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Plant growth, ion dynamics, and microbial communities in soils irrigated with treated produced water for sustainable agriculture

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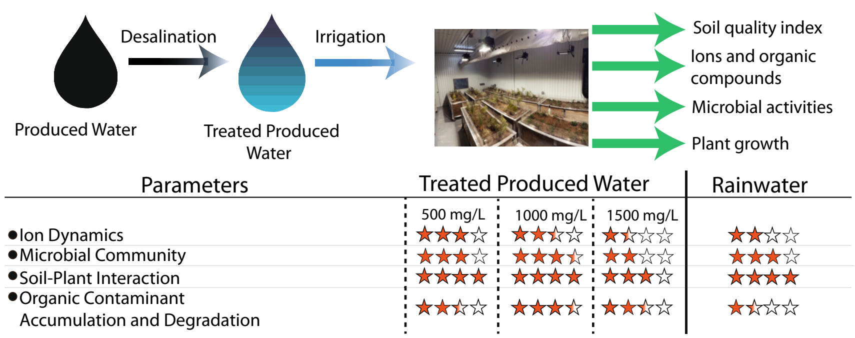
HIGHLIGHTS

- Treated PW with ≤1000 mg/L TDS sustains or improves soil health for irrigation.
- CBA soils retain ions better; WKA soils need ≤500 mg/L TDS tPW to reduce leaching.
- 1500 mg/L TDS tPW leads to organic accumulation and microbial community shifts.
- 1000 mg/L TDS tPW irrigation enhances alfalfa quality and plant productivity.

GRAPHICAL ABSTRACT



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ABSTRACT

The reuse of treated produced water (tPW) for irrigation is increasingly attractive in water-scarce regions, yet its impacts on plant performance, soil health, ion dynamics, and microbial communities are not fully explored. This study evaluated plant growth and soil response over a nine-month greenhouse experiment in the Permian Basin (Texas), using clay-rich and sandy-loam soils irrigated with tPW at total dissolved solids (TDS) concentrations of 500, 1000, and 1500 mg/L, alongside a desalinated-groundwater as the control. Soil quality index analysis showed that tPW at ≤1000 mg/L maintained and occasionally improved soil health relative to the control, whereas 1500 mg/L caused soil degradation by disrupting ion balance, increasing salinity stress, and shifting microbial communities. Moderate-salinity tPW preserved a balanced ion profile that supported nutrient retention, microbial activity, and soil structure; in contrast, higher TDS led to ion accumulation, salinization, nutrient depletion, and osmotic stress, which diminished water retention and fertility. Alfalfa irrigated with 1000 mg/L tPW produced forage with higher crude protein, lower fiber fractions, and improved digestibility, affirming its suitability for saline forage systems. Microbial analysis illustrated minimal impact on bacterial and fungal diversity at ≤1000 mg/L TDS, whereas 1500 mg/L TDS alters fungal composition in loamy soils, reducing richness and increasing pathogenic fungi in deeper layers. These results underscore the promise of tPW for sustainable irrigation, provided that salinity levels, ion accumulation, and microbial responses are carefully managed to safeguard soil health, optimize nutrient cycling, and sustain long-term productivity.

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1. Introduction

Water scarcity continues to challenge agricultural sustainability worldwide. As a response, various treated alternative water sources, such as reclaimed municipal wastewater, and desalinated brackish water and seawater, have been proposed and are currently utilized for agricultural irrigation (Jaramillo and Restrepo, 2017; Kesari et al., 2021; Martinez-Alvarez et al., 2020; Zolghadr-Asli et al., 2023). Reusing treated produced water (tPW) generated during oil and gas (O&G) production for agricultural irrigation has emerged as a strategic response to increasing water scarcity for non-potable applications (Du et al., 2025; Edirisooriya et al., 2024; Jiang et al., 2022a; Oetjen et al., 2018). In arid and semiarid regions such as the southwestern United States, large quantities of PW are generated annually, making tPW a potential resource for irrigation while reducing the environmental impact of O&G operations (Sabie et al., 2022; Scanlon et al., 2020). These regions, often characterized by limited rainfall and high evaporation rates, rely heavily on groundwater irrigation to sustain crop production. However, the demand for freshwater in these areas frequently exceeds supply, exacerbating competition for available water resources among agriculture, urban development, and industrial activities. Repurposing tPW for irrigation could alleviate pressure on freshwater resources, providing farmers with an alternative water source to sustain agricultural productivity. This is particularly relevant in inland areas where options for water reuse are limited, and agriculture represents the primary consumer of water resources.

With agriculture accounting for a significant proportion (~60 %) of freshwater consumption globally, tPW provides an opportunity to mitigate water shortages, particularly in top O&G producing regions such as Texas, New Mexico, California, Colorado, and Wyoming. Produced water is often rich in dissolved salts, organic compounds, heavy metals, and other contaminants (Jiang et al., 2022b), making its reuse in agriculture both an opportunity and a challenge. While tPW can serve as a supplementary water source for irrigation, the complex water chemistry makes effective treatment necessary to remove harmful constituents (Chen et al., 2024; Delanka-Pedige et al., 2024; Hu et al., 2022; Oetjen et al., 2018; Sedlacko et al., 2019; Tarazona et al., 2024b). In addition to the potential impacts of introduced ions and chemicals on soil and plants via tPW, the long-term viability of using tPW for irrigation depends on the soil's natural buffering capacity to preserve its structural integrity, nutrient balance, and microbial activity.

The chemical composition of tPW strongly depends on the geological formations from which it is extracted, the fracturing additives used during hydrocarbon recovery, and the desalination treatment process (Jiang et al., 2021; Jiang et al., 2022a; Oetjen et al., 2018; Sedlacko et al., 2019). This water profile significantly affects soil structure and nutrient availability, mainly when irrigation involves tPW with high total dissolved solids (TDS) concentrations (> 1000 mg/L) (Sedlacko et al., 2019). Studies have demonstrated that irrigation with highly saline water imposes osmotic stress on plants, thereby significantly reducing their ability to absorb water (Oetjen et al., 2018; Suvendran et al., 2024). Similarly, findings have illuminated that irrigation with tPW influences ion dynamics in soil profiles and alters cation exchange capacity (Ganjegunte et al., 2008; McAdams et al., 2019). Hence, examining the effects of tPW with varying TDS levels alongside conventional water for agricultural irrigation will offer valuable insight into the interplay among plant growth, soil health, nutrient dynamics, and microbial activity. While low-TDS tPW (≤1000 mg/L) supports stable organic carbon levels and essential nutrients such as calcium and magnesium, high-TDS waters (≥1500 mg/L) often lead to surface accumulation of chloride and sulfate, which can interrupt microbial activity and increase nutrient leaching (Oetjen et al., 2018; Sedlacko et al., 2019). Both ion composition and its concentration play a crucial role in soil health and crop yield. High concentrations of sodium and chloride are commonly present in PW and tend to elevate soil salinity, affecting plant growth and microbial diversity. In contrast, the presence of calcium and magnesium ions can mitigate soil sodicity, enhancing soil permeability and aeration. Therefore, understanding soil ion profiles when irrigating with tPW is essential for preserving soil health and sustaining agricultural productivity (Redmon et al., 2021; Shah et al., 2021).

In addition to ion composition, microorganisms in soils play essential roles in agriculture. They contribute to the carbon cycle by carbon fixation through photosynthesis and carbon decomposition, which are important for forming soil aggregates and producing products that might stimulate plant growth (Dobrovol'Skaya et al., 2015). Some organisms contribute to the nitrogen cycle by nitrogen fixation, nitrification, and denitrification to help plants to grow (Jacoby et al., 2017). The quality of reclaimed water used for irrigation could also impact the soil microbial communities, thereby affecting the growth of the crops. Even with the advanced engineered water treatment systems, tPW may still contain a certain amount of organic compounds, metals, and salts (Tarazona et al., 2024a). All of those residual constituents might alter soil microbial community structure and functions. Therefore, it is vital to assess the impact of tPW irrigation on soil microorganisms.

The interplay of ion/compound composition and microbial activity also depends on soil type, especially when irrigating with tPW (Kashani et al., 2024). Loamy soils, such as those represented in Whiskers (WK) regions, namely Texas, New Mexico, and Arizona, exhibit higher permeability and facilitate deeper ion penetration compared to clay-rich Cowboy (CB) soils, which tend to retain salts near the surface (Oetjen et al., 2018). These structural differences in soil underscore the importance of tailoring PW treatment and irrigation frequency based on the soil properties to optimize effects for soil health and plant growth. For instance, WK soil management strategies could prioritize optimizing irrigation depth and frequency to ensure sufficient nutrient delivery while minimizing the risk of valuable ions being leached beyond the root zone. Conversely, for clay-rich CB soils, attention must be given to managing salt accumulation and ensuring that irrigation practices minimize surface salinity and potential toxicity to plants. To maintain soil health, enhance microbial activity, and promote sustainable plant growth, it is crucial to thoroughly assess the interactions between soil characteristics, plant types, and the quality of the tPW used for irrigation. Hence, this study evaluates the dynamics of ions and microbial communities in soils irrigated with tPW at different TDS levels compared to conventional water sources. The tPW with various salinities were irrigated on different plant types, namely Mexican Feather Grass, Red Yucca Honey, Mesquite, and alfalfa on CB and WK soils. Among these plant types, the study will focus on alfalfa while also providing information about the soil comparison for the rest of the plant types. The findings aim to provide insights into the suitability of tPW as an irrigation resource, emphasizing its implications for long-term soil health, microbial ecology, and sustainable agricultural practices.

2. Materials and methods

2.1. Experimental setup and plant cultivation

The greenhouse experiments were conducted from July 11, 2023, to April 22, 2024, in Midland, Texas, where a large volume of PW is generated from unconventional tight oil production in the Permian Basin. During this period, the control water was the desalinated product water from Midland groundwater, treated via reverse osmosis (RO), followed by media filtration and disinfection to simulate rainwater quality. The tPW was sourced from a pilot-scale PW treatment plant located near the irrigation site. The PW treatment train consisted of multistage processes, including pretreatment to remove suspended solids, oil, iron, and H_2S, followed by fractional freeze desalination to partially remove dissolved solids. The desalted PW was filtered through greensand media and granular activated carbon (GAC) filters before being polished by a seawater RO (SWRO) system. The SWRO permeate and concentrate were blended to produce 500, 1000, and 1500 mg/L TDS streams (denoted as tPW-500, tPW-1000, and tPW-1500, respectively) for agricultural irrigation experiments.

In order to evaluate the impact of irrigation-water quality on different plant types, four plant species were selected, namely Mexican Feather Grass, Red Yucca Honey, Mesquite, and alfalfa (Fig. 1). Subsequent analyses focused on alfalfa due to its agronomic relevance and sensitivity to salinity. Additional information and results for Mexican Feather Grass, Red Yucca Honey, and Mesquite, are included in Supporting Information.

The soil types that were selected for this study are CB and WK, collected from Loving County and N. Reeves County, Texas, respectively. To minimize external influences, soil sampling sites were selected in areas with minimal disturbance from oil and gas activities. A vegetation survey was conducted at each site to assess the existing plant community, and soil samples were collected at 1 ft. (30 cm) intervals from the surface down to a depth of 42 in. (107 cm). Mexican Feather Grass, Red Yucca Honey, and Mesquite were planted in CB*i* and WK*i*, while alfalfa was planted in CB-alfalfa (CBA*i*) and WK-alfalfa (WKA*i*) (i = 1, 2, 3, and 4) sites. These plants were selected after the failure of mesquite trees, yucca, and sand dropseed grass due to root damage and mold development. In contrast, mesquite saplings, Mexican feather grass, red yucca, and mature alfalfa plants were transplanted, with seedlings pre-germinated in control water before all plants were acclimated for two weeks prior to tPW irrigation in September 2023.

