

DISSERTATION

SUSTAINABLE WATER MANAGEMENT ACROSS THE AGRICULTURAL LIFE CYCLE
AND REUSE OF OILFIELD PRODUCED WATER FOR IRRIGATION

Submitted by

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ABSTRACT

SUSTAINABLE WATER MANAGEMENT ACROSS THE AGRICULTURAL LIFE CYCLE AND REUSE OF OILFIELD PRODUCED WATER FOR IRRIGATION

Water scarcity presents an ever-growing challenge in global agriculture, with significant implications for food security. In the United States (US), the scale and complexity of the agricultural system magnify these challenges, calling for an integrated and adaptive approach to water management. Among emerging solutions, the reuse of produced water (PW), a byproduct of oil and gas extraction traditionally managed using percolation pits and subsurface disposal, is being explored as a potential alternative water source for agriculture. However, its safe and sustainable application requires a comprehensive understanding of its impact across agroecosystem. This dissertation addresses these challenges through a multi-scale investigation of agricultural water sustainability, combining system-level analysis with controlled and field-based experiments.

First, a review of six key strategies aimed at sustainable agricultural water management, including crop distribution optimization, soil management, modern irrigation technologies, water treatment and reuse, reduction of water demand in animal agriculture, and minimizing food loss and waste, provides a multidimensional perspective on optimizing water use in agriculture. In examining these strategies, several challenges emerged, including gaps in the current structure of incentives, psychological barriers, lack of awareness, reluctance to alter existing farming practices and consumption habits, and insufficient data on the effectiveness of certain water conservation measures.

Building on this broader context, a greenhouse study examined the effects of irrigation with conventional produced water (CPW) on crop production, soil health, microbial diversity and function, and plant metabolome. Results showed that wheat grain metabolite concentrations, above-ground biomass, and plant height showed no significant differences across treatments, while grain yield was highest in the PW treatment. However, PW irrigation increased soil salinity and pH while reducing aggregate stability, suggesting potential long-term implications on soil health. Additionally, microbial communities in CPW-irrigated soil demonstrated functional shifts, including greater numbers of hydrocarbon degradation genes, indicating subtle ecological changes that may not be immediately evident through crop performance alone.

To extend these findings to field conditions, a field study conducted in Orla, Texas evaluated the effects of treated produced water (TPW) irrigation on crop growth, soil chemistry, and the soil microbiome. Sorghum-sudangrass was grown under drinking water (DW) and TPW irrigation regimes. Post-harvest measurements showed no significant differences in aboveground biomass, and plant height was largely comparable across treatments. However, soil analysis revealed significantly elevated boron concentrations in TPW-irrigated soil and indicated limited microbial shifts in bacterial and fungal community composition. These results provide an important foundation for evaluating the viability of TPW irrigation and can support development of regulatory frameworks for TPW discharge in the Permian Basin.

Lastly, a seed germination test was developed to evaluate a remediation strategy of PW-contaminated soils. PW spills can impair soil quality, and while Prussian yellow (PY) is proposed to be used as a remediation agent, its effects on seed germination are not well understood. Seed germination tests were conducted using native grass species, and results indicated that PY did not

inhibit seed germination, and its addition to PW-contaminated soil did not increase cyanide toxicity in soil.

Overall, this dissertation integrates experimental evidence to advance understanding of PW reuse in agriculture. By linking broader agricultural water management strategies with greenhouse and field-scale evaluations, this work contributes to the development of scientifically informed approaches for sustainable water management in agriculture.

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DEDICATION

I dedicate this dissertation to my younger self, a little girl in a small mountain town in Pakistan who once dreamed of becoming a scientist and of traveling beyond the world she knew. It took courage, persistence and hard work, but we did it.

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CHAPTER 1: INTRODUCTION

‘پانی کی ہر بوند امانت ہے’ an Urdu expression meaning “*every drop of water is a trust placed in our care, to be preserved and passed on*”. Water is fundamental to human survival and shapes how societies develop and sustain themselves, influencing cultures and belief systems over time. It has been revered, feared, and even worshipped, reflecting how deeply it is embedded in human experience. As the most technologically advanced species, we have been largely able to explore, manipulate, and at times exploit it to our advantage. This ability has helped us achieve remarkable progress but has also led to unintended pressures on the very systems that sustain us. The same ingenuity that enabled us to harness water can now be directed towards its more sustainable and equitable use. Using water to meet present needs while maintaining the integrity of the systems that sustain it reflects that responsibility.

As an essential human enterprise and by far the largest consumer of freshwater resources, agriculture stands at the heart of the global water sustainability challenge. A significant share of global freshwater withdrawals is directed toward crop production and food systems,¹ making agriculture central to any discussion on sustainable water use. At the same time, it is also where some of the most complex challenges emerge, as water use is closely tied to ensuring food security for a growing global population² and to maintaining the integrity of water resources under climate change.^{3,4} Balancing these competing demands while ensuring long-term sustainability continues to be a critical challenge.

While water scarcity is a global concern, the United States presents a uniquely complex context due to its diverse climates, large-scale agricultural production,⁵ and fragmented water governance systems.⁶ Existing conservation efforts have often focused on individual components,

such as improving irrigation efficiency or soil management practices. However, these approaches, while important, may overlook the broader interactions across the agricultural system. Addressing water sustainability therefore requires a more integrated approach that considers multiple stages of the agricultural system and must account for region-specific constraints and policy environments.

To address this broader perspective, **Chapter 2** of this dissertation presents a system-level evaluation of sustainable water use across the agricultural life cycle in the United States. This chapter explores a diverse set of approaches, including crop and soil management, irrigation technologies, treatment and reuse of alternative water sources, and opportunities to reduce water use in animal feed production and food waste (**Figure 1**). It also examines the policy frameworks needed to support these strategies and identifies common barriers to implementation.

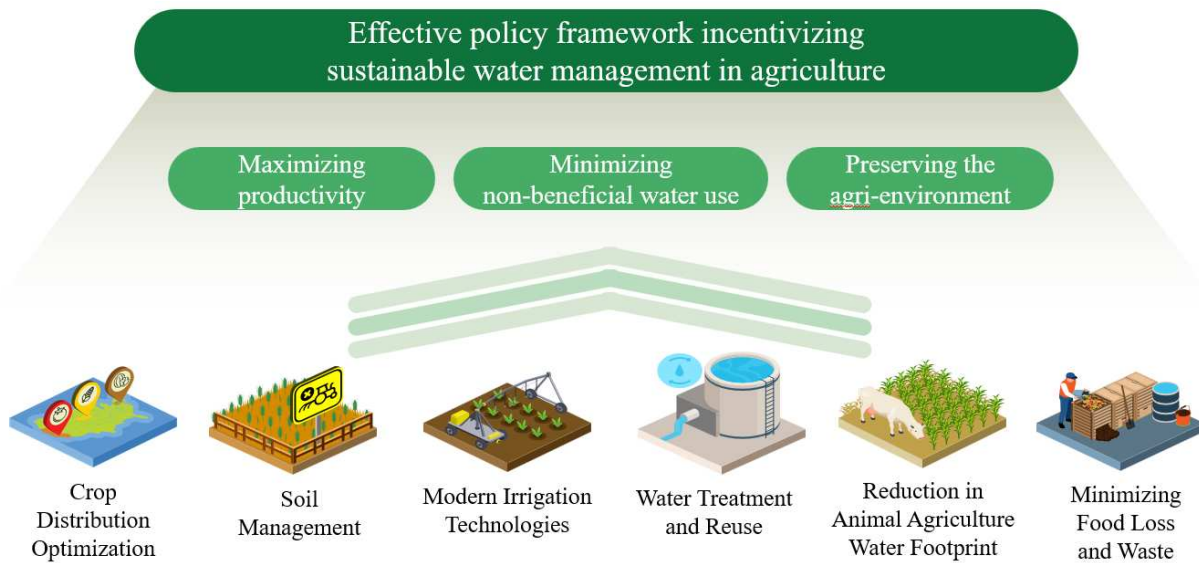


Figure 1. Strategies for sustainable agricultural water management aim to maximize water productivity, minimize non-beneficial consumption, and preserve the agri-environment.

Among the strategies discussed in Chapter 2, the treatment and reuse of alternative water sources represent a critical pathway for addressing water scarcity in agriculture. Building on this,

the following chapters focus on produced water (PW) as one such alternative source, examining its potential for reuse in agricultural systems. PW, the complex wastewater co-extracted during oil and gas (O&G) operations, is generated in enormous quantities worldwide, with global estimates exceeding 70 billion barrels annually.⁷ In the United States alone, PW production between 1985 and 2017 ranged from approximately 14 to 24 billion barrels per year, and cumulative volumes from 1933 through 2022 are estimated to have reached 1.8 trillion barrels,⁸ highlighting the immense scale of this O&G waste stream.

The large volume and continuous generation of PW presents significant management and environmental challenges as disposal methods such as percolation pits, sealed seepage pits and effluent injection⁹ have been linked with freshwater contamination (surface and ground)^{10,11} and induced seismicity,^{12,13} With increasing public and regulatory pressure to reduce the environmental impact of energy production, finding sustainable reuse pathways for PW has become more important.

In response, a variety of options for beneficial reuse of PW have been explored, both within and beyond the oil and gas sector.^{14,15} While a substantial portion of PW continues to be reinjected into high-permeability reservoirs to enhance oil recovery,¹⁴ there is growing interest in using excess volumes for non-industrial applications. One reuse option that has received growing attention is agricultural irrigation, especially in arid and semi-arid regions where freshwater resources are limited.¹⁶ Using PW for irrigation may help reduce the burden of disposal while also supplementing agricultural water supplies. However, PW is reported to contain a wide range of contaminants, including salts, metals, naturally occurring radioactive materials (NORMs), chemical additives including biocides and surfactants and organic pollutants such as benzene, toluene, ethylbenzene, xylenes (BTEX), polycyclic aromatic hydrocarbons (PAHs), and

phenols.^{17–20} These characteristics raise concerns about soil degradation, plant stress, and long-term impacts on agricultural systems.

Understanding how PW influences both soil quality and plant performance is central to evaluating its suitability for agricultural use. **Chapter 3** addresses this need through a greenhouse experiment that examines the effects of untreated conventional PW on soil chemical properties, plant growth, and early indicators of environmental stress using control (tap water), diluted PW (PW50), and undiluted PW (PW100) treatments, with an overview of the evaluated responses shown in **Figure 2**.

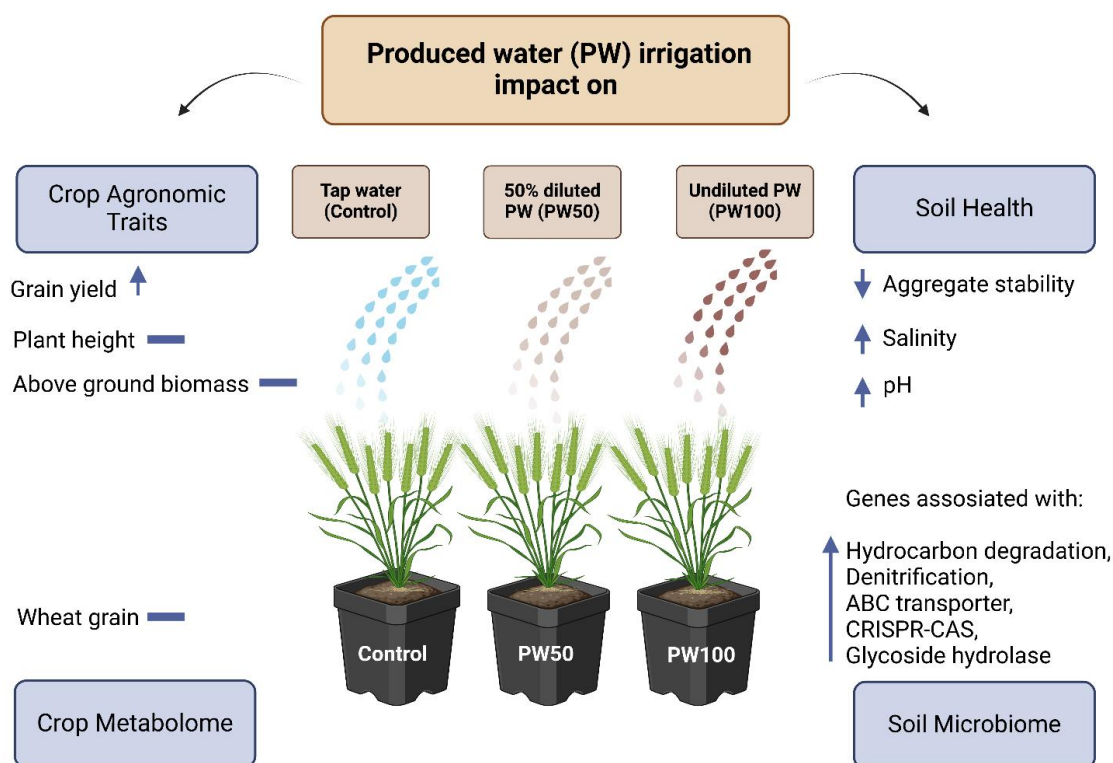


Figure 2: Overview of the greenhouse study illustrating the effects of produced water (PW) irrigation under control, diluted (PW50), and undiluted (PW100) treatments. Arrows indicate general trends across treatments.

While **Chapter 3** focused on the effects of raw PW under controlled greenhouse conditions, our next study extends this investigation to a field setting using treated PW (TPW).

Chapter 4 presents a field experiment conducted in Orla, Texas, within the Permian Basin, a major oil and gas producing region²¹ where substantial volumes of PW are generated.²² This study was designed to evaluate how TPW irrigation influences crop performance, soil chemistry, and the soil microbiome under realistic environmental conditions. By comparing TPW with drinking water irrigation, this study provides valuable insights into the potential viability and limitations of PW reuse in agricultural systems. It also serves to test whether treatment mitigates the concerns raised in the greenhouse experiment discussed in Chapter 3. Together Chapter 3 and 4 provide experimental evidence that can inform ongoing regulatory and policy discussions around the safe and sustainable reuse of PW in agriculture.

In addition to evaluating the impacts of PW on soil and crop systems, this dissertation also explores strategies to remediate salt-affected soils that may result from PW reuse or accidental releases. **Chapter 5** investigates a novel approach for facilitating salt removal using a crystallization modifier known as Prussian Yellow (PY), a potassium salt of ferrocyanide. PY promotes the upward migration and surface crystallization of salts by altering crystal formation dynamics during evaporation, potentially making salt removal more efficient.^{23,24} However, there are concerns about its environmental safety, particularly regarding the potential for cyanide release under ultraviolet light exposure.²⁵

To address both its remediation potential and environmental risks, **Chapter 5** examines the effects of PY-treated soils on the germination of native plant species. Through a series of greenhouse experiments, this study evaluates whether salt removal with PY compromises soil suitability for plant growth, providing important insight into the practicality and safety of using crystal modifiers for managing PW-induced salinity.

Overall, this dissertation explores broader strategies for advancing sustainable agricultural water use in the United States (Chapter 2), which was published in *Nature Water*.²⁶ It then examines the effects of produced water irrigation on soil and crop health in a greenhouse setting (Chapter 3), published in *Environmental Science & Technology Water*,²⁷ followed by an evaluation of treated produced water irrigation under field conditions (Chapter 4), which is ready to be submitted to *Environmental Science & Technology Water*. Finally, it investigates the potential effects of a crystal modifier used for salt remediation on plant germination and growth (Chapter 5), published in *Environmental Toxicology and Chemistry*.²⁸

My collaboration with other researchers involved two co-authored publications. The first contribution can be found in Appendix D titled: *Oil and Gas Produced Water for Cattle, Crops, and Surface Water Discharge: Evaluation of Chemistry, Toxicity and Economics* which was published in *Journal of Hazardous Materials*.²⁹ The second contribution can be found in Appendix E titled: “*Safety and Technical Feasibility of Sustainable Reuse of Shale Gas*” which was published in *Sustainable Chemistry & Engineering*.³⁰ Additionally, I presented Chapter 3 at the *American Geophysical Union (AGU)* in Fall 2023 and Chapter 4 at *Society of Toxicology and Chemistry (SETAC)* in Fall 2025.

In addition to my academic research, I was selected for several competitive fellowships and professional development programs. I was selected as a Sustainability Leadership Fellow with the School of Global Environmental Sustainability (SoGES) and as a Water Fellow with Colorado Water Center, both of which provided interdisciplinary training in sustainability, policy, and stakeholder engagement. I was also selected to participate in the Climate Reality Leadership Corps program led by former U.S. Vice President Al Gore. My engagement with global water challenges was further strengthened through attendance as an observer at the United Nations Water

Conference in New York. In addition, I was awarded a competitive scholarship to attend the Women in Agribusiness Summit in Nashville, USA. These experiences complemented my research by linking it to policy and real-world applications.

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CHAPTER 2: ADVANCING SUSTAINABLE WATER USE ACROSS THE AGRICULTURAL LIFECYCLE IN THE UNITED STATES¹

1. INTRODUCTION

Being an essential human enterprise and by far the largest consumer of freshwater resources, agriculture stands at the heart of the global water sustainability challenge. Agriculture is a major consumer of both ground and surface water, demanding the bulk of freshwater diversion worldwide.¹ Population growth and rising per capita food consumption necessitate continued increase in crop production,² intensifying reliance on already depleting freshwater resources. This dilemma is further compounded by climate change disrupting the water cycle,^{3,4} and addressing it requires a paradigm shift. Thus, the need for sustainable agricultural water management becomes imperative – an approach centered on efficient and effective utilization of water resources while minimizing adverse environmental impacts and ensuring long-term water availability.

Water scarcity in agriculture is a universal challenge, and its primary issues such as growing competition for water across sectors,^{29,30–33} diminishing water quality,^{30,31,33–35} over-abstraction of groundwater^{30–34} and emergent risks from climate change^{29,30–32} are shared globally. However, the drivers and solutions of agricultural water scarcity are deeply influenced by unique local geographic, climatic, and socio-economic conditions. In the US, water management challenges are amplified by the scale and complexity of its agricultural system, which spans 157 million hectares of cropland and 267 million hectares of grassland pasture and range⁵ spread over a multitude of climate zones, from desert to subtropical. These translate to a substantial weight in

¹Reproduced with permissions from Malik, H.T., Zvulunov, Y., Kinnebrew, E., Gates, T.K., Evett, S.R., VanderRoest, J.P., Radian, A., Chi, J., Abhijith, G.R., Mueller, N.D., Ostfeld, A., Fang, L., Borch, T. Advancing sustainable water use across the agricultural life cycle in the USA. *Nature Water* 2025, 3 (6), 655-667 Copyright 2025, Springer Nature.

the global food arena: in 2023, the US produced over 30% of the world's corn and soybean crops, was the largest exporter of rice, cotton and almonds and second-to-largest for corn, soy and chicken meat.³⁶ Water resources are regulated through several tiers including federal, state, and private entities, leading to varied approaches to pricing, subsidies, and allocation.⁶ The legal landscape further complicates water management as the coexistence of riparian and prior appropriation doctrines across states reflects the historical and geographical diversity shaping US water law.⁶ Hence, given the unique complexities of the US water management system and its significant role in the global food market, this review primarily focuses on the US, while incorporating relevant insights from other regions.

Traditionally, water conservation in agriculture has been largely focused on enhancing irrigation efficiency, but there is now a growing recognition of alternative approaches to alleviate water stress. The entire food supply chain is under scrutiny to highlight inefficiencies that exert avoidable strain on agricultural water resources. Hence, this review explores a broader range of strategies aimed at sustainable agricultural water management, encompassing key components along the food production pathway. These strategies follow the agricultural life cycle through the lens of water preservation, starting with optimizing crop selection to local conditions, implementing soil management practices, adopting efficient irrigation methods, utilizing alternative water sources, conserving water in crops used for animal feed, and finally, minimizing food waste. Collectively our recommended strategies aim to achieve shared objectives of maximizing water productivity (value per unit consumed), minimizing non-beneficial consumption, and preserving the agri-environment. This review also emphasizes that achieving these objectives depends on policies that effectively promote and incentivize the adoption of the recommended strategies.

Herein, we not only explore the potential of these strategies for enhancing water management in agriculture but also summarize critical factors limiting implementation or casting doubts on their utility in the US. Upon reviewing these strategies, several recurring challenges have emerged, highlighting areas for targeted policy efforts and further research.

2. CROP DISTRIBUTION OPTIMIZATION

In the US, water shortages are exacerbated by the spatial mismatch between precipitation and agricultural water consumption, which can differ substantially between crops and regions. Altering crop geographic distribution (also termed crop shifting) could therefore considerably reduce total agricultural water consumption. Differences in evapotranspiration rates between crops arise due to variability in phenology, leaf area, canopy cover, stomatal conductance, and crop height.³⁷ Crop management, including fertilization,³⁸ planting density,³⁹ and frequency of fallow⁴⁰ also alter evapotranspiration. Within a given crop type, different cultivars can vary in their water use,⁴¹ and the development and adoption of ‘limited transpiration’ cultivars could decrease water use during periods of high evaporative demand to increase yields in drought-prone regions.^{42,43}

A growing body of literature investigates the effects of “optimizing” crop distribution to decrease water use (**Table 1**), showing that it may be possible to decrease water consumption while simultaneously increasing caloric outputs and farmer incomes. For instance, global studies show that redistributing crops (under current climate conditions) could reduce rainwater (i.e., green water) and irrigation (i.e., blue water) consumption for agriculture by 14% and 12%, respectively,⁴⁴ while also feeding an additional 825-866 million people.^{44,45} National studies show similar trends; redistributing the 13 most common crops in China could reduce blue and green water consumption by between 4 and 19%, while also increasing farmer incomes, curbing greenhouse gas emissions, and reducing the need for fertilizers and pesticides (which additionally

require water to produce).⁴⁶ In the US, redistributing crops could increase calorie and protein production by 46 and 34%, respectively, double economic value, and decrease water consumption by 5%.⁴⁷ Notably, many of these optimization models indicated the need for a reduction in wheat and rice production and an increase in soybean and potato production to improve water conservation. For western US states, a study identified alfalfa and other cattle-feed crops as the major irrigation consumers that could be replaced to reduce irrigation volumes in these water-stressed regions.⁴⁸

While many optimization studies have examined crop shifts under static climate conditions,^{44–47} crop redistribution and crop water use will be influenced by climate change. The direct effects of climate change on evapotranspiration arise from the interacting effects of elevated CO₂ concentrations, increases in evaporative demand, and plant physiological responses, and the net effects of these mechanisms are uncertain and a topic of substantial debate.⁴⁹ However, the availability of irrigation water under climate change is a major concern. Irrigation water originating as snowpack, for example, has declined and is projected to further decrease in the coming decades due to changes in the volume and timing of snowmelt runoff.^{50,51} In the US, almonds, apples, rice, tomatoes, and walnuts face severe risk of water scarcity due to climate change, despite many regions already shifting away from water intensive crops and reducing irrigation water consumption.⁴⁸ Similarly, crop geography has changed in many other regions of the world, in some cases exhibiting poleward shifts consistent with adaptive crop migration.^{52,53}

Despite the potential benefits of crop shifting, there are many barriers that may deter farmers from switching crops. For instance, while crop redistribution could help farmers avoid future income losses due to water shortages, switching crops involves substantial financial investment (e.g., crop-specific infrastructure, machinery, and training) and inherent risk.⁵⁴ Also,

while climate change or the desire to help conserve water can be a factor in farmer decision making,^{55,56} other factors, such as income⁵⁷ and available markets,⁵⁸ may play a larger role in determining farmers' crop choice. Finally, it is important to consider that specific crops may relate to farmers' livelihoods, familial or cultural identities, and generational knowledge systems.⁵⁹

Achieving crop switching on a large scale would be extremely complex and expensive, requiring an organized effort across the US to incentivize behavior change and exchange knowledge. Financial incentives, like payment for ecosystem services programs or grants, would likely be necessary to encourage farmers to alter their cropping systems.⁶⁰ It is also possible that water conservation or regulatory programs, like California's Sustainable Groundwater Management Act or Kansas's Local Enhanced Management Area program, would motivate crop switching. The Local Enhanced Management Area program, for example, reduced groundwater use by 31% between 2013 and 2017, with 19% of these reductions resulting from farmers switching to less water intensive crops.⁶¹ In addition to this, educational programs, technical assistance, and outreach, such as through agricultural extension programs, would be essential to ensure that farmers have the skills and knowledge to successfully grow new crops in their specific geography. Research has shown the particular value in farmer networks and organizations to drive the adoption of new behaviors.⁶² Finally, expanding or subsidizing crop insurance is also important to buffer farmers' risk after switching crops.⁶³

Table 1. Summaries of existing studies on crop redistribution optimization. We only list studies that included water savings in their optimization conditions. The optimization conditions listed here refer to how variables were treated in the models. For example, “maintain” means that the value of this variable could not change; “meet or exceed” means that the model output for this value needed to be equal to or greater than its current value; “do not exceed” means that the model output for this variable needed to be less than or equal to the current value; “minimize” means that the model sought to find the lowest possible value for this variable; and “eliminate” means that the model was finding outcomes where the value of this variable equaled zero. “Geographic appropriateness” refers to efforts to distribute crops in areas wherein they have a history of

growing. “Major crops” refers to the crops with the greatest production in that region; examples of common major crops include rice, corn, wheat, soybeans, and tuber crops.

Article and geographic extent	Summary	Optimization conditions	Water reductions	Other environmental and social impacts
Beyer et al 2022; ⁶⁴ Global	Reallocate 25 major crops	Maintain: crop production (for each crop) Do not exceed: crop area Minimize: carbon emissions, biodiversity impacts Eliminate: irrigation	Irrigation consumption: –100%	Carbon emissions: –71% Biodiversity impacts: – 87% Crop area: –48%
Davis et al. 2017; ⁴⁴ Global	Reallocate 14 major crops/crop groups (optimization performed separately for rainfed and irrigated croplands)	Maintain: crop diversity, crop area Meet or exceed: calorie and protein production, crop value Minimize: green and blue water consumption	Irrigation consumption: –12% Rainwater consumption: –14%	Calorie production: +10% Protein production: +19% Feed production: +51% Additional people fed: 825 million
Xie et al. 2023; ⁴⁶ China	Reallocate 13 major (including altering crop rotations); optimizations were run multiple times, putting different weights on optimization conditions	Maintain: crop diversity, crop area, geographic appropriateness Meet or exceed: crop production (for each crop), farmer incomes Maximize: farmer incomes Minimize: water consumption, greenhouse gas emissions, fertilizer use, pesticide use	Irrigation consumption: –4.5% to –18.5% Rainwater consumption: –4.4% to –9.5%	Greenhouse gas emissions: –1.7% to –7.7% Fertilizer use: –5.2% to –10.9% Pesticide use: –4.3% to –10.8% Farmer incomes: +2.9% to +7.5%

Davis et al. 2018; ⁶⁵ India	Replace rice with alternative cereals (maize, wheat, finger millet, pearl millet, and sorghum); optimization performed separately for rainfed and irrigated croplands; this study ran several other scenarios not represented here	Meet or exceed: calorie production (OR crop replacement had to be finger millet, pearl millet, or sorghum) Minimize: blue water consumption,	Irrigation consumption: –33%	Protein production: +1% Iron production: +27% Zinc production: +13% Calorie production: “modest reduction”
Damerau et al., 2020; ⁶⁶ India	Reallocate 36 major crops	Maintain: crop diversity, geographic appropriateness Meet or exceed: calorie production Maximize: micronutrient production Minimize: crop area, irrigation consumption (for water stressed regions only)	<i>Optimizing to only reduce irrigation:</i> Irrigation consumption: –40% <i>Optimizing to reduce both irrigation and crop area:</i> Irrigation consumption: –16%	<i>Optimizing to only reduce irrigation:</i> Dietary GHG emissions: –26% to –34% <i>Optimizing to reduce both irrigation and crop area:</i> Crop area: –20% Dietary GHG emissions: –26% to –34%
Richter et al. 2023; ⁴⁸ Six regions in the US	Reallocate 30 major crops, allowing varying amounts of land fallowing	Maintain: crop diversity, geographic appropriateness Meet or exceed: farmer income Do not exceed: total irrigated area, 30% reduction in crop-specific area loss	<i>Allowing 30% land fallowing:</i> Irrigation consumption: –28% to –45% <i>Not allowing land fallowing:</i>	N/A

		Minimize: irrigation consumption	Irrigation consumption: -7% to -24%	
Davis, Seveso et al. 2017; ⁴⁷ US	Reallocate 11 major crops	Maintain: crop diversity, geographic appropriateness Meet or exceed: calorie production, protein production, and farmer incomes Minimize: water consumption	Combined irrigation and rainwater consumption: -5%	Calorie production: +46% Protein production: +34% Economic value: +208%

3. SOIL MANAGEMENT

Water preservation can be further promoted through soil management practices. Techniques such as cover crops, conservation tillage, and application of soil amendments may improve soil texture, elevate soil organic matter content and limit evaporation, contributing to higher water retention and increased irrigation efficiency.⁶⁷⁻⁶⁹ Wider adoption of water-conserving soil management regimes could ease the strain on farmers and the environment brought about by current and projected water shortages. Thus, understanding the benefits and challenges of implementing these practices is essential.

Cover crops can reduce soil erosion and loss of soil organic carbon (SOC), improving soil water retention.^{67,70} By utilizing excess soil nutrients, cover crops improve runoff quality; for example, full implementation of cover crops could reduce nitrate leaching by 20% in the Mississippi River Basin and by 69% globally.^{71,72} However, despite the documented advantages, only 3-7% of US farmers incorporate cover crops into their crop rotations.⁷³ Recent studies found that farmers' decisions to adopt cover crops are predominantly influenced by concerns over management complexity and economic viability.^{74,75} The impact of cover crops on primary crop

yield varies, with some reports finding a positive effect on primary crop yield and quality while others describe negligible or even negative effects.^{67,76} Furthermore, in arid regions where agricultural activity heavily depends on irrigation, cover crops might use more water than they conserve, making them less practical.⁷⁰ Incentive programs directly addressing these concerns by providing guidance and funding for the initial implementation steps have been instrumental in increasing adoption rates in the US over the last 15 years.⁷⁷

No-till farming improves both runoff quality (which translates to better irrigation water quality downstream) and soil water retention.⁶⁸ No-till field studies in Australia and Spain show marked increases in soil water content (approx. 7%) and available water content (25%) compared to conventional tillage.^{78,79} For the major cash crops in the US, the use of no-till farming ranges from about 20% of the planted acreage of cotton to approximately 40% for soy, wheat and corn; another 20-40% is farmed using other conservation tillage methods.⁸⁰ Despite the gradual increase in these numbers over the past two decades and proven long-term economic benefits over conventional tillage, no-till farming is still met with some reluctance.^{81,82} Common barriers to adoption include the increased herbicide use, lack of trust or eligibility restrictions pertaining to government incentives, and farmers' belief that conservation tillage is not advantageous for their specific operations.^{81,82} Governmental programs developed with farmers to reflect their needs, and increasing farmers' awareness to the monetary and soil health benefits, may help increase acceptance of the practice.

Soil amendments are commonly carbon-based, such as biochar, water-absorbing polymers, and raw or composted waste. Inorganic additives, often clays and zeolites, are also used, separately or mixed with organic substances.^{69,83,84} Amendments increase the soil water holding capacity and plant-available water by introducing properties that keep water in the root zone (apt porosity, high

surface area, polar functional groups) as well as through increased SOC and microbiome development.^{69,83,85} Thus, their beneficial effect is more substantial in soils having low SOC and/or sandy or coarse texture: biochar, for instance, has been found to increase plant-available water by an average of 45% in coarse-textured soils versus only 14% in fine-textured soils.^{83,86} Expanding the use of amendments for water conservation is complicated by the large variety of amendment types, physicochemical properties, application costs, and agricultural outcomes. Moreover, a thorough comparison of amendments focusing specifically on their water conservation potential is needed. A field study conducted in the Lao People's Democratic Republic concluded that high-cost amendments such as bentonite and biochar normally take 5-7 years to be economically viable, while low-cost additives (usually raw or processed waste) can return the investment in a year.⁶⁹ With water scarcity projected to increase, this calculation may shift to give higher weight to amendment-related drought resilience.

In addition to soil management methods that affect soil texture and percolation, water consumption can be directly reduced through rotational fallowing, where agricultural land is deliberately left unirrigated or uncultivated between growing cycles. The two largest fallowing programs in the western US include the Palo Verde Irrigation District and the Imperial Irrigation District in southern California, where approximately 20% and 4% of cropland are fallowed respectively, saving an average of $\sim 2100 \text{ m}^3 \text{ yr}^{-1}$ of water per hectare fallowed.⁸⁷ The loss of income associated with not cultivating crops for a growing season is a considerable barrier to adopting rotational fallowing. However, farmers can be financially compensated by selling the water saved during fallowing to public water-supply municipalities. For example, $\sim 40\%$ of the water supply in San Diego County is derived from the Imperial Irrigation District rotational fallowing program.⁸⁷ Such programs could be applied elsewhere in western US to address water scarcity. For example,

meeting the demand-management goal of reducing water consumption by 123,000,000 m³yr⁻¹ to avoid water shortages in the upper Colorado River basin could be achieved with temporary, rotational fallowing of 20% of cattle-feed crops.⁸⁷

4. MODERN IRRIGATION TECHNOLOGIES

Irrigated cropland contributes 54% of the \$400 billion value of US crops.⁸⁸ The 17 western US states, which hold 46% of the national harvested cropland, accounted for 82% of the irrigation volumes applied in 2023.^{89,90} Being dominated by semi-arid and mediterranean climates and lower precipitation averages, enhancing irrigation efficiency and productivity are imperative to sustain agricultural activity in these regions.

Traditional irrigation systems are characterized by significant inefficiencies, with 50–75% of appropriated water failing to reach the intended crops.⁹¹ To address this, modern irrigation technologies (MITs), such as pressurized irrigation systems (sprinkler and drip), canal lining and sealing, remote monitoring and control, and other advancements, are often promoted as effective solutions for improving water conservation in agriculture. By delivering water directly to the root zone and minimizing losses through evaporation and runoff, these technologies have the potential to optimize water application and improve crop water productivity. However, their impact on overall water conservation is not straightforward, as it depends on a multitude of complex and interrelated factors, which are discussed later in this section.

In recent decades, irrigation efficiency in the US has significantly improved, particularly with the widespread transition from gravity-fed or surface irrigation to pressurized systems.²⁹ According to USDA, water applied to the total irrigated land area across the western states decreased significantly over time as the proportion of land irrigated by pressurized systems increased.⁹² Although the U.S. has seen a 21% decrease in nationwide irrigation water withdrawals

since 1980 with increased MIT adoption,⁹³ a study found that the primary drivers of reduced water consumption (+0.5% to -45%) in six study areas across the Western U.S. were, a decrease in irrigated area (3–44%) and changes in crop mix.⁴⁸

Pressurized irrigation has also facilitated the adoption of precision irrigation (PI), which utilizes advanced technology including proximate and remote sensing systems along with specialized equipment to optimize water and nutrient use. These benefits have been observed in the US Great Plains,⁹⁴ Mississippi River Basin,⁹⁵ Southeastern Coastal Plain,⁹⁶ and California.⁹⁷ PI has benefitted from the rise in artificial intelligence (AI) and machine learning (ML) algorithms capable of processing large amounts of data to acquire insights, recognize patterns, and make predictions automatically. These tools have been demonstrated to predict water demand and identify water stress in real time to help control irrigation for various crops.^{98–100} Adoption of PI, however, is hindered by lack of effective policy^{101,102} and current technological limitations, such as the availability and interpretability of data to train ML models and the transferability of these models.⁹⁸ Nonetheless, the rapid developments in AI and ML show great promise for facilitating PI implementation.

