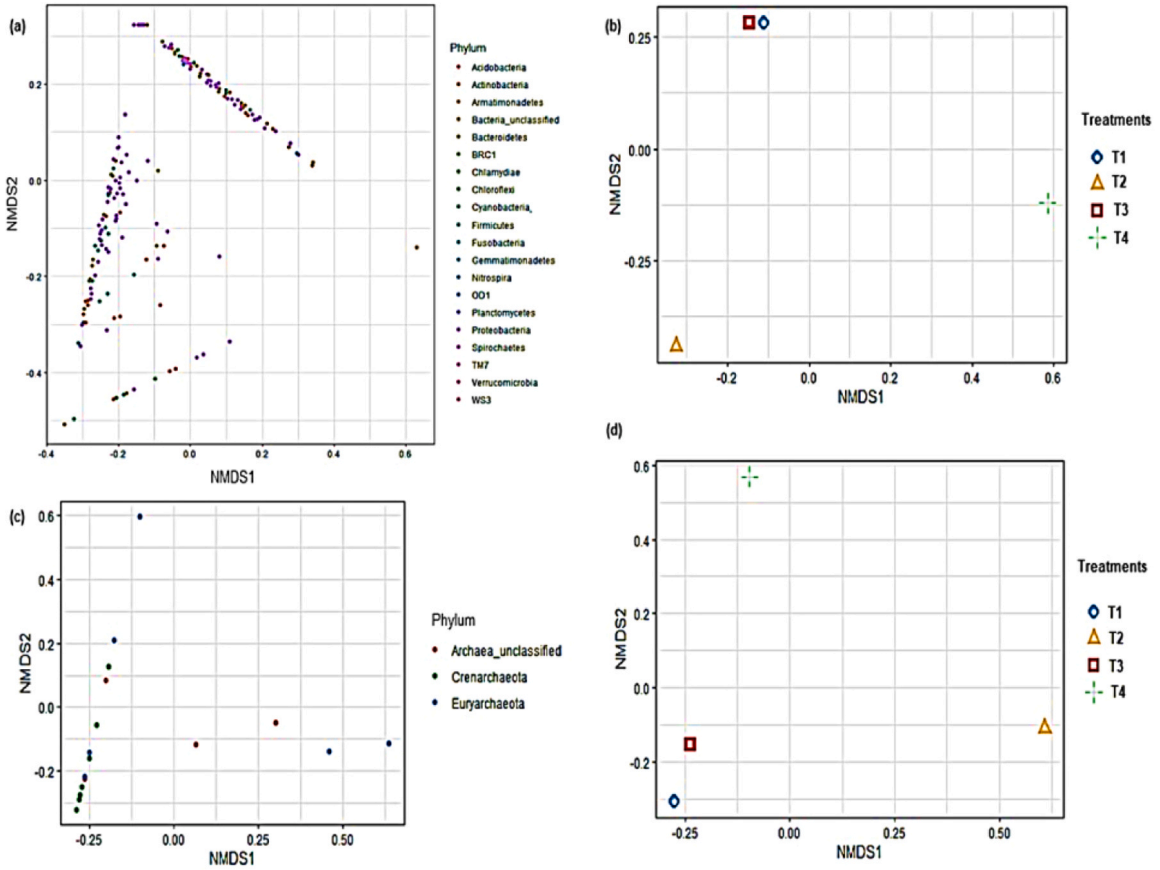

Fig. 7. Non-metric multidimensional scaling (NMDS) computed on the Bray-Curtis similarity index for (a and b): bacterial and (c and d): archaeal composition using the SILVA V4 hypervariable region and different salinity treatments. Treatments; T1: 4.7 dSm^{-1}, T2: 7.5 dSm^{-1}, T3: 11.25 dSm^{-1}, and T4: control (fresh water, 0.63 dSm^{-1}).