The irrigation was conducted using tPW with TDS concentrations of ~1500 mg/L ($i = 1$), 1000 mg/L ($i = 2$), and 500 mg/L ($i = 3$) (Table 1). The control water (CW) system maintained the TDS at 12 mg/L ($i = 4$) and was considered as the baseline study. Irrigation scheduling was tailored to the water requirements of each planting. Boxes CB*i* and WK*i*, which contained drought-adapted ornamentals (Mexican feather grass, red yucca, and honey mesquite), received a single watering per week delivered by three UV-stabilized micro-sprinklers per box (20 min at 0.585 mm/min). This low-volume regime satisfies the species' limited water demand while avoiding root hypoxia. In contrast, boxes CBA*i* and WKA*i* were seeded with alfalfa, a high-evapotranspiration forage crop, and were irrigated twice weekly through five identical sprinklers per box, a frequency sufficient to replace evapotranspiration losses every 3–4 days and maintain forage yield and quality. Two organic pesticides, Bonide 8066 Captain Jack's *Bacillus Thuringiensis* BT Organic Worm & Caterpillar Control and Bonide Captain Jack's Neem Oil, were applied twice a week for two weeks (from Aug. 30 to Sep. 13) and three times (from Nov. 10 to Nov. 25) during this study. These pesticides were applied to mitigate potential influences from pests on the plant and soil in this study.

The study was conducted under a controlled greenhouse environment with daytime temperatures maintained at 35 °C with nighttime temperatures dropping to ~29 °C. Humidity levels were controlled below 30 % throughout the study using industrial dehumidifiers to replicate the arid conditions in West Texas. Full-spectrum grow lights (1000 W HPS DE lamp) were installed and operated daily from 6:45 AM to 8:15 PM to ensure consistent light exposure for plant growth. The cultivated planter box measured 2.5 × 7 ft. (76.2 × 213 cm) with a depth of 3 ft. (90 cm) and was constructed using Plexiglass reinforced with aluminum frames (Fig. 1). To facilitate proper drainage, the bottom layer of each planter box was perforated with 0.5-in. (1.3 cm) vinyl and polypropylene holes, which were lined with burlap to prevent soil loss while allowing excess water to drain when necessary. To assess evaporation rates within the greenhouse, an evaporation pan test was conducted over a 24-h period (12 PM–12 PM), illustrating that 60 % of the initial water volume evaporated overnight. Additionally, a soil moisture test was performed using a saturated soil sample from the CB box. The sample was fully saturated with control water, weighed, and left in the greenhouse for 24 h before reweighing. Results indicated that 50 % of the water had evaporated during this period.

2.2. Water sources and treatment

Table 1 summarizes the water quality for agricultural irrigation. Detailed information on the PW treatment process will be discussed in a separate publication. The water samples (CW and tPW) analysis was conducted using pH and conductivity meters for pH and conductivity, inductively coupled plasma-optical emission spectrometry (ICP-OES) for element measurement, and high-pressure ion chromatography (HPIC) for the quantification of cations and anions. The remaining compounds were analyzed via an external laboratory stationed in Midland, Texas.

2.3. Soil sampling and analysis

Soil samples were collected at depths of 1, 2, and 3 ft. (30, 60, 90 cm)

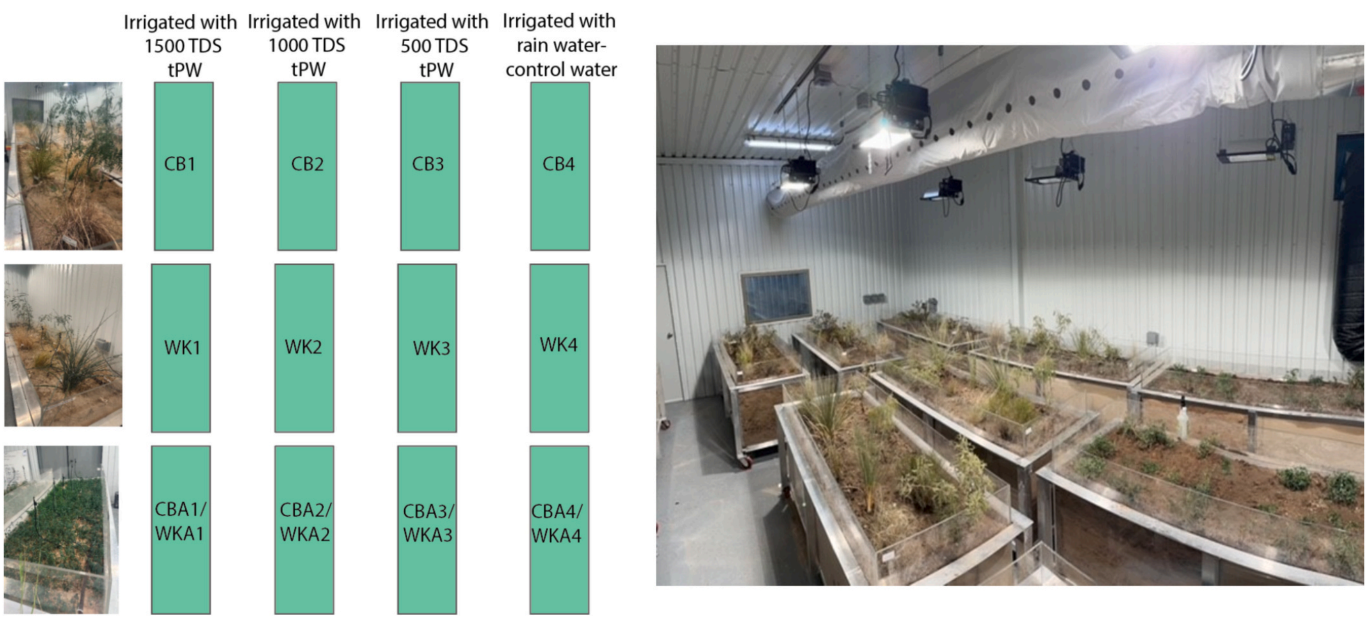

Fig. 1. Experimental layout of the agricultural site irrigated using tPW (with different TDS levels) and CW on Cowboy (CB) and Whiskers (WK). The irrigation treatments included tPW with TDS of 1500 mg/L (1), 1000 mg/L (2), and 500 mg/L (3) alongside CW (desalinated groundwater) with TDS of 12 mg/L to simulate rainwater quality (4). Mexican Feather Grass, Red Yucca Honey, and Mesquite were grown in CB and WK boxes, and alfalfa was grown in CBA and WKA containers.

Table 1
Physical and chemical composition of tPW at respective TDS levels (1500, 1000, and 500 mg/L) and desalinated groundwater as CW utilized in this study.

Parameter	Unit	tPW-1500	tPW-1000	tPW-500	CW
pH	–	6.28	6.38	6.25	7.44
Conductivity	μS/cm	2695	1589	843	63
Sodium adsorption ratio (SAR)*	–	21.1	16.0	8.2	0.8
Total Dissolved Solids (TDS) calculated	mg/L	1394	828	419	12
Analyte		**Value**			
1,2-Dichloroethane-D4	mg/L	ND	0.053	0.0831	NA
2-butanone (mek)		ND	0.072	ND	NA
2-methylphenol		ND	0.000137	ND	NA
3&4-methyl phenol		ND	0.000271	ND	NA
Acetone		ND	0.0599	ND	NA
carbon disulfide		ND	0.000314	ND	NA
Ethylbenzene		0.000336	ND	ND	NA
Xylenes, total		0.00128	ND	ND	NA
TOC (Total Organic Carbon)		1.06	0.78	0.51	NA
Total petroleum hydrocarbons (TPH) Chromatography with Flame Ionization Detection (GC/FID) High Fraction		0.0813	0.0769	0.0725	NA
TPH (GC/FID) Low Fraction		0.138	0.161	0.184	NA
Ammonia (NH_4 as N)		9.85	4.65	2.05	NA
Barium (Ba)		0.01	0.02	0.05	0.1
Boron (B)		1.80	1.18	0.56	ND
Bicarbonate (HCO_3^-)		4.9	4.9	4.9	7.3
Bromide (Br^-)		6.87	4.21	1.55	NA
Calcium (Ca)		37.7	21.8	5.9	1.0
Chloride (Cl^-)		903	528	153	3.0
Iron (Fe)		0.04	0.05	0.05	ND
Lithium (Li)		0.22	0.14	0.05	NA
Magnesium (Mg)		9.8	3.9	1.0	NA
Manganese (Mn)		0.02	0.01	0.02	ND
Nitrate (NO_3^-)		0.43	ND	ND	NA
Phosphorus, Total (P)		0.08	0.04	ND	NA
Potassium (K)		7.6	5.4	4.2	1.0
Silicon (Si)		0.2	0.2	0.2	ND
Sodium (Na)		766	309	82	3.0
Strontium (Sr)		9.9	3.9	0.8	ND
Sulfate (SO_4^{2-})		8.9	5.0	1.1	3.0
Zinc (Zn)		0.06	0.04	0.02	ND

ND: not detected, NA: not available/not analyzed

* $SAR = \frac{Na^+}{\sqrt{\frac{Ca^{+2}+Mg^{+2}}{2}}}$; here, the sodium ($Na^+$), calcium ($Ca^{+2}$), and magnesium ($Mg^{+2}$) concentrations are in meq/L.

within the irrigation bed profile to assess changes in soil composition over time. Initial samples were taken at the start of the experiment (week 0), and final samples were collected after 40 weeks of irrigation. All soil samples were analyzed for inorganic elements, including metals, metalloids, and trace elements, utilizing ICP-OES. Total petroleum hydrocarbons (TPH) and other organic compounds were analyzed using wet chemistry methods and gas chromatography–mass spectrometry (GC/MS), following protocols from the United States Environmental Protection Agency (USEPA) or the Texas Commission on Environmental Quality (TCEQ) (Table S1). During sample analysis, *o*-terphenyl, toluene-D8, 4-dichloroethane, 1,2-dichloroethane-D4, and α,α,α-trifluorotoluene were added as laboratory surrogates prior to extraction and GC analysis to assess analytical performance by calculating percent recoveries (0–111 % except *o*-terphenyl at 35–97 % due to matrix suppression). These compounds are not environmental detections and were not present in the original soil samples.

2.4. Alfalfa plant analysis

A comprehensive forage analysis was conducted to assess the influence of soil health on plant nutrient composition and overall growth. During the analysis, moisture; protein fractions such as crude protein (CP), acid detergent insoluble crude protein (AD-ICP), and neutral detergent insoluble crude protein (ND-ICP); fiber fractions including acid detergent fiber (ADF), neutral detergent fiber (NDF), ash-corrected NDF (aNDF), organic matter-corrected aNDF (aNDFom), and lignin; and digestibility parameters namely neutral detergent fiber digestibility at 30 and 240 h (NDFD30, NDFD240) as well as undigested NDF at 30 and 240 h (uNDF30, uNDF240) were measured. Additionally, carbohydrate fractions such as ethanol-soluble carbohydrates (ESC), water-soluble carbohydrates (WSC), and starch, along with fat levels (ether extract, EE; total fatty acids, TFA) and mineral concentrations, were analyzed. Energy values, including total digestible nutrients (TDN), net energy for lactation (NEl), gain (NEg), and Maintenance (NEm), as well as indices like relative feed value (RFV), relative forage quality (RFQ), and estimated Milk per ton, were calculated, providing a holistic understanding of forage suitability and nutritional balance.