Moreover, MIT advantages extend beyond the field level across watershed scales, especially in irrigated alluvial valleys.^{103–105} Shallow groundwater created by excessive deep percolation and canal seepage can be mitigated by MITs, thereby reducing waterlogging and salinity under irrigated lands.¹⁰⁶ MITs also can diminish the solute loading of nutrients, pesticides, and mobilized salts and trace elements, and can reduce gradients that drive these pollutants into receiving streams.^{104,107}

However, the shift to MITs has raised concerns that the resulting increase in irrigation efficiency may paradoxically increase water consumption at the watershed scale,¹⁰⁸ prompting

debate about their effectiveness for achieving tangible water savings.^{91,109} While agencies may promote MIT adoption to encourage water savings, farmers often prioritize maximizing profits.^{110,111} As increase in farm-level profit from adopting MITs fails to account for the costs of subsidies and environmental externalities⁹¹ farmers may view MITs as tools to expand irrigated acreage, cultivate water-intensive crops, or increase water application to boost yields.⁹¹ For example, a study analyzing 221 basins in the western US observed an increase in irrigation water use across much of the region, contradicting officially reported government statistics.¹¹² As a result irrigation intensification has been reported to impact streamflow sustainability in the western US.^{109,112} Such environmental implications are often overlooked when irrigation subsidy programs are developed,⁹¹ as seen in Australia's Murray-Darling Basin, where subsidized conversions to MITs faced criticism for encouraging reduced return flows and ecosystem degradation.¹¹³

It should be noted that this is not an inherent flaw of MITs but stems from the current structure of incentives pertaining to irrigation. It is also not an unavoidable failure of the subsidy system – subsidizing irrigation technologies carries important benefits, such as food security and reduced poverty – but a guideline to improve related policy making. One potential solution is to implement water quotas while subsidizing irrigation technology, which can help maintain positive social benefits while reducing environmental impacts.¹¹⁴ However, in the US, any reforms regarding water quotas is particularly complex due to significant regional variability in water prices, sources, and institutional frameworks.⁶ The banding together of irrigators in collaborative water users' associations, rather than government-mandated quotas, can contribute to enhanced water management, but has shown mixed success worldwide.^{115–117} A recent examination of historical and present-day irrigation advances in the Great Plains region showed that MITs could allow sustainable farm economies with reduced water consumption when public policy was

coordinated with effective technology transfer.¹¹⁸ Such policies have been implemented in Kansas^{119,120} and Nebraska¹²¹ with varying level of success.

Another important factor to consider is the hydrological properties of the individual river basin. In the western US, MITs can be beneficial in areas where water is lost through avoidable non-beneficial consumptive uses, while less efficient irrigation systems may benefit regions where irrigation increases the sub-annual flows despite climate aridification.⁶

Hence, the potential benefits of MITs for crop productivity and irrigation hydrology depend on their implementation within an appropriate policy framework. Customized solutions should be tailored to the specific social, economic, environmental, legal and climatic needs of each region.

5. WATER TREATMENT AND REUSE FOR IRRIGATION

Treated wastewater (TWW) is a crucial water resource, playing a pivotal role in addressing the needs of arid regions by satisfying agricultural demands, preserving freshwater reservoirs, and facilitating the reuse of organic matter, nitrogen, phosphorous, and other indispensable ions.^{6,10,97–99} Globally, an estimated ~1.7% of municipal wastewater is treated and reused, but this practice is widespread in regions with severe water scarcity. In Israel, over 87% of the TWW is reused, accounting for approximately 50% of agricultural demand.^{7,10} In the US, approximately 10% of TWW is estimated to be directly recycled, increasing by about 5% per year; the rest is typically discharged into natural waterways, often indirectly reused as water is withdrawn downstream for irrigation and potable use.^{100–102} Reusing TWW from the 16,000 existing plants (~63 billion gallons/day) could fulfill over 30% of US agricultural requirements.¹⁰¹ Specifically, local TWW supply varies across the US, with eastern states experiencing a greater excess of reclaimed water compared to western states in terms of irrigation needs. Nonetheless, an examination on a sub watershed scale indicates that a non-negligible demand could be offset by TWW generated within

reasonable distance.¹²² Direct recycling of TWW for irrigation also allows efficient utilization of residual nutrients by irrigated crops, whereas indirect reuse after discharge into surface water may compromise environmental health.

Recent survey data highlight three primary drivers for direct water reuse in the US: water scarcity, environmental constraints on wastewater effluent, and state-level mandates to develop and implement integrated water resource plans.¹²³ The leading states where these practices have been developed are Florida, California, and Texas. In Florida, approximately 48% of TWW is directly reused for industrial needs, agriculture, and irrigation of public areas. The high direct water reuse is supported by relatively few irrigation restrictions on reclaimed water during droughts and no reported illnesses related to its agricultural uses.¹²⁴ Similarly, California has high direct TWW reuse rates for application in agriculture (37%) and landscape and golf course irrigation (24%).¹²⁴ Still, public perception, the cost of water treatment to mitigate reuse risks, lack of consistent regulatory frameworks, and legal restrictions impede the expansion of directly recycled water use across the country.^{123,125–127} Challenges still exist concerning TWW quality, as detailed in **Table 2**, affecting both costs and farmers' perception. A survey from 2019 found that growers were apprehensive about irrigation with reclaimed water, especially for edible crops, although acceptance increased when provided information on the quality of TWW compared to their primary water sources.¹²⁸ Implementation of more stringent treatment policies is also expected to increase the share of TWW that meets quality criteria for irrigation.^{122,129} The cost of TWW may be inhibitive as well, in part because current water prices do not reflect true costs or scarcity.^{130,131} Allocating resources to develop cheaper and more efficient water treatment techniques may help balance this disparity over time.¹³⁰ Moreover, attaching value to reclaimed wastewater may reduce water treatment expenses and promote efficient irrigation techniques.^{10,99} In the short and medium

term, incentivizing optimized selection of crops based on local water supply and/or adjusting irrigation water prices (both from alternative and traditional sources) could promote the incorporation of TWW into irrigation schemes. For example, direct TWW use in Israel is integrated into a variable water tariff system that incentivizes irrigation with non-potable water and takes into account local water availability.¹³² Several European countries have instituted water pricing mechanisms and policies as well.¹³³

Lastly, many of the setbacks limiting the use of TWW apply to other non-traditional water sources, such as produced water and agricultural drainage and runoff.^{90,130} In addition to quality and treatment cost concerns, reuse of such waters may require modifications in the current water rights systems established in the western US states. These systems are mainly based on the prior appropriation doctrine, but in some cases, groundwater may also be treated as a mineral right tied to land ownership. The legal landscape is exceedingly complex and varies from state to state. For example, some downstream water rights are based on historical irrigation practices that produced significant volumes of runoff directly to streams and/or drainage to riparian aquifers that provided base flow to streams. These water volumes of streamflow were in some cases appropriated by users downstream whose water rights may preclude upstream farms from instituting irrigation practices that reduce drainage and runoff.¹¹⁸

To overcome the obstacles and increase the use of TWW and other alternative water sources in the US, technical, legislative, and social factors should all be addressed to increase public acceptance and trust.¹²⁵ This includes setting ambitious water reuse goals, increasing research and oversight on the environmental and health risks, advancing research and implementation of remediation technologies that meet both water quality and quantity standards, introducing water quality criteria that are tailored to the end user, and allocating appropriate

funding. Although difficult, the severity of water scarcity requires us to make the necessary adjustments and properly harness this invaluable water resource in a sustainable way.

Even with better utilization of alternative water sources, the western US states represent a disproportionate fraction of the national agricultural water demand, in part due to the needs of animal agriculture.^{87,130} Reducing the water footprint of animal agriculture is therefore an important aspect of overall minimization of agricultural water demand.

Table 2. Key challenges associated with poor quality of TWW.

Challenges	Negative impact	Current solutions and available technologies	Main gaps towards implementation	References
High salinity	Salinity causes osmotic stress which hinders water uptake by crops. High Na ⁺ and Cl ⁻ concentrations suppress uptake of essential nutrients. High SAR causes clay swelling, dispersion, and pore clogging.	Setting regulations regarding SAR and salinity with respect to the type of crop, soil, and duration. Mixing TWW with fresh or desalinated water. Ion exchange columns and membrane separation.	Cost efficiency related to treatment of saline water (mainly membrane separation technologies). Technologies that target specific mono-valent ions are missing.	134–138
Contamination by heavy metals and emerging organic contaminants (EOCs)	Reduction in soil fertility and health. Reduction in crop yield Uptake by plants, digestion by animals and humans and consequently negative health effects. Leaching and contamination of ground and surface water.	Tertiary treatment before irrigation: Current technologies for EOCs are advanced oxidation processes (mainly ozonation), or adsorption by activated carbon. Setting regulations regarding safe levels for irrigation with respect to the type of pollutant, crop and soil.	Cost efficiency of the tertiary treatment. The diversity and plentitude of EOCs makes them difficult to remove with one technology. A knowledge gap exists regarding EOCs fate in the WWTP and the agri-environment. Lack of legislation regarding safety levels in TWW.	124,135,139–142
Biocontaminants: Pathogens, antibiotic-	Breakout of disease through plant consumption.	Disinfection and membrane filtration	Disinfection does not eliminate the threat of ARGs and ARBs and the	135,137,138,141, 143,144

resistant genes (ARGs), and antibiotic-resistant bacteria (ARB)	Increase risk of spreading antibiotic resistant genes and bacteria. Deterioration of soil microbiome health	reduce risk of pathogen infection. Implementing physical barriers that minimize direct contact of the crop with TWW (such as drip irrigation and plastic mulch).	extent of their survival and dissemination in the agri-environment is unknown. Lack of legislation regarding ARG and ARB.	
Plastic contamination	Soil and groundwater contamination by microplastics	No regulatory or technological solutions are currently implemented.	The fate and related health risks are largely unknown. No specific technologies are available. No legislation is in place.	145–147

6. REDUCTION IN ANIMAL AGRICULTURE WATER FOOTPRINT

A large fraction of agricultural output is not consumed directly but dedicated instead to animal husbandry. Globally, animal production accounts for 29% of agricultural water usage, 98% of which is consumed in animal feed irrigation.¹⁴⁸ Cattle-feed crop irrigation is the greatest consumer of western US river water, accounting for 23% of total water consumption nationally and 55% of water consumption within the Colorado River basin.⁸⁷ Thus, curbing animal agricultural water demand could effectively decrease agricultural water usage.

In addition to optimizing crop distribution and rotational fallowing, prioritizing less water-intensive feed crops can reduce animal agricultural water demand. For instance, total water demand for feed concentrates (e.g. corn, cereals, pulses) is five times higher than that of roughages (i.e., pastures, straw).^{149–151} In Brazil, the water footprints of beef production for two separate cattle feedlots using 90% and 80% concentrates in feed were 6685-9673 m³kg⁻¹ and 4628-5236 m³kg⁻¹ of meat respectively,¹⁵¹ highlighting the water saving benefits when concentrates are substituted for roughages. Within North America, concentrate constitutes 5.1%, 26.9%, 72.8%,

77.4%, and 55.2% of the total feed for beef cattle, dairy cattle, broiler chickens, layer chickens, and pigs, respectively.¹⁴⁸ Thus, there is substantial room for improvement in shifting away from concentrates. Although decreasing reliance on feed concentrates in favor of roughages would help minimize feed crop water demand, the efficacy of this change depends on where roughages are grown, highlighting the importance of optimized crop distribution.¹⁵² For example, wildrye hay (a roughage crop) required more water compared to other feed crops in Northern China dairy farms since the hay was grown in a water scarce region of Northeast China.¹⁵² Lastly, while roughages are more water-efficient crops compared to concentrates, a substantial amount of water is still used to produce roughages for livestock feed: 28% of total water consumption in the western United States is used for alfalfa hay, grass hay, and other haylage irrigation.⁸⁷ Consequently, other practices beyond feed type selection are required to decrease agricultural water demand.

Beyond agricultural practices, over consumption of animal-based foods exacerbates animal agricultural water demand. Consumption of animal-based foods is increasing globally,¹⁵³ and US meat consumption exceeds the global average by almost threefold,¹⁵⁴ contributing to 37% of the food-related water footprint of an average American.¹⁴⁸ Different animal-based diets have varying resource demands, for instance, beef production requires eight times and eleven times more feed per kilogram of meat compared to pork and poultry, respectively, increasing the comparative water footprint of beef.¹⁴⁸ Thus, simply switching to a less water-intensive animal-based diet without reducing overall consumption could help alleviate some of the water demand. Moreover, producing animal-based foods generally requires more water compared to plant-based foods (**Figure 1a**) and producing animal-based calories is less water efficient compared to plant-based calories (**Figure 1b**). Replacing meat with equivalent quantities of plant-based foods, such as pulses and nuts, would decrease the average American food-related water footprint by 30%,

reducing agricultural water demand and allowing more food to be produced to feed an additional 1.8 billion people.^{148,155} Additionally, alternative protein sources, such as insects, merit consideration given that producing a kilogram of crickets requires ten times less water than beef and its amino acid content exceeds requirements provided by FAO and the World Health Organization.¹⁵⁶ Barriers to adopting more sustainable diets include factors such as affordability, deeply ingrained habits, lack of incentives and limited consumer knowledge.^{157,158} Community-based initiatives (such as the 2020 Meatless Monday Campaign in Bedford, MA), disseminating plant-based recipes, and subsidizing plant-based foods can help address these limitations.^{157,158} Nevertheless, additional behavioral changes from consumers, such as minimizing food loss and waste, are needed in conjunction with decreasing meat intake to further drive down agricultural water consumption.

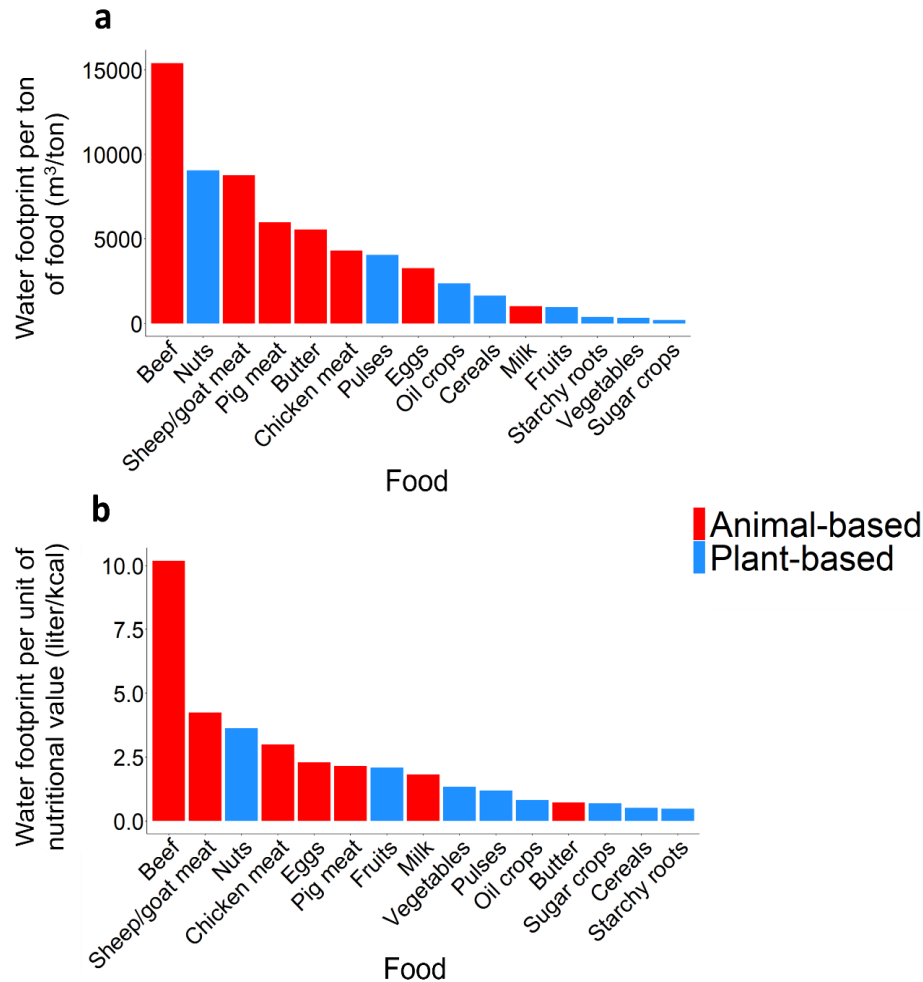


Figure 1. Comparing the water footprint of animal-based and plant-based foods. a) Volume of water required to produce one ton of food. b) Volume of water required to produce one kilocalorie of food. Foods with lower liter/kcal are more water resource efficient from a caloric standpoint, requiring less water to produce the same number of calories. Data are from Mekonnen and Hoekstra 2012.¹⁴⁸

7. MINIMIZING FOOD LOSS AND WASTE

The endpoint of the agricultural endeavor is the harvest, trade and consumption of its products. Here, too, opportunities arise to reduce agricultural water demand through the minimization of food loss and waste (FLW). Food loss refers to the reduction in food quantity or quality from harvest through processing, while food waste primarily occurs at the retail and consumer levels.¹⁵⁹

FLW contribute to a significant loss of resources, including the water used in production, highlighting the urgency of achieving Sustainable Development Goal 12.3, which calls for halving per capita global food waste by 2030.¹⁵⁹ Worldwide, an estimated 1.3 billion tons of food is lost or wasted annually,¹⁶⁰ with the US alone accounting for approximately 10% of this total.¹⁶¹ Additionally, the US has the highest annual per capita blue water footprint linked to FLW at 54.9 m³ while global average is 21 m³.¹⁶² Globally, FLW is responsible for 24% of freshwater used in food production,¹⁶³ while in the US, uneaten food accounts for 22% of all water use in the country.¹⁶⁴ Reducing global FLW by only half could decrease the water footprint of food production by 12-13%, benefiting over 720 million people by alleviating water scarcity.¹⁶⁵

As the cause and extent of FLW vary based on the production stage, countries' income levels and technological development, consumers' socio-economic status and dietary patterns, there is no one-size-fits-all solution, and various approaches have been adopted to address it.^{166–}¹⁶⁸ For instance, in developing countries, food loss is driven by limited resources for harvesting, storage, and transportation infrastructure, leading to higher post-harvest FLW.¹⁶⁰ In contrast, in high-income countries such as the US, substantial FLW occurs at retail and consumer levels because of higher purchasing power of consumers.¹⁶⁰

Countries differ widely in their progress towards enacting FLW regulations. For instance, supermarkets in France have a legal obligation to donate unsold food or repurpose it as animal feed, with potential fines for non-compliance.¹⁶⁹ In Italy, regulations encourage FLW mitigation by providing tax benefits to supermarkets engaged in donations.¹⁶⁹ The United Kingdom is the only country with data confirming a 27% reduction in FLW, attributed to its focused FLW reduction efforts.¹⁷⁰ Despite the US setting a goal in 2015 to reduce half of its FLW by 2030,

progress toward this target has significantly lagged, evident by increased per capita food waste since 2015.¹⁷¹

Greater federal involvement is needed to standardize FLW policies. Currently, US federal laws aimed at FLW reduction are limited and inadequate;¹⁷² however, several state-level legislations have been enacted (**Table 3**). In the absence of federal regulations, states exercising their discretion to regulate food date labels results in inconsistent regulations nationwide.¹⁷³ This lack of uniformity contributes to confusion over product safety or selling regulations resulting in 7% of all food waste in the US.¹⁷³

Another seemingly effective solution to reduce FLW is federally-funded, national-level awareness campaigns, such as *Love Food Hate Waste* launched in UK,¹⁷⁴ *Too Good for the Bin* started in Germany¹⁶⁹ and *Clean Plate Campaign (2020)* in China.¹⁷⁵ The US could immensely benefit from similar consumer education campaigns considering that households contribute to nearly 50% of all food waste generated in the US.¹⁶⁴

An alternative strategy could be to involve dominant companies in specific food sectors to participate in FLW reduction efforts through government regulations or societal pressure. For instance, with three companies controlling over 50% of the chicken and pig market and 75% of all cattle in the US, implementing FLW reduction policies by them can influence the entire supply chain.¹⁶⁷

Furthermore, in the US, meat and dairy account for the highest water footprint linked to FLW, while fruits and vegetables constitute the largest portion of wasted food. Therefore, shifting to a plant-based diet in isolation could increase the volume of wasted food and its associated water footprint. However, combining dietary changes with FLW reduction strategies can potentially be more effective in reducing the water footprint of an average American.¹⁶⁸

Although all the above-mentioned solutions to curb FLW are available, there are several constraints and barriers to adoption. For instance, inconsistencies in definitions and measurement methodologies of FLW among various entities hinder accurate estimation and comparison, affecting FLW policy development.¹⁶⁶ Another challenge is the inadequate monitoring of the progress of FLW reduction policies, making it difficult to gauge their effectiveness. The dissemination of information about current FLW policies also needs to be done more effectively. For example, in the US, despite the liability protection available through the Good Samaritan Act for food donors (Table 2), companies still fear consequences related to the condition of donated foods and prefer disposal over donation.¹⁷² Other barriers to adoption include insufficient collaboration between different stakeholders, handling costs of food donations, and extremely low landfill disposal fees.¹⁷⁶

A recent study discussed the possibility that lower food prices resulting from reduced FLW could potentially increase consumption, offsetting some of the benefits of reducing FLW, a phenomenon known as the 'rebound effect'.¹⁷⁷ Therefore, it is crucial to acknowledge that a major reduction in water usage tied to FLW can only be achieved by avoiding surplus food production,¹⁷⁸ which may have implications for food security. These complex trade-offs must be considered in policy making aimed at FLW reduction.

Table 3. Sample of policy efforts in the Unites States to reduce food waste.¹⁷⁹

Policy focus area	Policy category	Legislation/statue title	Legislation description	Jurisdiction
Prevention	Date labelling	CAL. FOOD & AGRIC. CODE § 82000-1	Mandates state department of food and agriculture to promote labels “use by” and “best if used by” to indicate safety and quality respectively.	California

Rescue/recovery	Tax incentive	CAL. REV. & TAX. CODE § 17053.12	Agricultural product businesses are eligible for a tax credit equivalent to 50% of transportation costs for donating crops to eligible nonprofits.	California
	Food Safety	25 TEX. ADMIN. CODE § 228.64	Detailed safety protocols for food donations, including storage temperature, shelf life, labeling, product damage, and distressed food.	Texas
	Liability protection	Good Samaritan Food Donation Act (amended by the Food Donation Improvement Act)	Provides legal protection to food donors and non-profit organizations against civil and criminal liability claims related to the condition of donated foods.	Federal
Recycling	Organic waste bans	VT. STAT. ANN. TIT. 10, § 6605K (Act 148 Universal Recycling Law)	Individuals and businesses are required to separate and recycle organic waste.	Vermont

8. OUTLOOK

While the challenges of water availability in agriculture present a pressing concern, there exists no 'silver bullet' solution, like many contemporary sustainability issues. Instead, a multifaceted approach integrating strategies across food production and consumption is required. The key to achieving sustainable water management through these strategies lies in policies that effectively incentivize progress toward their implementation. The development of such policies must adopt a multidimensional context-dependent approach with consideration of competing uses and potential caveats. Therefore, while this review explores global strategies for water conservation, it specifically addresses their caveats and implementation challenges within the US, highlighting areas for further development and research.

Here, policy reform is constrained by the cognitive inertia, where actors and stakeholders favor familiar perspectives, over emerging evidence challenging the status quo. Additionally, failing to account for and communicate the full cost of irrigation subsidies, including their externalities, such as reduced return flows, limits public awareness of the broader social and environmental impacts, thereby hindering demand for change. Psychological barriers due to insufficient awareness and education, such as negative public perception of TWW-irrigated crops and reluctance to modify farming practices or consumption habits, pose additional limitations. Another major challenge is the lack of adequate data assessing the efficiency of some water conservation measures, such as FLW mitigation programs and soil amendments. Government and regulatory bodies should prioritize efforts to overcome these obstacles and incentivize change.

While water-preserving measures such as water treatment, water-efficient irrigation equipment, soil amendments, long-distance produce transport, and AI and ML integration can

optimize water use, they may also increase greenhouse gas emissions. To mitigate this, energy-efficient designs or incentive programs may be needed. Therefore, it is critical to conduct life cycle analyses of proposed strategies to comprehensively assess their environmental and societal impacts and to identify measures for mitigation.

As the global water crisis deepens, there is a need to develop more sophisticated models that incorporate operational costs, educational requirements, technical support mechanisms, and data collection strategies to sustainably maximize productivity per drop of water in agriculture. If effectively applied, the diverse and interconnected strategies reviewed here, which combine efforts from various stakeholders, can establish a more sustainable and water-efficient agricultural system in better synergy with the hydrologic environment.

AUTHOR CONTRIBUTIONS

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CHAPTER 3: BIOLOGICAL AND SOIL INDICATORS OF ENVIRONMENTAL STRESS IN CROPPING SYSTEMS IRRIGATED WITH PRODUCED WATER²

1. INTRODUCTION

Oilfield produced water (PW) refers to the wastewater co-produced during the oil and gas (O&G) extraction process. Globally O&G exploration generates large volumes of PW, with the US producing over 21 billion barrels annually.^{15,180} The management of PW has primarily focused on using disposal methods such as percolation pits, sealed seepage pits and effluent injection.⁹ However, these techniques have been linked to freshwater contamination (surface and ground)^{10,11} and induced seismicity,^{12,13} which has sparked widespread concern among regulators and the general populace. Thus, a critical need for adequate management solutions and regulatory oversight to ensure that PW is handled in an environmentally responsible manner has emerged.

Various options for beneficial reuse of PW within and outside of the O&G sector have been proposed and, in some cases, adopted.^{14,15} Although a significant portion of PW volume is injected into high permeability reservoirs to improve oil recovery,¹⁴ excess PW generated by some wells may be used to supplement freshwater demand outside of the energy sector. One such option that has received considerable recent attention is the reuse of PW for crop irrigation. While the agricultural sector in arid and semi-arid regions faces significant water shortages,¹⁶ losing irrigated acreage to water scarcity is not an option given the expanding food demand of the world's growing

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population.¹⁸¹ Thus, there is a key need to explore alternate irrigation water sources such as PW in water scarce regions.

Although reusing oilfield PW for agricultural irrigation appears to be an intriguing possibility, the reported presence of contaminants, including salts, metals, naturally occurring radioactive materials (NORMs), chemical additives including biocides and surfactants and organic pollutants such as benzene, toluene, ethylbenzene, xylenes (BTEX), polycyclic aromatic hydrocarbons (PAHs), and phenols^{17–20} present challenges for irrigation applications. The chemical composition of PW can vary significantly based on the geological formation, reservoir conditions, and chemicals added during the O&G extraction process.^{19,182} Furthermore, PW can be divided into either conventional or unconventional based on the source rock of O&G resource, with extraction from highly permeable rocks such as sandstone referred to as conventional, and extraction from low permeability shale reservoirs referred to as unconventional.¹⁸³

High-salinity unconventional PW (UPW) is unsuitable for agricultural irrigation, as it stunts crop growth, reduces yields, impairs photosynthetic efficiency,¹⁸⁴ shifts soil microbial communities,¹⁸⁵ and suppresses plant immune systems¹⁸⁶ while altering primary metabolic profiles in wheat.¹⁸⁷ Conversely, the use of low-salinity CPW, such as that used for decades in Kern County, California, can serve as an encouraging model. Although a promising example of successful PW irrigation, there are still concerns regarding potential issues related to soil salinity and metals toxicity that should be thoroughly investigated.¹⁸⁸ While previous studies discussed above have examined the effects of produced water irrigation on specific aspects of soil and crop health, no study has comprehensively investigated the influence of untreated low-salinity CPW irrigation on various components of soil and crop health to assess the potential risks. In this study, we used a greenhouse-based crop irrigation experiment to perform an integrated assessment of the

effects of untreated PW irrigation on soil and crop health. By employing multiple analytical approaches, we aimed to detect underlying early-warning indicators of environmental stress that may not be immediately evident from crop performance alone. The decision not to treat PW was made to better capture the potential impacts of insufficiently treated PW.

2. MATERIALS AND METHODS

2.1 GREENHOUSE EXPERIMENT

Municipal tap water served as the control water in this study while the CPW was sourced from a 50-year-old well in an O&G extraction field in Colorado. Additionally, clay loam soil was excavated from a nearby farm, where hard red winter wheat, corn, proso millet, and milo are typically grown. The soil was passed through an 8mm sieve to remove large rocks and organic debris, before initiating the greenhouse experiment.

Ten spring wheat (*Triticum aestivum* L., Apogee) seeds were directly sown per 3.78 L pot (15.9 cm depth x 16.5 cm diameter and five drainage holes). When the plant reached the three-leaf stage, seedlings were thinned to five plants per pot, and the pots were then split into three irrigation treatments: 1) Tap water (Control), 2) 50% diluted CPW (PW50), 3) Undiluted CPW (PW100). The greenhouse environment was carefully regulated to support optimal plant growth conditions. It was equipped with a photoperiod lighting system set to provide approximately 16 hours of light and 8 hours of darkness daily, an ambient temperature maintained between 20–24 °C, and approximately 50% relative humidity. To address greenhouse variability, three irrigation treatments with ten replicates each were arranged using a randomized complete block design (RCBD). Plants were hand-irrigated three times a week with 150 mL of their designated treatments. Control treatment relied on the greenhouse's untreated water supply which complies with Colorado's agricultural water guidelines. The wheat was harvested 115 days after planting.

2.2 CHARACTERIZATION OF IRRIGATION WATER

Water quality was assessed for the Control and PW100 treatments, with electrical conductivity (EC) and pH also measured for PW50. EC and pH were measured by a pH/conductivity meter (Fisher Scientific, Hampton, NH). Total dissolved solids (TDS) and alkalinity was estimated using standard methods SM-2540C and SM-2320B, respectively. Ion chromatography (IC, Dionex ICS-2100, Sunnyvale, CA) quantified anion concentrations after each sample was filtered through a 0.22 μm poly(ether sulfone) (PES) membrane filter. A Dionex IonPac AS11-HC RFIC 4×250 mm analytical column (Sunnyvale, CA) was used for the IC analyses with a gradient anion method. Inductively coupled plasma optical emission spectroscopy (ICP-OES) was used to quantify irrigation water metal concentrations. Total Nitrogen (TN) and Total Organic Carbon (TOC) were analyzed using a carbon analyzer (Shimadzu TOC-L).

In order to determine polycyclic aromatic hydrocarbons (PAHs) in PW, samples were prepared following the procedure from Dórea et al., 2007.¹⁸⁹ Following the sample preparation, the analysis utilized an atmospheric pressure gas chromatography coupled with Waters Xevo G2 quadrupole time-of-flight mass spectrometer (APGC/QTOF/MS). Instrument analysis followed the procedure described by Jeong et al., 2023.¹⁹⁰

Benzene, toluene, ethylbenzene, xylenes (BTEX) and Oil and Grease were measured in the PW via a certified third-party laboratory (Technology Laboratory, Inc., Fort Collins, CO). The lab followed EPA methods EPA 8015B and 8260B respectively.

Acute 48-h median lethal concentrations (LC50) and effect concentrations (EC50) were determined in PW according to OECD guidelines (OECD, 2004).¹⁹¹ Absence of gill respiratory motion when viewed under a dissecting microscope was used to determine mortality for LC50 determination. Immobilization as defined by the inability of a daphnid to move over a period of

15 s under gentle agitation was used as the designated effect for EC50 determination. For all experiments, 5 neonates <24 h old, housed in 20 mL of the appropriate dilution of experimental test solution were used for each tested concentration and fasted for the duration of the experiment. A total of 6 replicates were performed for each test, scored by a researcher who was blind to the treatment identities. The OECD water without additions was used as the control to verify a lack of mortality in *Daphnia* not exposed to produced water. These exposures were housed in uncapped 20 mL glass scintillati vials, which were washed in 10% nitric acid (24 h) and 10% ethanol (24 h) and thoroughly rinsed with DI water prior to experimentation.

2.3 CROP AGRONOMIC TRAITS MEASUREMENTS

Wheat height was measured from the base of the plant to the tip of the most grown leaf on days 24, 35, 54 and 91 after planting. At harvest, total above-ground biomass was collected, dried for 96 hours at 60°C, and weighed. Then, grains were separated and weighed for yield determination. Statistical analysis on this data was performed using the ANOVA followed by Tukey's HSD test (R statistical package "emmeans").¹⁹²

2.4 SOIL CHARACTERIZATION AND HEALTH ANALYSIS

Following harvest, the soil from each pot was transferred into a separate 1-gallon zip-lock bag, thoroughly mixed, sieved to 8 mm, and 150 g was stored at 4°C for microbial biomass carbon analysis. Several hundred grams of 8-mm sieved soil was then passed through a 2-mm sieve, air-dried, then analyzed for organic carbon, pH, electrical conductivity, potentially mineralizable nitrogen, plant-available potassium and phosphorus, soil texture and betaglucosidase activity following the methods described in the previous study.¹⁹³ The remaining 8-mm sieved soil was then utilized to determine water stable aggregates.¹⁹³

The Soil Management Assessment Framework (SMAF) was used to determine soil physical, chemical, nutrient, biological, and overall soil health index (SHI). SMAF is a soil health assessment tool that incorporates soil indicators alongside site-specific conditions such as climate conditions, soil management practices, and soil and crop type in its scoring mechanism.

In this study water stable aggregates contributed to physical SI; pH and EC values contributed to chemical SI; microbial biomass carbon, potentially mineralizable nitrogen, beta-glucosidase, and soil organic carbon contributed to biological SI; phosphorus and potassium concentrations contributed to nutrient SI. The additive SHI combined all individual soil indicator scores into one metric. The output from SMAF produces indices that range from 0 to 1, with 1 representing the best soil health and 0 the worst. The complete methodology for the scoring of each soil indicator and the calculation of the SHIs is detailed in prior studies.^{194,195} Additional relevant soil metals and anions were measured through ICP-OES and IC techniques respectively. Statistical analysis on all soil characterization data was performed using the ANOVA followed by Tukey's HSD test (R statistical package "emmeans").¹⁹²

2.5 SOIL MICROBIAL COMMUNITY ANALYSIS

Rhizosphere and bulk soil were collected separately from 5 plant replicates of Control and PW100 treatments (20 samples) on harvest day for microbial community analysis. A 6 cm soil core with a 3.2 cm diameter was collected from bulk soil. Rhizosphere samples were collected by pulling the entire plant from the soil, shaking to remove excess soil, and then collecting root-associated soil material. Samples were immediately placed on ice and stored at -80 °C until analysis. Microbial DNA was extracted using the Quick-DNA Fecal/Soil Microbe Miniprep Kit (Zymo Research, CA, USA) following manufacturers protocol. Extracted DNA samples

underwent a single-step PCR to amplify DNA and attach Illumina adaptor sequences and barcodes following the Earth using the Earth Microbiome Primers.

The V4-V5 regions of the 16S rRNA gene used forward primer (5'-*AATGATACGGCGACCACCGAGATCTACACGCT* XXXXXXXXXXXXX *TATGGTAATT* GTGTGYCAGCMGCCGCGGTAA-') where italics represent the 5'illumina adaptor, Xs represent the unique golay barcode, followed by the forward primer pad, forward primer linker and finally the 515F primer.¹⁹⁶ The reverse primer used was: (5'- *CAAGCAGAAGACGGCATACGAGAT* AGTCAGCCAG CC GGACTACNVGGGTWTCTAAT-3') where italics represent the reverse compliment of the 3' Illumina adapter followed by the reverse primer pad, reverse primer linker and reverse primer (806R).¹⁹⁷

The amplification and library preparation of the ITS region was performed using forward primer (5'-*AATGATACGGCGACCACCGAGATCTACAC* GGCTTGGTCATTTAGAGGAAGTAA-3') where italics represent the 5'Illumina adaptor, followed by the forward primer linker and finally the ITS1f primer.¹⁹⁸ The reverse primer used was: (5'-*CAAGCAGAAGACGGCATACGAGAT* NNNNNNNNNN CG GCTGCGTTCTTCATCGATGC - 3') where italics represent the reverse compliment of the 3' Illumina adapter, Ns represent the unique golay barcode followed by the reverse primer linker and reverse primer (ITS2).¹⁹⁹ Libraries were normalized using the SequalPrep Normalization Kit (ThermoFisher, Waltham MA), and quality was assessed using Agilent 2100 Bioanalyzer DNA High Sensitivity assay, Qubit (dsDNA High Sensitivity assay) and library qPCR assay (NEB). The pooled libraries were sequenced on Illumina MiSeq Platform, using the v2-500 cycle kit, with 251 bp paired-end sequencing chemistry at the University of Colorado-Boulder.

Using the QIIME2 (v. 2021.2)²⁰⁰ pipeline, paired-end reads were demultiplexed, merged, trimmed, and binned inferring amplicon sequence variants (ASVs). Taxonomic identities were assigned using the naïve Bayes sklearn classifier trained with the SILVA database (v. 134).²⁰¹ The 16S rRNA gene data from the greenhouse study contained 15,663 ASVs from 20 samples. All samples were rarefied to 10,000 reads, resulting in the loss of 4 samples due to low read depth. The ITS data contained 5,467 ASVs from 20 samples which were rarefied to 10,000 reads, resulting in the loss of 2 samples.

Generalized linear models (GLMs) were used to assess changes in alpha diversity (Shannon Diversity Index, species richness (number of ASVs), and Pielou's Evenness) in the fungal and bacterial communities. Models were run using the lme4 package²⁰² in R and effects of each variable were subsequently determined using TukeyHSD (Tukey Honest Significant Differences).