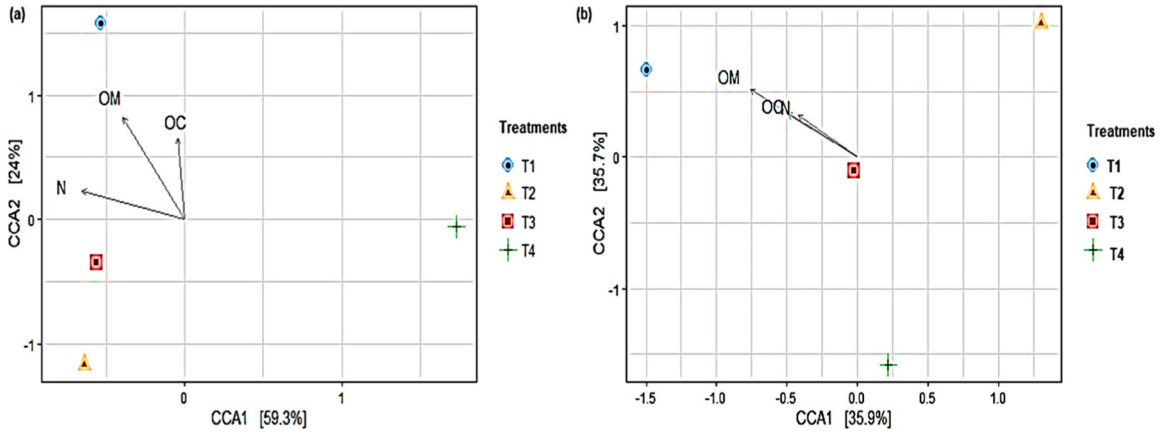

Fig. 8. Constrained ordination showing the influence of environmental factors on changes in (a): bacterial and (b): archaeal community composition. Treatments; T1: 4.7 dSm^{-1}, T2: 7.5 dSm^{-1}, T3: 11.25 dSm^{-1}, and T4: control (fresh water, 0.63 dSm^{-1}).

4. Discussion

Red beetroot (*Beta vulgaris* L.) is known to be a salt-tolerant crop whose salt tolerance level was reported to range between ~40–120 mM sodium chloride (NaCl) (Yolcu et al., 2021). Therefore, this crop can be grown in moderately saline conditions without major detrimental effects on its growth. In this study, we investigated the effect of salinity on the growth, yield, and nutritive composition of red beetroot cultivated under a saline integrated vegeculture-aquaculture system (IVAS). Highly saline conditions (i.e. 9000 ppm) reduced most of the growth and yield parameters, probably as a result of inhibition of photosynthesis, cell elongation, biosynthesis of sugars and their translocation (Bouras et al., 2021; Chourasia et al., 2022; Hao et al., 2021). The reduced plant growth and herbage yield parameters included plant heights, leaf length, leaf width, leaf fresh weight, and leaf dry weight. Similar results in the reduction of plant growth and herbage yield in beets have been reported by Bouras et al. (2021), Ghoulam et al. (2002), and Jamil et al. (2007). A reduction in plant height is one of the most observed