2.5. Microbial analysis

To evaluate the impact of tPW on soil microbial community composition, top soil samples (0–15 cm) and subsurface soil samples (15–30 cm) with alfalfa planted were collected. Due to the low microbial load of soil samples, total genomic DNA was extracted from 10 g of each soil sample using DNeasy PowerMax Soil Kit (Qiagen, 12988–10) followed by DNA concentration using 5 M NaCl and 100 % cold ethanol, according to the manufacturer's protocol.

Illumina MiSeq sequencing was conducted by a commercial company (GENEWIZ from Azenta Life Sciences, South Plainfield, NJ) to assess both bacterial/archaeal and fungal communities. The variable regions of 16S rDNA (V3 and V4) were amplified for bacteria/archaea identification using forward primers containing the sequence "CCTACGGRRBGCASCAGKVRVGAAT" and reverse primers containing the sequence "GGACTACNVGGGTWTCTAATCC". The Internal Transcribed Spacer 2 (ITS2) was amplified for fungi identification using specific forward primer containing sequence "GTGAATCATCGARTC" and reverse primer containing sequence "TCCTCCGCTTATTGAT". Libraries were constructed using purified PCR products before Illumina paired-end configuration sequencing. The two sequences of each read pair were merged according to overlapping sequences (>20 bp long). Primers and adapter sequences were removed by using Cutadapt (v1.9.1). Quality filtering on joined sequences was performed to remove poor quality sequences, and UCHIME algorithm was used to detect and remove chimera sequences by comparing with the reference database (RDP Gold database). The Quantitative Insights Into Microbial Ecology (QIIME 1.9.1) data analysis package was used for data analysis. The effective sequences were grouped into operational taxonomic units (OTUs) using the clustering program VSEARCH (1.9.6) against the Silva 119 database pre-clustered at 97 % sequence identity. The Ribosomal Database Program (RDP) classifier was used to assign taxonomic categories to all OTUs at a confidence threshold of 0.8 to obtain the bacterial community composition of each sample. Sequences were then rarefied prior to the calculation of alpha diversity indices (ACE, Chao1, Shannon and Simpson) and beta diversity statistics by using QIIME 1.9.1.

2.6. Calculation

In order to evaluate the overall soil health change over time and depths, the Soil Quality Index (*SQI*) (Eq. 1) was considered. The macroscale soil quality was evaluated utilizing the chemical (SQI_{chem}) and physical (SQI_{phy}) aspects of soil health (Andrews et al., 2004; Miller et al., 2020). The normalized pH and electrical conductivity (EC) of the soil were considered as the parameters for the SQI_{chem}, while total solids and SAR were considered when calculating the SQI_{phy}.

$$SQI = 0.6 \times SQI_{chem} + 0.4 \times SQI_{phy} \tag{1}$$

$$SQI_{chem} = \frac{\left(1 - \frac{|pH - 6.75|}{\frac{7.5-6.0}{2}}\right) + \left(1 - \frac{EC}{4000}\right)}{2}$$

$$SQI_{phy} = \frac{\frac{Total\ Solids - 50}{95-50} + \left(1 - \frac{SAR-6}{13-6}\right)}{2}$$

When calculating the *SQI*, a higher weight was assigned to the SQI_{chem} (60 % of *SQI*) to highlight the impact of salinity, identified as the primary factor influencing soil health due to the TDS in the irrigation water (Table 1: tPW and CW). The first term of the SQI_{chem} is related to the pH score, where 6.75 and (7.5–6.0)/2 are the midpoint values of the optimal pH range (6.0–7.5) and half of the optimal pH range, respectively, to normalize the interface of the pH change (Andrews et al., 2004). The second term is related to the EC score, where 4000 μS/cm is the threshold for high salinity in soil (Saha, 2022). Since both pH and EC of the soil are equally important, the average was taken when calculating SQI_{chem} (Service, 2015). The first term of the SQI_{phy} is related to the total solid score, where 50 and (95–50) are the lower limit of the total solid in soil (as a %) and the recommended total solid percentage range, respectively (Andrews et al., 2004). The recommended SAR for soil for aggregate is between 6 and 13; thereby, the influence of the SAR was normalized against the lower limit and recommended SAR range (María Isabel Zamora Zamora Re et al., 2022). Similar to SQI_{chem}, both physical parameters contribute equally to the soil structure and physical health; 1:1 weighting was applied when calculating SQI_{phy}. The details of the constants, ideal ranges, and scoring values utilized in *SQI* analysis are summarized in Table S2.

3. Results and discussion

3.1. SQI fluctuates over time and depth for different soil types

The influence of tPW irrigation on soil health was examined by assessing and comparing the *SQI* of alfalfa planted soils irrigated using tPW with different TDS against the CW as the baseline/control study (Figs. 2, S1, and Table S3). The effect of the salinity levels on soil health was evaluated using the Chemical *SQI* (SQI_{chem}: Table S4), with the impact on the soil structure being analyzed by Physical *SQI* (SQI_{phy}: Table S5). This approach allowed for a targeted evaluation of the influence of salinity and structural conditions contribute to overall *SQI* when irrigating with different water qualities (Table 1).

When irrigating CBA with tPW and CW, the total *SQI* between week 0 and week 40 underwent a measurable decline, whereas each of the four WKA samples displayed a net increase (Fig. 2A). This decrease was pronounced in soil samples collected at 1 ft., indicating a substantial decline in soil health near the surface (near the irrigation site), mainly driven by the reduction in SQI_{chem} (Fig. 2C). Interestingly, the influence of water quality on soil health exhibited an inverse relationship with soil profile due to the clay-like nature of CB, which limited ion permeability and nutrient uptake in lower soil layers (Oetjen et al., 2018). However, CBA2 (the system irrigated with tPW TDS of 1000 mg/L) outperformed the system irrigated with the CW system (CBA4). Despite the minor variations in SQI_{chem} and SQI_{phy}, the CBA3 and CBA4 (tPW with 500 mg/L TDS and CW, respectively) *SQI* followed similar behavior during the 40-week time period (Figs. 2C and D). Among the CBA systems, CBA1 (tPW 1500 TDS) exhibited the poorest soil health after 40 weeks,

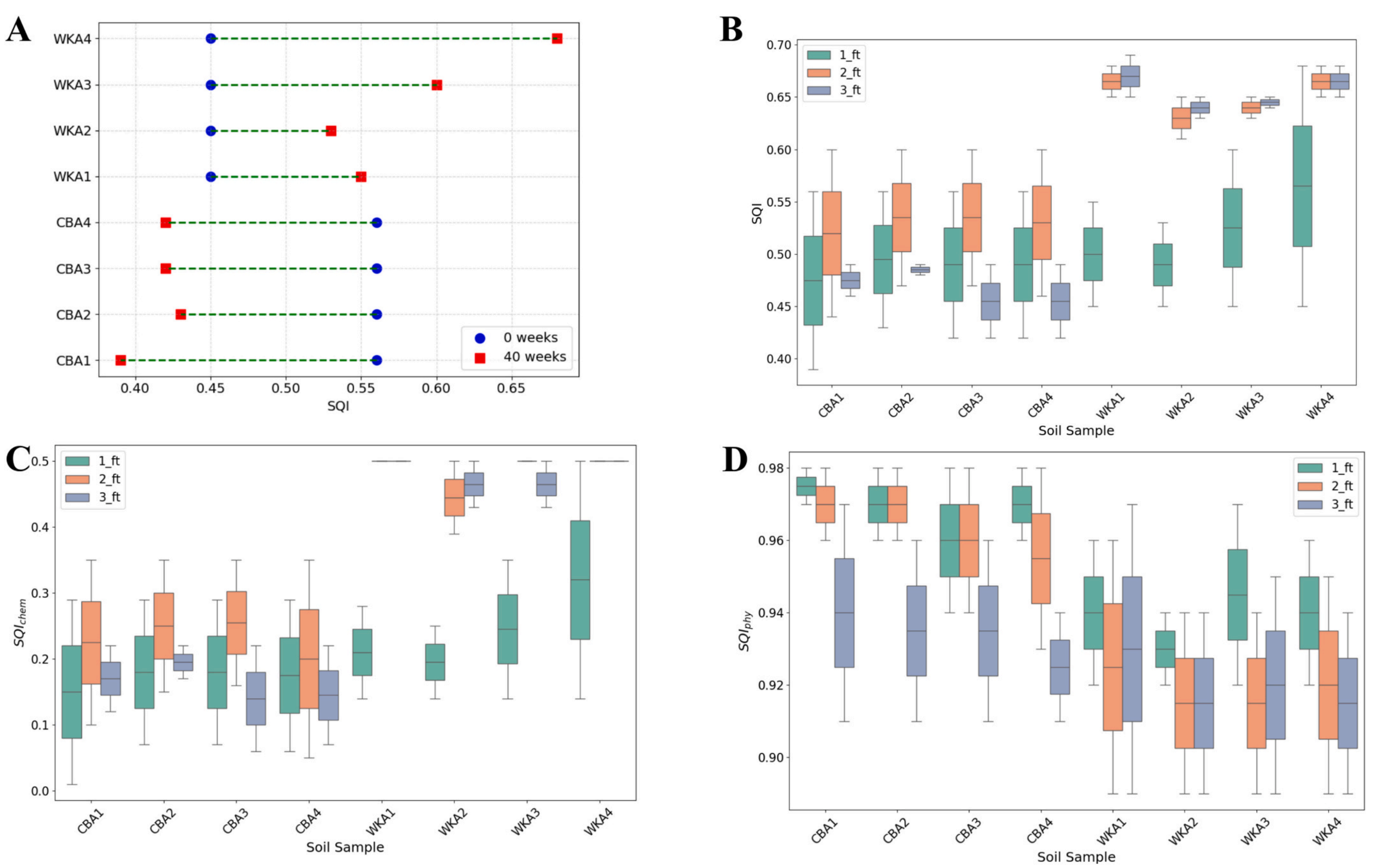

Fig. 2. The (**A**) *SQI* value at the 0th and 40th week of alfalfa soil samples and (**B**) its fluctuation. The variation of (**C**) SQI_{chem} and (**D**) SQI_{phy} at different depths over 40 weeks.

consistent with previous findings that high-salinity irrigation impairs clay soil structure and nutrient balance over time (Sedlacko et al., 2019).

In contrast, the chemical and physical components of *SQI* in WKA soils showed complementary trends driving overall soil quality gains within the experiment duration (Fig. 2). During the 40-week period, SQI_{chem} in WKA remained relatively high (compared to 2 and 3 ft. CBA), with a median increase of ~0.15 (Table S4), likely due to sustained organic–mineral inputs in tPW. However, physical *SQI* was maintained within a 0.1 range throughout the 40-week period (Table S5), reflecting minor losses in aggregation or porosity irrespective of the water quality (Table 1). The net increase in total *SQI* for all four WKA samples indicated that chemical resilience dominates over-compensation for physical attrition, reflecting the sustainability of chemical health in loamy type soils compared to clay soil types.

Depth-related patterns illustrated an improvement in chemical *SQI* when moving from the 1 ft. through the 3 ft., for both soil types, which can be attributed to the accumulation of minerals and organic constituents below surface layers. However, CBA soil started from a much lower chemical baseline (medians ~0.10–0.30) and displayed only marginal gains with depth. Conversely, WKA inherited lower chemical *SQI* with further enhancement with depth (Table S4). Physical *SQI* peaked in the 1 ft. for both soil series, where root networks and organic binding are the strongest, and gradually reduces toward deeper layers, with a pronounced change in WKA samples. In general, these vertical insights suggested that CBA should focus on subsurface chemical enrichment (e.g., deep incorporation of organic residues), whereas WKA likely benefited from balancing structural surface preservation with reinforcement of deeper physical integrity. However, overall fluctuations in organic and inorganic compounds can facilitate a better understanding of utilizing the tPW quality in irrigation.