To understand how community structure was impacted by water treatment, we conducted permutational analysis of variance (PERMANOVA) and analysis of multivariate homogeneity of group dispersions (PERMDISP2) using Bray-Curtis distances on bacterial and fungal communities. Results were visualized using non-metric multidimensional scaling (NMDS). Results from the soil and water analysis were correlated with community structure and visually overlaid in the NMDS plots using envfit. Analyses were conducted using the Vegan package²⁰³ and visualized using ggplot2 in R.²⁰⁴

2.6 SOIL METAGENOME ANALYSIS

A subset of 6 samples (3 Control and 3 PW100 rhizosphere details on collection and extraction provided above) underwent metagenomic sequencing to understand how microbial functional potential is altered following exposure to PW. Samples were sequenced at the CU

Anschutz sequencing facility. Libraries were prepared using Ovation Ultralow System V2 (Tecan) and sequenced on an Illumina NovaSeq 6000. Fastq files were trimmed using Sickle v1.33²⁰⁵ and assembled using MEGAHIT(v.1.2.9)).²⁰⁶ Scaffolds greater than 2500 bp were binned using MetaBAT2 (v2.12.1)²⁰⁷ for each assembly. Metagenome assembled genomes (MAGs) were retained if they had greater than 50% completion and less than 10% contamination, as calculated using checkM (v1.1.2).²⁰⁸ MAGs were dereplicated at 99% identity using dRep (v2.6.2),²⁰⁹ assigned taxonomy using GTDB-tk (v2.1.1)²¹⁰, and annotated for function using DRAM (v1.4)²¹¹. MAGs were assessed for hydrocarbon degradation capabilities using the CANT-HYD database²¹². Additionally, we characterized microbiome functional potential through a gene-centric approach. Genes from scaffolds >2500 bp were predicted using Prodigal (v2.6.3)²¹³ and clustered at 97% identity using MMseqs2.²¹⁴ Following annotation of the gene clusters using DRAM (v1.4), trimmed reads were mapped to the clusters using bbmap (v38.70)²¹⁵ to generate gene coverage values that were calculated for each sample using coverM (v0.6.0).²¹⁶ These analyses were performed in contig mode to retain genes with a minimum percent identity of 97% and at least 75% of the gene mapped to an assembly.

DESeq2 was used to identify genes that were differentially abundant between treatments. After identifying groups of genes that were differentially abundant between the control and produced water treatments, relative abundances of these genes were calculated based on coverage values. The relative abundances of genes within the same categories were summed up and compared across treatments. Gene relative abundances are displayed across five broad categories: (A) hydrocarbon degradation, (B) the nitrogen cycle (c) Transporters, (D) CRISPR genes, and (E) glycoside hydrolases.

2.7 PLANT METABOLOME ANALYSIS

2.7.1 METABOLITE EXTRACTION

Biphasic extraction was performed by adding 1 mL of 6:3:1 MTBE aqueous solution (MTBE:MeOH:H₂O) separately to 25 mg of homogenized grain sample and 10 mg of leaf-tissue into pre-chilled 2ml glass vials. Following brief vortexing, the samples were sonicated cold for 10 mins and were then shaken at 4 °C for 0.5 h. The samples were then centrifuged for 15 min at 3200 rpm before adding 350 µL of cold water to the solution, and then vortexed cold for 20 min. Aqueous and organic (non-polar) layers were separated by centrifuging for 10 min at 3500 rpm.

After separation, 0.35 mL of the non-polar fraction was transferred to a new 2 mL glass vial for subsequent characterization via LC-MS. To the remaining lower aqueous layer, 0.85 ml of acetonitrile was added followed by vortexing for 10 min at 4 °C. The solution was then incubated at -20°C overnight and centrifuged for 10 min at 3500 rpm at 4 °C. 1.35 ml of supernatant was then transferred to a new 2ml glass vial for GC-MS analyses.

2.7.2 LC-MS DATA ACQUISITION

Samples were separated and run according to sample type. One microliter was injected onto a Waters Acquity UPLC system. Separation was achieved using an Acquity HSS T3 C18 column (1.8 µM, 2.1 x 100 mm), using a gradient from solvent A (Water, 0.1% formic acid) to solvent B (Acetonitrile, 0.1% formic acid). Injections were made in 99% B, held at 99% B for 0.5 minutes, ramped to 95% A over 9.5 minutes, held at 95% A for 1 minute, and then returned to starting conditions over 0.5 minutes and allowed to re-equilibrate for 3.5 minutes, with a 400 µL/min constant flow rate. The column and samples were held at 45 °C and 6 °C, respectively. The column eluent was infused into a Waters Xevo G2-XS Q-TOF-MS with an electrospray source in positive

ionization mode, scanning 50-1200 m/z at 0.1 seconds per scan, alternating between MS (6 V collision energy) and MSE mode (15-30 V ramp). Calibration was performed using sodium formate with 1 ppm mass accuracy. The capillary voltage was held at 700 V, source temperature at 150 °C, and nitrogen desolvation temperature at 600 °C with a flow rate of 1000 L/hr.

2.7.3 LC-MS DATA ANALYSIS

RAMClustR version 1.2.4 in R²¹⁷ (version 4.2.2) was used to normalize, filter, and group features into spectra. XCMS^{218,219} output data was transferred to a ramclustR object using the `rc.get.xcms.data` function. Feature data was extracted using the `xcms.featureValues` function. Features with missing values were replaced with small values simulating noise. For each feature, the minimum detected value was multiplied by 0.5. Noise was then added using a factor of 0.5. The absolute value of this value was used as the filled value to ensure that only non-negative values were carried forward. Features were normalized by linearly regressing run order versus qc feature intensities to account for instrument signal intensity drift. Only features with a regression p-value less than 0.05 and an r-squared greater than 0.1 were corrected. Of 73557 features, 0 were corrected for run order effects. Features were filtered based on their qc sample CV values. Only features with CV values less than or equal to 0.5 in MS or MSMS data sets were retained.

Features were clustered using the ramclustR algorithm.²²⁰ Parameter settings were as follows: `st = 2.08`, `sr = 0.5`, `maxt = 208`, `deepSplit = FALSE`, `hmax = 0.3`, `minModuleSize = 3`, and `cor.method = pearson`. Molecular weight was inferred from in-source spectra²²¹ using the `do.findmain` function, which calls the `interpretMSSpectrum` package.²²² Parameters for `do.findmain` were set to: `mode = positive`, `mzabs.error = 0.005`, `ppm.error = 10`, `ads = default`, `scoring = auto`, and `use.z = TRUE`. MSFinder²²³ was used for spectral matching, formula inference, and tentative structure assignment.

2.7.4 GC-MS DATA ACQUISITION

The dried extract of the sample was resuspended in 50 μ L of pyridine containing 25 mg/mL of methoxyamine hydrochloride, incubated at 60°C for 45 min, vigorously vortexed for 30 s, sonicated for 10 min, and incubated for an additional 45 min at 60°C. Next, 50 μ L of N-methyl-N-trimethylsilyltrifluoroacetamide with 1% trimethylchlorosilane (MSTFA + 1% TMCS, Thermo Scientific) were added, and samples were vigorously vortexed for 30 s, incubated at 60 °C for 30 min. Metabolites were detected using a Trace 1310 GC coupled to a Thermo ISQ mass spectrometer. Samples (1 μ L) were injected at a 20:1 split ratio to a 30 m TG-5MS column (Thermo Scientific, 0.25 mm i.d., 0.25 μ m film thickness) with a 1.2 mL/min helium gas flow rate. GC inlet was held at 285°C. The oven program started at 80°C for 30 s, followed by a ramp of 15°C/min to 330°C, and an 8 min hold. Masses between 50-650 m/z were scanned at 5 scans/sec under electron impact ionization. Transfer line and ion source were held at 300 and 260°C, respectively.

2.7.5 GCMS DATA PROCESSING

The ADAP-BIG software was used to preprocess the raw GC-MS data. The computational workflow used by ADAP-BIG includes chromatogram construction, peak detection, spectral deconvolution, alignment, and ANOVA significance test.²²⁴ The resulting spectra with retention times was converted to .msp file and exported to ADAP-KDP for spectral matching and subsequent annotation.²²⁵

2.7.6 STATISTICAL ANALYSIS

For both LC-MS and GCMS data, metabolite abundance within each plant section (grain and leaf) underwent comparison via one-way ANOVA, incorporating a Benjamini-Hochberg false discovery rate adjustment and employing a p-value threshold of 0.05 in R through the p.adjust

function. To help identify any significant metabolic variation across treatments, Principal Components Analysis (PCA) analysis was performed on mean centered and pareto scaled metabolites data using prcomp function in R.

3. RESULTS AND DISCUSSION

3.1 PRODUCED WATER QUALITY REQUIREMENTS FOR IRRIGATION

The water quality data showed that while some metrics in PW100 such as EC, Cl and B exceeded recommended levels,²²⁶ only a few such as pH, Na and carbonate ions were above the usual range for irrigation water (**Table 1**). For reference, the PW50 treatment had an EC of 1.6 dS/m and a pH of 8.65. Though consistent with previous findings of PW contaminants surpassing irrigation guidelines, the ones observed in this study fall within the lower range for PW indicating potential for targeted treatment.¹⁹ Nonetheless, even at these relatively low levels, these ions can accumulate in soil over time, leading to soil degradation.²²⁷ This is particularly concerning in arid and semi-dry climates, where less efficient leaching of salts can cause irreversible damage to soil fertility.²²⁸ It is important to note that the PW used in the present study was untreated, which does not reflect standard practice for irrigating edible crops. A separate study analyzing CPW samples from 18 wells within the Denver-Julesburg Basin reported broadly comparable water quality, particularly among the 14 southern wells located in close proximity to the PW source well used in the present study, while the four northern wells had relatively higher salinity.²⁷ This provides regional context of CPW quality and supports our detailed water quality assessment.

3.2 PRODUCED WATER SHOWED NO ACUTE TOXICITY EFFECTS ON DAPHNIA MAGNA

BTEX, a group of volatile organic compounds commonly found in PW that are known for their toxicity and carcinogenic potential in humans²²⁹ and their harmful effects on plants²³⁰, were

found within drinking water standards (used as a reference due to the absence of irrigation guidelines). Oil and grease levels were also below federal effluent limits (**Table A1**).

PAHs are toxic environmental pollutants commonly found in crude oil, known to disrupt plant metabolism and reduce yield.²³¹ In PW100, 15 PAHs (either classified as Priority Pollutants by the EPA or commonly found in PW) were screened. Of these, seven were detected, but only two compounds, Acenaphthylene and 9,10-dimethylanthracene, were quantified at 0.78 ppb and 2.31 ppb, respectively (**Table A2**). Neither compound is classified as a Priority Pollutant PAH by the EPA, nor do they have established Maximum Contaminant Levels (MCLs).

The acute toxicity of PW100 was also assessed using the LC50 value for *Daphnia magna*, as chemical characterization alone does not fully predict toxicity due to potential synergistic effects and undetected analytes.²³² The lethality towards *Daphnia magna* in PW100 was too low to determine an LC50 (**Figure A1**). Overall, these findings suggest that PW100 meets certain safety standards and poses no immediate acute toxicity risk.²³³ However, the potential for chronic toxicity, which can occur even in the absence of acute effects,²³³ remains unknown and needs to be explored in future studies.

Table 1. Water quality of PW and Control compared to recommended level for irrigation water and usual range in irrigation water.²³⁴ *ND = ‘not detected’; NA= ‘not available’

Water quality indicator	Control	PW100	Recommended level for irrigation	Usual range in irrigation water	Units
Salinity					
EC	0.12	3	≤ 0.75	0-3	mS/cm
TDS	75.5	1710	< 450	0-2000	mg/l
Specific ion toxicity					
Sodium	ND*	782	≤ 70	0-450	mg/l
Chloride	3.55	339	≤ 140	0-350	mg/l
Boron	ND*	2.00	≤ 0.7	0-2	mg/l
Potential nutrients					
Phosphorous	ND*	0.03	< 5	0-2	mg/l

Potassium	0.78	18.4	NA	0-2	mg/l
NH ₄ -N	0.15	1.04	NA	0-5	mg/l
NO ₃ -N	ND*	ND*	<5	0-10	mg/l
Miscellaneous					
pH	6.9	9.4	6.5-8.4	6-8.5	
Bicarbonate	3	11	<1.5	0-10	meq/l
Carbonate	ND*	12.6	<0.5	0-0.1	meq/l
Calcium	18	ND*	40-100	0-400	mg/l
Magnesium	1.22	ND*	6-24	0-60	mg/l

3.3 PRODUCED WATER IRRIGATION RESULTED IN ENHANCED GRAIN YIELD

Crop agronomic traits were measured to characterize the response of wheat to PW irrigation. The PW100 treatment had the highest grain yield among the three treatments (**Figure-1A**) whereas above-ground biomass (**Figure-1B**) and plant height (**Figure-1C**) showed no significant differences between treatments. Previously, PW irrigation reported to results in stunted crop growth, decreased crop yield, and inhibited photosynthetic efficiency.¹⁸⁴ The previous study used diluted unconventional PW at 10 and 50% concentrations, with EC values of 2.4dS/m and 18dS/m, respectively. While the 10% treatment had lower salinity to the CPW used in the present study, it had elevated boron concentrations (3.19 mg/l) and acidic pH (5.81). Information on PAHs and toxicity was not provided, so their potential contribution to the observed effects cannot be ruled out. These differences in PW sources, which likely led to variations in composition, may have contributed to the contrasting results and highlight the need for case-specific evaluations to assess the viability of PW reuse in agriculture.

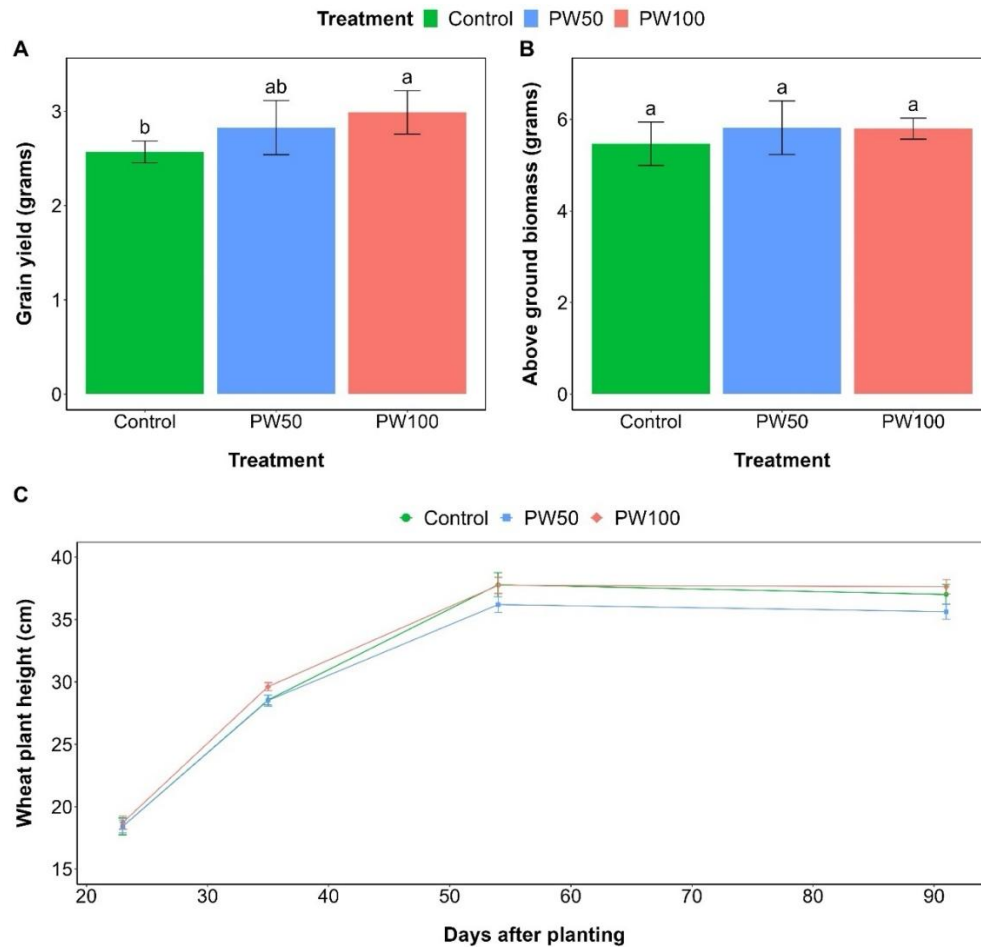


Figure 1. Wheat plant grain yield (A), Above ground biomass (B), and Plant height (C) comparison after harvest. Control: Tap Water, PW100: Undiluted Produced Water, PW50: 50% dilution of Produced Water. Different letters (a, b) denote statistically significant differences between treatments ($p < 0.05$); groups sharing at least one letter are not significantly different.

3.4 PRODUCED WATER IRRIGATION INCREASED SOIL SALINITY AND PH WHILE REDUCING AGGREGATE STABILITY

Multiple soil health indicators assessed at the conclusion of the irrigation trial showed several significant differences between the Control and both PW-irrigated treatments (**Table 2**). PW100 had statistically higher Na, Cl, and B, but lower Ca, Mg, Cu, Ni, Pb, Ti, and Be concentrations than the PW50 treatment. The significant increase in ions in the PW100 treatment

stems from the absence of dilution, while the decrease in certain ions such as Ca and Mg may result from conditions caused by elevated pH, high carbonates, bicarbonates, and Na ion concentration in PW100 (**Table 1**) conditions that can lead to displacement and subsequent precipitation of these ions.²³⁵ Additionally, we observed an increase in the concentration of some heavy metals in PW, though they remained well below regulatory standards for crop irrigation.^{236,237} While high Na disrupts soil structure and plant processes,²³⁸ chloride reduces photosynthesis,²³⁹ and boron toxicity stunts plant growth,²⁴⁰ we did not observe these effects in crop agronomic traits within one growing cycle despite high salinity affecting soil health.

The low chemical SHI (**Figure 2A**) for PW treatments was primarily influenced by high pH and EC. Soil pH was significantly higher in PW treatments than the Control (Control: 5.96; PW50: 7.48; PW100: 8.28) (**Table 2**). Previous studies show PW increases soil pH,^{241,242} which can impact plant growth by limiting the availability of crucial nutrients and micronutrients.²⁴³ Additionally, soil EC was significantly higher in PW treatments (PW50: 3.11 dS/m; PW100: 3.85 dS/m) compared to Control (1.72 dS/m) (**Table 2**). While soil EC increased significantly with PW irrigation, the difference between PW50 and PW100 was modest, given the nearly twofold difference in water EC. This limited increase may be due to ion precipitation under the high pH and carbonate-rich conditions of the PW100 treatment. Such conditions can lead to the removal of ions like Ca and Mg from the soil solution, reducing their contribution to soil EC. Notably, PW treatments' EC remained within the acceptable level for wheat (4 dS/cm)²⁴⁴ throughout the experiment and did not appear to impact crop growth (**Figure 1**). While crops have varying levels of salinity tolerance, continuous salt inputs into cropping systems can eventually lead to elevated soil salinity and impact all crops by reducing plant-available water.²³⁸

Table 2. Soil health Indicator means ($\pm$ SE mean) with ANOVA between water treatments. When present, different letters (a, b, c) denote statistically significant differences between treatments ($p < 0.05$); groups sharing at least one letter are not significantly different.

Soil Health Indicator	Control	PW50	PW100	ANOVA
General soil quality				
CEC (meq/100g)	-	22.8 $\pm$ 0.12 ^a	24.6 $\pm$ 0.96 ^a	NS
ESP (%)	-	18.6 $\pm$ 0.14 ^b	27.0 $\pm$ 1.09 ^a	**
Extractable elements (mg/kg)				
Na	ND*	967 $\pm$ 12 ^b	1645 $\pm$ 175 ^a	*
Cl	ND*	28.6 $\pm$ 0.7 ^b	55.0 $\pm$ 2.2 ^a	***
Ca	2068 $\pm$ 74 ^b	2414 $\pm$ 35 ^a	2179 $\pm$ 39 ^b	**
Mg	383 $\pm$ 20 ^b	474 $\pm$ 3.5 ^a	423 $\pm$ 7.5 ^b	**
B	ND*	1.3 $\pm$ 0.08 ^b	2 $\pm$ 0.16 ^a	**
Fe	82.8 $\pm$ 7.5	89.2 $\pm$ 1.9	85.2 $\pm$ 5.9	NS
Ba	11.6 $\pm$ 0.85 ^b	19.5 $\pm$ 0.4 ^a	19.5 $\pm$ 0.66 ^a	***
Cu	1.2 $\pm$ 0.14 ^b	1.86 $\pm$ 0.13 ^a	1.65 $\pm$ 0.2 ^{ab}	*
Cr	ND*	ND*	ND*	-
Mn	127 $\pm$ 10	148 $\pm$ 4.3	140 $\pm$ 4.1	NS
Zn	3.2 $\pm$ 0.4 ^b	4.3 $\pm$ 0.19 ^a	4.4 $\pm$ 0.23 ^a	*
Ni	0.86 $\pm$ 0.05 ^b	1.15 $\pm$ 0.03 ^a	1.01 $\pm$ 0.06 ^{ab}	**
Pb	3.07 $\pm$ 0.26 ^b	4.02 $\pm$ 0.09 ^a	3.69 $\pm$ 0.17 ^{ab}	**
Cd	0.08 $\pm$ 0.007 ^b	0.12 $\pm$ 0.002 ^a	0.10 $\pm$ 0.006 ^a	***
Al	543 $\pm$ 28 ^b	666 $\pm$ 9 ^a	646 $\pm$ 16 ^a	**
Sr	9.2 $\pm$ 0.30 ^b	11.6 $\pm$ 0.19 ^a	10.8 $\pm$ 0.23 ^a	***
V	0.9 $\pm$ 0.13 ^b	1.5 $\pm$ 0.03 ^a	1.4 $\pm$ 0.06 ^a	***
Be	0.07 $\pm$ 0.007 ^b	0.10 $\pm$ 0.001 ^a	0.09 $\pm$ 0.004 ^{ab}	**
Physical Indicator				
WSA, %	44.2 $\pm$ 3.4 ^a	21.1 $\pm$ 2.0 ^b	14.9 $\pm$ 1.1 ^b	***
Biological Indicators				
BG, ug/g/hr	31.1 $\pm$ 1.8	36.6 $\pm$ 7.1	31.7 $\pm$ 4.2	NS
PMN, mg/kg	49.4 $\pm$ 8.7	52.6 $\pm$ 5.5	45.0 $\pm$ 8.1	NS
MBC, mg/kg	172 $\pm$ 11	159 $\pm$ 26	212 $\pm$ 23	NS
SOC, %	1.55 $\pm$ 0.02	1.52 $\pm$ 0.02	1.55 $\pm$ 0.02	NS
Chemical Indicators				
pH	5.96 $\pm$ 0.03 ^c	7.48 $\pm$ 0.05 ^b	8.28 $\pm$ 0.04 ^a	***
EC, dS/m	1.72 $\pm$ 0.09 ^c	3.11 $\pm$ 0.05 ^b	3.85 $\pm$ 0.04 ^a	***
Nutrient Indicators (mg/kg)				

P	126 ± 15 ^b	174 ± 6 ^a	167 ± 12 ^{ab}	*
K	1038 ± 26	1057 ± 13	1078 ± 9	NS
Moisture Content	18.29 ± 1.34 ^b	25.3 ± 0.4 ^a	26.4 ± 0.74 ^a	***

WSA, water-stable aggregates; BG, β -glucosidase activity (pnp = *p*-nitrophenol); PMN, potentially mineralizable N; SOC, soil organic C; MBC, microbial biomass Carbon; EC, electrical conductivity; CEC, cation exchange capacity; ESP, exchangeable sodium percentage.

*Significant at the .05 probability level; **Significant at the .01 probability level; ***Significant at the .001 probability level; NS, nonsignificant; ND, not detected.

Water-stable aggregates (WSA) significantly decreased between the Control (44%) and PW treatments (PW50: 21%; PW100: 14.9%) (**Table 2**), leading to a significant reduction in the physical SHI for the PW treatments (**Figure 2B**). The combination of high sodicity and alkalinity likely contributed to the dispersion of clays leading to the reduction of WSA in PW treatments.²⁴⁵ This contrasts with Miller et al.'s study, which found no decline in soil WSA with PW irrigation, likely due to differences in soil texture; they diluted the soil to 4% clay with sand,¹⁸⁵ while our trial used unaltered field soil with 30% clay. While PW irrigation reduced physical and chemical SHIs, it had no significant effect on biological and nutrient SHIs (**Figure 2C & D**). However, the overall SHI decreased in response to PW irrigation (**Figure 2E**).

The significant reduction in overall SHI, driven by changes in soil pH, EC, and WSA, highlights the challenges of using untreated PW for irrigation. This also underscores the need for targeted treatment strategies to reduce pH and EC to ensure sustainable soil health. We believe SHI provides a valuable integrative assessment of underlying soil changes that could influence future productivity. However, we acknowledge that SHI, as a single summary value, simplifies complex soil processes and must be interpreted within the context of the system for meaningful interpretation.

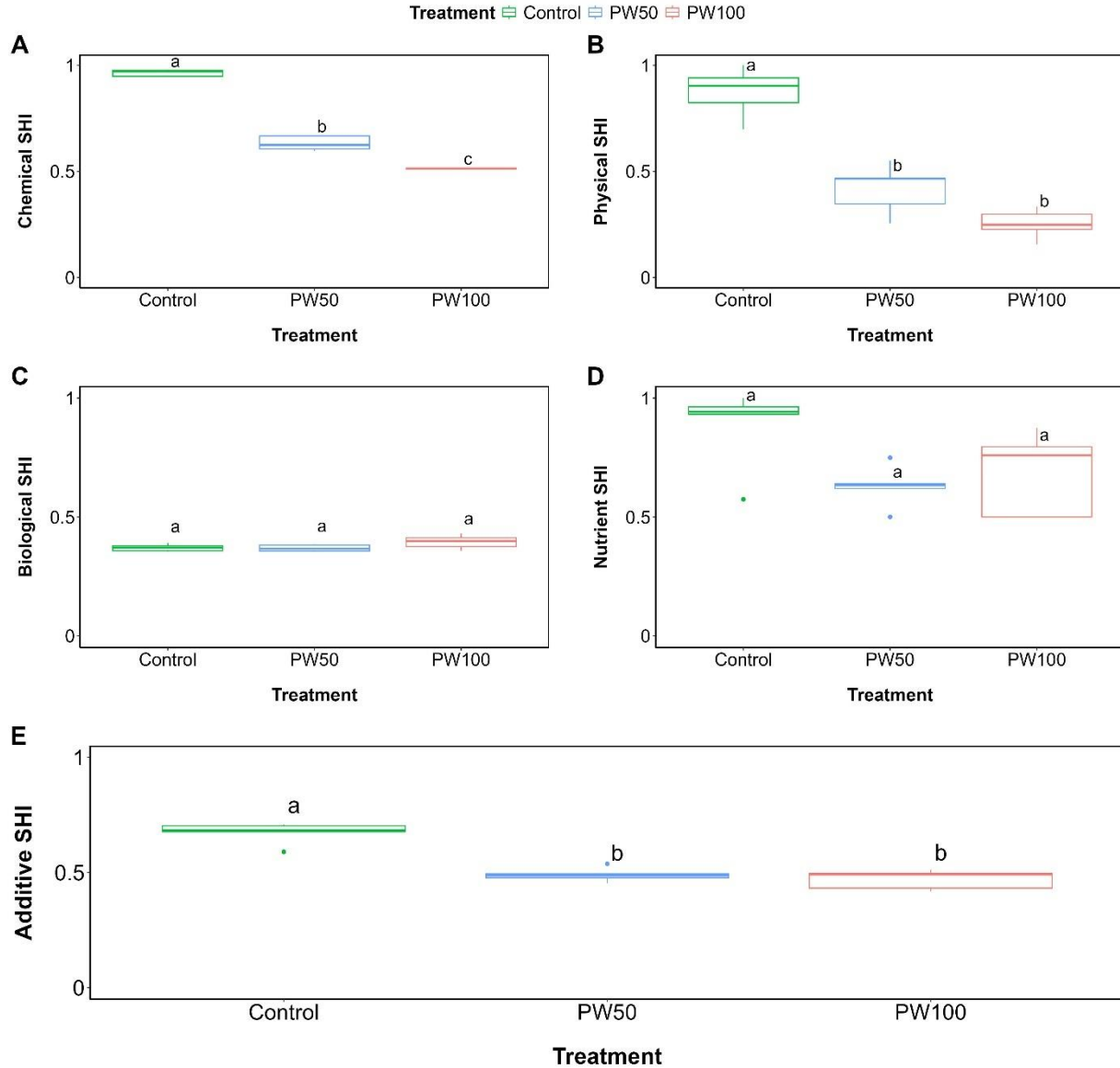


Figure 2. Individual soil health indices (SHI). Water stable aggregates contributed to physical SI (A). pH and EC values contribute to chemical SI (B). Microbial biomass carbon, potentially mineralizable nitrogen, beta-glucosidase, and soil organic carbon contribute to biological SI (C). Phosphorus and Potassium concentrations contribute to nutrient SI (D). The additive SHI (E) combines all soil indicator results into one metric. Control: Tap Water, PW100: Undiluted Produced Water, PW50: 50% dilution of Produced Water. Different letters (a, b, c) denote statistically significant differences between treatments ($p < 0.05$); groups sharing at least one letter are not significantly different.

3.5 PRODUCED WATER IRRIGATION CAUSED FEW OBSERVABLE CHANGES IN WHEAT PLANT METABOLOME

In leaf tissue a significant increase in a metabolite tentatively annotated as *Harzianumol E* was detected in PW100 samples using LCMS metabolomics (**Figure A3**). This compound is a bioactive secondary metabolite of fungus *Trichoderma harzianum*.²⁴⁶ *Trichoderma* species including *Trichoderma harzianum* have been reported to exhibit antagonistic effects against plant fungal pathogens.²⁴⁷ Further studies would be required to confirm this metabolite annotation. However, no significant differences ($P < 0.05$) or discernible trends in grain samples between Control and PW100 treatments were observed (**Figure A4**).

It is important to note that alterations in leaf tissue metabolome were measured only two weeks after initiating PW irrigation, warranting further investigation into the effects of longer-term exposure. The reason for sampling leaf tissue only after two weeks was to capture data before the crop's reproductive stage, ensuring that any variations during that stage would not mask the treatment effects.

No significant effects of PW100 treatment on leaf tissue or grain was detected using GC-MS metabolomics (ANOVA; $P > 0.05$) (**Figure-A5**). These results were inconsistent with previous work where PW irrigation altered the primary metabolic profiles of both wheat leaf and grain.¹⁸⁷ While, as noted earlier, the previous study used diluted unconventional PW with differences in water chemistry,¹⁸⁴ they also sampled leaf tissue at maturity. Differences in PW source, composition and sampling time likely contributed to observed variations in metabolic responses, underscoring the need for context-specific evaluations of PW reuse.

3.6 MICROBIAL COMMUNITIES SHOWED AN INCREASED CAPACITY FOR HYDROCARBON DEGRADATION

Microbial communities in the rhizosphere and bulk soil were investigated at harvest. Bacterial and fungal diversity measured using Shannon Diversity Index, between water treatments (PW and Control) and soil types (rhizosphere and bulk) showed no significant differences (**Figure 3A, 3B**). However, PW irrigation was found to impact community composition of the rhizosphere (PERMANOVA: $R^2=0.501$, $F=6.019$, $P=0.019$) and bulk soil (PERMANOVA: $R^2=0.409$, $F=4.151$, $P=0.033$) of the bacterial community (**Figure 3C, 3E**). Bacterial communities in PW-irrigated bulk and rhizosphere soils correlated with higher pH, moisture content, and EC. Bulk soil also associated with increased microbial biomass carbon (MBC), whereas Control communities were associated with higher WSA. The separation of bacterial communities by different soil physicochemical properties is expected; soil pH has long been recognized as a crucial factor influencing soil microbial diversity²⁴⁸ while salinity can alter the composition and diversity of soil microbial communities²⁴⁹ by inducing osmotic stress^{250,251} and interfering with nutrient uptake and enzyme production.²⁵²

Interestingly, no significant differences based on treatment were observed in the fungal communities (**Figure 3D, 3F**). Previous work in soils contaminated with crude oil and petroleum showed that while changes did occur in fungal communities, they were smaller than what occurred in the bacterial communities.^{253,254} Although the exposure levels and associated stresses in these previous studies were much higher than in the present study, the general pattern of greater bacterial community shifts relative to fungal communities is consistent with our findings. While no community level change was detected, PW-irrigated soil showed an increase in Olpidiomyces, a fungal class associated with fungal pathogens (**Figure A2**). Other microbial groups may produce metabolites in response to enrichment of pathogens; indeed, as discussed earlier, we measured an increase in the concentration of *Harzianumol E*, a metabolite associated with *Trichoderma*

*harzianum*²⁴⁶, in PW-irrigated soils. This fungal species has previously been shown to act as a fungicide in plants²⁴⁷ and the presence of this metabolite may reflect microbial antagonistic interactions within the soil microbiome.

Linear Discriminant Analysis (LDA) was used to assess changes in rhizosphere bacterial communities due to PW irrigation. Several taxa were enriched in the PW100 treatment (**Figure 3G**), which could suggest a natural role in bioremediation of the soil. One such enriched taxa was the genus *Devosia*, for which we generated 3 unique MAGs. Two of the MAGs were found to encode naphthalene dioxygenase (*ndoB*), a gene involved in aromatic hydrocarbon degradation further suggesting that the microbial communities could help mediate the effects of the contaminants before they impact crop growth.^{255–257}

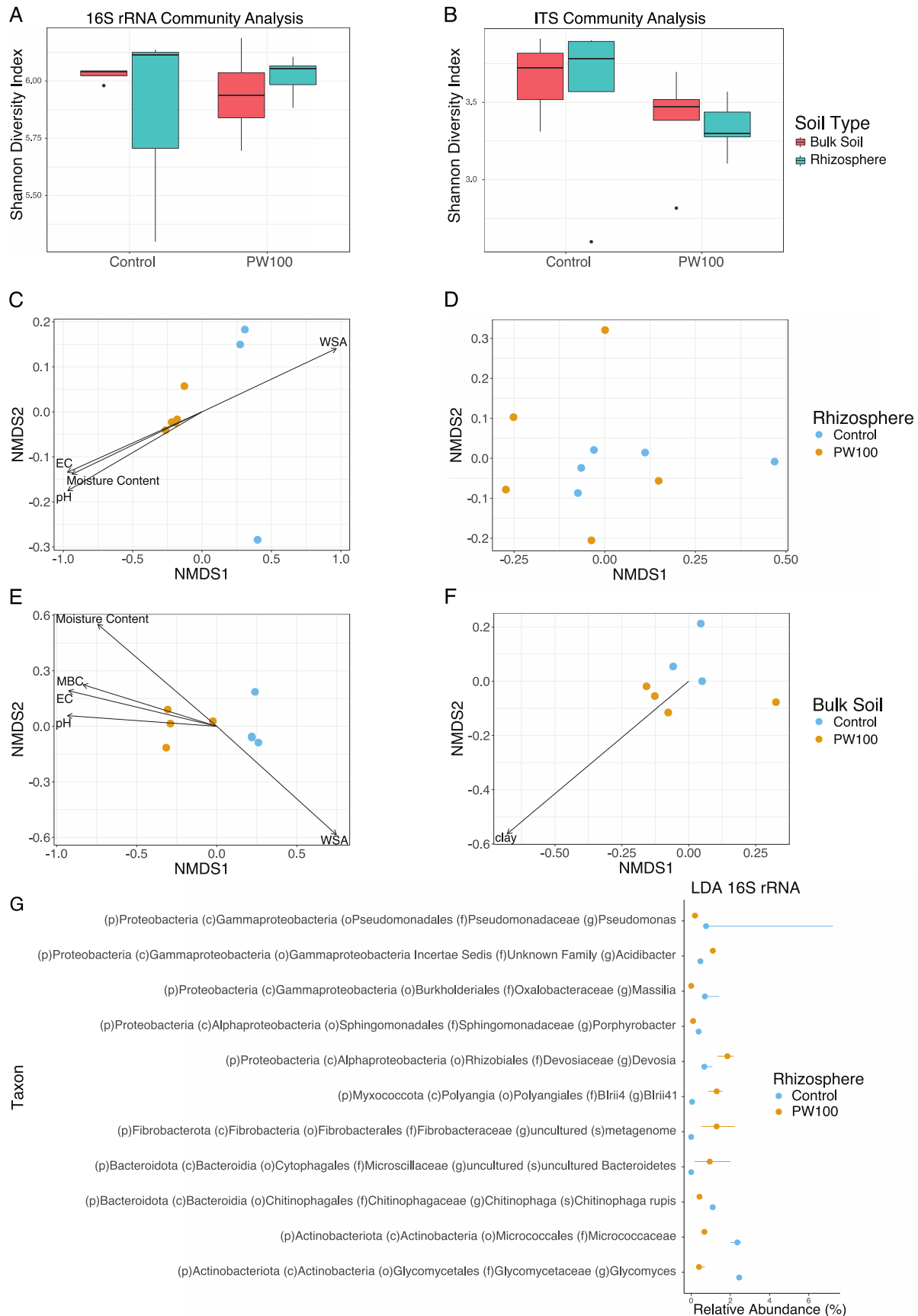


Figure 3. Bacterial/archaeal (16S; A) and fungal (ITS; B) species showing no significant differences in alpha diversity between irrigation treatments and soil types. NMDS plots showing bacteria/archaeal (16S) community dissimilarities between irrigation treatments for rhizosphere (C) and bulk soil samples (E). NMDS plots (C, E) also indicating the correlation of soil bacterial communities with soil health parameters. NMDS plots of fungal (ITS) community showing no significant differences between irrigation treatments for rhizosphere (D) and bulk soil samples (F). (G) Relative abundance of bacterial ASVs in the rhizosphere that were significantly different between treatments, identified using an LDA analysis. Letters in parentheses represent taxonomic classification, where each ASV is provided down to the lowest taxonomic level that was identified.