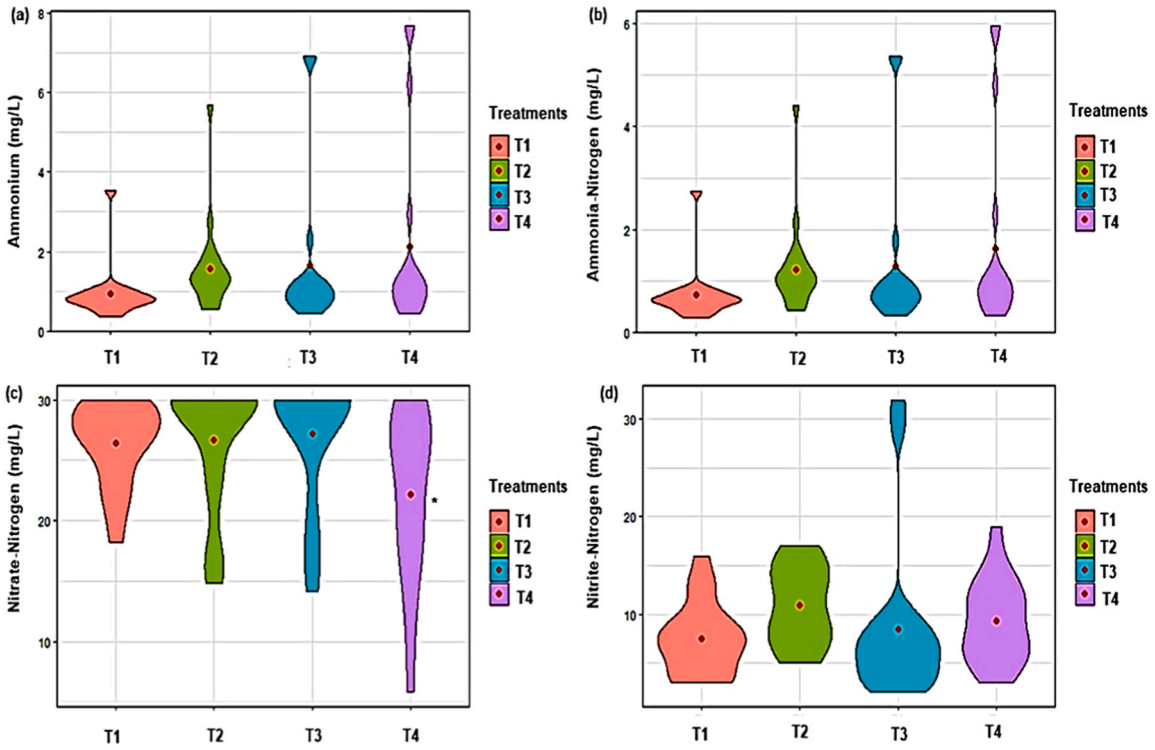

Fig. 9. Violin plots of fish effluent quality parameters recorded throughout the study, (a): Ammonium, (b): Ammonia-Nitrogen, (c): Nitrate-Nitrogen, and (d): Nitrite-Nitrogen. The red dots indicate the mean. Asterix indicates significant differences at $p < 0.05$. Treatments; T1: 4.7 dSm^{-1}, T2: 7.5 dSm^{-1}, T3: 11.25 dSm^{-1}, and T4: control (fresh water, 0.63 dSm^{-1}).

Table 4
Fish growth performance under different salinity treatment groups.

Treatment	BWG (g)	SGR	FCR	Survival (%)
T1	$482.25^{a} \pm 112.34$	$1.20^{a} \pm 0.18$	$0.85^{a} \pm 0.15$	$85.25^{a} \pm 4.50$
T2	$462.25^{a} \pm 61.00$	$1.17^{a} \pm 0.10$	$0.87^{a} \pm 0.15$	$83.25^{a} \pm 6.95$
T3	$388.75^{a} \pm 134.24$	$1.03^{a} \pm 0.26$	$1.10^{a} \pm 0.44$	$87.25^{a} \pm 8.50$
T4	$383.00^{a} \pm 85.51$	$1.01^{a} \pm 0.15$	$1.08^{a} \pm 0.32$	$87.50^{a} \pm 10.85$

Data presented as mean ± SD. Treatments within the same column having different letters are significantly different ($p < 0.05$). BWG: body weight gain, SGR: specific growth rate, FCR: feed conversion ratio. Treatments; T1: 4.7 dSm^{-1}, T2: 7.5 dSm^{-1}, T3: 11.25 dSm^{-1}, and T4: control (fresh water, 0.63 dSm^{-1}).

phenomena in salt-stressed plants and is probably linked to hormonal signals produced by the roots (Szymanska et al., 2020). Although roots are in direct contact with the soil, it would be expected that they would be more susceptible to salinity stress, but a higher sensitivity of stems to salinity stress compared to the roots has been reported (Ramoliya et al., 2006; Szymańska et al., 2019). Furthermore, the study results indicated no severe effect of salinity on the leaf number of plants. This could be one of the beet's adaptive responses to salinity stress. The findings of this study also indicated that irrigation water salinity significantly impacted root yield in terms of root fresh weights and diameter. Similar results in beets have been previously reported by Bouras et al. (2021), Gadelha et al. (2021), and Zaki et al. (2014). Regarding the relative water content (RWC), a decrease in the RWC with increasing salinity was noted at 15 and 30 days after transplanting (DAT). Similar results have been reported in other crops, such as *Cucumis sativa* (El-Shraiy et al., 2011; Turan et al., 2022), *Solanum lycopersicum* (Ali et al., 2021), and *Lactuca sativa* (Bres et al., 2022; Sardar et al., 2023). Water uptake by plants is severely affected by salinity stress due to the osmotic effect. Accumulation of salts in plant tissues reduces the water potential and thus water loss from plant tissues (Ahmed et al., 2019; Polash et al., 2018).