3.2. Overview of ions and organic compounds change over time and depth

Analysis of soil metal and nonmetal composition illustrated that across all depths and time intervals (Fig. S2 and SI2), the dominant element is Ca (Figs. 3 and S2A) in all soil samples (CB, WK, CBA, and WKA). Among these soil-plant combinations, cowboy alfalfa–cultivated soils (CBA) showed the highest Ca concentrations and the lowest Na accumulation (Fig. S3), even though the tPW contained elevated levels of monovalent ions (Table 1). The high Ca concentration indicated that the soil maintained low sodicity (SAR < 1) and very high physical *SQI* (≈ 0.96–0.98) consistently 40-week period, confirming the favorable soil structure supported by abundant Ca ions (Section 3.1). However, during the experimental period, divalent cations (Ca and Mg) decreased uniformly (Ca by ~12 % and Mg by 14–40 %), whereas concentrations of potassium and trace elements (Cu, Zn, Pb, B) increased by 20–40 % and 23 % respectively (Fig. 3). These trends reflected controlled lysimeter findings in which alfalfa removed Na as efficiently as gypsum and far better than bare soil, owing to deep-root exclusion of Na from shoots and rhizosphere-driven $CaCO_3$ dissolution that liberates Ca for Na—Ca exchange (Bhattarai et al., 2022; Qadir et al., 2003). Studies have illustrated that for each tonne of harvested alfalfa hay exported, ~112 lb. Ca and 32 lb. Mg (50.8 kg Ca and 14.5 kg Mg) were exported, explaining the observed depletion and signaling the need for periodic Ca/Mg amendments to sustain long-term chemical fertility under the tPW irrigation (Kelling, E.E.S.a.K.A., 2014).

Whiskers alfalfa soils (WKA) illustrate ion-flux dynamics analogous to the CBA plots with a noticeably attenuated Na build-up, again confirming alfalfa's buffering capacity (Fig. S3). Similar to CBA, Ca remained the dominant ion and declined by less than 2 % over the 40-week time, maintaining a high physical *SQI* (Section 3.1; ~ 0.91–0.95). Magnesium and Fe changed by <8 % and <9 %, respectively, whereas K accumulates 10–12 % (Fig. 3). However, WKA cation profiles showed greater ion accumulation at 3 ft., indicating that the sandy soil did not retain salts in the upper soil profile, thereby allowing downward leaching toward the lower root zone. This pattern aligns with observations that, as plants extract water from surface layers, salts migrate deeper into the profile (Bai et al., 2024). However, long-term experiments irrigated with high Na concentration (Table 1) had shown lower surface Na accumulation and more balanced Ca/Mg ratios in alfalfa-cropped soils than in fallow cropland systems, illustrating the potential of utilizing tPW directly for irrigation without remineralization (Hou et al., 2023). Consequently, WKA soils retain good chemical *SQI*; however, CBA required remineralization of Ca/Mg to offset continuous plant uptake while exploiting alfalfa's demonstrated capacity to moderate sodicity.

The analysis of soil anion composition across all depths and time (SI2) highlighted PO_4^{3-} (indicated in light blue in Figs. 4 and S4) as the

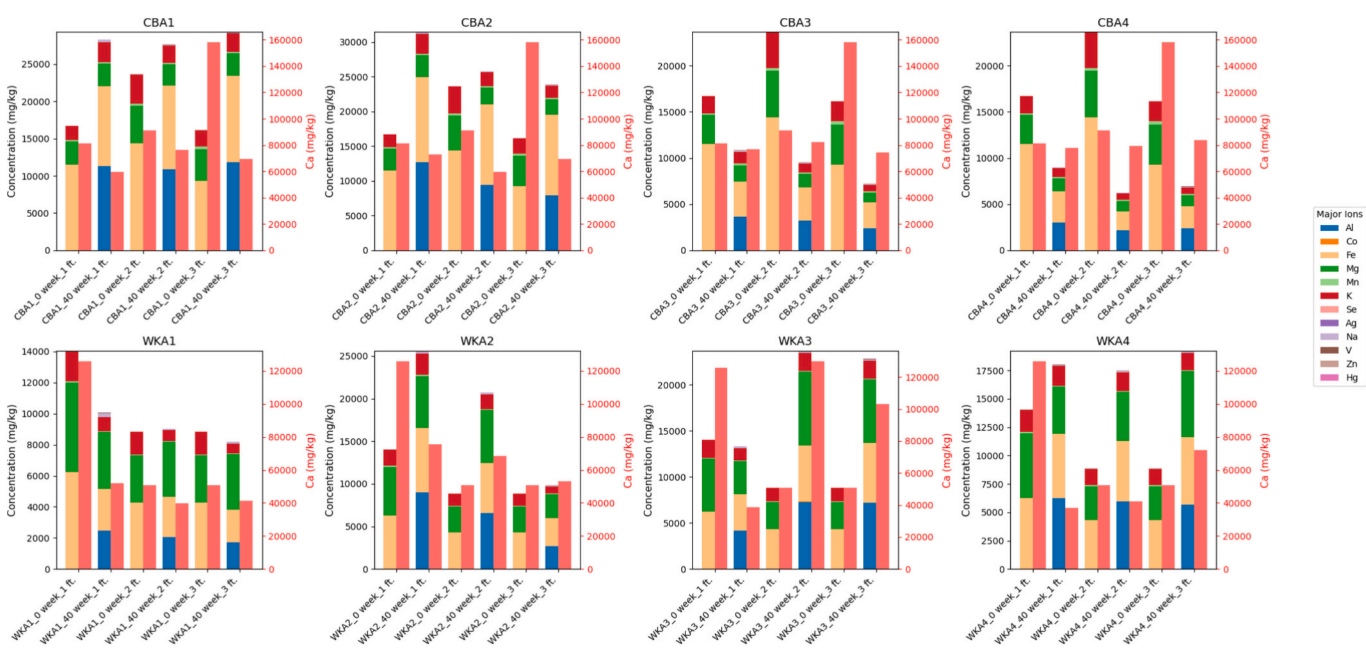

Fig. 3. The major metal and metalloid composition at 1 ft., 2 ft., and 3 ft. of CBA*i*, and WKA*i* (*i* = 1, 2, 3 and 4) fluctuates over time. The ion concentration of the 0th week and 40th week is shown for all the depths, and the compounds are color-coded.

dominant ion in initial soil samples (CB, WK, CBA, and WKA). The initial P concentration remained consistent throughout the experimental time period, especially in samples irrigated with tPW, which can be credited to the added fertilizers during this study. Importantly, the data indicated that fluctuations in PO_4^{3-} concentration, which is a crucial ingredient for plant growth, behave similarly when irrigating with tPW as with CW. However, in CBA1 and CBA2, a notable increase in PO_4^{3-} concentration (~20–40 %) (Fig. S3) was observed at deeper layers (3 ft.) after 40 weeks, indicating possible leaching or redistribution within the soil profile. The Cl^- showed a general trend of accumulation in the upper soil layers (1 ft.) in CBA samples, similar to Na after 40 weeks. This surface build-up was consistent with sprinkler placement, which led to the accumulation of Cl^- near the soil surface. In contrast, WKA samples exhibited a more uniform distribution of Cl^- along the soil profile, reflecting higher ion mobility and permeability in WKA soils compared to CBA soils. This pattern aligned with the behavior observed for cations and previous studies, highlighting the unique structural and transport characteristics of WK soils (Oetjen et al., 2018; Sedlacko et al., 2019). The F^- ion exhibited negligible changes across all soil samples and depths, with the exception of WKA3 and WKA4, where slight increases (~10–15 %) were observed at 3 ft. depth.

Among the divalent anions, SO_4^{2-} concentrations exhibited a significant increase (>100 %) in CBA samples, a result attributed to its high concentrations in tPW (especially at the 1500 mg/L TDS level), which facilitated deeper penetration. By comparison, SO_4^{2-} in WKA soils irrigated with CW showed minimal change (Fig. 4), underscoring the link between sulfate input from tPW and soil accumulation. The NO_3^- levels remained low in all soils and depths, apart from modest rises (~15–20 %) in WKA1 and WKA3 after 40 weeks (Fig. S5), suggesting that nitrate retention in WKA was influenced by both irrigation quality and plant uptake.

In general, tPW treatments displayed pronounced fluctuations in anion concentrations, primarily SO_4^{2-} and Cl^-, relative to CW systems (Fig. S5). These anion trends corroborated the *SQI* results (Fig. 2A; Table S3) and illustrated that irrigation water quality played a critical role in anion transport and retention, thereby identifying both limited nutrient availability and potential leaching risks. Excessive anion leaching posed potential threats to groundwater quality and long-term soil nutrient management.

Oil & gas (hexane-extractable) (O&G) dominated the organic composition at 40 weeks in all the depths for both soil types, followed by GC/FID high- and low-fraction TPH remained minor (generally ≤8 and ≤3 mg/kg, respectively) (Fig. 5). Across CBA and WKA, O&G range from 50 to 130 mg/kg, indicating the presence of hydrophobic, largely non-volatile material. The weak and relatively depth-invariant TPH response suggests that the more labile/volatile fractions did not accumulate appreciably by 40 weeks, potentially sorption and biological attenuation limiting TPH accumulation (Fig. 5).

Depth patterns illustrate that CBA1 (tPW-1500) showed a surface-skewed profile (1 ft. > 2 ft. > 3 ft.), whereas CBA2 (tPW-1000) and CBA3 (tPW-500) exhibited deeper maxima at 3 ft., indicating a higher risk of hydrophobic organic accumulation in the top soil when irrigating 1500 TDS tPW. This can cascade to organic retention on the surface and limited downward transport of the necessary nutrient, limiting the plant growth and spiral degradation of soil health. In WKA, accumulation tended to be deeper or more uniform: WKA1–WKA2 (tPW-1500/1000) peaked at 3 ft., and only WKA3 (tPW-500) showed a distinct surface maximum. This distribution aligns with the higher intrinsic permeability of the WKA profile, which facilitates vertical migration once hydrophobic phases are mobilized. Notably, the controls (CB4 and WKA4) also exhibited substantial O&G at 40 weeks, implying that raw soil (at 0th week) might contain these organics, as the soil originated from or near the Permian Basin. Additionally, organics such as TPH were present in anti-seize used between brass and galvanized fittings, or potentially from the PTFE tape or pipe dope used at connection points, can contribute to the organic profile in soil that is irrigated using CW. Regardless of the potentially present O&G and TPH compounds in the pristine soil, the soil irrigated with tPW behaves similarly to the CW system, indicating no TPH contamination from tPW irrigation.