Metagenomic analysis with DESeq2 revealed significant impacts on multiple gene groups in microbial communities responding to PW irrigation. Several hydrocarbon degradation genes were enriched in the PW treatment (**Figure 4A**), suggesting greater functional potential within the soil microbiome for processing compounds present in PW. One such gene encoded toluene degradation; although toluene was present at low concentrations in PW (**Table S1**), increased gene abundance suggests that these degrading mechanisms may start at very low concentrations.

Furthermore, an increase in coverage of denitrification genes in PW-irrigated soil was observed (**Figure 4B**), suggesting the potential for greater nitrate transformations under this irrigation regime. Multiple studies show increased denitrification rates following oil exposure.^{258–260} This is typically attributed to the abundance of organic carbon in the oil, serving as a substrate for denitrifying bacteria.^{258,259} However, enhanced hydrocarbon degradation (**Figure 4A**), could lead to increased substrate availability for denitrifying bacteria.

Increased coverage of ABC transporter genes in PW-irrigated soil (**Figure 4C**) could aid in detoxification processes, plant defense against biotic and abiotic stress, and nutrient uptake resulting in promotion of plant growth.^{261,262} Additionally, several studies demonstrated the involvement of ABC transporters in hydrocarbon metabolism^{263,264} often linked to the bioremediation pathways of oil-contaminated soils.²⁶⁵

PW-irrigated soils also displayed an increase in CRISPR-CAS genes, a defense mechanism against viruses (**Figure 4D**). Viruses present in PW may have stimulated the abundance of CRISPR-Cas genes since previous studies highlighted the presence of viruses in the deep subsurface hydrocarbon systems.^{266,267} Finally, an increase in some glycoside hydrolase genes (GH) was observed in the PW-irrigated soil (**Figure 4E**) that perform a variety of activities including degradation of plant material and recalcitrant aromatic compounds.^{268,269}

Overall, microbial communities showed both a taxonomic and functional shift in response to PW irrigation. In this system, the microbial community may act as an early detection system showing changes in response to low levels of contaminants. The increased presence of hydrocarbon degradation genes and taxa may indicate a role for microbial community in bioremediation of the PW, thus reducing the impact on the crops.

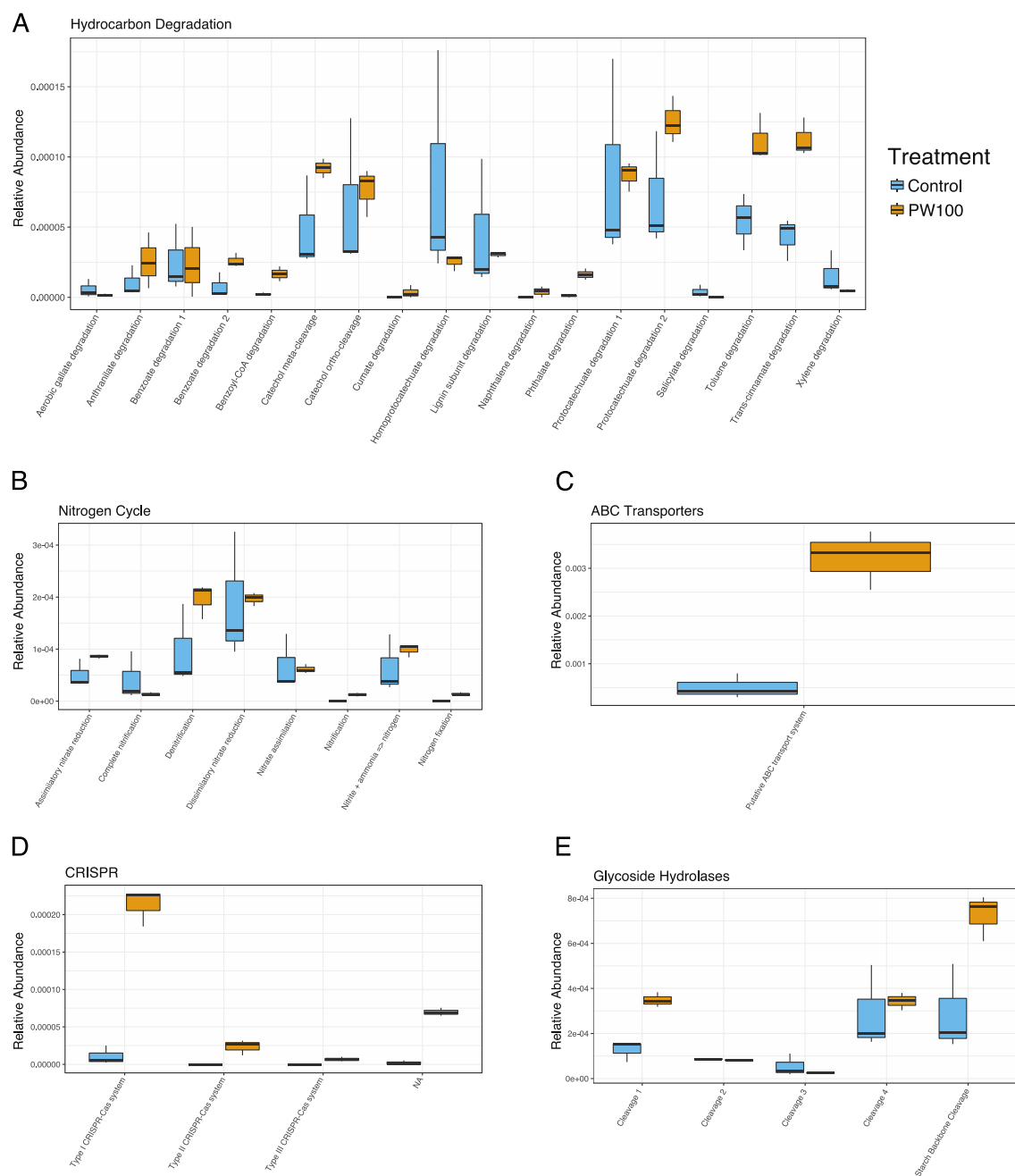


Figure 4. Relative abundance of genes that were identified as differentially abundant using DESeq2 and placed into functional categories using DRAM annotations: (A) hydrocarbon degradation, (B) the nitrogen cycle (C) Transporters, (D) CRISPR genes, and (E) glycoside hydrolases.

4. CONCLUSION

The discharge of treated PW (oilfield wastewater) into streams and its use for irrigation are rapidly increasing in the United States, with regulators issuing permits. However, public concerns persist over the potential impacts of PW on water, soil, crop quality, and ultimately human health. Thus, this study provides a comprehensive, multifaceted characterization of the impacts of low-salinity CPW irrigation on soil and crop health. Overall, PW affected soil health and drove changes in both bulk and rhizosphere bacterial communities, leading to increased abundances of certain genes associated with hydrocarbon degradation and pathogenicity. However, these changes did not translate into observable impacts on crop agronomic traits during the first growing cycle.

Microbial communities are remarkably sensitive, readily responding to even slight environmental shifts. These communities therefore may act as early detection systems for contaminants, with the presence of hydrocarbon degradation genes suggesting a natural attenuation potential of the investigated soils. These findings highlight the significance of integrated assessments to capture environmental stress and emphasize microbial monitoring as a valuable tool for evaluating soil and water quality in agricultural and environmental contexts. This study occurred over one growing season using PW from a single well, thus future work evaluating multi-season impacts are warranted to confirm these findings and assess potential longer-term effects on crop performance and soil health. Based on findings here and in a parallel study,²⁷ we conclude that reuse of low salinity CPW for agricultural irrigation is a viable water management strategy that can be accomplished sustainably via an economical water treatment approach.

AUTHOR CONTRIBUTIONS

Huma Tariq Malik: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft.

Emily K. Bechtold: Investigation (microbial analysis), Data curation, Formal analysis, Visualization, Writing – review & editing.

Corey Broeckling: Investigation (Assisted with metabolomics analysis), Data curation, Formal analysis, Writing – review & editing.

Michael J. Wilkins: Investigation (Assisted with microbial analysis), Writing – review & editing.

Cassidy Buchanan: Investigation (Assisted with soil health analysis).

Marin Wiltse: Investigation (Assisted with Water quality analysis), Data curation.

James A. Ippolito: Investigation (Assisted with soil health analysis), Writing – review & editing.

Thomas Borch: Conceptualization, Funding acquisition, Project administration, resources, supervision, writing-review & editing.

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CHAPTER 4: ORLA STUDY

1. INTRODUCTION

The Permian Basin, spanning western Texas and southeastern New Mexico, is one of the most productive oil and gas regions in the world. As of the fourth quarter of 2024, oil production in the Permian Basin reached 6.5 million barrels per day,²¹ accounting for nearly 50% of total US output.²⁷⁰ This high level of production generates large volumes of produced water (PW), a byproduct of oil and gas extraction²² that is predominantly managed through subsurface disposal, a practice linked to induced seismicity.²⁷¹ As a result, sustaining oil and gas operations increasingly requires coordinated efforts to manage PW injection volumes and pressures under evolving regulatory constraints.²⁷² Therefore, there is increasing interest in exploring alternative PW management strategies, such as surface water discharges and land application of treated produced water (TPW; produced water that has undergone treatment to reduce salinity and other constituents), to decrease reliance on subsurface injection.²⁷³

Among these alternatives, the reuse of TPW is increasingly being considered for agricultural irrigation, especially in water-limited regions where freshwater availability constrains crop production. The Permian Basin is characterized by low annual precipitation²⁷⁴ and limited surface water availability; consequently, groundwater supplies support municipal, agricultural,²⁷⁵ and industrial uses,²⁷⁶ leading to increasing competition for water resources. In this context, the year-round availability of TPW in oil-producing areas offers a potential supplemental source to offset a portion of irrigation demand.

However, raw untreated PW is chemically complex, containing elevated concentrations of salts, radionuclides, trace metals, hydrocarbons, and fracturing fluid additives^{17–20} and has been

shown to exhibit toxicity often due to the combined effects of these constituents.^{277,278} Such constituents can pose risks to soil quality and plant health if not properly managed, and the water treatment processes needed to mitigate such risks are still under active development.

With salinity ranging from 70,000 to 150,000 mg/L total dissolved solids (TDS),²⁷⁹ Permian Basin PW is several times more saline than seawater and therefore requires desalination and additional treatment to remove constituents such as ammonia and hydrocarbons for beneficial reuse beyond the oil field. Advanced treatment technologies capable of achieving these removals have been documented in prior studies.^{273,280,281}

Moreover, toxicity evaluations of TPW demonstrate substantial reduction in toxicity following treatment using pre-treatment, desalination, and post-treatment polishing.^{277,281–288} Of the chemicals that exhibits toxicity and is difficult to remove with both thermal and membrane-based treatments, is ammonia.²⁸⁸ Residual ammonia can be observed in distillates and reverse osmosis (RO) permeates can require additional polishing to pass whole effluent toxicity tests.²⁸⁸ The sensitivity to residual ammonia varies among organisms, with terrestrial organisms including crops, generally exhibiting lower sensitivity to ammonia in PW than aquatic organisms.^{282,283,288,289} This has been demonstrated through both field applications and greenhouse studies using standardized test protocols. However, these evaluations have been limited to a small subset of crop species and source waters, and relatively few have focused specifically on desalinated TPW from the Permian Basin.

More broadly, the impacts of PW irrigation on soil and crop health remain poorly characterized. Only a limited number of greenhouse studies have evaluated these effects^{184–187,290} and most have relied on untreated PW that was often diluted to reduce salinity or acute toxicity. Of the small number of studies that have evaluated TPW, one study in China applied advanced-

treated PW to examine physiological and biochemical responses of wheat to PW irrigation.²⁸ More recently, a long-term greenhouse study using TPW from the Permian Basin examined irrigation across controlled salinity levels, evaluating alfalfa plant growth, soil chemical properties, and microbial community responses.²⁹¹ However, both studies were conducted under controlled greenhouse conditions, which limits their ability to capture the spatial variability, soil heterogeneity, and management complexities inherent to field-scale agricultural systems.

To address this gap, the present study compared TPW and municipal DW for irrigation of sorghum-sudangrass, evaluating crop performance, soil chemical properties, and soil microbial community composition under field conditions near Orla, Texas. By integrating these complementary lines of evidence, this study strengthens the scientific foundation for risk assessment of desalinated produced water irrigation in the Permian Basin and informs the design of future evaluations of TPW reuse in agriculture.

2. MATERIALS AND METHODS

2.1 SITE DESCRIPTION

The field experiment was conducted in Orla, Texas (31.825° N, 103.912° W), located in the arid Permian Basin region of the southwestern United States. The region is characterized by hot summers, limited rainfall, and high evaporative demand. Daily temperature (**Figure B1**), precipitation data (**Figure B2**), and moisture data (**Figure B3**) were collected on-site throughout the growing season to monitor environmental conditions.

2.2 CHARACTERIZATION OF IRRIGATION WATER

Irrigation water samples including drinking water (DW; municipal drinking water sourced from Carlsbad, New Mexico) and TPW were analyzed at Eurofins (USA) for general water quality

parameters, major ions, nutrients, trace metals, and organic compounds (VOCs, SVOCs, and hydrocarbons) using standard methods.

2.3 FIELD EXPERIMENT

The experimental plot in Orla, Texas, covered approximately one-quarter acre. The site was excavated and lined with a non-permeable, 60-mil HDPE liner, with panels fusion welded to form a continuous impervious barrier to prevent seepage and release of irrigation water to the surrounding environment. The plot was then backfilled with soil to a minimum depth of 3 feet and subdivided into four quadrants separated by waterproof barriers (**Figure 1a**). Two diagonal quadrants, referred to as DW1 and DW2, received DW irrigation, while the remaining two, labeled TPW1 and TPW2, were irrigated with TPW. The PW was treated on site through a treatment train that combined industry-standard and proprietary pretreatment processes (Produced Water Resources, LLC), followed by an eleven-stage, low-salt-rejection reverse osmosis (LSRRO)16 system for bulk desalination, and subsequently a final polishing step.

The TPW and the DW were stored in 38 m³ (10,000 gal) HDPE tanks. The DW and PW tanks were connected to two dedicated pumps and piping networks that conveyed the water to the respective irrigation plots (DW1/DW2 and TPW1/TPW2). The DW and TPW were connected to their own drip irrigation lines (Drip Depot, USA) that were installed over each planting row (**Figure 1b**). The system was designed to ensure uniform water applications and to prevent cross-contamination between irrigation quadrants.

Sorghum-sudangrass (*Sorghum × drummondii*), cultivar Sweeter N Honey II (Richardson Seeds, Ltd.) was selected as the test crop because its growing season aligned with the study timeline and due to its documented resilience to drought and other abiotic stressors^{292–294}, allowing clearer attribution of potential observed effects to irrigation water quality. The crop was hand-

seeded on July 22, 2024, with rows spaced 12 inches apart, and harvested on October 27, 2024, for a total growth period of 97 days. At the end of the study period, surface ponding occurred in the TPW2 quadrant due to a breakage in the irrigation line; soil moisture data indicate that this occurred on day 95, approximately two days prior to soil sampling (**Figure B3**).

In each quadrant, the three outermost rows and a 3-ft buffer on both sides perpendicular to the crop rows were excluded from sampling to minimize edge effects. The remaining area was divided into a grid of 3×3 -ft squares using ropes, from which five squares were randomly selected for further sampling.

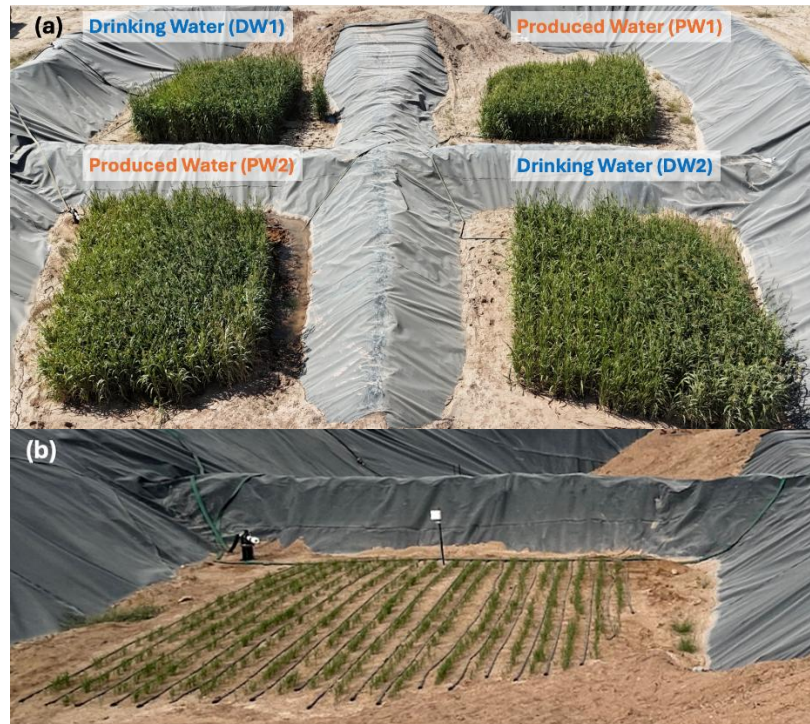


Figure 1. (a) Layout of the quarter-acre experimental plot in Orla, Texas, divided into four quadrants by waterproof barriers, and (b) drip irrigation lines dedicated to the crop rows.

2.4 CROP AGRONOMIC TRAITS MEASUREMENTS

Plant height measurements were taken at harvest (97 days after planting) by starting from one corner of each quadrant and traversing diagonally across it, recording the height of a single plant within each of the designated sampling squares. This process was then repeated along the opposite diagonal to ensure comprehensive sampling coverage. For biomass analysis, whole sorghum plants were collected at harvest, roots were removed, and the remaining above-ground biomass was oven-dried at 60 °C for 72 h and weighed.

2.5 SOIL CHARACTERIZATION AND HEALTH ANALYSIS

Soil samples were collected from DW and TPW irrigation quadrants using a shovel at randomly selected points within the designated sampling squares. Samples were placed in resealable plastic bags, stored on ice, and analyzed at the Soil, Water, and Plant Testing Laboratory, Colorado State University (Fort Collins, CO, USA), using standard procedures summarized in **Table B1**.

2.6 MICROBIAL COMMUNITY ANALYSIS

Five quadrants were randomly sampled within each irrigation plot, resulting in 10 quadrants sampled per irrigation treatment. Within each quadrant, the bulk and rhizosphere were each sampled 3 times, resulting in 60 samples of each bulk and rhizosphere (30 per irrigation treatment). Bulk soil samples were collected from three randomly selected spots within each designated sampling square. Surface soil cores were taken to 5 cm deep, soil was then homogenized, and 5 g was placed in RNALater. From the same square, rhizosphere samples were collected from 3 distinct plants by extracting the whole plant, removing the bulk soil from the roots, and then placing 5 g of rhizosphere into RNALater, used in 2:1 ratio of preservative to soil. To analyze the phyllosphere microbial community, five swabs were collected per quadrant by

dipping each swab in sterile water and swabbing the upper and lower surfaces of at least five individual leaves. All samples were then immediately flash frozen using dry ice and ethanol before being placed on dry ice for up to 30 hours and then kept in a -80 °C freezer until extraction. Phyllosphere samples were extracted from swabs instead of whole leaf samples to reduce chloroplast contamination and allow sequencing of all samples using the same primer set.

Total microbial DNA was extracted using the Qiagen DNeasy Power Soil Pro Kit following manufacturers' protocol for bulk and rhizosphere soil samples. For phyllosphere, 3 swabs per quadrant were placed in a bead beating tube and then extraction proceeded following the same protocol used for soil samples. Amplicon libraries were prepared using a single-step PCR to amplify either bacterial/archaeal communities targeting the V4 region of the 16S rRNA gene with primers 515F/806R,^{197,295} or fungal communities targeting the ITS region using primers ITS1f/ITS2.^{296,297} PCR products were cleaned and normalized using SequalPrep Normalization Kits (ThermoFisher), ensuring consistent DNA concentrations across samples. Pooled libraries were sequenced on the Illumina MiSeq platform using 251 bp paired-end chemistry at the Colorado State University Next-Generation Sequencing Core.

Sequence reads were processed using QIIME 2 (v2023.9).²⁹⁸ For 16S rRNA gene data, samples were demultiplexed, and forward and reverse reads were trimmed to 220 bp before merging. Amplicon sequence variants (ASVs) were inferred from the merged reads. For ITS data, reverse reads were discarded due to poor quality, and only forward reads were used for ASV inference. Taxonomic classification was performed using a naïve Bayes classifier trained on the SILVA 138 database for 16S rRNA²⁹⁹ and the UNITE database for ITS.

Chloroplast and mitochondrial sequences were removed from the 16S dataset. To ensure sufficient sequencing depth, only bulk soil and rhizosphere samples with at least 10,000 reads and

phyllosphere samples with at least 4,000 reads were retained. For each quadrant, triplicate bulk and rhizosphere samples were averaged at the ASV level to account for within-plot heterogeneity and avoid pseudo-replication. Alpha and beta diversity analyses were performed on rarefied datasets, with samples rarefied to the corresponding minimum read thresholds.

Statistical Analyses

Differences in alpha diversity (Shannon Diversity Index and observed species richness (number of ASVs)) were calculated in the *vegan* package in R Studio and compared among treatments using analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) tests.³⁰⁰ Permutational multivariate analysis of variance (PERMANOVA) based on Bray–Curtis dissimilarity matrices, also calculated in *vegan*, was used to determine whether microbial community composition differed among treatments. Results were visualized using non-metric multidimensional scaling (NMDS). PERMANOVA and NMDS were conducted using the *vegan* package,²⁰³ and visualized using *ggplot2*.³⁰⁰ Additionally, Bray-Curtis distances were correlated with environmental variables using *envfit* from the *vegan* package. PERMANOVA was conducted to compare between treatments and within treatments to assess if variation across plots within treatments was greater than variability between plots across different treatments.

3. RESULTS AND DISCUSSION

3.1 LOW RESIDUAL CONCENTRATIONS OF METALS AND ORGANIC COMPOUNDS IN TREATED PRODUCED WATER

Table 1. Water quality of DW and TPW compared to recommended irrigation water guideline values. Samples were collected at five time points over the irrigation season, and values are presented as seasonal averages. Guideline values are based on general, conservative irrigation water quality recommendations for most sensitive crops rather than regulatory standards.^{226,234}

Water quality indicator	Drinking water	Treated PW	Irrigation water guideline for sensitive crops	Usual range in irrigation water	Units

TDS	1580	676	<450	0-2000	mg/l
pH	8.0	7.3	6.5-8.4	6-8.5	
TOC	0.50	1.18	NA	NA	mg/l
Alkalinity (bicarbonate)	4.57	1.02	<1.5	0-10	meq/l
Sodium	94.7	136	≤70	0-450	mg/l
Chloride	237	132	≤140	0-350	mg/l
Boron	0.167	5.78	≤0.7	0-2	mg/l
Calcium	219	2.30	40-100	0-400	mg/l
Magnesium	71.2	4.76	6-24	0-60	mg/l
Phosphorous	<0.005	3	<5	0-2	mg/l
Potassium	4.38	2.42	NA	0-2	mg/l
NH₄⁺-N	0.05	8.925	NA	0-5	mg/l
NO₃-N	4.07	0.092	<5	0-10	mg/l
Arsenic	ND	0.001	<0.10	NA	mg/l
Nickel	ND	0.002	<0.20	NA	mg/l
Chromium	0.0008	0.003	<0.10	NA	mg/l
Mercury	ND	ND	NA	NA	mg/l
Lead	ND	ND	<5	NA	mg/l
Copper	ND	ND	<0.20	NA	mg/l
Cadmium	ND	ND	NA	NA	mg/l
*ND = 'not detected'; NA= 'not available'					

Overall, TPW met general irrigation water quality guidelines for most measured parameters, with some exceptions (**Table 1**). The TPW exhibited lower total dissolved solid compared to DW, and a slightly lower pH. Alkalinity was also substantially lower in the TPW relative to DW. However, TPW contained elevated concentrations of certain constituents, particularly boron (5.78 mg L⁻¹) and sodium (136 mg L⁻¹). While sodium concentrations remained within ranges commonly observed in irrigation waters, boron exceeded irrigation guideline values and usual concentrations reported for irrigation water.²³⁴ Boron is required in trace amounts (0.5-0.7 mg L⁻¹) for optimal plant development,³⁰¹ however excess boron has been reported to cause stunted plant growth and reduced yields by disrupting cell division and altering energy metabolism.²⁴⁰ It is important to note that critical concentrations defining boron deficiency or toxicity are not universal but vary substantially depending on soil characteristics, crop type, and

climatic conditions.^{301,302} For example, sorghum is reported to have irrigation water tolerance thresholds of approximately 4–6 mg L⁻¹ for boron,²³⁴ however, specific tolerance data for sorghum–sudangrass hybrids are limited.

Calcium and nitrate–N concentrations were lower in the TPW than in DW, whereas ammonium concentration was slightly elevated relative to typical range for irrigation water but is not expected to adversely affect most plants growth.²³⁴ Ammonia is a recognized constituent of PW and can occur at high concentrations, averaging approximately 432 mg/L in raw PW in the Permian Basin.²⁷⁹ Given its regulated status in wastewater, removal of ammonia from PW has been investigated as a key treatment consideration for surface discharge and reuse.³⁰³

Trace metals were present at very low concentrations in both water sources (**Table 1**), with most analytes below detection limits. Nickel, chromium, and arsenic were detected in TPW at the concentration on the order of 10⁻³ mg/L and remained well within established irrigation guidelines,²³⁴ while mercury, lead, copper, and cadmium were below detection limits in both irrigation treatments.

Benzene, toluene, ethylbenzene, and xylenes (BTEX) were below detection limits, except for toluene, which was present at a low concentration (3.28 µg/L) (**Table B2**), well below the U.S. EPA drinking water limit (1000 µg/L).³⁰⁴ Because there are no specific irrigation water standards for BTEX, the acceptable limits for drinking water have been used as a reference. Moreover, all analyzed polycyclic aromatic hydrocarbons (PAHs) were not detected in both DW and TPW samples (**Table B3**), indicating effective removal of these compounds during treatment.

Overall, the TPW met general irrigation quality criteria and showed minimal organic or metal contamination. Similar substantial reductions in dissolved constituents and organic

contaminants following treatment have been reported in previous studies evaluating advanced treatment of PW for reuse.^{28,281} However elevated boron concentration remains a potential constraint for long-term agricultural reuse for this specific treatment train that used a membrane-based desalination method.

3.2 TREATED PRODUCED WATER SUPPORTED SORGHUM GROWTH COMPARABLE TO DRINKING WATER

Plant height was comparable among three of the four quadrants, with DW1 exhibiting significantly greater height than the remaining quadrants (Tukey's test, $p < 0.05$) (**Figure-2A**). However, no significant differences were observed in aboveground biomass among treatments (**Figure-2B**). The isolated increase in plant height observed in DW1 was not replicated in DW2 quadrant, indicating that the response is unlikely to indicate a treatment effect. Instead, it may reflect potential site-level differences explored in the **soil section below**. In field settings, small-scale heterogeneity can influence morphological traits³⁰⁵ such as height without necessarily translating to differences in aboveground biomass.³⁰⁶

The limited number of studies evaluating PW reuse for crop irrigation have reported mixed plant growth responses. For example, a greenhouse study using advanced-treated PW reported reduced wheat biomass relative to tap water, while no significant differences in plant height were observed.²⁸ In contrast, studies using untreated PW have documented negative effects on plant growth primarily due to elevated TDS,^{184,307} whereas another study, reported no significant differences in plant height and biomass under low-salinity PW irrigation.²⁹⁰ Together, these findings indicate that plant growth responses to PW irrigation are highly context dependent and influenced by water quality, crop type, treatment level, and experimental conditions.

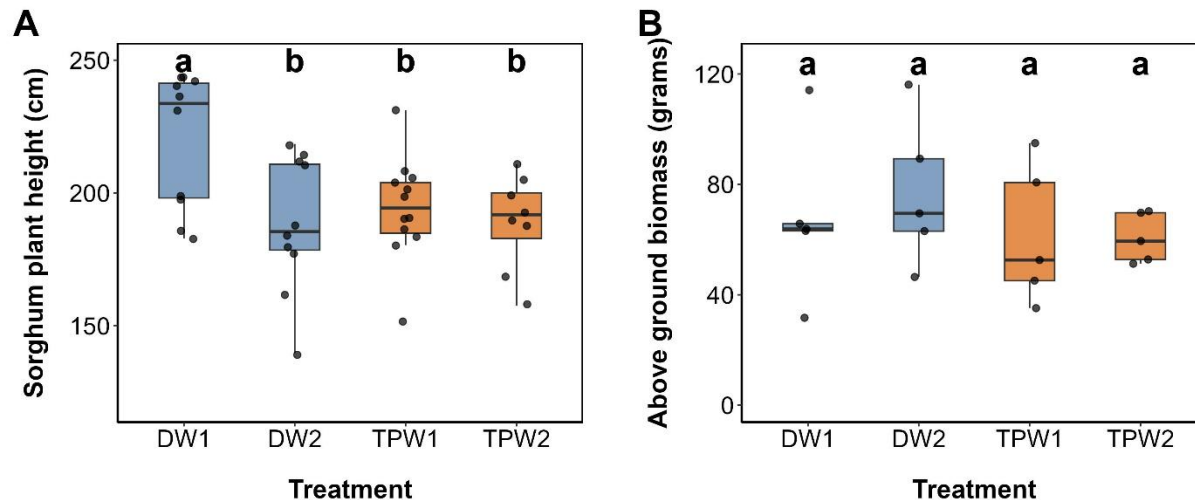


Figure 2. Sorghum growth response under different irrigation treatments. (A) Plant height and (B) above-ground biomass for sorghum irrigated with drinking water (DW1, DW2) and treated produced water (TPW1, TPW2). Different letters above boxes indicate statistically significant differences among treatments ($p < 0.05$) based on post-hoc multiple comparisons. Treatments sharing one or more letters are not significantly different from each other.

3.3 SOIL PROPERTIES REMAINED LARGELY STABLE UNDER TREATED PRODUCED WATER IRRIGATION

Prior to planting, several soil indicators showed notable spatial heterogeneity (**Table B4**). Extractable sodium and chloride concentrations displayed within- and between-quadrant variability, indicating patchy baseline salinity distribution across the site. Extractable calcium concentrations were relatively high across all quadrants, consistent with calcareous soil conditions typical of arid-region systems. Several trace elements (e.g., cadmium, lead, arsenic) were also detectable at baseline, with spatial variability indicative of inherent field heterogeneity. In addition, soil texture differed across quadrants, with DW1 classified as clay loam and the remaining quadrants as sandy clay loam, a contrast that may influence local water availability and solute movement and provide context for the isolated height response observed in DW1 (**Figure 2**).

Following the growing season, several soluble constituents declined across all treatments relative to pre-planting conditions (**Table 2**), consistent with irrigation-related redistribution or

leaching processes. Extractable sodium and chloride concentrations were lower post-season than at baseline in both freshwater and TPW plots, while soil pH and electrical conductivity (EC) remained within a narrow range.

Most soil chemical and biological indicators remained comparable across irrigation treatments post-season; however, select elements exhibited treatment-associated responses. Extractable boron increased significantly in TPW-irrigated soils relative to DW treatments, representing the most pronounced difference from baseline and consistent with boron inputs from TPW (**Table 2**). Although no adverse effects on sorghum growth were observed during the study period, elevated boron may become phytotoxic under longer-term irrigation or for highly sensitive crops.

Additionally, magnesium was lower in TPW treatments than in DW plots, consistent with higher magnesium inputs from DW irrigation (**Table 2**). Moreover, elevated extractable iron and manganese were observed only in a single TPW2 quadrant that experienced localized ponding prior to sampling; given that these elements were quantified using DTPA extraction (**Table B1**), the increases likely reflect changes in the soluble fraction under reducing conditions rather than whole-soil metal content.

Overall, irrigation with TPW resulted in minimal changes to bulk soil properties over the study period, consistent with the advanced treatment applied in this study. This pattern aligns with previous work showing that advanced treatment²⁸ or higher-quality PW²⁹⁰ is associated with limited soil responses, whereas irrigation with untreated PW can adversely affect soil health.¹⁸⁵

Table 2. Soil health Indicator means ($\pm$ SE mean) from soil samples collected at the end of the experiment. Different letters (a, b, c) indicate significant differences among treatments based on one-way ANOVA followed by post-hoc comparisons ($p < 0.05$); groups sharing at least one letter

are not significantly different. N=5. TPW2 plots experienced surface ponding approximately two days prior to soil sampling.

PMN, potentially mineralizable Nitrogen; NO_3^- -N, nitrate nitrogen; NH_4^+ -N, ammonium nitrogen; EC, electrical conductivity; OM, Organic matter.

Soil health indicator	DW1	DW2	TPW1	TPW2
Extractable elements (mg kg⁻¹)				
Sodium	161 ± 20 ^a	184 ± 18 ^a	213 ± 46 ^a	259 ± 68 ^a
Chloride	538 ± 107 ^a	499 ± 108 ^a	461 ± 97 ^a	681 ± 226 ^a
Calcium	8439 ± 1111 ^a	9428 ± 440 ^a	11471 ± 679 ^a	8135 ± 1296 ^a
Magnesium	439 ± 45 ^a	518 ± 46 ^a	231 ± 34 ^b	200 ± 26 ^b
Sulfur	281 ± 33 ^a	380 ± 48 ^a	289 ± 56 ^a	394 ± 50 ^a
Zinc	0.25 ± 0.09 ^a	0.25 ± 0.02 ^a	0.36 ± 0.08 ^a	0.34 ± 0.04 ^a
Boron	0.16 ± 0.02 ^a	0.25 ± 0.10 ^a	6.18 ± 0.98 ^b	4.32 ± 1.11 ^b
Arsenic	1.82 ± 0.33 ^a	1.62 ± 0.14 ^a	1.52 ± 0.36 ^a	1.71 ± 0.18 ^a
Iron	1.18 ± 0.27 ^a	2.39 ± 0.48 ^{ab}	2.28 ± 0.35 ^{ab}	10.84 ± 2.54 ^b
Copper	0.60 ± 0.20 ^a	0.67 ± 0.02 ^a	1.02 ± 0.28 ^a	1.05 ± 0.17 ^a
Manganese	3.69 ± 1.08 ^a	3.18 ± 0.20 ^a	4.58 ± 0.33 ^{ab}	18.14 ± 5.00 ^b
Cadmium	0.27 ± 0.02 ^a	0.27 ± 0.04 ^a	0.27 ± 0.02 ^a	0.27 ± 0.01 ^a
Chromium	6.73 ± 0.47 ^a	6.02 ± 0.15 ^a	6.35 ± 0.23 ^a	6.25 ± 0.17 ^a
Lead	0.97 ± 0.18 ^a	0.84 ± 0.22 ^a	0.73 ± 0.26 ^a	0.53 ± 0.24 ^a
Biological Indicators				
PMN, mg/Kg (NO_3^- -N)	10.1 ± 2.0 ^a	14.6 ± 2.3 ^a	16.6 ± 0.3 ^a	10.7 ± 2.5 ^a
PMN, mg/Kg (NH_4^+ -N)	1.88 ± 0.37 ^a	1.48 ± 0.20 ^a	0.90 ± 0.36 ^a	1.40 ± 0.51 ^a
Respiration (CO ₂), µg/g soil/day	4.34 ± 0.7 ^a	5.82 ± 1.30 ^a	6.10 ± 0.90 ^a	7.76 ± 0.88 ^a
Chemical Indicators				
Total Carbon (%)	2.59 ± 0.06 ^a	2.58 ± 0.05 ^a	2.60 ± 0.04 ^a	2.57 ± 0.02 ^a
Total Nitrogen (%)	0.055 ± 0.00 ^a	0.057 ± 0.001 ^a	0.058 ± 0.002 ^a	0.056 ± 0.001 ^a
Carbon-to-Nitrogen Ratio	46.5 ± 0.6 ^a	45.5 ± 1.6 ^a	44.6 ± 1.2 ^a	45.8 ± 0.9 ^a
pH 1:1	7.98 ± 0.04 ^a	7.92 ± 0.04 ^a	7.88 ± 0.04 ^a	7.94 ± 0.04 ^a
EC 1:1	1.85 ± 0.14 ^{ab}	2.03 ± 0.07 ^a	1.89 ± 0.04 ^{ab}	1.60 ± 0.13 ^b
OM (%)	1.89 ± 0.04 ^a	1.96 ± 0.03 ^a	1.95 ± 0.05 ^a	1.93 ± 0.08 ^a
Nutrient Indicators (mg kg⁻¹)				
NO_3^-	5.28 ± 2.04 ^a	7.40 ± 1.63 ^a	9.10 ± 1.63 ^a	3.20 ± 0.95 ^a
NH_4^+	1.08 ± 0.61 ^a	0.32 ± 0.20 ^a	0.66 ± 0.34 ^a	2.06 ± 1.39 ^a
Phosphorus	3.00 ± 0.63 ^a	1.20 ± 0.34 ^a	3.00 ± 0.55 ^a	1.80 ± 0.72 ^a
Potassium	369 ± 48 ^b	524 ± 28 ^a	534 ± 43 ^a	474 ± 27 ^{ab}

3.4 SURFACE PONDING ALTERED SOIL MICROBIAL COMMUNITIES IN TPW 2 QUADRANT

In TPW2, surface ponding occurred due to a breakage in the irrigation line two days before soil sampling. Water saturation is known to alter soil chemistry and microbial processes by limiting oxygen diffusion and inducing redox-driven transformations.³⁰⁸ In line with this, TPW1–TPW2 comparisons revealed that chemical shifts (**discussed in the previous section**) were also accompanied by microbial community changes (**Figure B4**). TPW2 was enriched in taxa commonly associated with flooded or water-saturated environments (e.g., *Flavobacterium*,³⁰⁹ *Aeromonas*,³¹⁰ *Vogesella*³¹¹) as well as putative anaerobic and facultatively anaerobic taxa including *Clostridia*³¹² (*Clostridium sensu stricto*) and *Azoarcus*,³¹³ suggesting the presence of localized low-oxygen zones following inundation. These changes justified the exclusion of TPW2 from primary microbial analyses to minimize confounding effects.