Salinity influenced the nutrient content of red beetroot. The study results showed increased concentrations of magnesium (Mg), zinc (Zn), and manganese (Mn) in saline treatments compared to the control. Mg, Zn, and Mn are cofactors of several metalloenzymes and have been reported to activate the antioxidant defense system of plants under abiotic stress conditions, improving their stress tolerance (Lv et al., 2024; Shao et al., 2023; Zheng et al., 2023). Furthermore, Zn stabilizes the integrity and permeability of cell membranes, thus conferring protection to cells against oxidative damage (Aravind and Prasad, 2004; Shao et al., 2023). As such, the exogenous application of Mg, Zn, and or Mn has been shown to improve salinity stress tolerance in different plant species (Al-Zahrani et al., 2021; Eisakhani et al., 2023; Galal, 2019; Rahman et al., 2016; Sharavdorj et al., 2022).

Irrigation water quality influences changes in the soil's physical, chemical, and microbial properties (Comino et al., 2019; Guo et al., 2022). The soil analysis results revealed higher concentrations of NPK in T3 compared to other treatments. These changes in the soil's chemical properties could be attributed to the quality of fish effluents used to irrigate plants. There is a direct relationship between the fish's metabolic processes and the quality of fish effluent. Under salinity stress, certain fish species exhibit increased excretion of ammonia, leading to increased N concentrations in rearing water (Rahmah et al., 2020; Uliano et al., 2010). On the other hand, P is one of the major nutrients in aquaculture feed formulations due to its importance in energy metabolism, bone formation, and cell functions (Nathanailides et al., 2023). However, this nutrient is also an environmental pollutant whose high concentrations cause negative impacts on aquatic ecosystems (Nathanailides et al., 2023; Varol and Balcı, 2020). Under certain environmental stress factors such as high salinity and high temperature, freshwater fish reduce their feed intake (Dawood et al., 2021; Martins et al., 2022; Pandit and Nakamura, 2010). This often leads to the accumulation of uneaten feed that can lead to P accumulation in fish effluents, thus, explaining why P concentrations were higher in soils

irrigated with highly saline fish effluents.

The soil rhizobiome profile indicated *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *Actinobacteria* as the dominant phyla across all treatments. *Proteobacteria* are highly opportunistic and thrive in nutrient-rich environments (Chen et al., 2017). Moreover, they are known to encompass an enormous level of physiological, morphological, and metabolic diversity, and as such, they are significant drivers of global carbon cycling (Chen et al., 2017; Yergeau et al., 2012). The study results also indicated an increased abundance of *Proteobacteria* in saline treatments compared to the control, indicating that *Proteobacteria* are highly tolerant to salinity. Our results agree with previous studies by Zhu et al. (2023), Loganathachetti et al. (2022), Xie et al. (2017), and Miao et al. (2015). Among the *Proteobacteria*, *Gammaproteobacteria* were the most dominant class, followed by *Alphaproteobacteria* and *Betaproteobacteria,* hence in line with a previous meta-analysis of bacterial sequences in salt-affected soils (Ma and Gong, 2013). The study results also revealed a decrease in the relative abundance of *Actinobacteria* with increasing salinity, hence in line with previous studies by Laiq et al. (2024), Li et al. (2021), and Yang et al. (2021). Although saline conditions recorded less bacterial diversity than the control, there were no significant differences in the microbial richness and evenness as per the Kruskal-Wallis test. Similar results on microbial richness and evenness in saline and non-saline soils have been reported (Xie et al., 2017). For *Archaea*, *Euryarchaeota* was the dominant phylum in all the treatments, followed by *Crenarchaeota* and unclassified *Archaea*, respectively. *Thermoprotei* and *Halobacteria* were the dominant classes of archaea observed in the treatments, and their abundance was greater in saline conditions than in the control. *Thermoprotei* are obligate anaerobes involved in the nutrient cycling of N, Fe, S, and carbon (C) and are predominantly found in saline environments (Cao et al., 2011; Sanka Loganathachetti et al., 2016; Wuchter et al., 2006). Likewise, *Halobacteria* thrive in saline conditions due to their ability to utilize salts (i.e. sodium chloride) in the surrounding environment (Elshahed et al., 2004), and certain species have been reported to possess plant growth-promoting attributes (Naitam et al., 2023; Yadav et al., 2019). *Methanomicrobia* was detected in extremely saline conditions (T3; 9000 ppm). This class of archaea mainly depends on methane and methanol for survival and is responsible for methane production (Sanka Loganathachetti et al., 2016). An increase in salinity leads to a reduction in oxygen concentrations (Mariu et al., 2023). Low oxygen availability stimulates the proliferation of methanogens (Wilmoth et al., 2021), and thus, a possible attribution to the observed results in this study. We conducted a constrained ordination analysis to elucidate the influence of soil physiochemical analysis on microbial distribution and observed that organic carbon (OC), organic matter (OM), and N were the main factors that influenced the distribution of microbes. Similar results have been previously reported on saline soils by Xie et al. (2017), Chi et al. (2021), and Wang et al. (2021).