The CBA and WKA soils irrigated using different TDS tPW after 40 weeks showed that the organics maintain the respective concentrations well below typical agricultural/residential screening levels (Loyzim,

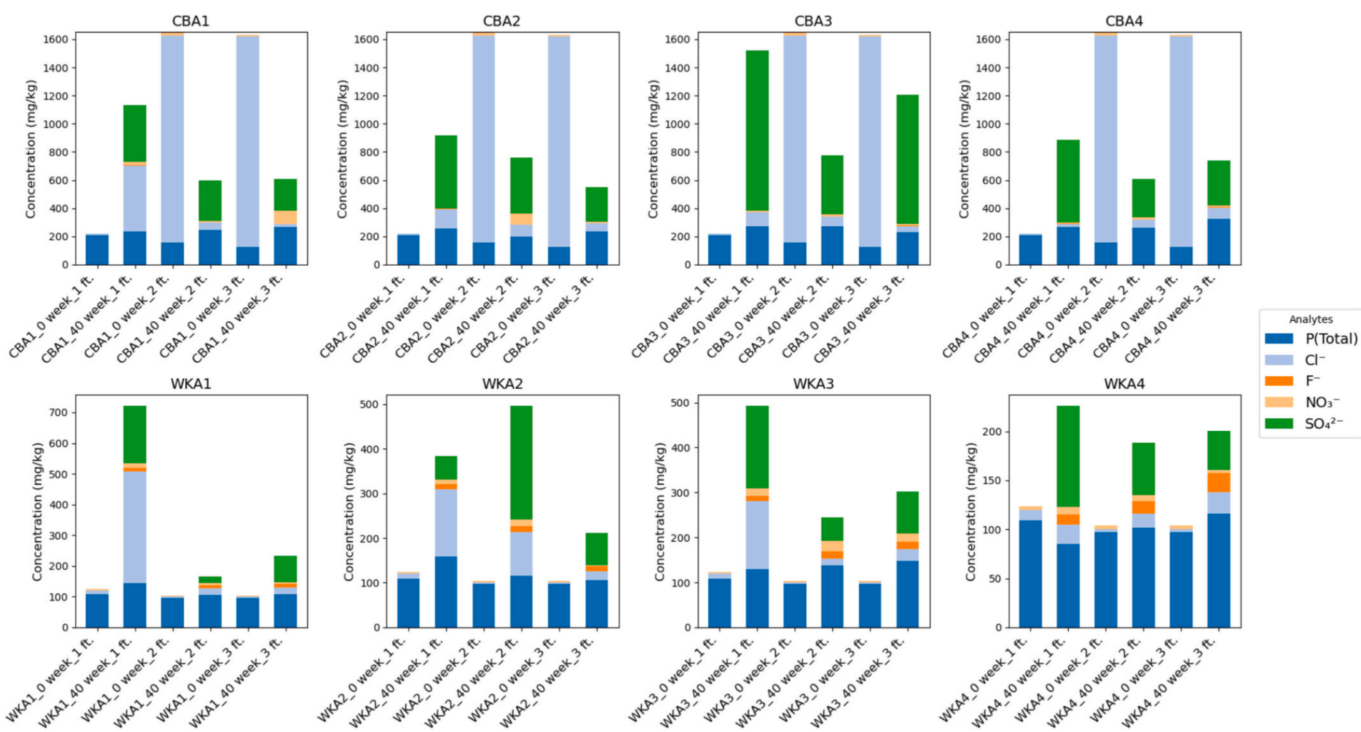

Fig. 4. The anion composition at 1 ft., 2 ft., and 3 ft. of CBA*i*, and WKA*i* (*i* = 1, 2, 3, and 4) fluctuates over time. The ion concentration of the 0th week and 40th week is shown for all the depths, and the compounds are color-coded.

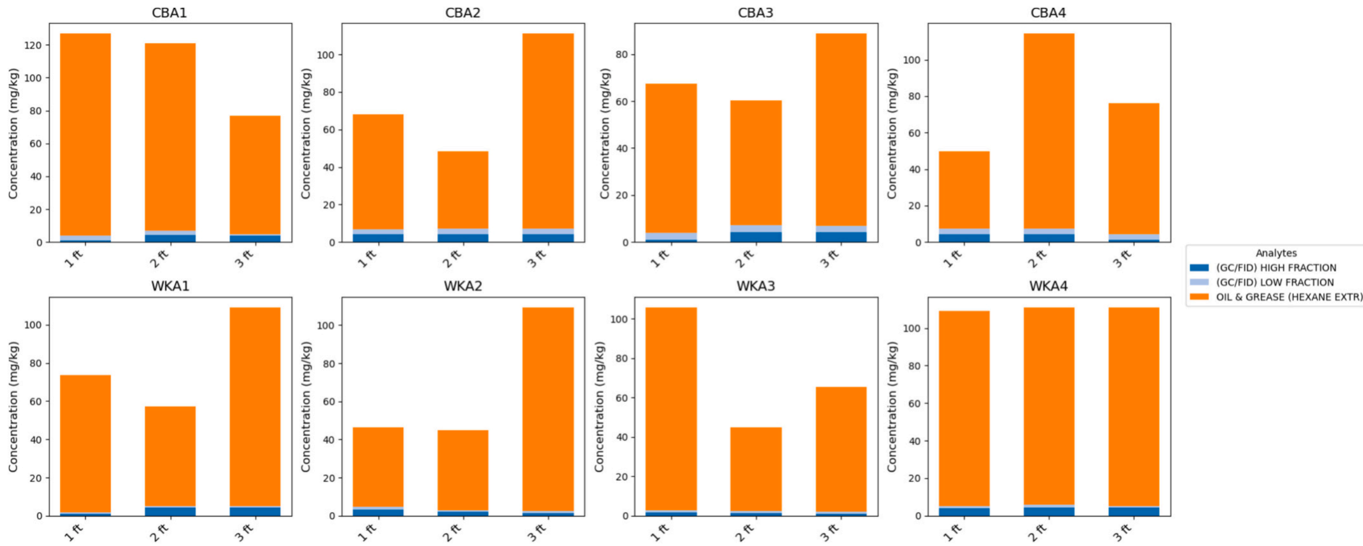

Fig. 5. The concentration/cumulative organic concentration at 40th week at 1 ft., 2 ft., and 3 ft. depths (CBA*i*, and WKA*i* (i = 1, 2, 3, and 4). All the organic compounds are color-coded.

2009; Protection, 2010). At the detected concentrations (TPH-high ≤9.31 mg/kg; TPH-low ≤3.03 mg/kg; O&G 41–123 mg/kg), adverse effects during long-term irrigation namely, phytotoxicity, microbial inhibition, or groundwater migration are unlikely, due to the fact that light-range hydrocarbons are scarce and expected to volatilize and biodegrade, whereas heavier fractions are strongly sorbed, exhibit low aqueous solubility, and therefore have limited mobility (Environmental, 2008). Overall, the comparison showed the organics present in the tPW (Table 1) did not influence the soil organic composition even when irrigating with high TDS (1500 mg/L), as the concentrations remained orders of magnitude below typical agricultural/residential screening levels and below common cleanup/action thresholds. These changes were consistent with limited surface retention (strong sorption of heavier constituents) rather than progressive accumulation or vertical migration, while any light-range hydrocarbons present were expected to volatilize or biodegrade.

3.3. Influence of inorganic and organic compounds fluctuation on soil health and plant growth

Irrigation with different water qualities (Table 1) required evaluation of ion and compound accumulation or depletion in the soil to preserve soil health. Additionally, understanding recommended soil concentration ranges was essential for microbial activity and plant growth (Table S6). Among the variations in cations, Ca and Mg, which are essential minerals, showed a concentration decline of 10–25 % and 14–40 %, respectively, in both soil types. At the same time, Na had increased under the tPW-1500 irrigation, thereby raising SAR and potentially threatening clay dispersion. Fluctuations in mono- and divalent ion concentrations directly impacted soil stability and structure, with implications for nutrient retention and permeability. The decrease was more prominent in CBA soil due to its clay-like nature, which promoted the ion-exchange mechanism. Organic amendments had been shown to buffer ion exchange by adding exchange sites; however, studies had illustrated that alfalfa absorbed these divalent ions, accelerating Ca/Mg depletion (Qadir et al., 2008). In contrast, the sandy texture of WKA promoted downward water flow, and Na-excluding root physiology led to only one-third as much exchangeable Na at 1 ft. (Bhattarai et al., 2022). Additionally, K concentrations in both soils illustrated an upward trend (20–40 %) over 40 weeks, which is known to improve water uptake and enzyme activation (Johnston et al., 2011; Shah et al., 2021). Although B concentrations had increased at every depth after 40 weeks (Fig. S2B), B levels remained below the agronomic guideline of 0.1–5.0 mg/kg (Table S6).

Among CBA anions, Cl^- and SO_4^{2-} concentrations had increased by more than 15 % in the top 2 ft. under tPW-1500, consistent with evaporative concentration in slowly draining clay (Ganjegunte et al., 2008; Miller et al., 2020). However, Cl^- levels remained below recommended thresholds except in CBA1 and CBA2, illustrating the influence of irrigation salinity and ion composition (Table S6). Conversely, WKA soils had migrated 60 ± 10 % of added Cl^- below 2 ft., aligning with advective transport in coarse soils (Miller et al., 2020). Phosphate behavior showed retention of PO_4^{3-} in the upper CBA profile via sorption and likely due to Ca-phosphate precipitation, whereas WKA leached 0.8 ± 0.2 mg/kg PO_4^{3-} to 3 ft., increasing competition for sorption sites (Miller et al., 2020; Pan et al., 2023). Such leaching could have affected nutrient availability for microbes and plants (Johnston et al., 2011). Both soils reduced semi-volatile organics by 30–50 % at depth over 40 weeks, attributed to amendment-enhanced aerobic biodegradation (Ali et al., 2024). However, hydrophobic TPH accumulated (~4 mg/kg) in CBA's top soil (1 ft.), reflecting weaker percolation in clay-rich media. This pattern was likely due to organic contaminants, experimental setup, and/or pesticide additions, potentially leading to microbial degradation that may have been limited by compound structure and environmental conditions (Ali et al., 2024; Ben Ali et al., 2022).

Soil texture and cation-exchange capacity (CEC) strongly influenced how irrigation salinity reshaped the rhizosphere, cascading to microbial function and crop performance. Accumulation of soluble salts and exchangeable Na led to elevated electrical conductivity (EC) and SAR, conditions repeatedly linked to declines in microbial biomass C, enzyme activity, and N mineralization once EC approached ~4 dS/m (Rietz and Haynes, 2003). Therefore, managing the Ca:Na balance was critical in clayey CBA: Ca amendments (gypsum, phosphogypsum; 2–5 t/ha) displaced Na, promoted flocculation, and restored infiltration, improving root aeration and sustaining microbes under marginal-quality irrigation (Gharaibeh et al., 2010; Lacolla and Cucci, 2008; Mahdy, 2011). In contrast, low-CEC WKA soils permitted rapid salt leaching, delaying EC buildups but increasing nutrient export, thus necessitating split fertilizer and organic-matter additions to maintain K, Ca, and P in the root zone (Lacolla and Cucci, 2008; Mahdy, 2011). These findings reinforced that biological responses to tPW irrigation depended on soil type: clayey soils mandated proactive sodicity management, whereas sandy soils required nutrient-retention strategies to exploit their leaching advantage (Lacolla and Cucci, 2008; Rietz and Haynes, 2003).

In general, soil irrigated with tPW ≤ 1000 mg/L produced balanced ion and organic-compound profiles relative to CW and illustrates a possibility of sustained microbial activity and alfalfa performance, especially in the clay-rich CBA soil. In contrast, 1500 mg/L promoted pronounced salt accumulation (Na^{+}, Cl^{-}, SO_4^{2-}), which potential leads to microbial shifts and nutrient imbalances, predominantly in the sandy-loam WKA soil, which shows amplified permeability leading to down-profile transport and deeper salinity exposure. This generally showed higher microbial activity and nutrient uptake, whereas deeper layers (2–3 ft.) were prone to leaching and reduced oxygen under high-TDS irrigation. Therefore, long-term irrigation can lead to enhanced salinity near the surface, thereby raising SAR, which could lead to depressing microbial biomass/activity and deteriorating structure unless proper soil-specific management is deployed (e.g., Ca/Mg additions to control SAR in CBA; split nutrient additions and depth-aware monitoring in WKA).