Ponding events can reasonably be expected in field-scale agricultural systems. In this study, it occurred approximately two days prior to soil sampling (**Figure B3**); yet measurable microbial responses were observed. These observations provide useful context for future TPW reuse scenarios, where short-term saturation events may temporarily influence soil microbial composition independent of irrigation water quality.

3.5 TREATED PRODUCED WATER IRRIGATION ENRICHED AMMONIA-OXIDIZING AND NITRIFYING TAXA

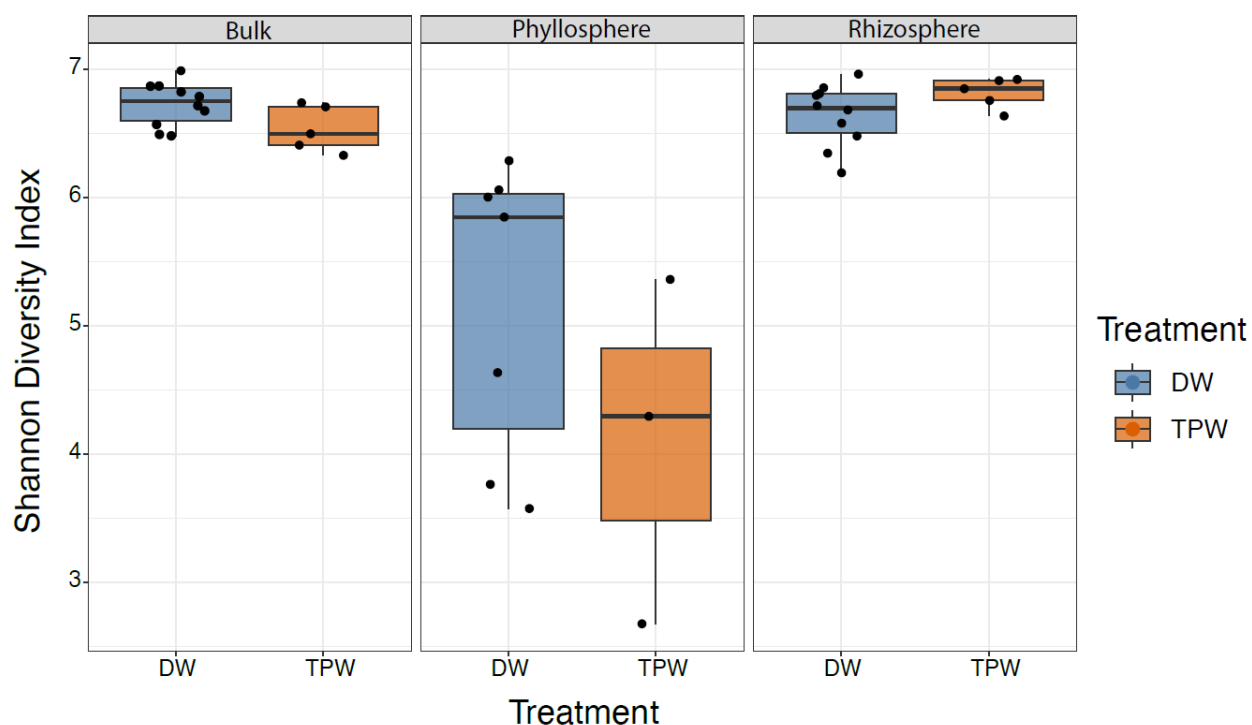


Figure 3. Bacterial/archaeal (16S) species showing no significant differences in Shannon Diversity Index across bulk soil, phyllosphere, and rhizosphere compartments under DW and TPW irrigation.

Across bulk soil, rhizosphere, and phyllosphere compartments, no significant differences in bacterial alpha diversity measured by Shannon Diversity Index were observed between DW and TPW treatments (**Figure 3**).

However, TPW had a modest influence on microbial community composition, with spatial heterogeneity explaining some portion of the observed variation. These relatively subtle treatment effects were most apparent in bulk soil (**Figure B5**) and phyllosphere samples (**Figure B6**), while rhizosphere communities (**Figure B7**) were more strongly structured by spatial variability. The limited microbial responses observed in the present study highlights the effectiveness of treatment in minimizing soil biological stress.³¹⁴

While some TPW-associated microbial community shifts aligned with changes in soil chemistry (e.g., boron and magnesium) in the bulk soil and phyllosphere communities, patterns in the rhizosphere were less strongly associated with measured variables. This likely reflects the ability of plants to moderate the rhizosphere environment through selective nutrient uptake, root exudation, and local modification of soil microenvironments. Boron taken up by roots may be sequestered in plant tissues or redistributed within the rhizosphere, reducing the concentrations available to microorganisms and buffering microbial community composition.^{315,316} Similarly, root exudates can chelate ions or provide alternative substrates, further dampening the direct chemical influence of irrigation water. As a result, rhizosphere communities may appear more stable compared to bulk soil, where chemical inputs are not moderated by plant activity.^{317,318}

From a human health perspective, plant uptake of boron could influence its concentrations in edible tissues; however, at the levels measured in the treated PW used in this study, uptake is unlikely to pose a risk to consumers.³¹⁹ However, monitoring of crop tissue concentrations is recommended when using PW with elevated ion content, to ensure that long-term irrigation does not lead to accumulation beyond safe dietary thresholds. Elevated boron ($>1 \text{ mg L}^{-1}$) in irrigation water has been shown experimentally to influence soil microbial activity and community composition, including reductions in microbial respiration under high doses.³¹⁴ For a detailed description of multivariate analyses and ordination results, see **Appendix B**.

Differential abundance analysis revealed distinct taxonomic shifts in bulk soil (**Figure 4A**) and rhizosphere communities under TPW irrigation (**Figure 4B**). Specifically, nitrifying taxa such as *Nitrosomonas*, *Nitrospira*, and *Candidatus Nitrocosmicus* were enriched in PW soils, consistent with elevated ammonia concentrations in the TPW (8.9 mg/L in TPW vs $<0.05 \text{ mg/L}$ in DW). Ammonia availability is a well-established driver of nitrifier community composition in soils and

likely contributed to the increased representation of ammonia-oxidizing microorganisms under TPW irrigation.^{320,321}

In the absence of fertilizer application, nitrogen inputs from TPW may have been rapidly assimilated by plants, resulting in no significant increase in residual soil ammonia at the end of the crop cycle (**Table 2**) while still producing a microbial community signature indicative of prior ammonia exposure.

Fungal community composition did not differ significantly among treatments (PERMANOVA, $p > 0.05$). This muted response is consistent with the generally lower sensitivity of fungi to environmental perturbations relative to bacteria, owing to their greater physiological resilience, slower growth rates, and ability to utilize a wider range of carbon sources.³²² This finding aligns with a previous study using higher-quality PW, which reported detectable shifts in bacterial communities but not in fungal communities.²⁹⁰ Similarly, studies in environments exposed to crude oil and petroleum contamination have often shown less pronounced responses in fungal communities compared to bacterial communities.^{253,254}

While these results suggest that TPW does not strongly alter soil or plant-associated microbial communities over a single growing season, several caveats should be noted. This study covers only one growing season, and longer-term effects could emerge with repeated applications. Even with relatively high-quality TPW, subtle shifts in microbial composition were observed, reflecting the sensitivity of microbial communities to changes in chemical inputs. Compared with bulk soil chemistry or plant physiological measures, microbial communities may serve as early indicators of environmental change. Continued monitoring across multiple crop cycles will be important to better understand the potential ecological impacts of TPW irrigation.

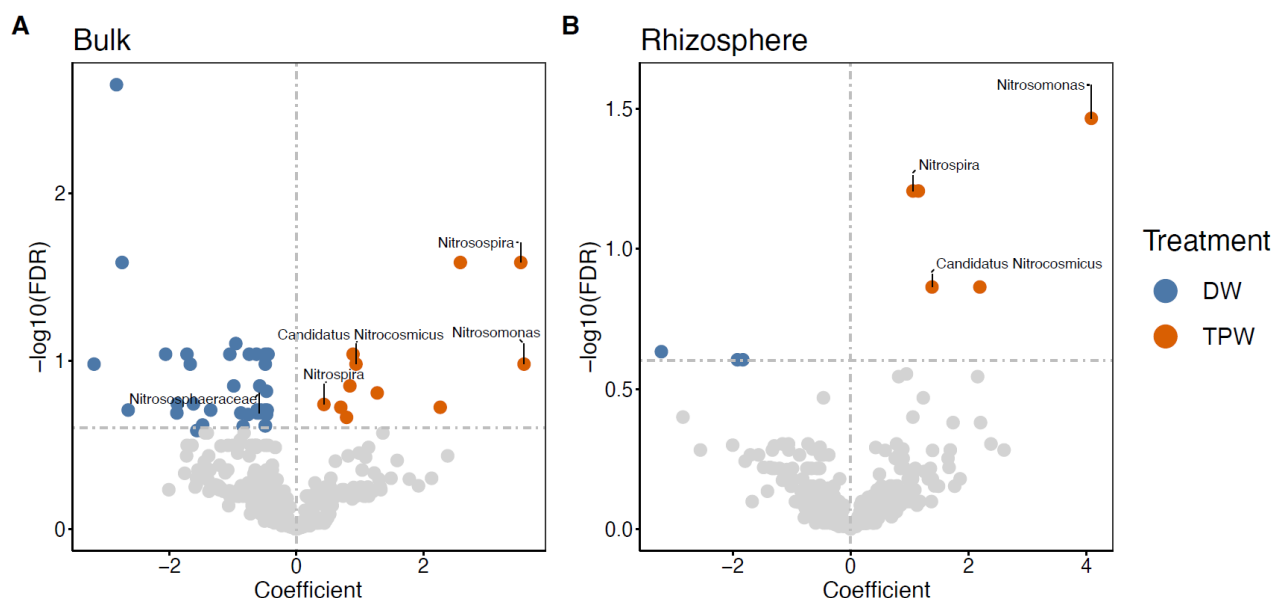


Figure 4. Volcano plots showing differential abundance of bacterial/archaeal taxa in (A) bulk soil and (B) rhizosphere soil. The x-axis shows model coefficients (log-fold change), with positive values indicating enrichment under DW irrigation (green) and negative values indicating enrichment under TPW irrigation (orange), while non-significant taxa (NS) are shown in gray. Key nitrifying taxa enriched in irrigation waters are labeled.

4. IMPLICATIONS FOR TREATED PRODUCED WATER REUSE

Overall, results from a single growing season indicate that irrigation with TPW resulted in minimal changes to soil chemistry and did not adversely affect crop growth, while inducing modest shifts in soil microbial community composition.

By integrating field-based plant and microbial responses with detailed chemical characterization, this study provides an integrated assessment of TPW under realistic field conditions rather than controlled greenhouse settings. Chemical analyses revealed generally low concentrations of organic compounds and metals. The absence of adverse plant responses is consistent with the lower sensitivity of terrestrial plants to ammonia at the concentrations observed.

Collectively, these findings indicate that PW, when treated to address key constituents such as ammonia, may represent a viable supplemental irrigation water source especially in water-limited regions such as the Permian Basin. Future research should evaluate TPW reuse across multiple growing seasons, crop types, and soil conditions to better assess long-term implications for soil health, crop productivity, and ecosystem function.

AUTHOR CONTRIBUTIONS

Huma Tariq Malik: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft.

Emily K. Bechtold: Investigation (microbial analysis), Methodology, Data curation, Formal analysis, Visualization, Writing – review & editing.

James Rosenblum: Conceptualization, Funding acquisition, Investigation, Writing – review & editing.

Tzahi Y. Cath: Investigation, Methodology, Writing – review & editing.

Kaylee Cuskey: Investigation, Methodology.

Michael J. Wilkins: Investigation (Assisted with microbial analysis), Writing – review & editing.

Aaron Redman: Investigation, Writing – review & editing.

Ryan Hall: Methodology, Writing – review & editing.

Thomas Borch: Funding acquisition, Project administration, resources, supervision, writing-review & editing.

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CHAPTER 5: ASSESSING PRUSSIAN YELLOW-ASSISTED REMEDIATION OF SALT AND PRODUCED WATER CONTAMINATED SOILS USING NATIVE GRASS SEED GERMINATION TESTS³

1. INTRODUCTION

Increasing production of on-shore oil and gas has led to the generation of large volumes of produced water (PW) on land that must be managed.^{15,180} Produced water is a byproduct of extraction typically characterized by high salinity.¹⁹ Elevated salt concentrations in PW pose a risk to soil health, especially in the event of accidental spills or improper disposal.³²³ Such spills can result in severe soil degradation, as the high salinity can alter soil structure,²³⁸ disrupt microbial communities,^{249,324} and impede plant growth.²³⁹ As salinity increases, osmotic forces hinder water transport to plant roots causing plant impairment.³²⁵ These effects can lead to long-term declines in soil health and fertility, ultimately reducing the soil's capacity to support vegetation and disrupting local ecosystems.³²⁶ Conventional approaches to remediate salt-affected soils, such as “dig and haul” and the use of soil amendments to leach salts out can be time-consuming, resource-intensive, or ineffective under certain conditions.³²⁷ Hence, there is growing interest in innovative remediation strategies that can facilitate more efficient salt removal at lower cost.

The use of enhanced evaporative flux, facilitated by a crystallization modifier, has been reported to provide a novel approach for remediating soil impacted by salts from PW.^{23,24} Salts typically migrate through the soil via evaporation and capillary action. As water evaporates, salt

³ Reproduced with permissions from Malik, H. T.; Gieger, J. K.; Parkerton, T. F.; Borch, T. Assessing Prussian Yellow-Assisted Remediation of Salt and Produced Water Contaminated Soils Using Native Grass Seed Germination Tests. *Environmental Toxicology and Chemistry* 2026, vga038, Copyright 2026, Society of Environmental Toxicology and Chemistry.

concentration increases, and without intervention, salts may precipitate below the surface, potentially clogging soil pores. The addition of a crystallization modifier alters this process by delaying crystallization and reducing the size of salt crystals. This modification promotes the upward migration of salts, allowing crystallization at the soil surface rather than within the soil profile, where salts can be more easily removed and disposed.

Sodium chloride (NaCl) is typically the dominant salt in PW,³²⁸ which makes ferrocyanide, known as a potent halite crystal modifier, a suitable choice for mitigating salt accumulation in soil. Prussian yellow (PY), a potassium salt of ferrocyanide ($K_4[FeII(CN)_6]_3 \cdot 3H_2O$) has been identified as the most effective form of ferrocyanide due to its higher aqueous solubility and ability to dissociate more readily compared to the less soluble Prussian blue form.²⁴ Although these characteristics make PY promising for remediating PW-contaminated soils, it is crucial to also consider its potential impacts on the surrounding soil environment, including effects on any native vegetation.

While ferrocyanide complexes are considered among the most stable inorganic complexes and practically nontoxic because of the strong bonding between iron and cyanide,³²⁹ studies have shown that ferrocyanide complexes can undergo photodissociation under ultraviolet (UV) light, releasing free cyanide²⁵ which has been found to be phytotoxic at elevated concentrations.^{330,331} Therefore, understanding the potential photo-transformation of PY in soil is an important research need for determining the environmental acceptability of this new remediation approach.

The aims of this research were addressed in three sequential phases. The first phase was designed to evaluate the germination of two native grass species, Brewster sideoats grama (BSG, *Bouteloua curtipendula*) and Santiago silver bluestem (SSB, *Bothriochloa laguroides*), in control soil and greenhouse soil media. These grass seed species are native to New Mexico and southwest

Texas and overlaps with the Permian Basin which in 2021 was responsible for 60% of the on-shore produced water generated in the United States.³³² Based on the 10-day test duration and germination success in Phase 1, results were used to select a preferred plant species for subsequent toxicity studies. In the second phase, concentration-response relationships for seed germination in field-collected soil contaminated with different concentrations of NaCl and PW were determined. These experiments were designed to specifically test if contaminants other than NaCl that were present in the PW contributed appreciably to seed germination inhibition. In the third and last phase, the effect of PY amended soil alone and in combination with NaCl and PW contamination was examined. This experiment tested whether UV exposure of PY in soil releases free cyanide at levels harmful to seed germination and subsequent growth of native grass. This study aims to establish native seed germination tests as a pragmatic basis for evaluating the acceptability of soil quality obtained by remediation strategies using PY.

2. MATERIALS AND METHODS

2.1. GENERAL EXPERIMENTAL CONDITIONS

All three phases were conducted in a controlled greenhouse. To mitigate potential variations in temperature, light, and airflow, treatments with replicates were arranged using a randomized complete block design. The greenhouse was equipped with a photoperiod lighting system to provide 16 hr of light and 8 hr of darkness daily. Natural sunlight was the primary light source during the day, supplemented in the late afternoon and evening by high-pressure sodium lamps (Plantmax PX-LU400, 2000K). These bulbs primarily emit light in the visible spectrum, with only a negligible portion in the UV range. Light intensity was recorded at 15-min intervals using sensors installed in the greenhouse and varied diurnally from 0 to 1009 $\mu\text{mol m}^{-2} \text{s}^{-1}$, with a

mean daytime intensity of approximately $298 \mu\text{mol m}^{-2} \text{s}^{-1}$ during the experimental period. The full light intensity time series is provided in **Figure C1**.

Irrigation was supplied using the control water consisting of municipal drinking water supplied to Colorado State University. Produced water used in the study was sourced from Midland Basin, Texas. Water quality parameters for Control and PW are provided in **Table 1**. The soil used was a sandy loam field soil (control soil) collected from Andrews, Texas. This soil was analyzed by a commercial laboratory for a range of soil properties (**Table C1**).

Table 2: Water quality parameters of PW and Control water used in this study. **ND** = not detected; **NA** = not available; **Alk as CaCO_3** = alkalinity expressed as calcium carbonate; **$\text{NH}_4\text{-N}$** = ammonium-nitrogen; **$\text{NO}_3 + \text{NO}_2$** = nitrate plus nitrite; **EC** = electrical conductivity; **TDS** = total dissolved solids; **ppm** = parts per million; **mS/cm** = millisiemens per centimeter.

Water quality parameter	PW	Control	Unit
Barium	2.38	NA	ppm
Boron	31.13	ND	ppm
Calcium	1530	18	ppm
Iron	<0.5	ND	ppm
Magnesium	175	1.22	ppm
Manganese	0.83	NA	ppm
Potassium	624	0.78	ppm
Sodium	38100	ND	ppm
Strontium	537	0.017	ppm
Titanium	<0.05	NA	ppm
Alk as CaCO_3	100	ND	ppm
Bromide	665	0.64	ppm
Chloride	76600	3.55	ppm
Fluoride	<200	0.61	ppm
$\text{NH}_4\text{-N}$	1050	0.15	ppm
$\text{NO}_3 + \text{NO}_2$	<0.1	NA	ppm
Phosphorus	<0.05	ND	ppm
Sulfate	<2000	ND	ppm
pH	7.52	6.9	
EC	110	0.12	mS/cm
TDS	106,000	75.5	ppm

2.2. PHASE 1: SPECIES SELECTION FOR SEED GERMINATION TEST

Two warm season perennial bunch grass species native to Texas were investigated: BSG and SSB. These species were identified via Texas Native Seeds Program, a statewide initiative focused on enabling large scale native plant restoration through the development and release of regionally adapted native seed sources for commercial production. Seeds were sourced from Douglass King Seed Co, San Antonio, Texas and were planted in two types of uncontaminated soil: Texas field soil and greenhouse soil (Pro-mix BX Biofungicide + Mycorrhizae). Seeds were placed in T.O. Plastics SVT450 square pots with four drainage holes to assess seed germination. Four replicates with 15 seeds per replicate were planted in both soils. For the first 3-4 days, soil moisture was maintained by incrementally applying fine mist. After germination began, pots were watered on alternate days with approximately 25 mL of water per pot. Seed germination was assessed at 5 and 10 days after planting. The germination endpoint was established through a pre-experiment pilot study (**Figure C2**) which indicated that most seeds visibly germinated by Day 5. To account for any delayed germination, a second observation was conducted on Day 10 to capture any additional emergence.

2.3. PHASE 2: SEED GERMINATION SENSITIVITY TO PW AND NaCl

Produced water was diluted with distilled water to 0.3%, 1%, 3%, 10%, and 30% to assess concentration-response relationships for seed germination with the selected plant species. Sodium chloride dilutions were similarly prepared using a 2.16 M (126.23 g/L) stock solution to approximately match PW chloride concentration for evaluating salinity stress alone. The procedure used for spiking soil with the PW and NaCl dilutions was as follows. The control soil was screened using a #10 sieve to remove larger roots and plant matter. For each treatment, 750 mL of diluted

solution was added to 3 kg of dry soil. The wet packing process involved the following protocol. An empty container was weighed, and approximately 1 cm of solution was added. The container was re-weighed to calculate the solution's mass. Soil was then added gradually, packing it evenly after each addition to ensure consistent moisture distribution. The process continued until the top of the soil appeared damp but without standing water. The container was weighed again to calculate the soil mass. This process was repeated until all the soil was used. Finally, the soil was dried at 50°C until all moisture was removed, with weight loss monitored to confirm consistency with the calculated water content. The soil was mixed daily during the drying process and thoroughly mixed again once drying was complete to prevent the stratification of contamination.

In this phase a total of 11 soil treatments were tested, including control field soil and individual dilutions of NaCl and PW (0.3%, 1%, 3%, 10%, and 30%). Soil electrical conductivity (EC) of all treatments was measured prior to the greenhouse experiment using a 1:1 soil-to-water extract. The soil and deionized water were thoroughly mixed and allowed to settle until the supernatant was clear, and EC was measured by inserting the probe (Fisher Scientific, Hampton, NH) into the supernatant while avoiding soil disturbance. Drainage holes in the pots used for seed germination tests, described previously, were sealed to prevent salt leaching. Twelve BSG seeds were planted in each of the 11 treatment soils, with 5 replicates per treatment. The pots were covered with transparent plastic lids for the first 4 days after germination to maintain adequate moisture for seedling growth. Watering during this phase followed the same regime as described in the first phase. The germination count was done on day 10 post-planting as described previously.

2.4. PHASE 3: SEED GERMINATION SENSITIVITY TO PY ALONE AND IN COMBINATION WITH PW OR NACL

In this phase we tested 21 treatments, including uncontaminated field soil (control) and individual dilutions (0.3%, 1%, 3%, and 5%) of the treatments including; uncontaminated field soil spiked in the lab with PY (PY), salt contaminated field soil (NaCl), salt contaminated field soil after the addition of PY (NaCl+PY), PW contaminated field soil (PW) and PW field soil after the addition of PY (PW+PY). Prussian yellow (Potassium hexacyanidoferrate, CAS No. 14459-95-1) was obtained from Fisher Scientific as the hydrated potassium salt ($K_4[Fe(CN)_6] \cdot 3H_2O$) with >98% purity. A stock solution of 10 mM PY was prepared or added at this concentration to undiluted PW or NaCl solutions to simulate use as a crystal modifier in remediation applications. This assumes comparable PY and salt removal following remediation, consistent with preliminary data²⁴. The soil spiking followed the same method used in the second phase. In all treatments (e.g. PY, NaCl+PY and PW+PY) the nominal soil concentrations of ferrocyanide were 1.6, 5.3, 15.9, and 26.5 mg/kg at 0.3%, 1%, 3%, and 5% dilutions respectively.

For the greenhouse experiment, ten BSG seeds were planted in each of the 21 treatments, with 3 replicates per treatment. As in the phase 2 study, drainage holes in the pots were sealed to prevent salt leaching and covered with transparent plastic lids for the first 4 days after germination to maintain adequate moisture for seedling growth. Germination count was performed on day 10 post-planting and soil EC was measured after harvest (i.e., following completion of the germination assay and removal of germinated seedlings), in all treatments using the same method as described previously. Free cyanide was also measured in all soil treatments using a before planting and after harvest using a pyridine barbituric acid method with a Hach kit (TNT 862) and a UV-Vis spectrophotometer (DR1900). To apply this method, soil samples were extracted using a 1:2.5 soil to water ratio and filtered through a 0.22 μm filter prior to analysis.

2.5. DATA ANALYSIS

Germination data for Phase 3 treatments were analyzed using non-parametric methods since the assumptions for normality were not met. Instead, the Kruskal-Wallis test was applied to assess overall treatment effects, followed by Dunn's post hoc test for pairwise comparisons. Multiple comparison corrections, including Bonferroni and Holm adjustments, were tested. However, under these conservative corrections, no significant pairwise differences were detected. To explore potential treatment effects, results from the unadjusted Dunn's test are reported in the main figures.

To evaluate the concentration-response relationship between soil EC and seed germination, a non-linear dose-response model was fitted using the Weibull function. This approach allows for flexible modeling of the decline in germination with increasing salinity levels across all treatments involving PW, NaCl, PY, and their combinations. The model was implemented using the `drm()` function from the 'drc' package³³³ in R (R version 4.4.3),³³⁴ specifying a three-parameter Weibull type 2 function (W2.3).

Germination data from Phase 2 (5 replicates) and Phase 3 (3 replicates) were combined, only for treatments that were present in both phases, to calculate the mean germination percentage for each treatment. For PY treatments that were tested exclusively in Phase 3, only the three available replicates were used. The final dose-response dataset consisted of mean germination values per treatment, plotted against the mean after-harvest soil EC values from Phase 3, which served as the salinity exposure metric.

3. RESULTS and DISCUSSION

3.1. PHASE 1: SPECIES SELECTION FOR SEED GERMINATION TEST

Brewster sideoats grama exhibited significantly higher germination than Santiago silver bluestem in both control soil and greenhouse media (**Figure 1**). Although the greenhouse mix

appeared to support slightly better germination, results were not significantly different and the field-collected soil (**Figure C3**), which is classified as sandy loam and has low electrical conductivity (0.16 dS/m) and sodium levels (10 ppm) (**Table C1**), was selected for subsequent phases to better simulate post-remediation field conditions.

The superior performance of BSG in this study aligns with the prior research reporting that sideoats grama exhibits rapid germination under favorable conditions: achieving 50% germination within 22 hr in one study,³³⁵ while another study reported under optimal conditions, the majority of sideoats grama seed germinate within a week.³³⁶ Furthermore, certain BSG genotypes have been reported to maintain comparatively high germination rates under osmotic stress.³³⁷ These results collectively support the utility of BSG in short-term toxicity assays and potential field-scale revegetation trials.

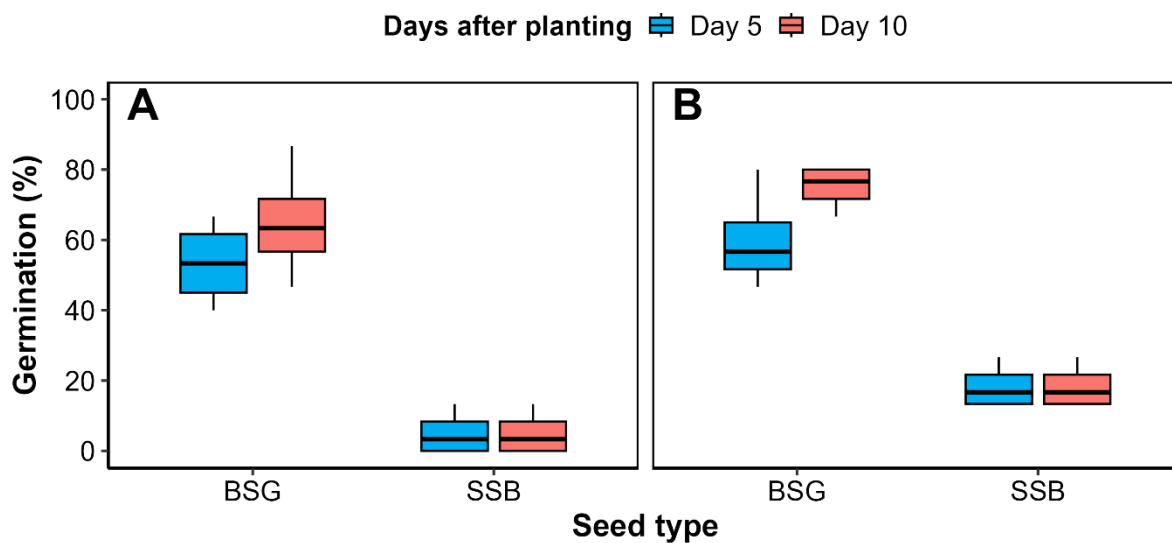


Figure 1. Comparison of seed germination between BSG and SSB in field soil (A) and greenhouse media (B) at 5 (early assessment)- and 10-days (final assessment).

3.2. PHASE 2: SEED GERMINATION SENSITIVITY TO PW AND NACL

The Soil EC results indicated no significant differences in soil EC between the corresponding dilutions of PW and NaCl, except at the 30% level (**Figure C2**). The low variability among replicates, as reflected by small standard deviations represented by error bars, suggests that the soil spiking process achieved a relatively homogenous distribution throughout the soil.

Seed germination declined progressively with increasing concentrations of both PW and NaCl, indicating a clear dose-dependent response (**Figure 2**). The similarity in germination patterns between PW and NaCl treatments suggests that salinity was the primary stressor limiting germination, rather than other potential contaminants included in the PW.

No seed emergence was observed at 10% and 30% dilutions of NaCl and PW, suggesting that these salinity levels were too high to support germination under the tested conditions. These concentrations were therefore excluded in Phase 3 tests.

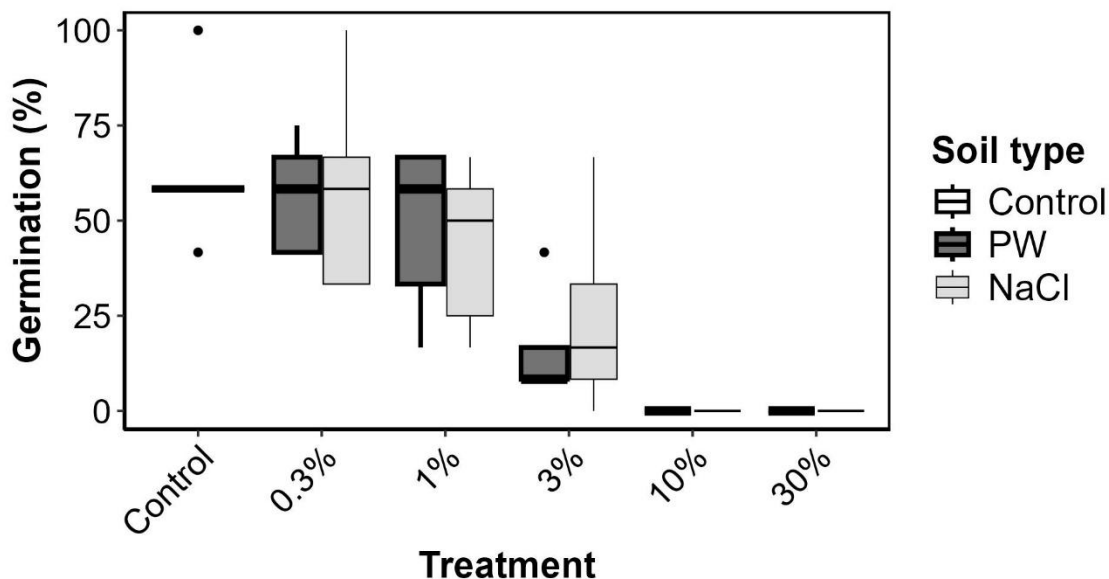


Figure 2. Comparison of 10 d seed germination (%) for PW and NaCl treatments. Boxes represent the interquartile range (25th–75th percentiles), the horizontal line within each box indicates the median, and whiskers extend to the minimum and maximum. Points indicate individual observations outside the whisker range. Each treatment includes 5 replicates.

3.3. PHASE 3: SENSITIVITY TO PY ALONE AND IN COMBINATION WITH PW OR NaCl

A key goal of this study was to determine whether PY, a crystal modifier proposed for use in salt-affected soil remediation, has unintended phytotoxic effects. To assess the potential for PY phytotoxicity, we quantified free cyanide in soils before planting and after harvest (**Table C2**). Free cyanide concentrations in the soil were generally below detection limits before planting in most treatments, though detectable levels were observed in two PY-amended soils, 3% (NaCl + PY) and 3% PY, which measured 160 and 360 ng/g respectively. Because these soils had not been exposed to natural sunlight, which is required to induce photodecomposition of ferrocyanide to free cyanide, the low-level detections are likely attributable to analytical variability rather than to true cyanide formation.

After harvest, free cyanide was detected in a few treatments at low concentrations (**Table C2**), including some that did not receive any added PY including 0.3% and 3% NaCl, as well as 1%, 3%, and 5% PW treatments. In contrast, treatments with the highest concentrations of PY such as 5% PY, 3% NaCl+PY, and 3% PW+PY exhibited free cyanide levels below the detection limit. Thus, results indicate that sunlight exposure (a known pathway for ferrocyanide photodecomposition) during the seed germination test did not result in a significant increase in soil cyanide levels. The lack of correlation between PY application rate and cyanide concentration further suggests that reported results reflect background interference associated with applying this colorimetric method to soils and were likely below detection for the analytical method employed.

In both PW and NaCl-based treatments without PY, a clear concentration-dependent decline in germination is observed with increasing salinity (**Figure 3A and 3B**), consistent with

the well-established inhibitory effects of salt stress on early plant development.^{338,339} The corresponding EC measurements for all treatments are available in the **Table C3**.