The fish growth performance and survival were assessed during the experimental period, and our results showed no significant differences in body weight gain (BWG), specific growth rate (SGR), feed conversion ratio (FCR), and survival across the treatment groups. Nile tilapia (*Oreochromis niloticus*) can tolerate a wide range of salinity, reaching up to 20,000 ppm (El-Leithy et al., 2019; Rairat et al., 2022; Withyachumnarnkul et al., 2017). However, the tolerance of this fish species to salinity stress depends on several factors, such as age, sex, body weight, and strain (Kimera et al., 2023). The salinity treatments in this study did not negatively impact the growth response of fish, hence, in line with previous studies (Kimera et al., 2023; Thomas et al., 2019, 2021).

4.1. Study limitations

- 16S rRNA sequencing for the V3-V4 region was used for microbial phylogeny. This approach does not capture the entire DNA of the microbes, allowing for a broader view of the microbes present, their functional genes, and metabolic pathways.
- Whereas the SILVA database presents a significant advancement in unifying microbial data through a single reference tree, it still presents certain limitations in the taxonomic coverage for less well-characterized environments or organisms not extensively represented in the underlying databases, as well as the inability to classify organisms to a species level.

5. Conclusion

Freshwater scarcity is among the major global challenges affecting food production. As such, the use of brackish water for the cultivation of salt-tolerant crops and fish in an integrated system could be a feasible alternative to maximize food production amidst the growing food demand. The current study showed that red beetroot (*Beta vulgaris* subsp. vulgaris conditiva) can be cultivated in a saline integrated vegeculture-aquaponics system of water salinities not exceeding 7.5 dSm^{-1} with minimal adverse effects on root and leaf biomass yield. Furthermore, the irrigation of saline fish effluents influences changes in the diversity and abundance of the rhizosphere microbiome, favoring the proliferation of salinity-tolerant microbes that could be of great benefit to plant growth. On the other hand, rearing Nile tilapia (*Oreochromis niloticus*) in water salinities reaching up to 11.25 dSm^{-1} does not negatively affect the survival and growth performance of fish. Overall, the integration of red beetroot production with *O. niloticus* in brackish water conditions not exceeding 7.5 dSm^{-1} results in better yield for both fish and crops.

Ethics approval

This study followed the guidelines and approval of the Committee of Animal Welfare and Research Ethics, Faculty of Agriculture, Kafrelsheikh University, Egypt.

Author contributions

Hani Sewilam and **Fahad Kimera**: conceptualization**. Fahad Kimera, Muziri Mugwanya, Walaa Ahmed, and Mahmoud Dawood**: research design and data collection. **Muziri Mugwanya**; data analysis and software. **Fahad Kimera, Hani Sewilam, Muziri Mugwanya, Walaa Ahmed, and Mahmoud Dawood**: paper writing, reviewing, and editing. **Hani Sewilam**: supervision. All authors reviewed the manuscript.

Declarations

None

Plant material

All plant materials and related procedures in this study were done per the guidelines of the Institutional Review Board of the American University in Cairo and the Ministry of Agriculture and Land Reclamation in Egypt.

Consent to participate

Not applicable.

Consent to publish

Not applicable.

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CRediT authorship contribution statement

Fahad Kimera: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Data curation, Conceptualization. **Walaa Ahmed:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Muziri Mugwanya:** Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation. **Hani Sewilam:** Writing – review & editing, Writing – original draft, Supervision, Resources, Conceptualization. **Dawood Mahmoud A.O:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation.

Declaration of Competing Interest

The authors have nothing to declare.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agwat.2025.109900.

Data availability

Data will be made available on request.

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