In order to assess the impact of soil health on plant nutrient composition and overall forage quality, a comprehensive analysis was conducted on alfalfa plants from CBA and WKA (Section 2.4). The results illustrate that forage quality and digestibility fluctuate with the salinity level of irrigation water (Table 2). The alfalfa irrigated with tPW-1000 (CBA2, WKA2) generally produced forage with a more balanced profile, combining relatively high crude protein levels (17.66 % in CBA2 and 21.67 % in WKA2) and lower fiber contents. Acid Detergent Fiber (ADF) values were 31.57 % (CBA2) and 27.48 % (WKA2), while Neutral Detergent Fiber (NDF) levels were 36.43 % (CBA2) and 33.06 % (WKA2). These lower fiber values reflect improved digestibility and energy availability, providing nutrient-rich feed for livestock. In contrast, the samples irrigated with lower TDS water at about 500 mg/L (CBA3, WKA3) displayed improved fiber digestibility, reflected in their higher NDFD values (NDFD 30: 41.89 % in CBA3, 48.18 % in WKA3). However, overall nutrient density was lower, particularly in crude protein, which was recorded at 18.42 % in CBA3 and 18.55 % in WKA3. While improved digestibility is beneficial, the slightly reduced protein levels suggest that these plants may not provide as complete a nutritional profile as those grown under ≤1000 mg/L TDS conditions.

When irrigating with the highest TDS level water (tPW-1500 in CBA1, WKA1), the results show that protein and sugar levels exhibit minimal fluctuation, with crude protein values of 19.09 % (CBA1) and 18.88 % (WKA1). The ADF values of alfalfa irrigated with treated PW with 1500 mg/L were 30.18 % (CBA1) and 29.80 % (WKA1), while NDF levels were 38.50 % (CBA1) and 34.66 % (WKA1). Although the higher TDS does not appear detrimental, the salinities of this water do not offer the same optimal balance of nutrient density and digestibility that the tPW-1000 delivers. The control samples (CBA4, WKA4), irrigated with rainwater quality, exhibited the lowest forage quality. These alfalfa samples had elevated ADF values of 31.56 % (CBA4) and 31.85 % (WKA4) and higher NDF values (37.26 % in CBA4 and 39.95 % in WKA4). Furthermore, the lignin content at 18.27 % (CBA4) and 17.17 % (WKA4) reduces overall digestibility and energy availability for livestock. Therefore, 500 and 1000 mg/L TDS levels in tPW appear to offer the most beneficial conditions for forage quality, even with higher SAR values (Table 1). This intermediate level provides a suitable balance of protein and digestibility, surpassing the nutritional composition of both higher (1500 mg/L) and lower (12 mg/L) TDS waters.

Relative to tPW treatments, the CW systems (CBA4/WKA4) showed higher fiber and lignin with lower energy yield, consistent with the lowest overall forage quality in the dataset. For instance, ADF was 31.56/31.85 % DM, and NDF was 37.26/39.95 % DM for CBA4/WKA4, exceeding tPW-1000 TDS in WKA (27.48 % ADF; 33.06 % NDF) and matching or exceeding tPW-1000 in CBA (31.57 % ADF; 36.43 % NDF). Lignin was also elevated under CW (CBA4/WKA4: 18.27/17.17 % NDFom) compared with tPW-1000 (CBA2/WKA2: 15.62/16.09 % NDFom), indicating reduced digestibility potential under CW. NDFD30 under CW was 43.65 % (CBA4) and 38.31 % (WKA4), which underperformed compared to tPW-1000 in WKA (38.74 %) and tPW-500 in WKA (48.18 %), while CBA showed similar digestibility (CW 43.65 % vs. tPW-500 41.89 % and tPW-1000 39.65 %). Consistent with these patterns, milk-per-ton was 2646/2580 lb./ton for CW (CBA4/WKA4) while it was 2498/3184 lb./ton at tPW-1000 (CBA2/WKA2), underscoring inferior energy yield under CW in WKA and no advantage in CBA. Altogether, moderate salinity (≤1000 mg/L) in tPW can match or

Table 2
Key cultivation parameters for alfalfa in CBA1–CBA4 and WKA1–WKA4 include Crude Protein, ADF, NDF, Lignin, and NDFD (30 and 240). Data compares irrigation with 1500 TDS (CBA1/WKA1), 1000 TDS (CBA2/WKA2), 500 TDS (CBA3/WKA3), and CW (CBA4/WKA4).

Parameter	Unit*	CBA1	CBA2	CBA3	CBA4	WKA1	WKA2	WKA3	WKA4
Moisture	%	57.79	43.30	60.37	52.92	54.38	71.24	54.84	57.91
Dry Matter	%	42.21	56.70	39.63	47.08	45.62	28.76	45.16	42.09
Crude Protein	%CP	19.09	17.66	18.42	19.10	18.88	21.67	18.55	17.85
ADF	%DM	30.18	31.57	34.14	31.56	29.80	27.48	34.77	31.85
NDF	%DM	38.50	36.43	39.61	37.26	34.66	33.06	40.81	39.95
Lignin	%NDFom	15.74	15.62	17.07	18.27	15.53	16.09	16.46	17.17
Lignin (Sulfuric Acid)	%DM	5.84	5.04	6.02	5.99	4.37	5.01	5.24	5.43
NDFD 30	%NDFom	41.30	39.65	41.89	43.65	45.45	38.74	48.18	38.31
NDFD 240	%NDFom	53.27	44.36	47.05	48.08	50.03	53.44	52.48	55.11
ESC (Sugar)	%DM	8.44	6.82	6.58	6.55	5.91	5.93	5.05	5.59
WSC (Sugar)	%DM	8.32	7.12	7.96	8.74	7.87	7.10	6.10	7.44
Starch	%DM	4.98	3.75	4.10	2.83	4.37	5.10	2.91	2.38
Fat (EE)	%DM	3.16	3.06	2.77	2.94	1.56	2.99	3.08	3.14
Ash	%DM	14.25	16.78	14.66	15.71	17.18	12.87	18.73	18.59
Calcium	%DM	1.48	1.14	1.52	1.58	1.57	1.53	1.33	1.49
Phosphorus	%DM	0.23	0.35	0.34	0.32	0.35	0.50	0.41	0.38
Magnesium	%DM	0.32	0.32	0.32	0.33	0.25	0.39	0.35	0.33
Potassium	%DM	1.83	3.12	2.76	2.70	3.01	0.40	3.01	2.79
Sulfur	%DM	0.30	0.27	0.26	0.26	0.17	0.30	0.23	0.21
Chloride	%DM	1.13	1.47	0.85	0.73	1.24	1.01	0.85	0.61
TDN (ADF)	%DM	65.39	64.31	62.30	64.31	65.69	67.49	61.81	61.42
TDN (OARDC)	%DM	61.02	58.01	57.49	57.39	59.09	63.12	55.72	55.65
TDN (MLK 2013)	%DM	62.29	56.61	57.75	58.71	59.51	65.83	57.56	58.61
Milk per ton	lbs/ton	2920	2498	2583	2646	2700	3184	2507	2580
NFC	%DM	35.67	33.21	32.66	33.12	35.14	35.85	31.85	33.03
NSC	%DM	14.37	12.85	11.99	11.57	12.24	12.99	9.62	9.82
RFV	–	175.39	164.02	146.33	160.53	176.19	189.91	142.79	142.84
RFQ	–	174.87	138.44	132.37	145.66	160.27	204.11	133.41	140.14

* DM:-Dry Matter, NDFom:-Neutral Detergent Fiber measured on an organic matter, and lbs./ton:-Pounds per Ton.

surpass CW by lowering fiber/lignin and improving energy yield (especially in WKA), provided salinity is managed agronomically.

Across all the tPW TDS ranges, alfalfa irrigated at 1000 mg/L TDS produced forage with balanced crude protein (CP) and fiber fractions, whereas irrigation at 500 mg/L TDS improved neutral-detergent fiber digestibility (NDFD); however, this resulted in slightly lower CP in both soil types. In contrast, forage grown under conventional low-TDS water (CW; 12 mg/L) had higher lignin and fiber fractions and lower milk-per-ton estimates, demonstrating that moderate salinity in tPW could outperform CW in nutritive value when soil salinity was controlled. This trade-off aligned with salinity-mediated shifts in nitrogen assimilation and carbon partitioning. Under moderate salinity, biomass accumulation slowed, allowing plants to maintain or even increase tissue nitrogen as nitrate reductase (NR) and glutamine synthetase (GS) activities stayed high enough to assimilate available N. Conversely, under minimal salinity, rapid growth diluted tissue N concentration despite comparable or greater total N uptake (Bertrand et al., 2015; Bertrand et al., 2020; Raffrenato et al., 2017; Singh et al., 2022; Warnke and Ruhland, 2016). Fiber digestibility primarily depended on the extent of lignification and lignin–hemicellulose cross-linking. Salinity altered these cell-wall assembly processes via osmotic stress signaling and secondary metabolism: mild to moderate salinity reduced lignin cross-linking or directed carbon toward soluble osmolytes (e.g., sucrose, pinitol, proline), thereby increasing NDFD, whereas higher or prolonged salinity induced earlier or more extensive lignification, which reduced fiber degradability by rumen microbes (Anderson et al., 2023; Ferreira et al., 2015; Sandhu et al., 2017). Different alfalfa cultivars varied in their capacities to exclude Na^+ and retain K^+, influencing NR/GS enzyme activities and cell-wall deposition. Moreover, salinity levels and cutting intervals were shown to change stem lignocellulose content and overall forage quality, highlighting the importance of adjusting TDS targets and harvest schedules to specific cultivars and production objectives (Anderson et al., 2023; Ferreira et al., 2015; Sandhu et al., 2017).

Across different tPW TDS, the CP-NDFD trade-off reflects carbon–nitrogen partitioning and cell-wall dynamics under salinity. When irrigated with 1000 mg/L tPW, results lead to the speculation of moderated growth and maintained NR/GS activity, preventing nitrogen dilution while restraining lignification, yielding balanced CP with lower ADF/NDF. At 500 mg/L TDS tPW, reduced wall cross-linking increases NDFD; however, it can slightly reduce CP (especially in WKA). Osmotic stress can promote more extensive lignification that depresses the digestibility of plants irrigated with 1500 mg/L tPW. Therefore, tPW with 1000 mg/L salinity is more likely to maintain nutrient balance in alfalfa. At this TDS level, plants exhibited minimal osmotic stress, avoiding nitrogen dilution and declines in crude protein. Simultaneously, 1000 mg/L TDS suppressed excessive lignification and preserved nitrogen-assimilation enzyme activity. Lower TDS (500 mg/L) improved fiber digestibility by reducing cell-wall cross-linking but modestly decreased protein concentration. Nevertheless, the optimal TDS target depended on whether the production goal prioritized protein content or fiber digestibility and was fine-tuned according to soil texture, cultivar, and nutrient management.

3.4. Soil microbial community profiles under the influence of tPW irrigation

The soil microbial community analyses focused on the first foot of the soil given this layer has the highest microbial abundance and richness. The alpha diversity indices of microbial communities in both top soil (0–0.5 ft) and subsurface soil (0.5–1 ft) with alfalfa are shown in Fig. 6 and Table S7.