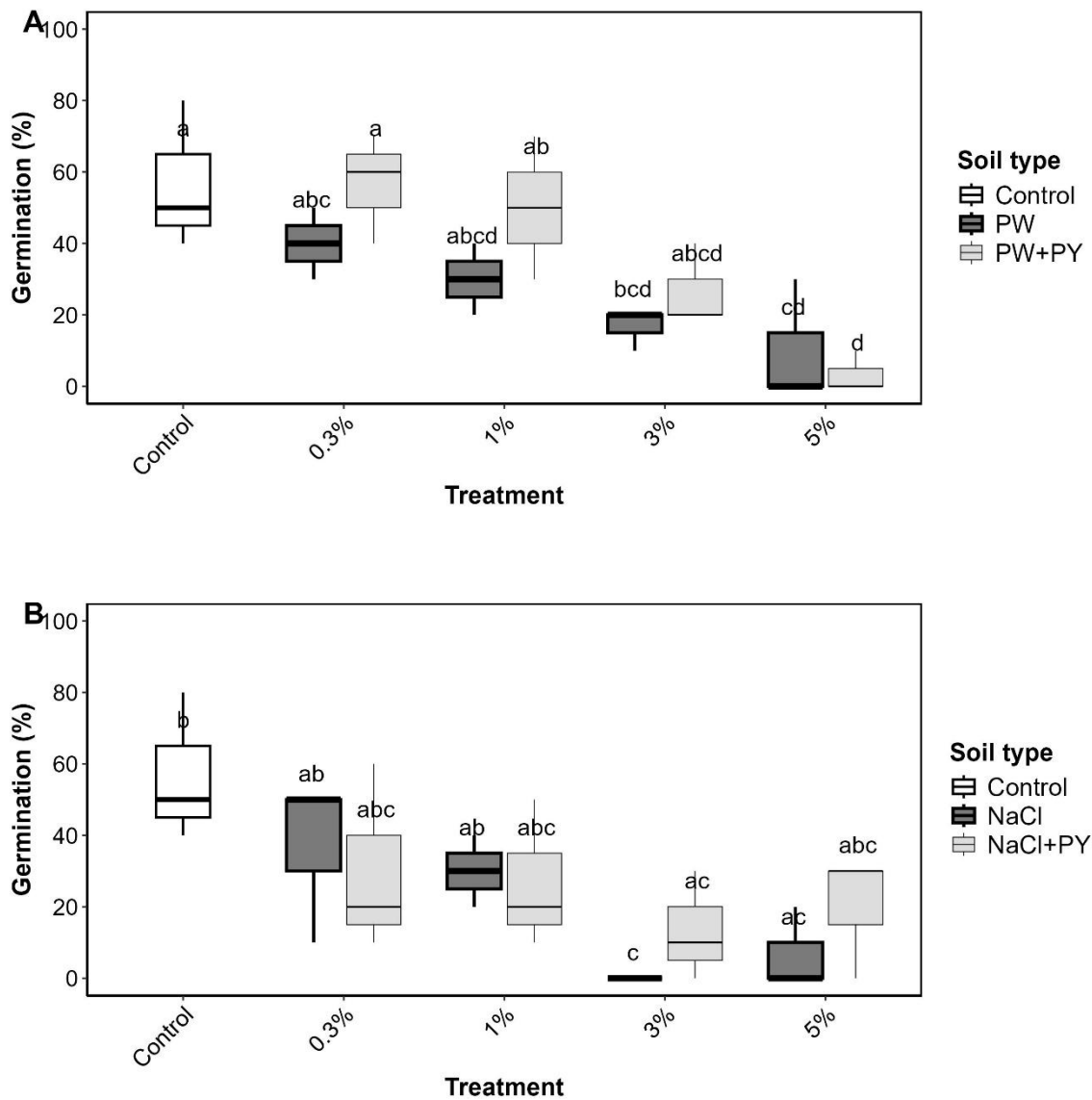


Figure 3: Comparison of 10 d seed germination (%) across soil treatments during phase 3 of the study. (A) Comparison PW and PW+PY across increasing concentrations. (B) Comparison of NaCl and NaCl+PY treatments across increasing concentrations. Different letters above the boxes indicate statistically significant differences among treatments ($p < 0.05$) based on post-hoc multiple comparisons. Treatments sharing one or more letters are not significantly different from each other. Each treatment includes 5 replicates.

Increasing PW concentrations led to a marked reduction in germination, with the 5% PW treatment showing the lowest germination rate (**Figure 3A**). Importantly, the addition of PY to PW did not show a consistent decrease in germination compared to their corresponding PW-only treatments, it rather appeared to slightly mitigate salinity stress at lower concentrations. For example, 0.3% and 1% PW+PY treatments had slightly higher germination than their PW-only counterparts. However, at higher concentrations (e.g., 3% and 5%), germination in PY treatments appeared to be comparable with PW-only treatments, indicating that PY did not exacerbate salinity stress

Sodium chloride treatments served as a salinity control revealing a similar concentration-dependent decline in germination as PW (**Figure 3B**). At lower concentrations (0.3% and 1%), PY did not significantly reduce germination, and in some cases, germination rates were higher than with NaCl alone. However, at higher concentrations (3% and 5%), germination remained low in both NaCl and NaCl+PY treatments. These patterns reinforce the interpretation that salinity is the dominant stressor limiting seed germination in PW. Furthermore, the lack of a significant inhibitory effect on seed germination across PY alone treatments (**Figure C3**) coupled with limited evidence of free cyanide formation (**Table C2**) indicates that use of PY in soil remediation does not pose an environmental concern based on native grass seed germination tests.

To better understand the relationship between salinity and seed germination across all treatments included in study phases 2 and 3, we plotted germination percentage against post-harvest EC and fitted a dose-response curve (**Figure 4**). This approach allowed us to evaluate the effect of salinity, regardless of salt source or amendment, on early plant development. As expected, germination declined with increasing EC, consistent with known inhibitory effects of salt stress.^{338,339} Importantly, treatments that included PY did not deviate from this general trend, suggesting that germination was primarily driven by salinity rather than from PY or other contaminants present in PW.

The estimated EC₅₀ (the electrical conductivity at which germination was reduced by 50%) was 0.87 dS/m (95% CI: 0.35–1.38 dS/m). This value provides a quantitative benchmark for characterizing the sensitivity of BSG native grass seed germination to salinity stress.

Results from other studies indicate that established plant species generally tolerate soil ECs in the range of 0.5 to 1.25 dS/m when EC measurements were determined using a 1:2 soil to water ratio.³⁴⁰ Since we used a 1:1 soil to water ratio, the adjusted EC₅₀ derived from our study would fall at the lower end of this range. However, seed germination is more sensitive to salinity stress than other stages of plant growth. As a result, sprigging, sodding, or transplanting of plants is often used in revegetation programs to avoid the sensitivity of the seedling stage.³²⁵ Thus, while BSG seed germination tests may be an effective tool to assess the efficacy of soil remediation involving PW spills, these assays may be less predictive for determining the success of field revegetation particularly for other salt tolerant species. Previous studies have reported the successful use of halophytic species for phytoremediation and revegetation of saline and sodic soils, where plant tolerance and persistence are primary objectives.^{341,342}

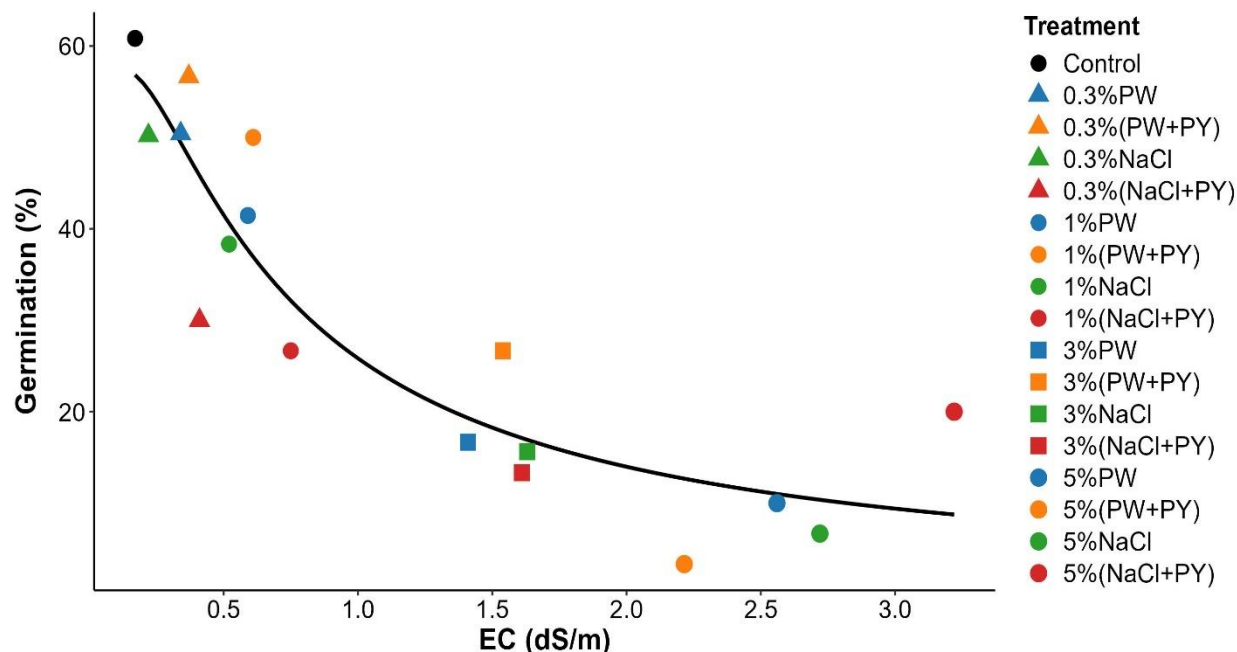


Figure 4: Dose-response relationship between soil EC and seed germination using combined data from second and third phases. Each point represents the mean germination for a given treatment as denoted by different colored symbols for PW, NaCl, PY or tested combinations. A non-linear regression (Weibull model) was fitted to describe the overall decline in germination with increasing soil salinity.

The use of PY as a soil amendment for remediating salt contaminated soils is intended to enhance the passive transport of salts trapped in the soil column by evaporative flux. In principle, the fate of the PY added to the soil should be transported and incorporated into the crystallized salts at the soil surface that are removed. Previous lab soil column studies suggest that up to almost 90% of the PY was in fact found in the recovered surface salts.²⁴ Consistent with this mechanism, visible salt crust formation was observed at the soil surface in our PY-treated pots, further supporting the upward mobilization and crystallization process (**Figure C6**). While further work is needed to optimize PY recovery in field applications of this technology, residual concentrations of PY may remain in soil following remediation.

Previous studies suggest that ferrocyanide exhibits low to moderate aquatic toxicity. For example, the EC50 for two early life stages of freshwater mussel were reported to be > 1000 mg/L

to sodium ferrocyanide.³⁴³ In the ECHA registration dossier for potassium ferrocyanide, a 96 h LC50 in carp and 48 h EC50 in *Daphnia magna* are both reported as > 100 mg/L. In contrast, the 72 h EC50 of 3.1 mg/L is given for the alga, *Pseudokirchneriella subcapitata*.³⁴⁴ In another study involving potassium ferrocyanide, the 72 h EC50 for *P. subcapitata* and the 7 d EC50 for *Ceriodaphnia dubia* was 2.3 and 1.8 mg/L³⁴⁵ respectively, when expressed as ferrocyanide. Earthworms were also tested and showed no mortality up to soil concentrations of 1068 mg/kg dry weight. These authors also conducted tests in the dark and found no difference in toxicity under standard laboratory light conditions. In a study investigating the aquatic toxicity of fire-fighting chemicals, the toxicity of PY in the absence and presence of UV-B light was investigated. The 96 h LC50 for rainbow trout and southern leopard frogs without UV-B light was found to be 2.4 and 99.3 mg/L, respectively. In contrast, co-exposure to UV-B light increased toxicity of PY by a factor of 12 and 38, respectively. The increased toxicity was associated with elevated levels of free cyanide that resulted from photodissociation of PY during the first 24 h of the test.³⁴⁶ Therefore, these authors concluded that toxicity of fire-fighting chemicals containing PY in the absence of UV light may understate risks in the field.

To address the potential for PY photodissociation during field application, we included PY in phase 3 of our greenhouse study. At the spiking concentrations investigated, there appeared to be little evidence of free cyanide formation. Our results are consistent with conceptual model proposed by³⁴⁷ that describes the fate of ferrocyanides in soil. Due to limited light penetration in soil, the generation of free cyanide is expected to occur slowly. Free cyanide that is formed may undergo a number of reactions including formation of iron ferrocyanide (i.e. $\text{Fe}_4[\text{Fe}(\text{CN})_6]_3$) also known as prussian blue. Additional soil detoxification pathways associated with iron ferrocyanide has raised interest in possible phytoremediation strategies.³⁴⁷ Thus, the potential risk of residual

PY in soil appears kinetically limited and further mitigated by revegetation efforts after salt removal.

4. CONCLUSION

This study highlights the utility of using a native grass species, Brewster sideoats grama, as a bioindicator for assessing phytotoxicity in soils exposed to PW and associated remediation amendment, PY. Across both study phases, salinity was confirmed as the primary stressor inhibiting seed germination, and the addition of PY as a crystal modifier did not lead to consistent, measurable increases in free cyanide in soil or inhibit seed germination. These findings support the potential use of PY for remediation of PW contaminated soils. Additional work is needed to better understand the long-term fate of residual PY that is not captured in the recovered salts that are removed during remediation of the PW contaminated soil. Such efforts can include the development of improved methods to detect trace concentrations of free cyanide that may be formed via photodegradation pathways. Future studies should also evaluate plant establishment following PY aging to better reflect full remediation timelines. While germination-based toxicity assays using native species can serve as a rapid and field-relevant tool to evaluate the efficacy of salt remediation technologies including use of PY amendments. The predictive ability of these assays to determine soil suitability for revegetation is unclear. Future work should explore the ability of native grass seed germination tests to predict revegetation success at PW remediated sites.

AUTHOR CONTRIBUTIONS

Huma Tariq Malik: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft.

Jason K. Geiger: Methodology, Writing – review & editing

Thomas F. Parkerton: Conceptualization, Writing – review & editing

Thomas Borch: Conceptualization, Funding acquisition, Project administration, resources, supervision, writing-review & editing.

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CHAPTER 6: SUMMARY

The purpose of this dissertation was to examine the sustainable water use in agriculture under increasing water scarcity, with a focus on both system-level strategies and the use of alternative water sources. This was accomplished by evaluating water use across the agricultural life cycle in the United States; assessing the impacts of produced water (PW) irrigation on soil health, crop performance, and microbial communities under controlled greenhouse conditions; investigating the use of treated produced water (TPW) under field conditions; and evaluating a remediation approach using a crystallization modifier to mitigate salinity impacts in PW-contaminated soils.

Chapter 2 provides a comprehensive synthesis of water use across the agricultural life cycle in the United States, highlighting key opportunities to improve efficiency and sustainability. It emphasizes that water challenges in agriculture are not confined to irrigation alone but span the entire system, from crop selection and soil management to irrigation practices and consumption patterns, emphasizing the need for integrated approaches rather than single-point solutions.

Chapter 3 investigates the use of untreated conventional produced water (CPW) for irrigation under controlled greenhouse conditions. Results show that while crop performance metrics such as biomass and plant height were largely unaffected, grain yield increased under undiluted PW irrigation. However, this apparent agronomic benefit was accompanied by changes in soil properties, including increased salinity and pH and reduced aggregate stability, alongside shifts in microbial community composition and function. These findings suggest that short-term crop performance may not fully capture underlying soil and ecological changes.

Chapter 4 extends this investigation to a field-scale study in Orla, Texas, evaluating the effects of treated produced water (TPW) irrigation on crop performance, soil chemistry, and the soil microbiome. Results showed no significant differences in aboveground biomass, and plant height was largely comparable between TPW and drinking water treatments. However, TPW irrigation led to elevated boron concentrations in soil, while microbial community composition exhibited only limited shifts. These findings provide field-scale evidence on the viability of TPW irrigation.

Chapter 5 focuses on remediation of PW-impacted soils using Prussian yellow (PY), a crystallization modifier designed to enhance salt removal. Using native grass seed germination as a bioindicator, the study demonstrates that salinity is the primary driver of toxicity in PW-contaminated soils, while PY does not introduce additional toxicity or significantly increase cyanide risk under the tested conditions. These results support the potential of PY as a viable tool for remediation of salt-affected soils.

Overall, this work reflects the need to think about water use in agriculture as part of a connected system rather than a set of isolated decisions. It cannot be evaluated through a single dimension, as outcomes that appear effective in one aspect may carry unintended consequences in others. Decisions around crop performance, soil health, and environmental impacts are closely linked, and considering these together is essential for developing more sustainable approaches. These insights can help guide regulators and policymakers in designing frameworks that account for both short-term performance and long-term system impacts when evaluating alternative water use practices. At the same time, this work points to the need for longer-term, field-scale studies that span multiple growing seasons and include a diversity of crops to better understand sustained system responses of produced water irrigation.

APPENDIX A: Supplementary Information for Chapter 3

Table A1: Concentrations of BTEX and oil and grease in PW100.

Compound	Concentration in PW (ppb)	Acceptable limits (ppb)
Benzene	3.8	5*
Toluene	2.2	1000*
Ethylbenzene	0.6	700*
Xylenes	37.2	10000*
Oil and Grease	22.9	35**

**The values provided here only represent the acceptable limits of BTEX in drinking water according to the US EPA. The maximum contaminant goal for BTEX in drinking water is zero.*

*** Federal Effluent Limitation Guidelines (ELGs) for oil and gas (40 CFR 435.E ELG).*

Table A2: Concentration of PAHs in PW100. LOQ = “limit of quantitation”; LOD = “limit of detection. *EPA High priority pollutants.

PAH	Concentration (ppb)
Acenaphthylene	0.784
9,10-dimethylanthracene	2.314
Fluoranthene*	<LOQ
Chrysene*	<LOQ
Perylene	<LOQ
d9-methylanthracene	<LOQ
1-methylnaphthalene	<LOQ
1-phenylnaphthalene	<LOD
2,6-diisopropylnaphthalene	<LOD
2,3-dimethylnaphthalene	<LOD
Fluorene*	<LOD
Acenaphthene*	<LOD
Anthracene*	<LOD
Benzo[a]pyrene*	<LOD
Pyrene*	<LOD

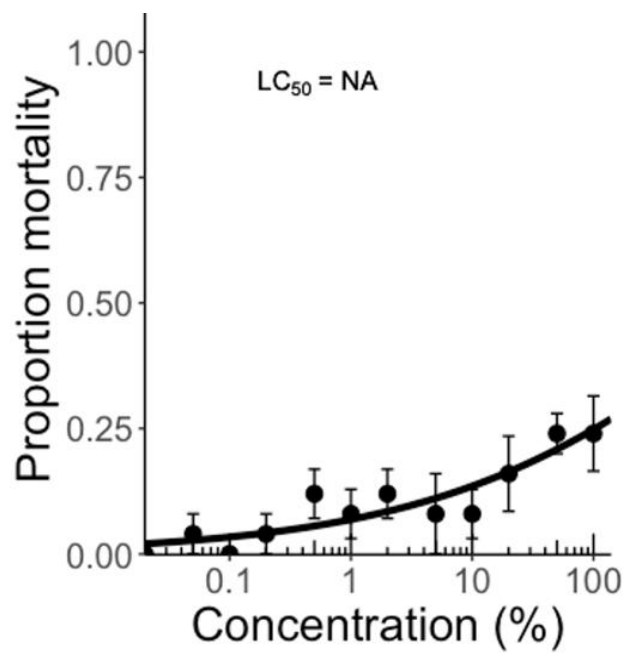


Figure A1: 48-h median lethal concentration (LC₅₀) for *Daphnia* after exposure to raw conventional produced water.

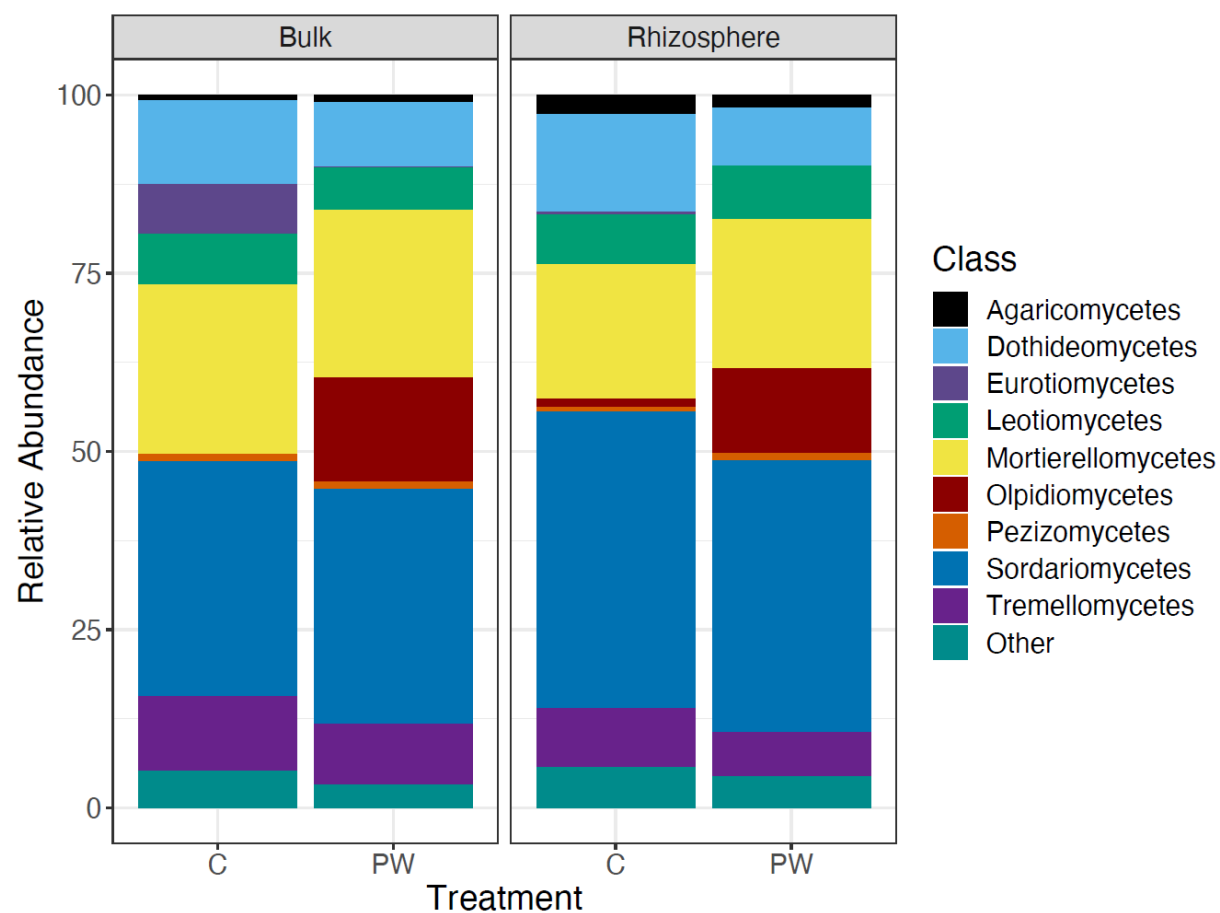


Figure A2: Relative abundance of fungal communities in Control and PW100-irrigated soils.

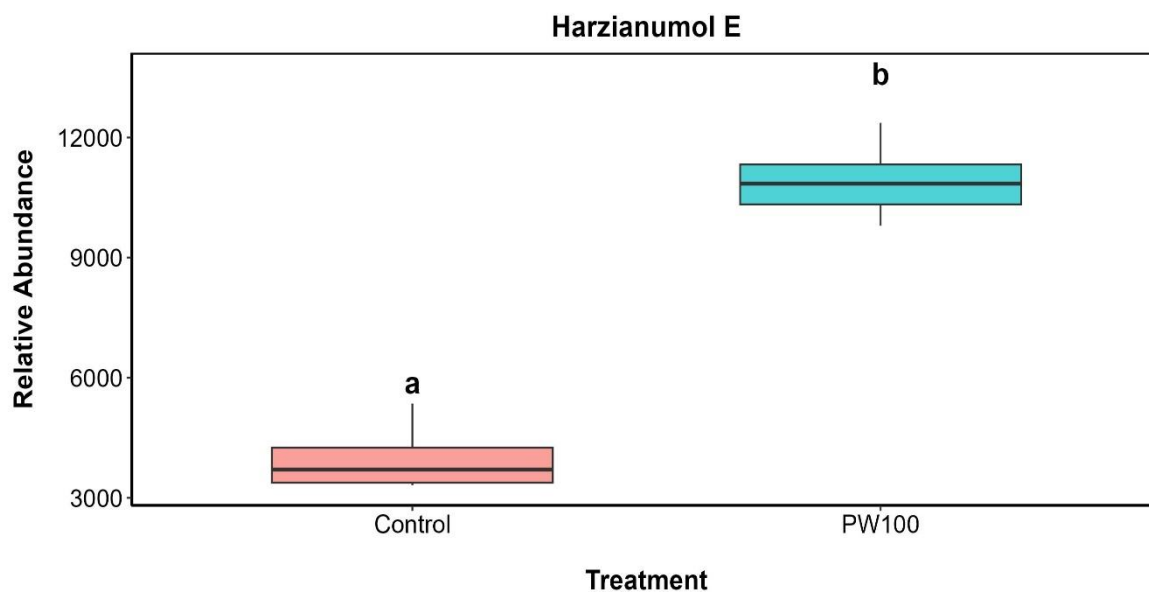


Figure A3: Normalized abundances of annotated Harzianumol E peaks in leaf tissue under LCMS metabolomics. Different letters (a, b) denote statistically significant differences between treatments ($p < 0.05$).

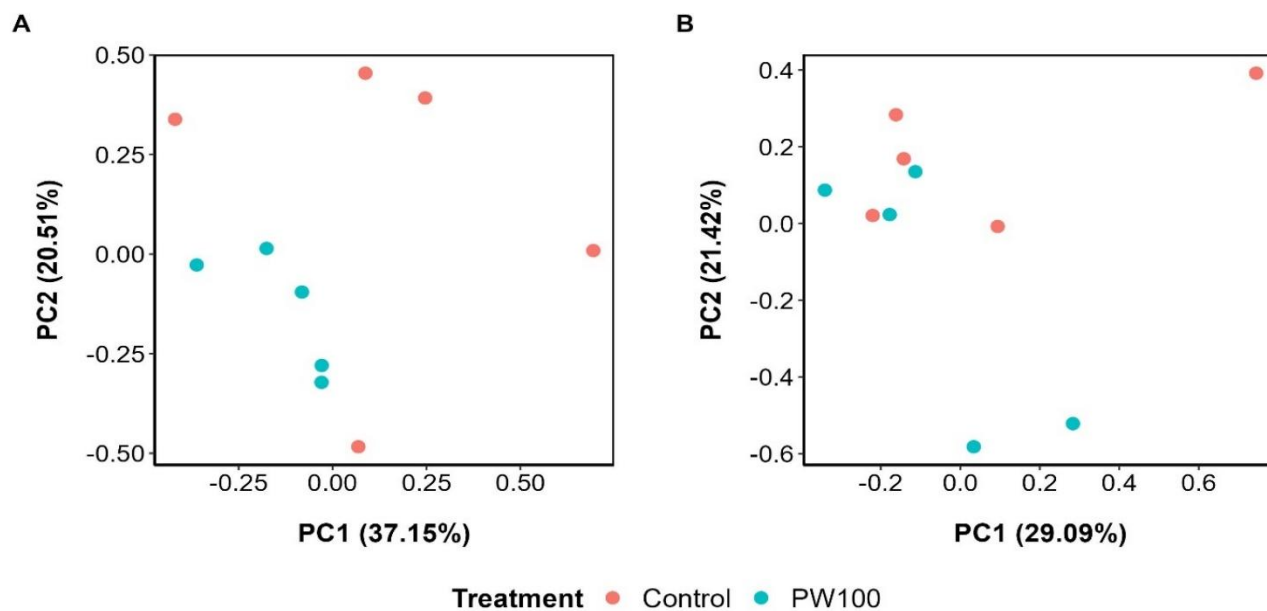


Figure A4: Principal component analysis (PCA) score plots of a nontargeted LC-MS data set of leaf tissue (A) and wheat grain (B).

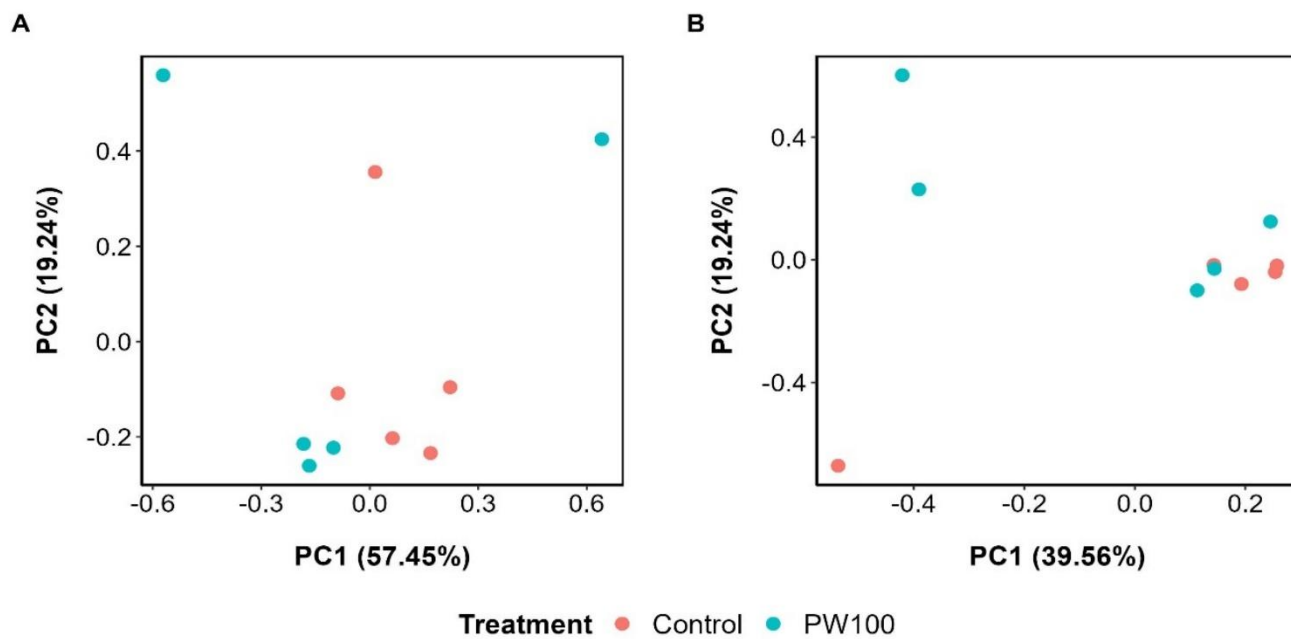


Figure A5: Principal component analysis (PCA) score plots of a non-targeted GC-MS data set of leaf tissue (A) and wheat grain (B).

APPENDIX B: Supplementary Information for Chapter 4

Section 1. Supporting Tables and Figures

Table B1. Soil-quality parameters and analytical methods used for both drinking water (DW) and treated produced water (TPW) irrigated soils.

Parameters	Analytical Method
pH, EC	1:1 soil-to-water suspension
B	Hot water extraction
Ca, Mg, Na, K	Ammonium acetate extraction
Fe, Mn, Zn, Cu	DTPA extraction
NO ₃ ⁻	KCl extraction
P	Olsen Bicarbonate extraction
S	Calcium phosphate extraction
Pb, Se, Cr, Cd, As	Total digestion
OM %	Loss-on-ignition method
Total C, Total N, C:N ratio	Combustion method using dry combustion
Texture (sand, silt, clay)	Hydrometer method
Soil respiration	24-hour incubation and measurement of CO ₂ release
Potential Mineralizable Nitrogen	28-day aerobic incubation followed by KCl extraction

Table B2: Concentrations of BTEX in DW and TPW.

Analyte	DW	TPW	Acceptable limits for drinking water	Units
Benzene	<0.460	ND	5*	ug/L
Toluene	<0.475	3.28	1000*	ug/L
Ethylbenzene	<0.385	ND	700*	ug/L
Xylenes	<1.240	ND	10000*	ug/L

**The values provided here only represent the acceptable limits of BTEX in drinking water according to the US EPA. The maximum contaminant goal for BTEX in drinking water is zero.³⁰⁴*

Table B3: Concentrations of PAHs in DW and treated PW.

Analyte	DW	TPW	Units
Pyrene	<0.0846	ND	ug/L
Phenanthrene	<0.134	ND	ug/L
Naphthalene	<0.0942	ND	ug/L
Indeno[1,2,3-cd]pyrene	<0.0997	ND	ug/L
Fluorene	<0.0945	ND	ug/L
Fluoranthene	<0.0881	ND	ug/L
Dibenz(a,h)anthracene	<0.0507	ND	ug/L

Chrysene	<0.0813	ND	ug/L
Benzo[k]fluoranthene	<0.0471	ND	ug/L
Benzo[b]fluoranthene	<0.0662	ND	ug/L
Benzo[a]pyrene	<0.0299	ND	ug/L
Benzo[a]anthracene	<0.0285	ND	ug/L
Anthracene	<0.0935	ND	ug/L
Acenaphthylene	<0.0994	ND	ug/L
Acenaphthene	<0.107	ND	ug/L
2-Methylnaphthalene	<0.0601	ND	ug/L
2-Chloronaphthalene	<0.377	ND	ug/L
2-Acetylaminofluorene	<1.26	ND	ug/L
1-Methylnaphthalene	<0.0624	ND	ug/L

Table B4: Soil health Indicator means ($\pm$ SE mean) from soil samples collected before planting. Different letters (a, b, c) indicate significant differences among treatments based on one-way ANOVA followed by post-hoc comparisons ($p < 0.05$); groups sharing at least one letter are not significantly different. N=3.

PMN, potentially mineralizable Nitrogen; NO_3^- -N, nitrate nitrogen; NH_4^+ -N, ammonium nitrogen; EC, electrical conductivity; OM, Organic matter.

Soil health indicator	DW1 (SW)	DW2 (NE)	TPW1 (NW)	TPW2 (SE)
Extractable elements (mg/kg)				
Sodium	89.3 ± 7^a	226 ± 175^a	191 ± 108^a	482 ± 397^a
Chloride	375 ± 100^a	2486 ± 2235^a	402 ± 131^a	3114 ± 2824^a
Calcium	6690 ± 401^b	10199 ± 241^a	6792 ± 904^b	7545 ± 696^{ab}
Magnesium	260 ± 22^a	229 ± 13^a	253 ± 14^a	214 ± 19^a
Boron	0.04 ± 0.01^a	0.10 ± 0.06^a	0.02 ± 0.12^a	0.92 ± 0.92^a
Sulfur	272 ± 33^a	409 ± 26^a	298 ± 71^a	298 ± 37^a
Zinc	1.00 ± 0.14^a	0.55 ± 0.13^a	0.57 ± 0.12^a	0.64 ± 0.11^a
Iron	1.65 ± 0.05^a	1.26 ± 0.17^a	1.50 ± 0.16^a	1.50 ± 0.15^a
Manganese	5.88 ± 1.36^a	4.19 ± 0.27^a	5.33 ± 1.52^a	7.13 ± 1.28^a
Copper	5.60 ± 1.03^a	3.57 ± 0.79^a	2.96 ± 0.62^a	4.00 ± 0.75^a
Arsenic	0.31 ± 0.20^{bc}	0.75 ± 0.03^{ab}	1.09 ± 0.16^a	ND
Cadmium	0.93 ± 0.43^b	3.69 ± 0.06^{ab}	4.99 ± 1.26^a	5.48 ± 1.10^a
Chromium	ND	ND	ND	ND
Lead	1.11 ± 0.62^b	3.82 ± 0.21^a	3.80 ± 0.55^a	2.76 ± 0.36^{ab}
Selenium	ND	ND	ND	ND
Biological Indicators				
PMN, mg/Kg (NO_3^- - N)	58.9 ± 24^a	80.3 ± 20^a	50.6 ± 15^a	59.8 ± 27^a
PMN, mg/Kg (NH_4^+-N)	2.92 ± 0.25^a	6.66 ± 1.79^a	3.62 ± 1.72^a	2.66 ± 1.21^a
Respiration (CO_2), $\mu\text{g/g}$ soil/day	4.59 ± 0.63^b	1.95 ± 0.36^a	2.14 ± 0.73^{ab}	3.22 ± 0.40^a
Chemical Indicators				
Total Carbon, %	2.53 ± 0.07^a	2.46 ± 0.08^a	2.43 ± 0.14^a	2.63 ± 0.14^a
Total Nitrogen, %	0.06 ± 0.002^a	0.07 ± 0.01^a	0.06 ± 0.001^a	0.07 ± 0.005^a
Carbon to Nitrogen Ratio	40.5 ± 1.94^a	38.1 ± 2.75^a	39.4 ± 2.42^a	37.3 ± 1.93^a
pH 1:1	7.97 ± 0.03^a	7.93 ± 0.03^a	7.97 ± 0.03^a	7.93 ± 0.03^a
EC 1:1*, dS/m	1.65 ± 0.12^a	1.88 ± 0.42^a	1.64 ± 0.13^a	2.15 ± 0.33^a
OM, %	1.69 ± 0.04^{ab}	1.89 ± 0.05^a	1.64 ± 0.07^b	1.68 ± 0.02^{ab}
Nutrient Indicators (mg/kg)				
NO_3	36.3 ± 15^a	55.4 ± 28^a	37.7 ± 12^a	46.4 ± 23^a

Phosphorus	4.33 ± 0.88^a	2.00 ± 1.50^a	3.33 ± 0.67^a	5.67 ± 0.88^a
Potassium	800 ± 5.93^{ab}	669 ± 25.85^a	814 ± 44.43^b	745 ± 36.36^{ab}
Soil Class	Clay Loam	Sandy Clay Loam	Sandy Clay Loam	Sandy Clay Loam

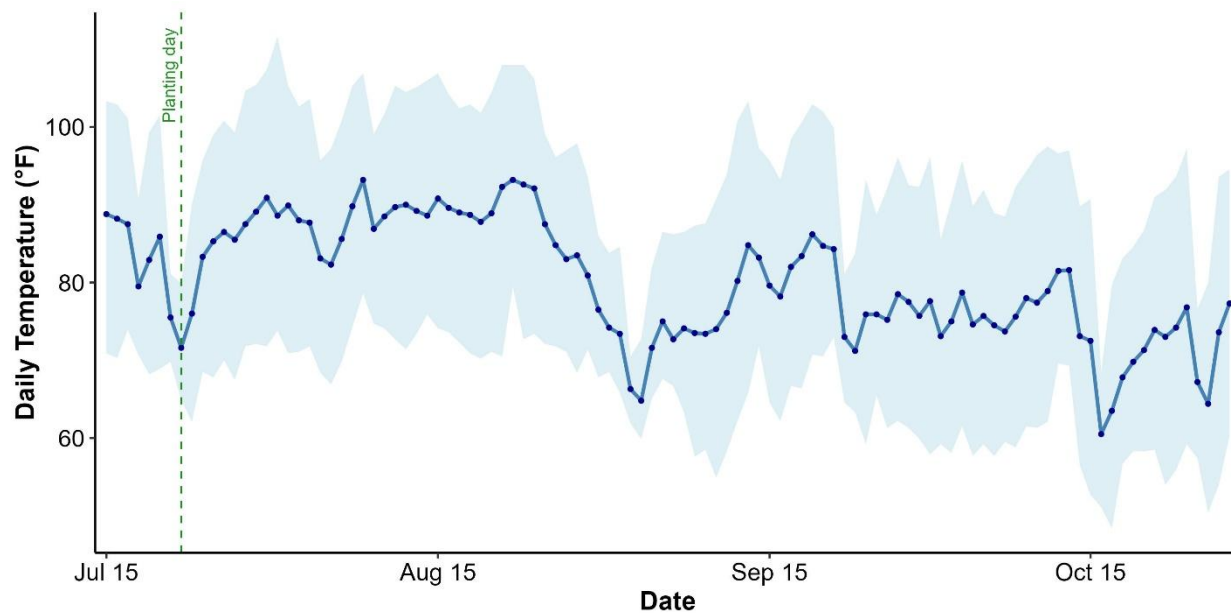


Figure B1. Daily average temperature (solid line) overlaid on a shaded ribbon representing the daily minimum and maximum temperatures recorded at the field site during the growing season (July 15 - October 28, 2024).