Both the ACE (Abundance-based Coverage Estimator) and Chao 1 indices were used to estimate species richness; thus, the same trends were observed for both ACE and Chao 1 indices. For all the soil samples, there were more bacterial/archaeal species than the fungal species and the top soils have more bacterial richness than subsurface soils. Compared with control soil (CBA4), tPW with the highest salinity (1500 mg/L TDS) resulted in the lowest ACE and Chao 1 bacterial richness in CB soils, whereas the fungal richness was not impacted by tPW. Clay-rich CB soils tend to retain salts in the surface soils, which could inhibit the growth of some salt-sensitive species and result in reduced richness in CB soils. The accumulation of anions such as Cl^-, SO_4^{2-}, and F^- was observed at 1 ft. of CBA (Fig. 4) soil after 40-week irrigation. Compared with the control (CBA4), the tPW-irrigated soils (CBA1, CBA2 and CBA3) had much higher Cl^- accumulation (200–500 mg/kg), which may contribute to reduced bacterial richness. It was reported that the high concentrations of Cl^- can negatively impact soil microbial communities, affecting their diversity, function, and overall health (Gryndler et al., 2008; Zhang et al., 2018). For WK soils, although high Cl^- accumulation (150–500 mg/kg) was also observed at 1 ft. soil (Fig. 4), tPW increased the bacterial richness of the soils. Compared with loamy WK soils, the clay-rich CB soils were impacted by high concentration of Cl^- more due to high clay content. High abundance of sodium and chloride can cause clay dispersion in soil, negatively impacting soil structure and water infiltration, thereby reducing microbial richness (Abbaslou et al., 2020). Surprisingly, Bacterial Shannon and Simpson diversity indices showed no remarkable changes between control and tPW irrigated soils. Compared with bacteria, the response of fungal richness to salinity is more complex. While some studies indicate an increase in fungal richness with salinity, potentially due to the emergence of salt-tolerant fungal species, others showed a decrease in fungal diversity, especially in extreme salinity conditions (Lin et al., 2023; Zhang et al., 2024). In this study, tPW with the highest salinity (1500 mg/L TDS) decreased the fungal richness in the subsurface soils (0.5–1 ft) while it increased the fungal richness in the top soils (0–0.5 ft). At the same time, tPW-1500 reduced the fungal diversity of the subsurface WK soils while tPW increased the fungal diversity of the top WK soils. Overall, tPW with ≤1000 mg/L TDS has no impact on soil microbial richness and diversity.

Fig. S7 shows the most dominant bacterial classes for CBAi and WKAi soils. Similar bacterial profiles at class level were observed for all the soil samples in both CB and WK soils, suggesting the tPW irrigation has no impact on soil bacterial community. Both CBAi and WKAi have the typical bacteria in the desert agriculture soils. For instance, the most abundant *Thermoleophilia* are most abundant bacteria class in both CB and WK soils and it is thermophilic organisms are abundant in the desert-grassland ecological transition zone (Cui et al., 2018). Class MB-A2–108 adapts to soil with low nutrient content with a high ability to tolerate adverse environment (Megyes et al., 2021). The class of *Rubrobacteria* is enriched in the desert oligotrophic environment (Zhang et al., 2019). The essential classes responsible for biogeochemical cycles were also abundant in all the soil samples, such as *Actinobacteria* and *Acidobacteria, Proteobacteria, Acidimicrobiia*. In all the top and subsurface soils, the most dominant bacterial family was *Gaiellaceae* (relative abundance of 6.3–14.5 %), which could utilize several organic compounds for carbon cycling (Albuquerque et al., 2011). The family *Rubrobacteraceae* can tolerate high levels of ionizing radiation and can tolerate the salinity and desiccation (Albuquerque et al., 2014; Mason et al., 2023). It was found that *Rubrobacteraceae* had the highest relative abundance (14 %) in the top CB soil irrigated with the most saline tPW (1500 mg/L TDS), which may be attributed to the accumulated salts in the top layer of clay-rich CB soils. *Nitrospira* play a crucial role in making nitrogen available to plants by converting ammonia to nitrite and then to nitrate. There was considerable changes in *Nitrospira* abundance for all the conditions, indicating its resistance to salts and organics in tPW (Sepehri and Sarrafzadeh, 2018; Zou et al., 2023). All the other abundant bacterial families had no substantial differences with the control soils.

The most dominating fungal classes was *Eurotiomycetes* for all the soils (Fig. S8). *Eurotiomycetes* can decompose the organic residues of plants and thus play an essential role in the carbon cycle of the ecosystem and also contribute to nutrient cycling (Liang et al., 2021).

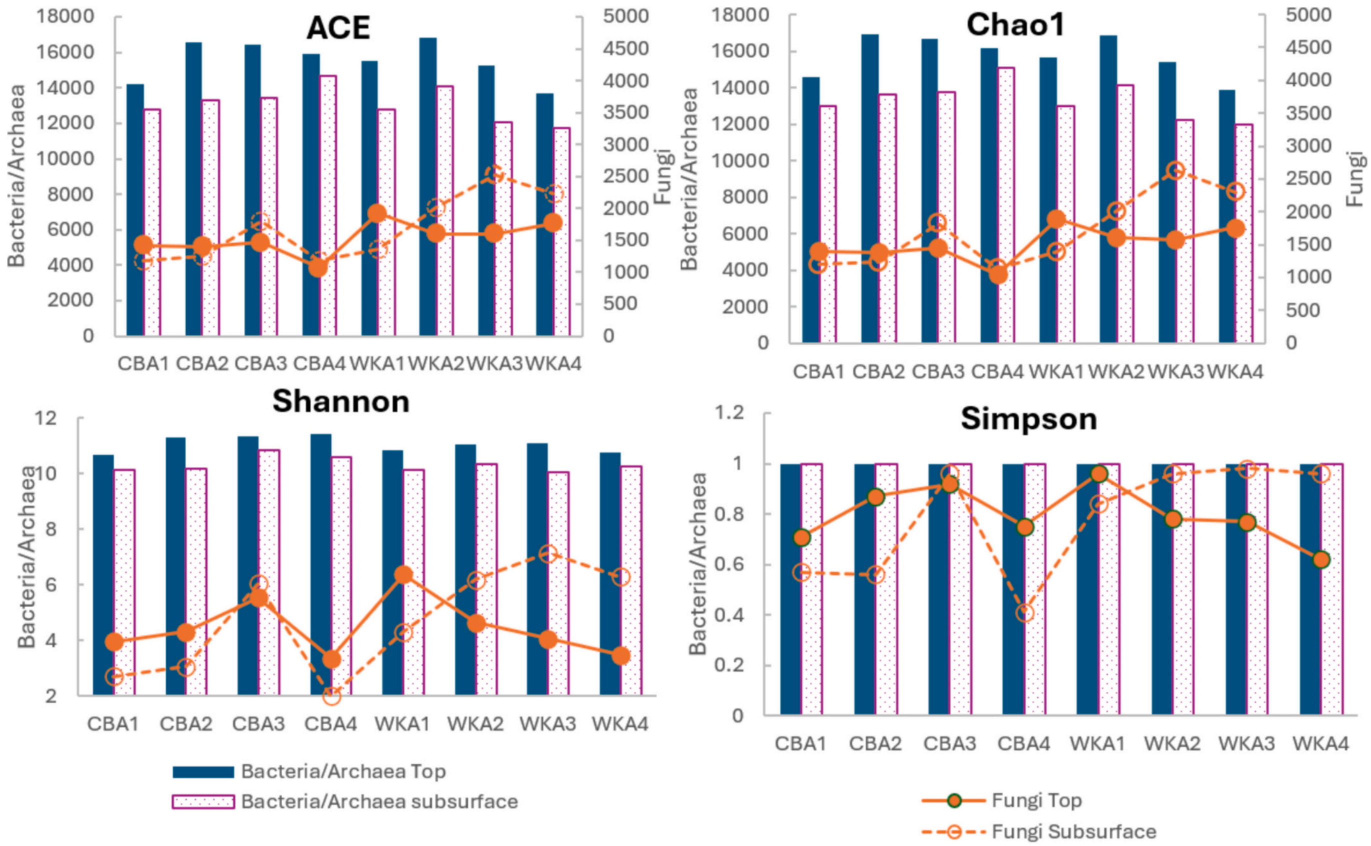

Fig. 6. The richness and diversity indexes of both top and subsurface soil of CBAi and WKAi (i = 1, 2, 3 and 4).

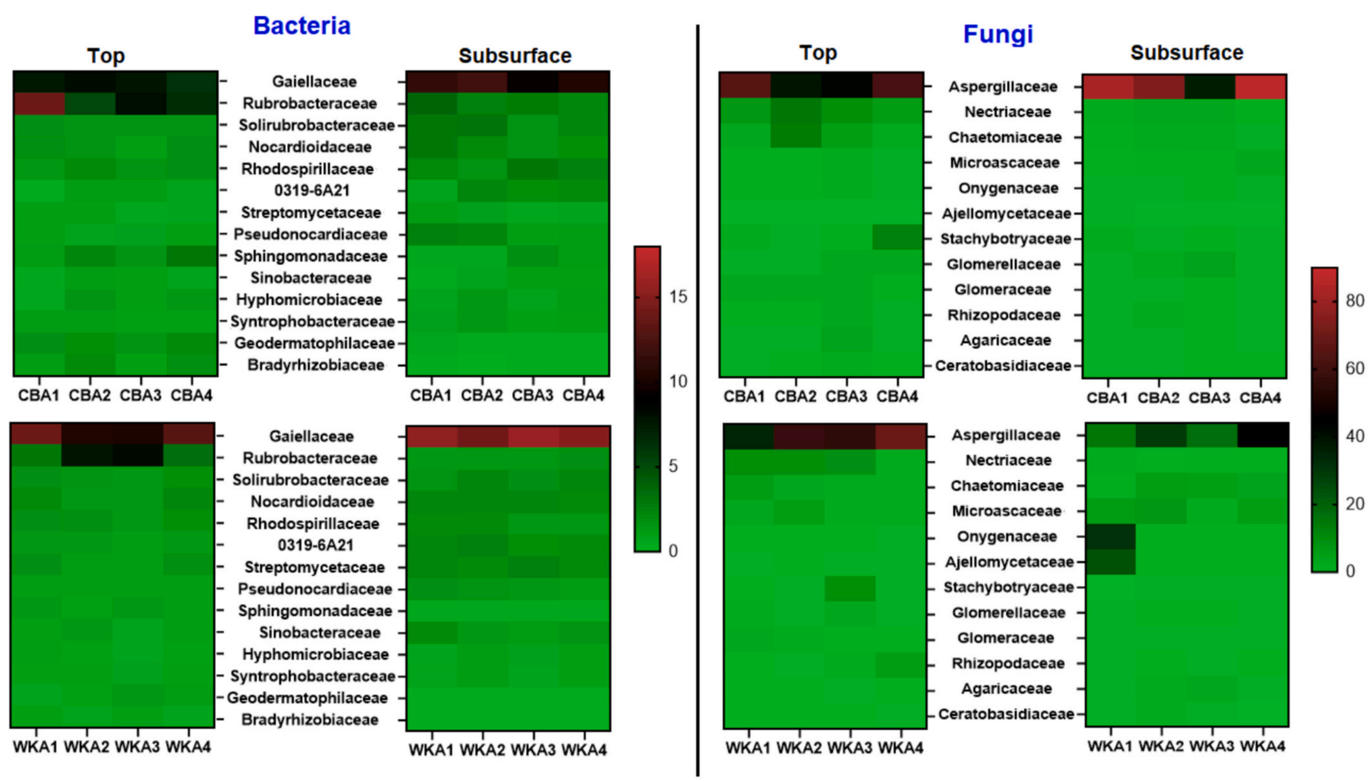

Fig. 7. The most dominant bacterial and fungal families for CBAi and WKAi (i = 1, 2, 3 and 4).