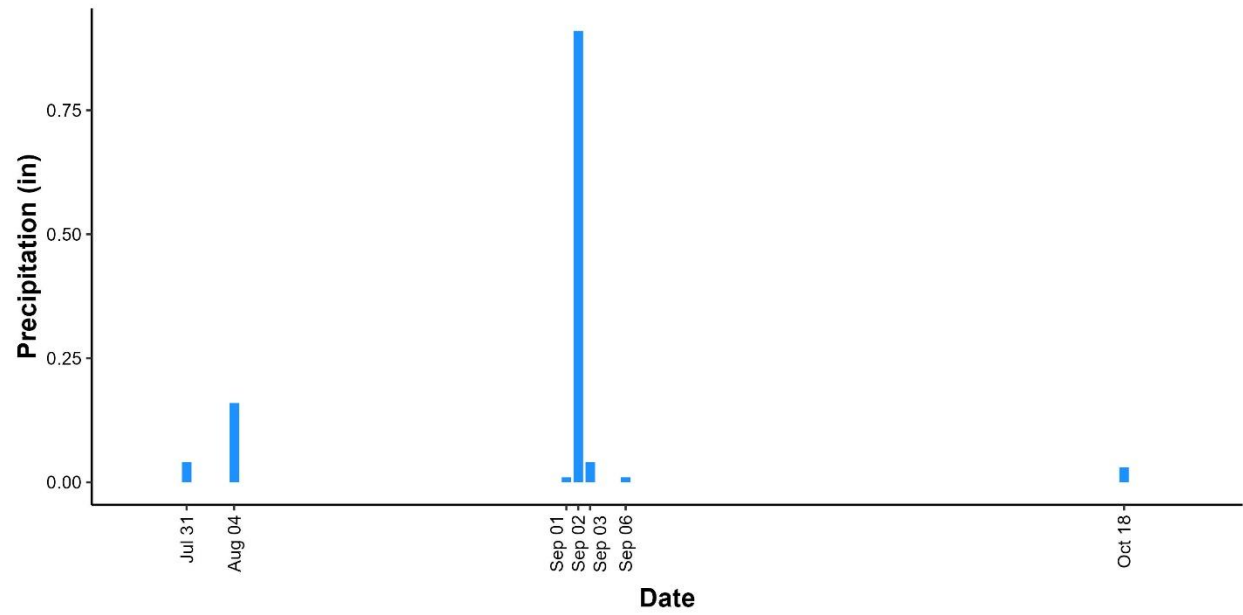


Figure B2: Daily precipitation measured at the field site in Orla, Texas during the 2024 sorghum growing season. Rainfall events were infrequent and concentrated in early September, with a major rain event recorded on September 2.

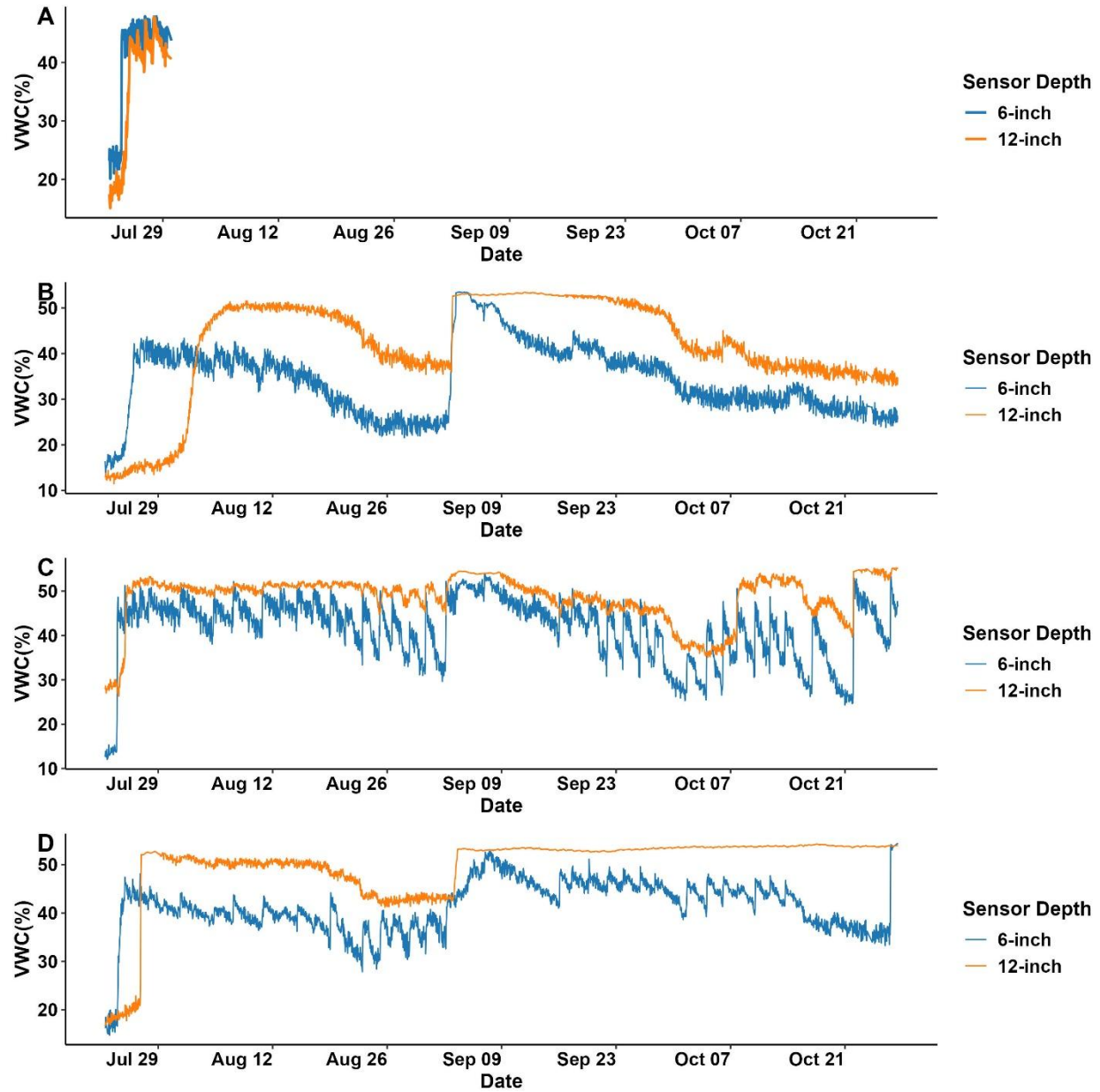


Figure B3: VWC (Volumetric water content) measured at 6-inch and 12-inch soil depths across four plots over time. Panels A–D represent time series data from different irrigation plots: (A) DW1, (B) TPW1, (C) DW2, and (D) TPW2. Measurements were recorded using soil moisture sensors, fluctuations reflect irrigation events and precipitation. The sensor in DW1 (Panel A) stopped functioning few days after installation, resulting in a truncated data record.

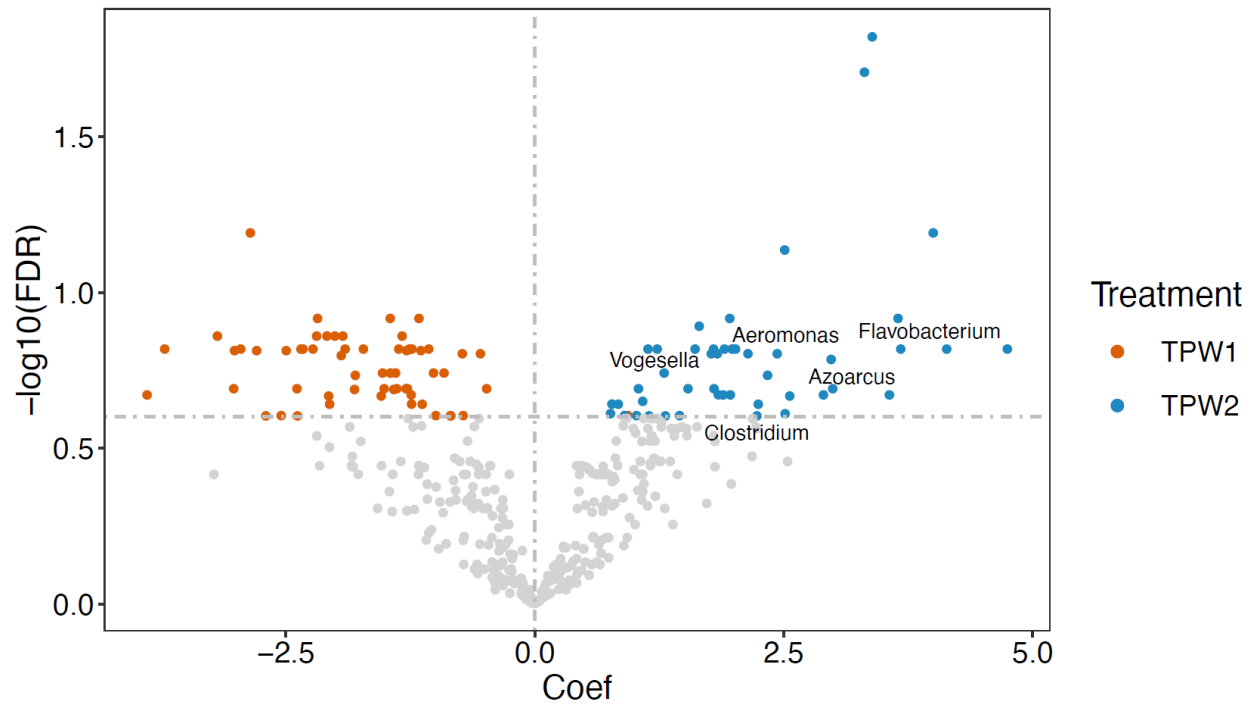


Figure B4: Volcano plot showing differential abundance of bacterial and archaeal taxa in bulk soil. The x-axis shows model coefficients (log-fold change), with positive values indicating enrichment under TPW2 irrigation (blue; subject to surface ponding) and negative values indicating enrichment under TPW1 irrigation (orange). Taxa that did not meet the significance threshold are shown in gray. Selected taxa enriched under TPW2 surface ponding conditions are labeled.

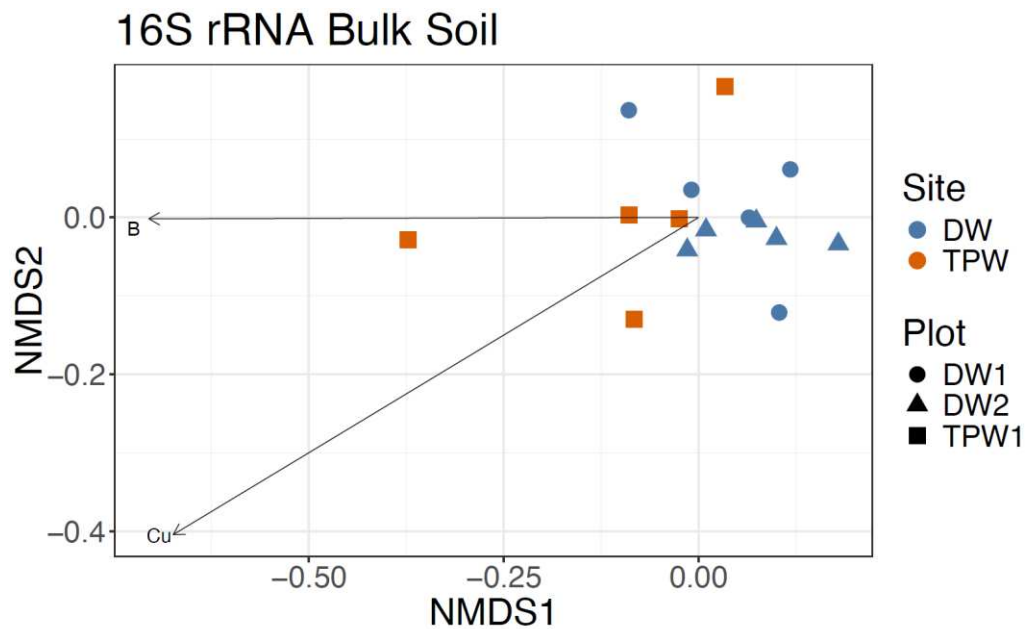


Figure B5: NMDS ordination of bacterial (16S rRNA gene) community composition in bulk soil under DW and TPW irrigation. Points represent individual samples colored by treatment and

shaped by plot. Vectors indicate soil parameters significantly correlated with community composition ($p < 0.05$).

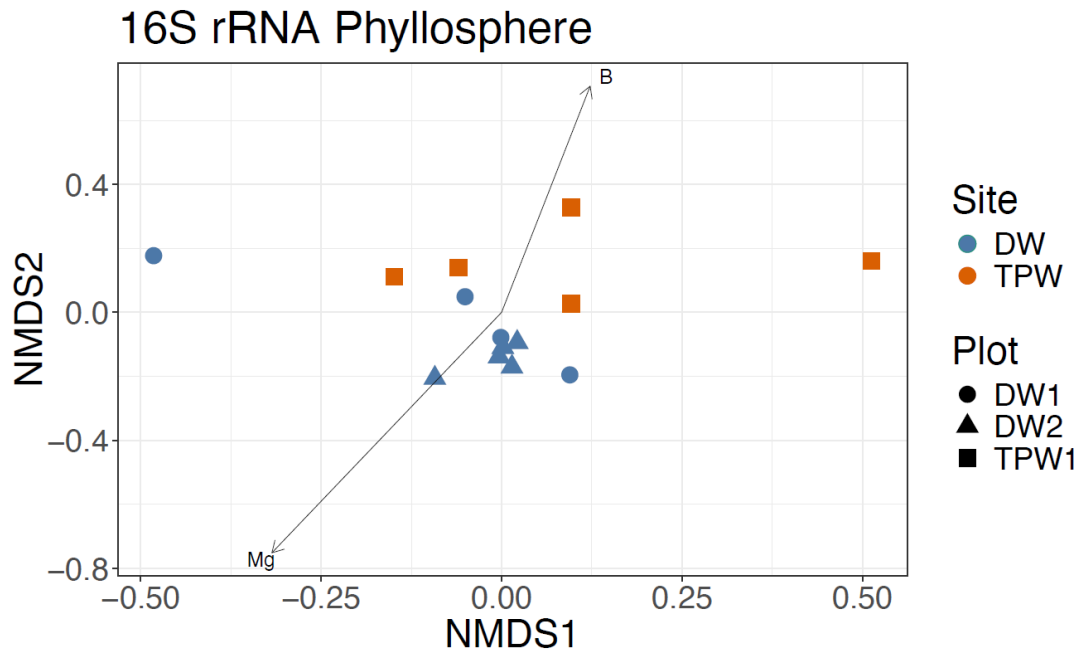


Figure B6: NMDS ordination of bacterial (16S rRNA gene) community composition in phyllosphere samples under DW and TPW irrigation. Points represent individual samples colored by treatment and shaped by plot. Vectors indicate soil parameters significantly correlated with community composition ($p < 0.05$).

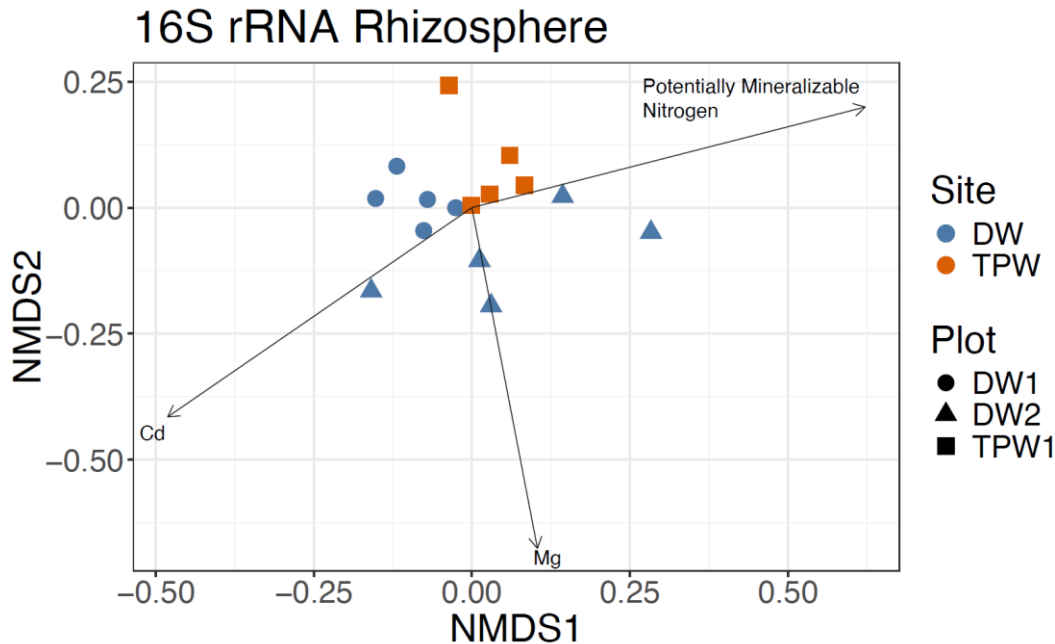


Figure B7: NMDS ordination of bacterial (16S rRNA gene) community composition in rhizosphere soil under DW and TPW irrigation. Points represent individual samples colored by treatment and shaped by plot. Vectors indicate soil parameters significantly correlated with community composition ($p < 0.05$).

Section 2: Detailed Description of Multivariate Analyses and Ordination Results

In bulk soil, PERMANOVA analysis indicated significant differences both among treatments ($p = 0.001$, $R^2 = 0.13$, $F = 1.9$) and among quadrants ($p = 0.001$, $R^2 = 0.21$, $F = 1.6$). Pairwise comparisons showed that all quadrant pairs differed significantly ($p < 0.05$), with stronger separation between TPW and DW quadrants than between the two DW quadrants indicating greater treatment effects than spatial heterogeneity. Consistent with these results, NMDS ordination revealed that TPW1 formed a moderately distinct cluster from the DW plots which clustered more closely together. This pattern reflects a moderate treatment-driven separation in microbial community composition. NMDS ordination with fitted soil parameters indicated that boron (B) and copper (Cu) were associated with the separation of TPW samples. Given the large increase in soil boron under PW irrigation boron enrichment likely contributed to the observed

microbial shifts. Copper concentrations showed a modest (but not significant) increasing trend under PW irrigation, which may have contributed weakly to community variation.

In the rhizosphere, PERMANOVA analysis revealed significant differences among treatments ($p = 0.031$, $R^2 = 0.11$, $F = 1.5$) and among quadrants ($p = 0.001$, $R^2 = 0.23$, $F = 1.8$). Pairwise comparisons showed all quadrants differed significantly ($p < 0.05$), with slightly greater dissimilarity between DW1 and DW2 than between either DW quadrant and TPW1, suggesting that spatial variability exceeded treatment effects. NMDS ordination also reflected this pattern, with DW and TPW samples partially overlapping, indicating higher spatial heterogeneity and a weaker treatment signal. NMDS ordination with fitted soil variables indicated associations between rhizosphere microbial composition and soil magnesium (Mg), cadmium (Cd), and nitrate-nitrogen (NO_3^- -N). The Mg vector corresponded with the separation of DW and TPW samples, consistent with significantly lower Mg concentrations under PW irrigation ($231 \pm 34 \text{ mg kg}^{-1}$) than in DW plots ($439\text{--}518 \text{ mg kg}^{-1}$). In contrast, soil Cd and NO_3^- -N showed no significant differences among treatments, suggesting weaker or spatially driven associations with microbial community patterns.

In the phyllosphere, PERMANOVA analysis showed significant differences among treatments ($p = 0.016$, $R^2 = 0.12$, $F = 1.7$) and among quadrants ($p = 0.007$, $R^2 = 0.20$, $F = 1.6$). Pairwise comparisons indicated that while quadrants differed significantly ($p < 0.05$) except DW1 vs TPW1, the strongest separation occurred between DW2 and TPW1, suggesting that treatment effects were evident but somewhat variable across quadrants. NMDS ordination also showed moderate separation between TPW and DW samples reflecting partial treatment effect driven mainly by DW2's separation. NMDS ordination with fitted soil parameters showed that boron (B) and magnesium (Mg) were strongly associated with community separation in the phyllosphere. The B

vector aligned with TPW samples, reflecting elevated soil boron concentrations under PW irrigation, whereas Mg pointed toward DW plots, consistent with lower Mg levels in TPW-treated soils. These opposing gradients suggest that boron enrichment and magnesium depletion jointly contributed to the moderate treatment-driven differences observed in phyllosphere microbial composition.

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APPENDIX C: Supplementary Information for Chapter 5

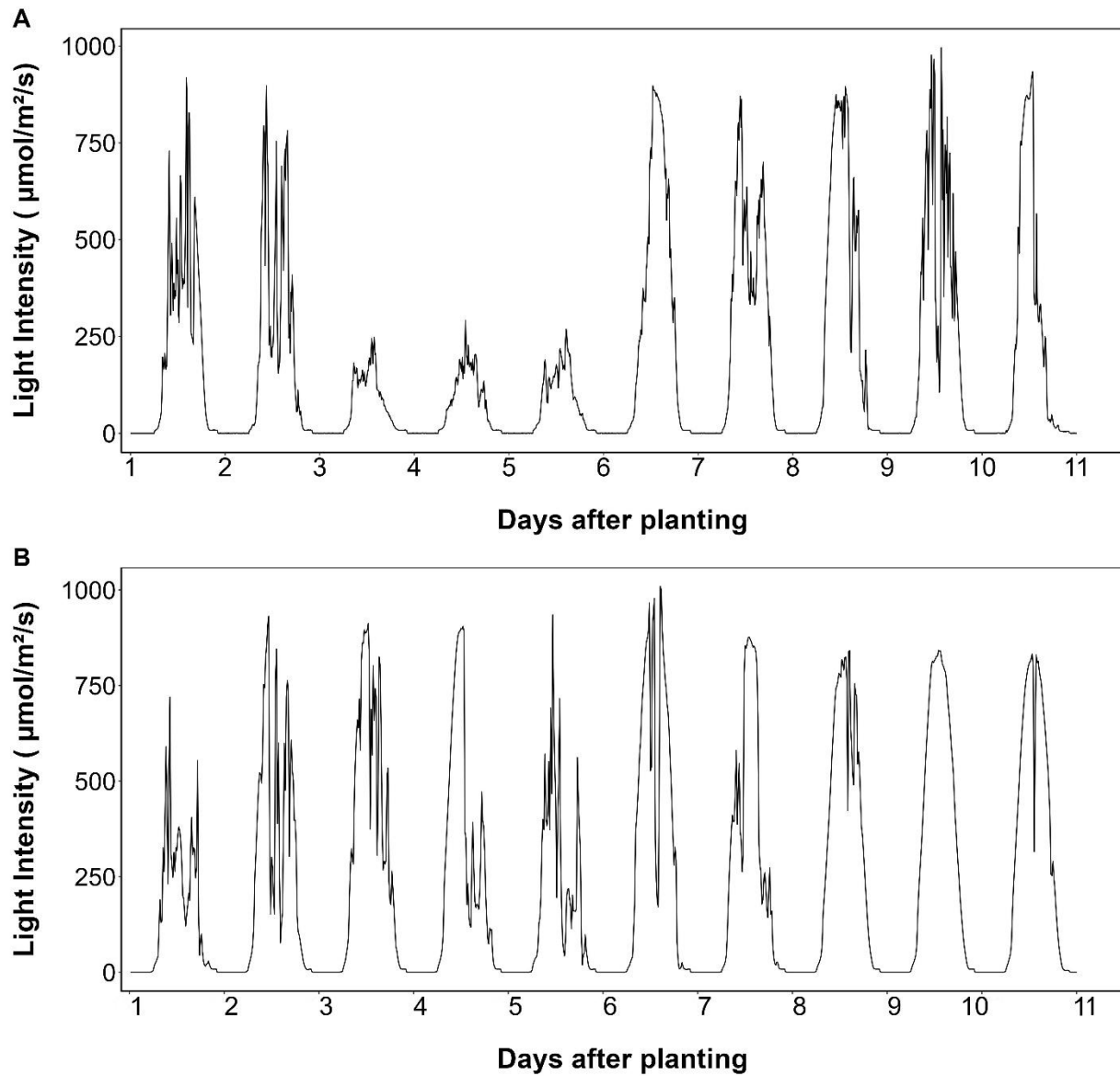


Figure C1. Light intensity measured in the greenhouse during (A) the second phase (B) the third phase. Light intensity ($\mu\text{mol m}^{-2} \text{s}^{-1}$) was recorded every 15 minutes using sensors installed in the greenhouse. Each panel shows the first 11 days after planting.



Figure C2. Pilot experiment conducted to determine the germination endpoint for two native seed types (BSG and SSB) in two soil types: greenhouse potting media (bottom tray) and field-collected control soil (top tray).

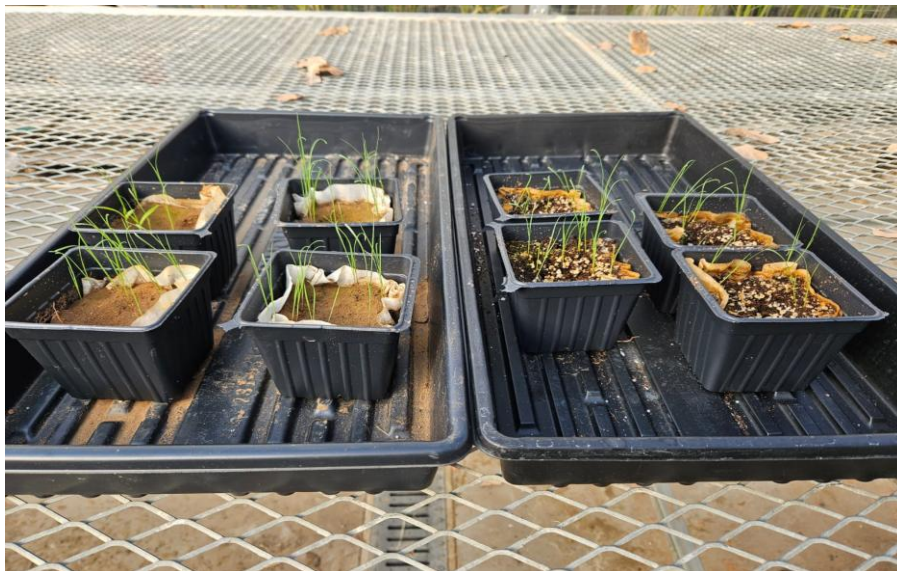


Figure C3. Seed germination observed in two soil types during Phase 1. The pots on the right contain greenhouse potting media, while those on the left are filled with field-collected control soil.

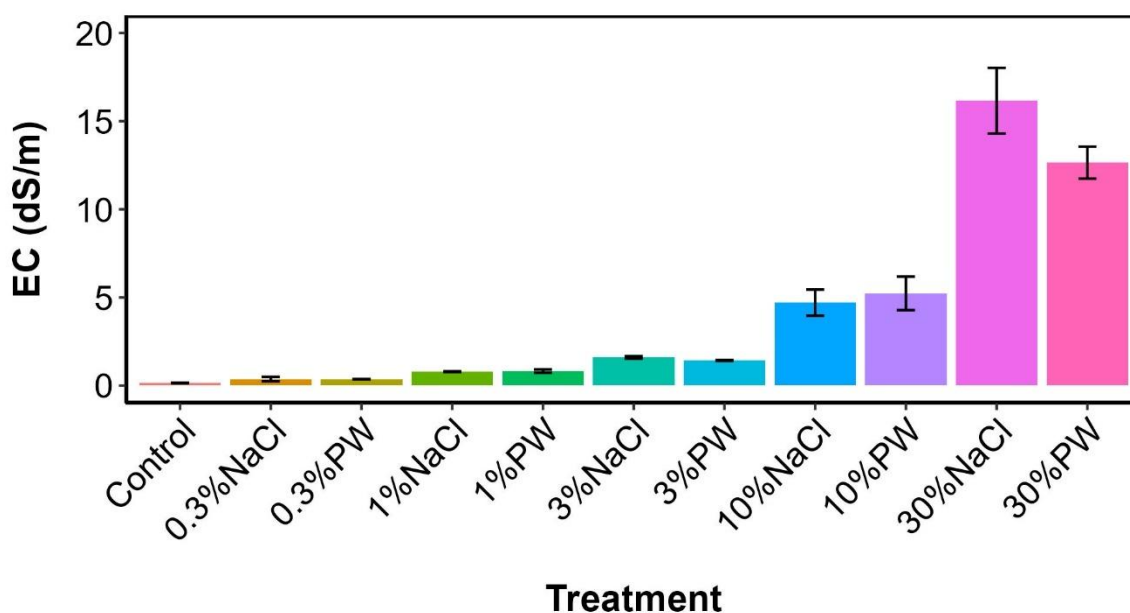


Figure C4: EC comparison in soils spiked with different dilutions of PW and corresponding NaCl solutions matched for salinity, measured before planting in Phase 2. Bars represent mean EC values; error bars indicate standard deviation across replicates (n=3). EC was measured using a 1:1 soil-to-water extraction method.

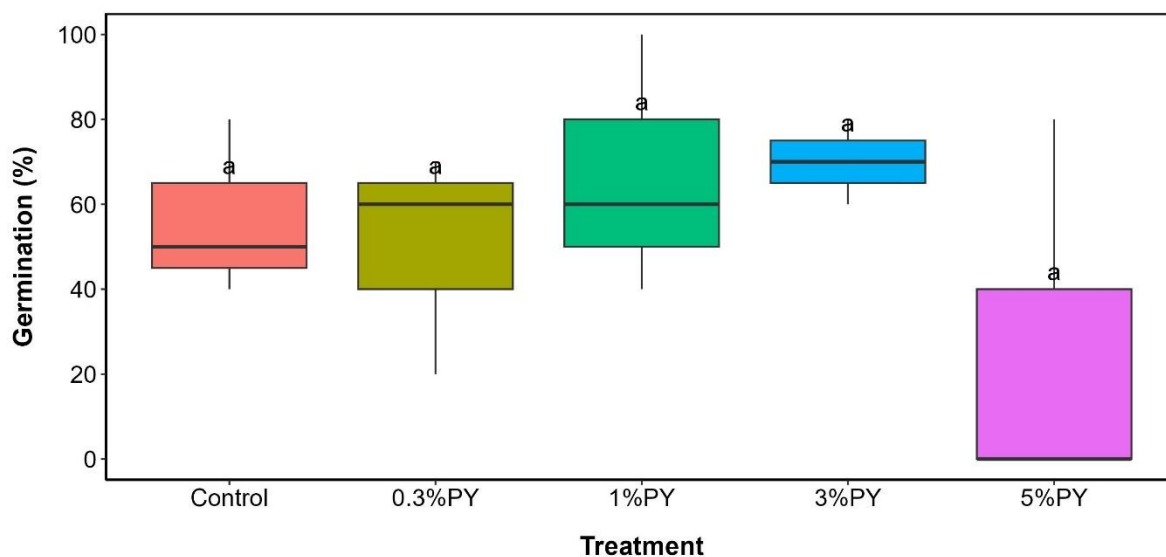


Figure C5: Percent germination of seeds under different concentrations of PY treatments. Boxplots represent the distribution of germination (%) across replicates for each treatment group. Treatments sharing the same letter (a) are not significantly different ($p > 0.05$).



Figure C6: Salt crust formation in PY-amended treatments.

Table C3: Soil quality parameters of the control soil utilized in the study.
EC, electrical conductivity; CEC, cation exchange capacity, OM; Organic matter.

Soil quality indicator	Value	Unit
pH 1:1	7.5	-
EC 1:1	0.16	dS/m
OM	0.9	%
NO ₃ -	44.5	ppm
P	8.2	ppm
S	21.4	ppm
K	198	ppm
Ca	2027	ppm
Mg	224	ppm
Na	10	ppm
CEC	12.5	meq/100g
Cl	10.2	ppm
B	0.31	ppm
Zn	0.42	ppm
Fe	2.3	ppm
Mn	1.2	ppm
Cu	0.24	ppm
Sand	71	%
Clay	11	%
Silt	17	%
Class	Sandy Loam	-

Table C4: Cyanide concentrations across soil treatments before planting and after harvest.

Treatment	Before planting (ng/g soil)	After harvest (ng/g soil)
Control	-	BDL
0.3%PY	BDL	BDL
1%PY	BDL	37.5
3%PY	BDL	60
5%PY	360.00	BDL
0.3%NaCl	BDL	92.5
1%NaCl	BDL	BDL
3%NaCl	BDL	82.5
5%NaCl	BDL	BDL
0.3%(NaCl+ PY)	BDL	BDL
1%(NaCl+ PY)	BDL	187.5
3%(NaCl+ PY)	160.00	BDL
5%(NaCl+ PY)	BDL	BDL
0.3%PW	-	BDL
1%PW	-	127.5
3%PW	-	32.5
5%PW	-	37.5
0.3%(PW+PY)	BDL	BDL
1%(PW+ PY)	BDL	BDL
3%(PW+PY)	BDL	297.5
5%(PW+ PY)	BDL	BDL

The lower detection limit for cyanide measurement in soil using the Hach kit for a 1:2.5 soil to water ratio is estimated to be 25 ng/g based on reported results for water sample analysis.

Table C5: EC of soil measured after harvest in phase 3 under different treatment conditions. Values are expressed as mean $\pm$ standard error (SE) based on replicate measurements ($n = 3$), using the 1:1 soil-to-water extraction method.