Sordariomycetes are the second largest family in the soil, which could help with organic decomposition, nutrient cycle, and pest control (Lee et al., 2019; Zhang et al., 2006). Interestingly, *Glomeromycetes* had an increased abundance in top soils irrigated with tPW. *Glomeromycetes* could colonize in the cortical cells of the plant root to form arbuscular mycorrhizae, a symbiosis between plants and fungi, which enhances the supply of water and nutrients to the host plant (Parniske, 2008; Redecker and Raab, 2006). The residual constituents in tPW may benefit the crop by promoting the formation of arbuscular mycorrhizae. From the fungal family profile (Fig. 7), it was observed that *Aspergillaceae* had the highest abundance in all CB and WK soils, and it plays a beneficial role in carbon and nutrient cycling for agriculture. Most fungal families have similar abundance in all soils, regardless of the salinity of PW. Differently, the abundance of *Onygenaceae* and *Ajellomycetaceae* increased to 31.3 % and 23.9 % from less than 1 % in the WK subsurface soils when the soils were irrigated with the tPW with the highest salinity (TDS 1500 mg/L) (Fig. 7). Both *Onygenaceae* and *Ajellomycetaceae* families include pathogens that impact mammals (Jiang et al., 2018; Van Dyke et al., 2019). A recent study also demonstrated that continued saline groundwater irrigation elevated the occurrence of pathotrophic fungal communities (Chandran et al., 2025). Saline water irrigation could reduce saprotrophic fungi due to salinity-induced physiological inhibition, leading to lower plant litter, soil carbon, and organic matter (Schmidt et al., 2019). Conversely, pathotrophic fungi could increase under these conditions due to their salinity tolerance and reduced competition among fungal groups, potentially increasing the risk of severe fungal disease outbreaks in saline-irrigated soils (Boumaaza et al., 2022). Therefore, using the tPW with high salinity for irrigation could potentially increase the risk of fungal infection from soils.

NMDS (Non-metric Multidimensional Scaling) plots visualize the similarity of bacterial and fungal communities in different soil samples (Fig. 8). The soils irrigated with the high saline tPW (CBA1 and WKA1) are not the outliners from other CBAi and WKAi (i = 2, 3 and 4), revealing the high similarity of the bacterial community. This means irrigation with treated PW has no remarkable impact on the soil bacterial community. The stress value in bacteria/archaea NMDS plot is less than 0.1, indicating the good fit between the original dissimilarity matrix and the fitted distance in the plot. For the fungal NMDS plot, both top and subsurface soil of WKA1 had a long genetic distance from WKAi (i = 2, 3 and 4), suggesting the tPW with the highest salinity (1500 mg/L TDS) had a considerable impact on the fungal community of the soils.

Soil texture governs the fate of salts introduced with tPW and, subsequently, the microbial and plant responses. In the clay-rich CBA soil, lower permeability and higher exchange capacity retain salts in the upper layers (1 ft.), elevating EC and SAR near the surface of the soil profile. This collectively leads to suppressing bacterial richness, especially at higher TDS, which led to a balance in cultivation metrics for alfalfa when irrigating with 1000 mg/L tPW. In the sandy-loam WKA soil, greater permeability promotes down-profile transport, limiting surface accumulation; however, increasing salinity exposure in deeper horizons, with subsurface fungal shifts most evident at 1500 mg/L tPW. These texture-driven exposure pathways (surface retention in CBA versus depth leaching in WKA) explain the observed differences in microbial stability and forage quality, and support a practical TDS ceiling of ≤1000 mg/L for agronomic use of tPW, with soil-specific amendments as needed.

4. Conclusion and recommendations

The study showed that tPW with TDS ≤1000 mg/L can serve as an alternative irrigation source, sustaining soil health equal to or better than that achieved with the simulated rainwater control (Fig. 9). In contrast, tPW with TDS >1000 mg/L intensified salinization, nutrient imbalance, and osmotic stress, especially in the upper layers of soil. Soil texture modulated these effects: clay-rich CBA soils benefitted from ≤1000 mg/L tPW through improved ion retention, whereas the highly permeable WKA soils were better suited to ≤500 mg/L tPW (or control water), which minimized leaching to deeper layers. Although Ca, Mg, and Fe decreased under ≤1000 mg/L tPW, K increased, enhancing water uptake and enzyme activation. Irrigating with tPW did not cause soil TPH contamination as compared to the control water, and the soil organic concentrations remained orders of magnitude below typical agricultural/residential screening levels.

Microbial analysis reveals that low TDS tPW (≤1000 mg/L) maintains bacterial and fungal community stability; however, tPW with 1500 mg/L TDS reduces fungal richness and increases pathogenic fungi in

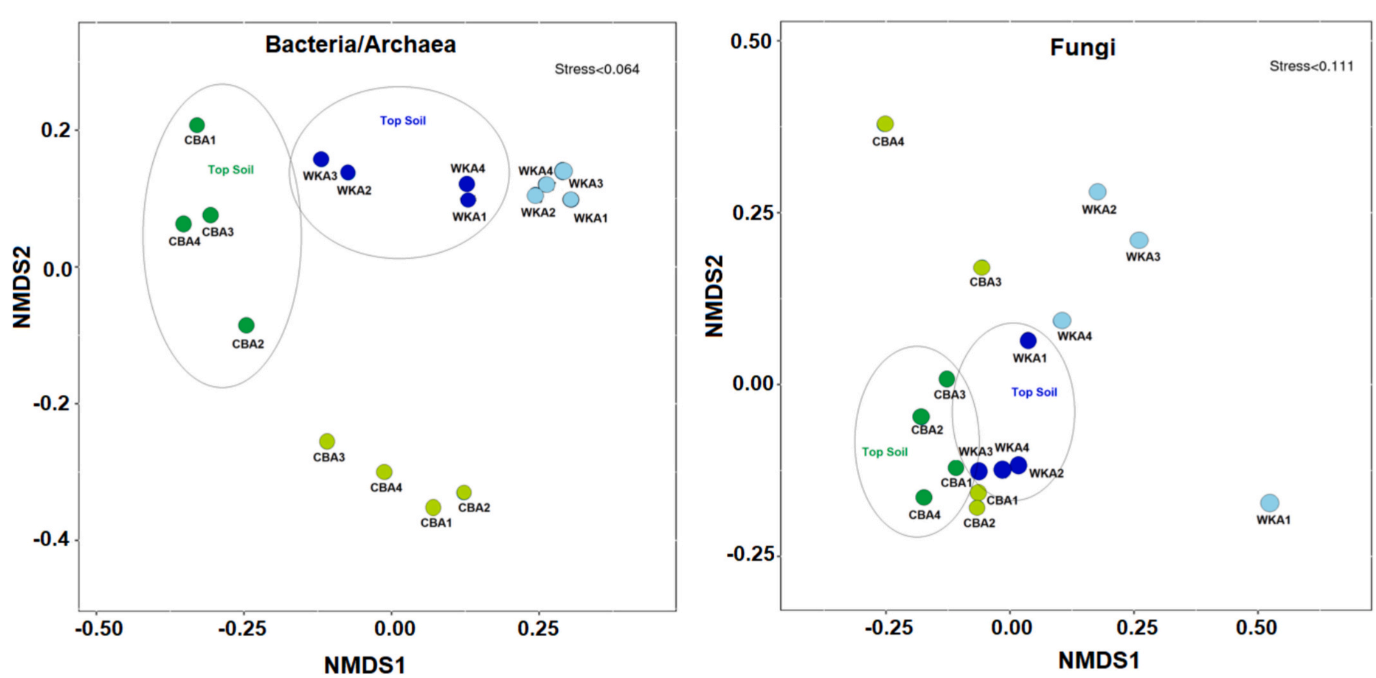

Fig. 8. The NMDS figures to show the similarity of the bacterial/archaeal and fungal community for CBAi and WKAi (i = 1, 2, 3 and 4). (green: top CB soil; light green: subsurface CB soil; blue: top WK soil; light blue: subsurface WK soil).

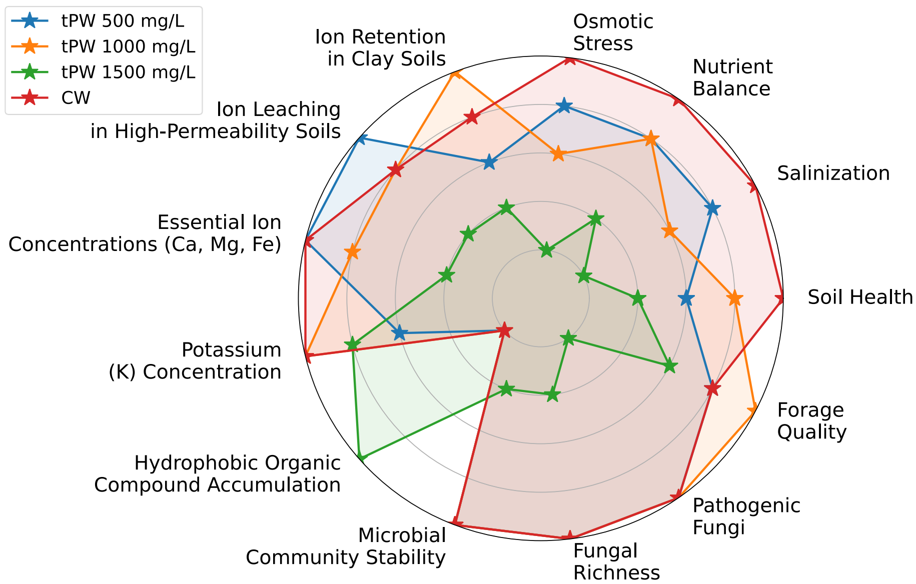

Fig. 9. Radar chart comparing the effects of different irrigation water types (tPW 500 mg/L, tPW 1000 mg/L, tPW 1500 mg/L, and CW) on various soil and forage parameters. Higher values indicate better performance for a given parameter.

loamy soils, particularly in the WK region. Alfalfa irrigated with 1000 mg/L tPW produced forage with higher crude protein, lower fiber content, and greater digestibility (Fig. 9), indicating that moderate salinity maximizes nutritional value and yield. Collectively, these results underscore the importance of tailoring tPW salinity to soil texture and crop requirements, ideally around or below 1000 mg/L to safeguard soil health, optimize nutrient cycling, and sustain long-term alfalfa productivity.

In order to optimize the use of tPW for sustainable irrigation, several measures should be implemented, such as remineralization of tPW with TDS ≤1000 mg/L using Ca and Mg. These divalent ions are essential to maintain soil structure, mitigating salinization risks, and preserving nutrient retention. The remineralization process with earth-alkaline materials is important for clay-like CB soils, which are more prone to ion imbalances. Additionally, monitoring soil ion concentrations, optimization of irrigation frequency, and tailoring irrigation water quality to soil type will assist in maintaining a stable ion profile and prevent nutrient leaching. Loamy soils in the WK region require tailored strategies to address their sensitivity to salts in tPW. High TDS tPW (1500 mg/L) reduces fungal richness and beneficial fungi abundance while increasing pathogenic fungi. Implementing targeted interventions, such as lower TDS irrigation water or periodic remineralization, can mitigate these effects and support fungal diversity for sustainable soil health.

This nine-month greenhouse study advances our understanding of plant growth, ion dynamics, and microbial community responses in soils irrigated with tPW of varying salinities. The findings underscore the potential of tPW as an alternative irrigation source, offering critical guidance for promoting long-term soil health, microbial stability, and sustainable agricultural practices in water-scarce regions. Future extended field application across successive growing seasons is recommended to further validate its long-term viability and investigate the potential progressive accumulation of residual salts and trace organic constituents in tPW on soil physicochemical characteristics and microbial dynamics, thereby influencing soil health and crop productivity.

CRediT authorship contribution statement

Punhasa S. Senanayake: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Yanyan Zhang:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation, Conceptualization. **E.M.N. Thiloka Edirisooriya:** Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **Adrianne A. Lopez:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Data curation, Conceptualization. **Danielle Smith:** Writing – review & editing, Resources, Methodology, Investigation, Data curation, Conceptualization. **Pei Xu:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Formal analysis, Conceptualization. **Huiyao Wang:** Writing – review & editing, Supervision, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2025.180520.

Data availability

Data will be made available on request.

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