Treatment	EC (mean $\pm$ SE) dS/m
Control	0.17 $\pm$ 0.01
0.3%(NaCl+PY)	0.40 $\pm$ 0.02
0.3%(PW+PY)	0.36 $\pm$ 0.005
0.3%NaCl	0.21 $\pm$ 0.01
0.3%PW	0.33 $\pm$ 0.01
0.3%PY	0.18 $\pm$ 0.01

1%(NaCl+PY)	0.74 ± 0.01
1%(PW+PY)	0.61 ± 0.03
1%NaCl	0.51 ± 0.05
1%PW	0.58 ± 0.04
1%PY	0.23 ± 0.01
3%(NaCl+PY)	1.61 ± 0.08
3%(PW+PY)	1.54 ± 0.11
3%NaCl	1.62 ± 0.07
3%PW	1.41 ± 0.11
3%PY	0.17 ± 0.01
5%(NaCl+PY)	3.22 ± 0.03
5%(PW+PY)	2.21 ± 0.24
5%NaCl	2.720 ± 0.08
5%PW	2.562 ± 0.19
5%PY	0.198 ± 0.01

APPENDIX D: CO-AUTHOR CONTRIBUTIONS TO OIL AND GAS PRODUCED WATER
FOR CATTLE, CROPS, AND SURFACE WATER DISCHARGE: EVALUATION OF
CHEMISTRY, TOXICITY AND ECONOMICS

Reprinted from *Wiltse, Marin E., Brooke Ballenger, Connor B. Stewart, Tamzin A. Blewett, Claire Wadler, Holly K. Roth, Maelle Coupannec et al. "Oil and gas produced water for cattle, crops, and surface water discharge: Evaluation of chemistry, toxicity and economics." Journal of Hazardous Materials 494 (2025): 138581.*

According to CRediT criteria, my co-author contributions to this work included data visualization, writing – review & editing.



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Oil and gas produced water for cattle, crops, and surface water discharge: Evaluation of chemistry, toxicity and economics

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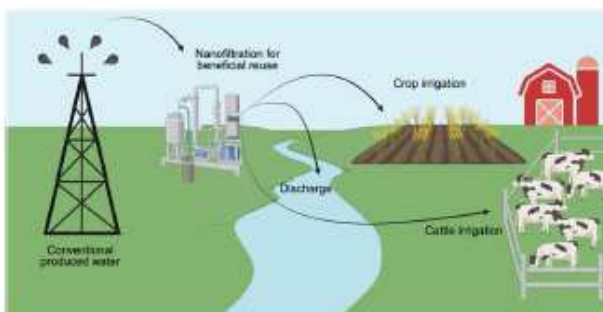
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HIGHLIGHTS

- PW had low concentrations of inorganic/organic compounds, near reuse guidelines.
- EC₅₀ showed little effect in evaporation ponds; organics likely controlled toxicity.
- AhR bioassay illustrated a level of toxicity not captured by the EC₅₀ test.
- Radium levels were generally low, but a few samples exceeded regulatory limits.
- TEA showed the PW can be beneficially reused for \$5.38/m³ (\$1.42/barrel) by NF.

GRAPHICAL ABSTRACT



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ABSTRACT

Oil and gas produced water (PW), may help alleviate regional water scarcity affecting agriculture, but is often rich in salts and organic compounds that constrain agricultural applications. The specific objective is to assess the reuse potential of conventional PW through a comprehensive assessment of chemistry, toxicity, and economics by investigating PW from 18 conventionally drilled wells from sandstone formations in the Colorado Denver-Julesburg Basin. Ammonium, total dissolved solids, boron, sodium, and chloride were all close to recommended guidelines for livestock and crop irrigation and surface water discharge. Diesel and gasoline range organics and polycyclic aromatic hydrocarbons were detected in low concentrations in evaporation ponds

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compared to oil water separators, suggesting volatilization or degradation of organic compounds. Radium levels were generally low, but select samples exceeded the regulatory 5 pCi/g threshold, categorizing them as Non-Exempt TENORM (Technologically Enhanced Naturally Occurring Radioactive Material) waste. EC₅₀ with *Daphnia magna* (*D. magna*) showed little to no toxicity for PW sampled in evaporation ponds in contrast to EC₅₀ values of 12 % at the oil water separator, indicating that volatile organics controlled toxicity. However, the Aryl Hydrocarbon Receptor (AhR) bioassay illustrated toxicity not captured by the EC₅₀ test. After chemical and toxicological analyses, it is clear that treatment is required, which informed our techno-economic assessment (TEA). Current PW volumes result in a treatment cost of \$5.38/m³ (\$1.42/barrel) by nanofiltration, but a scenario with increased volumes will result in a lower cost of \$3.83/m³ (\$0.60/barrel). Our chemical, toxicological, and economic assessment indicates that the PW in this study has potential to be discharged to surface water or reused for cattle and crop irrigation.

1. Introduction

Produced water (PW) is a byproduct of the oil and gas extraction process that is heavily influenced by the surrounding geology. [1] PW is often comprised of high salts and numerous contaminants of concern, such as hydrocarbons (including polycyclic aromatic hydrocarbons; PAHs), chemical additives such as surfactants and biocides, and other inorganic contaminants. [2–8] The inorganic fraction is typically dominated by sodium chloride, but can also contain metals, carbonates, and sulfates. Salts often contribute a large fraction of the total dissolved solids (TDS), ranging anywhere from 1,000 mg/L to 300,000 mg/L. [3] Studies have shown that the formation from which the PW originates dictates the types of contaminants and their concentrations in the water. [3,9] Oil and gas recovery from unconventional shale reservoirs often generates highly saline PW coupled with elevated levels of organic compounds. In contrast, conventional reservoirs, typically found in highly permeable rocks (e.g., sandstone) at shallower depths, produce PW with lower salinity and organic constituents. [10,11]

The type and concentration of organic contaminants in PW spans a wide range. Dissolved organic carbon (DOC) can range anywhere from 0.1 mg/L to 11000 mg/L. [9] The majority of DOC is contained within a colloidal suspension. [9] A large fraction of DOC is often comprised of hydrocarbons including gasoline range organics (GRO; C₆–C₁₂) and diesel range organics (DRO; C₁₀–C₂₆). The wide range of hydrocarbons presents difficulties for detection and removal due to the variability in size and molecular weight, but also creates differences in volatility and mobility. PW also contains PAHs, which are highly correlated with toxicity, even at low concentrations. [12–15] Surfactants, biocides, and other hydraulic fracturing fluid additives may also be present in PW and can change over time. Although these additives may appear harmless in their original form, surfactants, such as nonylphenol ethoxylates (NPEOs), can degrade into nonylphenols, which have been linked to endocrine disruption. [16]

Stewart et al. emphasized the difficulty in predicting PW's aquatic risk due to the complexity of organics and inorganics. [17] Significant spatiotemporal variation of PW has been reported based on LC₅₀ toxicity with *Daphnia magna*. [17] Other studies on PW's toxicity towards *Daphnia magna* reveal persistent effects beyond the 48 hr window of exposure, showing that PW not only affects reproduction, molting, and maturation, but also on metabolism and protein transport systems. [18]

The regulatory framework for PW management in the U.S. is anchored by the National Pollutant Discharge Elimination System (NPDES) and the Underground Injection Control (UIC) program. NPDES, under the Clean Water Act, regulates PW discharge to surface waters. East of the 98th meridian, the direct discharge of PW to the surface water is prohibited. West of the 98th meridian, discharge of treated PW to surface water for agriculture or wildlife beneficial reuse is allowed based on the Oil and Gas Extraction Effluent Guidelines and Standards (40 CFR Part 435 Subpart E). The UIC program, under the Safe Drinking Water Act, governs Class II injection wells to protect underground drinking water, primarily supporting reinjection practices in the water-scarce western regions. [19–21] The findings of this study provide critical insights into the water quality of PW effluents in Colorado and

the anticipated costs of achieving acceptable quality levels for applications such as surface water flow augmentation and irrigation of salt-tolerant crops.

The large volumes of PW being generated and the need for more sustainable management strategies have prompted governing agencies to advocate for oil and gas reform, while motivating the oil and gas industry to explore new methods for managing this water. [2,11,22–24] Currently over 95 % of PW is managed through underground injection. However, evolving challenges, such as fluid injection-induced seismicity and regional freshwater scarcity, have brought significant focus to the need for more diversified and sustainable management strategies. [20, 21,25] Several states including California, Colorado, New Mexico, Texas, and Wyoming have implemented or are considering reuse of treated PW outside oil and gas field activities, such as in-stream augmentation, agricultural irrigation, land application, and industrial uses. [7,26] Deep well injection increases seismicity and can cause groundwater contamination. [4,22,23,27,28] Evaporation ponds can similarly cause groundwater contamination, be hazardous for surrounding wildlife, [29,30] and release large amounts of volatile contaminants (e.g., PAH, BTEX; benzene, toluene, ethylbenzene, and xylenes) into the atmosphere. [29,31,32] To address the current challenges of PW management, new strategies for reusing PW outside the oilfield must be evaluated. The volumes of PW generated, along with typical well pad locations, makes PW reuse appealing for crop production, cattle industries, and surface discharge, but high concentrations of sodium and chloride pose challenges for agricultural use.

Sodium can displace essential ions like calcium and magnesium, leading to soil sodicity, which in turn causes degradation of soil structure. [33–35] Boron concentrations may be as high as 60 mg/L, [5,36] far exceeding the threshold of 3 mg/L at which plants may exhibit negative effects. [37] Additionally, carbonates in PW are often elevated, contributing to a more alkaline pH, which has long-term implications for soil and crop health. [38] While raw PW cannot be reused directly after oil recovery, it can be treated to a higher water quality standard enabling potential reuse outside the oilfield. [23,27,39,40] Treatment and desalination techniques such as nanofiltration, reverse osmosis, thermal distillation, and electrochemical treatment are capable of treating PW for beneficial reuse, but often at a very high cost reflective of high energy demand. [5,24,41–43] Treatment trains have also been investigated to combine techniques including coagulation, bioreactors, adsorption, ion exchange, and membrane distillation. [44–47] Such techniques may remove up to 99 % of constituents and greatly reduce toxicity, but still require high operating costs, which can make them unappealing. [5,48] While previous studies have investigated the economics of unconventional PW treatment, little to no studies have investigated treatment of conventional PW of low salinity.

In Colorado, there are over 50,000 active wells that produce over 170 million barrels of oil and 200 million barrels (44.5 million m³) of PW a year. [49,50] The Denver-Julesburg (DJ) basin, which stretches across Colorado, Nebraska, and Wyoming, is the fifth-largest producing basin in the U.S. In this study, we investigated conventionally drilled sandstone wells across a 50-mile radius in the DJ Basin in Colorado to determine the variation in PW quality and the potential for economical

PW treatment and reuse outside the oilfield. In order to evaluate potential safe management strategies, the water needs to be properly characterized both chemically and toxicologically. Key constituents of concern include TDS, pH, ammonium, boron, metals, naturally occurring radioactive material (NORM), and nonpurgeable organic carbon (NPOC). We assert that it is imperative that water quality analyses expand into more specific subsets, including PAHs, surfactants, and DRO/GRO, which are often associated with toxicity. Oil and gas wells in eastern Colorado are often located in areas with dryland agriculture due to limited freshwater, thus access to new water sources has the potential to improve crop production or establishment of animal agriculture such as dairy farms. The overarching goal of this paper is to evaluate the reuse potential of conventional PW through chemical, toxicological, water treatment, and economic evaluation for agriculture and surface water discharge.

2. Methods

2.1. Sampling

The PW samples were collected from 18 conventionally drilled well sites in eastern Colorado (Figure S1) in July and September 2023, totaling two trips. The wells were subcategorized by cardinal direction: north wells (indicated by the letter N), and south wells (indicated by the letter S). Each well was assigned a number to differentiate individual wells located within the same cardinal direction. At each well site, water was sampled from the oil/water separator (A), the pit outlet (B), and the evaporation pond (C), to help elucidate potential chemical release due to evaporation or breakdown (Fig. 1). At the evaporation ponds, sampling was consistently done close to edges of the evaporation pond (within 5–6 feet of an edge) and within one foot of the surface. Samples for organic analysis were collected in one liter acid-washed and combusted glass bottles. For inorganic analysis, samples were collected in methanol-rinsed and heated high density polyethylene bottles. All wells originated from sandstone in the DJ basin, and north and south wells were approximately 50 miles apart. All south wells were in the J Sand formation, while the north wells bordered or were located in the D Sand formation. The D Sands have pyritic sandstone, while the J Sands is composed of glauconite, which due to natural weathering can influence the concentrations of inorganics found in PW. [51] Four blanks were prepared for each sampling trip, (two trip blanks, which were glass liter bottles filled with ultrapure water and travelled to each well pad, but were never opened, and two field blanks, which also were glass liter bottles filled with ultrapure water and opened at various well pads), totaling 8 blanks from two sampling trips.

2.2. Analytical Methods

2.2.1. Chemical Analyses

A battery of chemical analyses was completed to elucidate which common PW constituents are present at each step of the PW lifecycle and should be included in future PW reuse guidelines. For each chemical analysis, there were method blank quality controls. These included both field and trip blanks (8 total blanks). Electrical conductivity (EC) and pH were measured by a dual pH/EC meter. TDS were measured using an evaporative approach in which 15 mL of PW were placed in Erlenmeyer flasks and then dried in an oven at 180°C for 16 hours or until dry. [52] The weight was measured before and after drying. Nitrate, nitrite, and ammonium were measured via O.I Analytical 3700 Automated Chemistry Analyzer. NPOC, total nitrogen (TN), and dissolved inorganic carbon (DIC) were measured with a Shimadzu TOC-L total organic carbon analyzer combined with a TNM-L total nitrogen unit.

Samples were filtered with a 0.22 µm poly(ether sulfone) (PES) membrane filter and acidified with 2 % trace metal grade nitric acid for metal analysis. Boron (B), sodium (Na), magnesium (Mg), aluminum (Al), copper (Cu), sulfur (S), calcium (Ca), chromium (Cr), manganese (Mn), zinc (Zn), arsenic (As), selenium (Se), strontium (Sr), barium (Ba), lead (Pb), and iron (Fe) were quantified via ICP-MS.

Samples for ion chromatography (IC) were all filtered with 0.22 µm PES membrane filter before analysis. Chloride (Cl⁻), fluoride (F⁻), and sulfate (SO₄²⁻) were all quantified by a Dionex ion chromatograph.

DRO (decane, dodecane, tetradecane, hexadecane, octadecane, eicosane, docosane, tetracosane) and ORO(3-methylpentane, 2,2,4-trimethylpentane, benzene, toluene, ethylbenzene, m-xylene, o-xylene, 1,2,4-trimethylbenzene) were analyzed via EPA method 8015D using gas chromatography flame ionization detection (GC-FID). These samples were prepared using liquid-liquid extraction (10 mL sample: 1 mL dichloromethane) resulting in a tenfold concentration.

PAHs (fluorene, acenaphthene, fluoranthene, anthracene, naphthalene, chrysene, acenaphthylene, pyrene, perylene, benzo[a]pyrene, benzo[a,h]anthracene, 2,6-diisopropylanthracene, 2,3-dimethylnaphthalene, 9-methylantracene, 9,10-dimethylantracene, 1-phenylnaphthalene, 2-ethylnaphthalene, 1-methylnaphthalene) were extracted by manually shaking 100 mL of PW with 3 mL hexanes for 10 min, followed by extraction of the organic layer. After repeating this three times, the organic layer was evaporated until it was less than one mL and then resuspended in 1 mL dichloromethane. [53] According to Jeong et al., samples were analyzed using atmospheric pressure gas chromatography (APGC) coupled with a WatersXevoG2 quadrupole time-of-flight (QTOF) mass spectrometer where one microliter of sample was injected into an Agilent DB-5MS column (30 m × 250 µm × 0.25 µm) under a pulsed splitless mode. The chromatography temperature gradient was set to

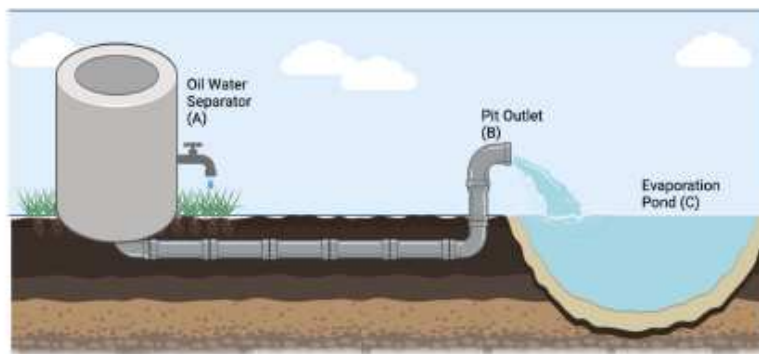


Fig. 1. Schematic diagram showing the three produced water collection locations from each well. Water was sampled from the oil water separator (A), pit outlet (B), and lined evaporation pond (C).

ramp at 18 °C/min, and the final temperature was 330 °C creating a total run time of 17.0 min. The mass spectrometer was run at a collision energy of 4 V with 0.1 s of scan time in positive mode. The analyzer was set to an API source and a scan range of 50–1200 Da. [5]

To analyze the presence of organic surfactants, 250 mL of each PW samples were filtered through a 0.7 µm glass fiber filter and then a 0.22 µm PES membrane filter. Solid-phase extraction (SPE) was conducted using Supel Select HLB cartridges (200 mg/3 mL, Supelco, Bellefonte, PA) to concentrate the surfactants and remove salts. Extracts were stored at –20 °C before the analyses. Surfactants were characterized using an Agilent 1100 series liquid chromatograph coupled with an Agilent G3250AA time-of-flight mass spectrometer (LC/TOF/MS) equipped with a ZORBAX RR Eclipse XDB-C8 (4.6 mm × 150 mm, 3.5 µm) column, according to Thurman et al. and Jeong et al. [5,54]

Radium analysis was performed on 6 different wells at both the oil water separator and evaporation pond (12 samples total) using gamma spectrometry with a High Purity Germanium detector (HPGe). This subset was chosen based on varying salts and NPOC concentrations. A 500 mL sample was placed in a PETE jar (Alpha Packaging, Greenwood, AR). To ensure a radon-tight seal, the jar lid was wrapped with parafilm, and the sample was vacuum sealed in a plastic bag. [55] Prior to measurement, the samples were stored for 28 days to allow Ra-226 to reach secular equilibrium with its progeny, Pb-214 and Bi-214. All samples were counted for 4 days on a 70 mm, 65 % efficiency coaxial HPGe detector (GEM-65200-P-S, Ortec, Oak Ridge, TN). Radium-226 activity was determined based on the Bi-214 peaks, while Ra-228 was quantified using the Ac-228 peaks. Spectrum analyses were performed with the Genie 2000 V3.4.1 software (Mirion Technologies, Atlanta, GA). Each spectrum was corrected for background radioactivity using a blank consisting of deionized water, counted under the same condition as the samples. The Minimum Detectable Activities (MDA) were calculated according to ISO 11929. [56] A 500 mL standard was prepared gravimetrically from NIST traceable stock solutions (Eckert & Ziegler, Atlanta, GA) for energy and efficiency calibrations specific to this geometry. The standard contained Eu-152 (286 ± 7 Bq), Ba-133 (44.5 ± 0.8 Bq), Cs-137 (164 ± 3 Bq), and Co-60 (8.9 ± 0.1 Bq). Coincidence summing in the standard was corrected through ISOCS geometry modeling. The measured activities were converted from pCi/L to pCi/g using the TDS (g/L) of each sample, as described in the Implementation Guidance for 6 CCR 1007-1 Part 20 – Technologically Enhanced Naturally Occurring Radioactive Material (TENORM). [57]

2.3. Toxicity Methodology

2.3.1. *Daphnia* colony maintenance

Daphnia magna obtained from Aquatic Research Organisms (ARO, USA, November 2023) were cultured at the University of Alberta in the Department of Biological Sciences aquatics facility following Organization for Economic Cooperation and Development (OECD) guidelines. [58,59] Organisms were cultured under a 16:8 light:dark cycle and 20 ± 0.7 °C using previously described methods. [17] *Daphnia* were fed a daily ration of 3 mL of 3×10^7 cells mL⁻¹ freshwater green algae (*Rhodocyclis subcapitata*; ARO) and 3 mL of yeast, cereal leaf and trout chow mix (YCT; ARO). Full renewal of colony water occurred every 2–3 days and the colony was continually seeded by < 24 hour old neonates from the second brood or later, maintained for maximum of two months. Colony health was monitored throughout the study via toxicity assays using copper as a reference toxicant to ensure the *D. magna* did not display unexpected sensitivity.

2.3.2. Toxicity testing

Acute median lethal toxicity testing (LC₅₀) and acute median effect toxicity (EC₅₀) tests with *D. magna* were executed in order to obtain standard acute toxicity in an aquatic environment. 3 wells, 2 from the south sites and 1 from the north sites, were tested from both oil water separator and evaporation locations. The subsets of samples were chosen

by having a range of salt and NPOC concentration. Acute toxicity tests were conducted over 48-hours according to OECD guidelines, with slight modifications to determine the concentration of treated PW causing 50 % lethality (LC₅₀) or 50 % immobility (EC₅₀) in *D. magna*. [58] More details concerning the methodology of LC₅₀ and EC₅₀ can be found in the [supplementary information](#). All statistical analysis was carried out using R version 4.0.3. [60] LC₅₀ and EC₅₀ analyses were performed using the probit method from the “ecotox” package performed using R version (4.3.2). LC₅₀ values are considered significantly different when the 95 % confidence intervals (CIs) do not overlap.

2.3.3. AhR Toxicity

Aryl Hydrocarbon Receptor (AhR; INDIGO Biosciences, State College, PA) assays were used to evaluate acute toxicity of the samples with the manufacturer's proprietary mammalian cells that are engineered to provide constitutive expression of the AhR. 12 samples from oil water separator locations and 3 samples taken from both oil water separator and evaporation pond were tested. Samples were stored at 4 °C before solid phase extraction was performed using HyperSep C18 cartridges (Thermo Scientific, Rockwood, TN). A more detailed description about AhR toxicity sample preparation can be found in the [supplementary information](#).

The background activity, found from method blank samples, was subtracted from the sample activity. The percent relative activation of AhR was found by dividing the sample activity by the highest activity on the standard curve. The standard curve was plotted using GraphPad Prism using a 4-parameter variable slope, least squares method. The percent relative activations were mapped onto the standard curve and assigned a biological equivalent (BEQ) of the 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) standard. The actual BEQ value in the original water sample was back calculated based on the dilution and concentration of the sample.

2.4. Economic Analysis Method

A techno-economic analysis (TEA) was conducted to estimate the cost-effectiveness of treating the sampled PW via nanofiltration, a treatment technique that is very likely to remove necessary levels of organics and inorganics for agricultural reuse based on our previous study. [5] The TEA evaluated a base scenario with a water processing capacity of 130 m³/day and a future scenario with a capacity of 3440 m³/day. The economic analysis considered capital costs for solar and nanofiltration (NF) equipment, as well as operation and maintenance (O&M) expenses for these units. Additionally, costs include transporting the treated water 30 miles to a centralized elevated storage tank where farmers can access the water for their livestock. The tank has a 5-day storage capacity for both scenarios. [61] Capital costs, labor, and maintenance expenses were sourced directly from the literature, averaging reported values when multiple sources were available.

The NF system utilizes thermal energy generated by a flat panel solar collector with a 20 % efficiency, operating under solar radiation of 410 W/m² for 9.7 hours per day, corresponding to January conditions in Washington County, Colorado. [62,63] The solar unit consists of a flat panel solar collector, a lithium-ion battery for energy storage, and a converter, as the system is assumed to be grid-connected. Key system inputs for cost determination include the area of the solar collector and the yield of clean water.

O&M costs for the NF process encompass thermal energy, pre-filter and membrane replacement, and labor. Energy is required to increase pressure at the NF membranes, with the pressure range expected to be 15–20 bar, and membrane water permeability standardized at 10 °C. [64,65] The system's anticipated recovery rate is 75 %. Non-membrane costs for the NF unit include equipment necessary to support membrane use, such as NF vessels, high pressure pumps, and treated water pumps. Fixed costs are accounted for by adding 2 % of the NF unit's capital costs per year. [66] Additionally, NF membrane replacement costs are

estimated at 5 % of the membrane cost annually.

The net present value (NPV) was calculated using parameters and assumptions detailed in Table S1, which includes costs, revenues, annual interest rate, and plant lifetime. The NPV provides an overall balance between revenues and costs, offering a comprehensive assessment of the economic viability of the system.

3. Results and discussion

3.1. Inorganic constituents

DIC, pH, TN, and ammonium were measured (Figure S2A-D) and are all important parameters to prevent adverse impacts for crop and cattle production and surface water discharge (Figure S2A-D). The pH, seen in Figure S2B, was approximately 8 and remained consistent across both north and south sites, and the slight basicity was likely due to the high concentration of inorganic carbon present. The north wells (324 mg/L) had significantly higher DIC concentrations compared to the south wells (211 mg/L) (Figure S2A). Alkaline irrigation water can result in limited availability of soil nutrients necessary for crop and plant growth due to the resulting increase in soil pH resulting in considerations towards long term soil quality and management strategies. [67–69]

Total nitrogen, nitrogen found as ammonium, organics, nitrite, and nitrate, was primarily driven by ammonium (50–90 % ammonium) (Figure S2C and D). Due to the pH of the PW being between 7 and 8 on average, ammonium ions are more present than ammonia. Ammonium is not considered as toxic as ammonia in aquatic organisms and requires high concentrations for plant toxicity, unlike its unionized form of ammonia. [70–73] The decrease in concentration from oil water separator to the evaporation pond is likely a result of uptake by plants, volatilization, and microbial transformation. Ammonium (average concentration of 1.5 mg/L) was well below toxic concentrations (3.14 mg/L) in the PW, even when it exits the oil-water separator, where levels are expected to be highest. [74] The levels of ammonium, TN, pH, and DIC illustrate levels of low risk or hazard for agriculture, cattle irrigation, and discharge, but these just represent general water quality parameters and not specific PW constituents. It is important to determine the potential presence of specific toxic constituents, such as metals, boron, NORM, and organics, since they often drive the risk and hazard associated with PW.

Typically, PW has a high TDS concentration (1000–300,000 mg/L), which often limits the treatment and reuse potential. [3] At our sites, TDS differed significantly between the north and south wells, with the north wells being significantly higher (Fig. 2 A). The same trend was observed for sodium (Na) and chloride (Cl) concentrations (Figs. 2 B and 2 C), possibly due to differences in bed layers, mineral compositions, and porosity of the formations. [2,3,24] While the two geographic locations differ significantly in the concentration of TDS, Na, and Cl, the concentrations at both geographic locations were towards the lower end of previously studied PW in the DJ-Basin. [3,75–77] However, TDS, Na, and Cl for the north wells were slightly above (10–30 % higher) recommended guidelines for livestock and crop irrigation or discharge requirements while the south wells were below these (Table 1). [76–80] Boron is a critical metalloid to look at, especially when it comes to crop production. Boron concentrations remained similar across all locations (1.36 mg/L average and ranging from 0.55 to 3.25 mg/L) (Fig. 2D) and were slightly above recommended discharge requirements and irrigation guidelines. While boron is an important micronutrient for plants at low concentrations (0.1–1 mg/L) it becomes toxic above these levels. [87,81] The long-term agricultural reuse guideline for boron, established by the USEPA, is 0.7 mg/L. [82] Given the concentrations determined in the north and the south sites, the effluents may result in slight to moderate restrictions (0.7–3.0 mg/L). The determined boron levels may still be suitable for boron-tolerant species, such as grasses, which can tolerate concentrations up to 10 mg/L. [88] Additional inorganic elements, were analyzed by ICP-MS and described in the supplementary information, but all concentrations were below levels of concern for irrigation (Table S2).

Non-Exempt TENORM-produced fluid wastes are defined as containing radium concentrations, excluding natural background, greater than 5 pCi/g for both Ra-226 and Ra-228. The approved background values for the state of Colorado are 1.4 pCi/g for Ra-226 and 1.3 pCi/g for Ra-228. [94] Radium levels measured in samples N1, S3, and S5 exceeded the 5 pCi/g threshold, categorizing them as Non-Exempt TENORM waste and prohibiting their release (Figures S3 and S4). For most samples, the radium activity measured in the produced water collected from the oil-water separator was higher than in those collected from the evaporation pond. This slight decrease in activity may be explained by TENORM attaching to particles that settle in the ponds or the formation of a precipitate. In contrast, for other wells from northern

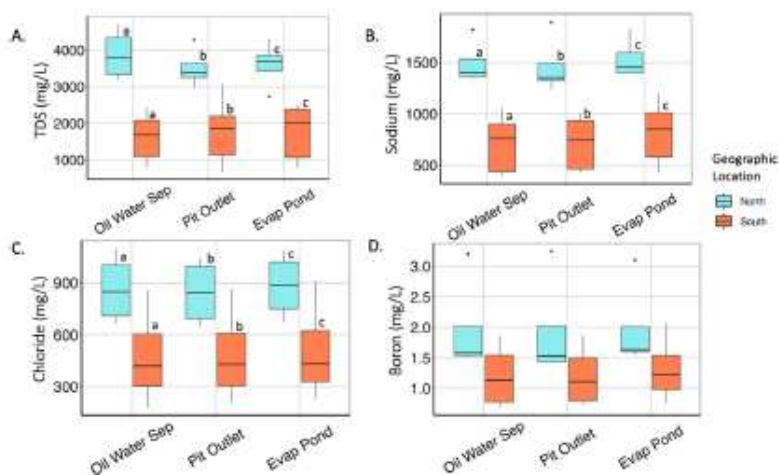


Fig. 2. (A) Total Dissolved Solids (TDS mg/L), (B) sodium (Na mg/L), (C) chloride (Cl mg/L), and (D) boron (B mg/L) observed at North ($n = 4$) and South ($n = 14$) sites and across the three sampling locations (i.e., oil water separator, pit outlet and evaporation pond). The lowercase lettering indicates two points significantly different from each other, using a student *t*-test.

Table 1

Regulations and guidelines of various water quality parameters compared to site concentrations. All values in mg/L unless otherwise specified. "A" indicates oil water separator, while "C" indicates evaporation pond sampling location.

	Average North A Concentration	Average South A Concentration	Average North C Concentration	Average South C Concentration	Regulation for Discharge	Guidelines
TDS	3878	1618	3601	1788	3500 ^a	450 ^c , 3000 ^b
Na	1492	715	1534	805	–	1000 ^c , 460 ^b
Cl	666	516	708	546	250 ^b	1500 ^c , 700 ^b
B	1.35	1.36	1.41	1.48	0.75 ^b	0.7 ^c , 1.0 ^b
BTEX	0.256	0.768	0.040	0.016	2.66 ^a	–

^a Produced Water Treatment Facilities COG840000

^b Evaluation of water quality and nutrition for Dairy Cattle, DAIREXNET

^c Irrigation water quality criteria - 0.506

^d EPA 2012 Guidelines for Water Reuse

and southern sites, no radium activity above the MDA reported in Table S3 was detected in the produced water after a four-day counting time. Although this indicates generally low radium activity, the classification of these fluids as Non-Exempt TENORM wastes cannot be definitively concluded since the MDA for Ra-226 and Ra-228 exceeded the limit of 5 pCi/g for select samples.

According to the regulations reported in Table 1, most south sites tested meet the recommendations/requirements for reuse in all inorganic categories, while the north sites tested were slightly above recommendations. It is important to note that current EPA guidelines indicate that TDS should be less than 450 mg/L for unrestricted irrigation, which none of the sampled sites meet. However, irrigation of more salt tolerant crop types can still be considered. [82] While TDS, Na, and Cl were slightly high for plants like wheat (the most common crop in Colorado), there are numerous cost-efficient management practices a farmer can employ to ensure the soil salinity is below the tolerance for the crop. The addition of calcium and magnesium is a common practice for managing sodic soils, and can help lower the sodium adsorption ratio (SAR) and thus improve the soil health. [85] Other management techniques include using cover crops to increase water permeability and infiltration, and adding organic amendments to encourage aeration and porosity of the soil. [85,86]

3.1.1. Organic Constituents

DRO, GRO, and PAHs are large compound groups commonly present

in PW. These organic compounds are all toxic, but the physicochemical properties of these organics (such as volatility, mobility, and chemical stability) could potentially reduce bioavailability and exposure, thereby minimizing adverse effects on potential receptors. DRO, GRO, and PAHs concentrations varied greatly across sampling locations and between all sites regardless of geographic location. Typically, the south sites exhibited higher concentrations of organic compounds (Fig. 3), but the median values are comparable between the north and south sites. The NPOC (Fig. 3 A) concentrations were relatively low and remained similar at both the oil-water separator and the evaporation pond indicating that the NPOC was primarily made up of nonvolatile species. [39, 87,88]

Biocides and surfactants are often added to wells to help separate oil and water and maintain the health of the well, [89–91] and likely comprise a substantial fraction of the measured NPOC. Figure S5 shows a select group of surfactants found in the water and their relative abundances. Biocides, such as benzyltrimethyl tetradecylammonium, were present but not very abundant, so this discussion is limited to the presence and potential toxicity of the more prevalent surfactants. For example, NPEOs and octylphenol ethoxylates (OPEO) are prevalent in oil and gas wells, and can be broken down into nonylphenols and octylphenols. [92] Nonylphenols and octylphenols can contribute to toxicity, [93] which could be a concern for agricultural reuse. [94] Many NPEO and OPEO formulas were assigned and present in both the A and C locations, but the number of formulas or their relative abundance were

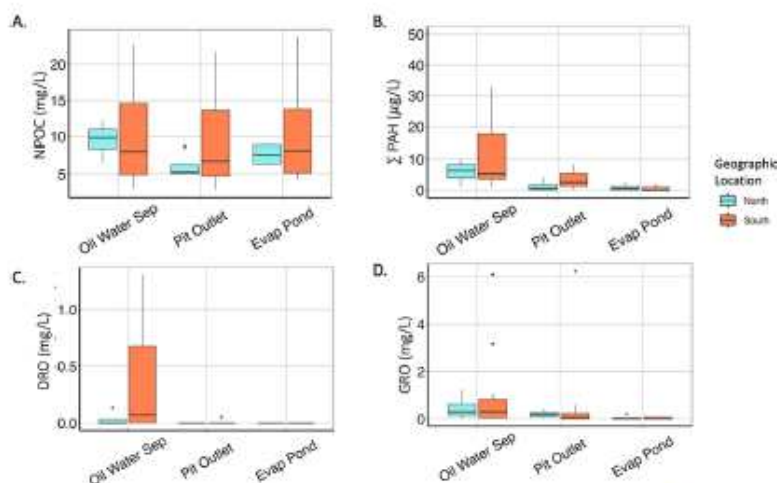


Fig. 3. (A) Nonpurgeable organic carbon (NPOC; mg/L), (B) sum of polycyclic aromatic hydrocarbons (ΣPAH; μg/L), (C) diesel range organics (DRO; mg/L), and (D) gasoline range organics (GRO; mg/L) observed at North (n = 4) and South (n = 14) sites across the Oil Water Separator, Pit Outlet, and Evaporation Pond sample locations.

typically lowest within the evaporations ponds. The decrease could be due to microbial breakdown of the surfactants in the evaporation ponds, [95] or may be attributed to surfactant sorption to sediments or algae in the ponds.

While a large proportion of the NPOC was likely surfactants, there were numerous other compound groups that can be a concern even at low concentrations. DRO and GRO encompass a wide range of hydrocarbons including BTEX. [12,76,96] As seen in Fig. 3 C and 3D, the sum concentrations of both DRO and GRO were low, but GRO was present in higher concentrations than DRO, particularly at the oil water separator (A location). Typically, DRO are reported at higher concentrations than GRO due to their comparatively lower volatility. However, the A samples were collected from a closed system, and it is thus unlikely that the GRO compounds would volatilize under those conditions. Additionally, the concentrations of both DRO and GRO are heavily dependent on the amount of oil present. In this case, the water to oil ratio is high, resulting in low concentrations of DRO and GRO in the PW. In general, the DRO and GRO concentration ranges for the south wells was much wider than the north wells but the median concentration was similar between both geolocations, like the observation for NPOC. For both DRO and GRO, the concentration decreased across the A, B, and C locations due to the high volatility or biodegradation of the compounds. The evaporation ponds were nonuniform and their depth varied significantly, which may have contributed to the chemical variation observed between sampling sites. However, biodegradation of organic pollutants in the evaporation ponds is likely a major removal pathway in addition to sorption.

PAHs, a compound group highly correlated with toxicity, [13,14,97] naturally occur in crude oil and are common in PW. [97,98] Fig. 3B shows a summation of 18 PAHs present in the PW (individual PAHs compounds can be found in Table S4). Some PAHs (e.g., benzo[a]pyrene, pyrene, and acenaphthene) are toxic at the hundreds of $\mu\text{g/L}$ [13, 14,98,99] levels, however, most well sites and sampling locations (50 out of 54 sampling locations) tested were below a cumulative concentration of 20 $\mu\text{g/L}$, indicating there was low risk of PAHs toxicity. [100] While PAHs can be considered toxic at low concentrations, when discharging into rivers and streams, the concentrations will be further diluted, minimising the concerns associated with PAHs toxicity. McLaughlin et al. demonstrated that with low concentrations of PAHs in discharged PW, there was no increase in developmental toxicity for zebrafish. [100] However, PAHs are a concern due to their low water solubility, which limits mobility but promotes binding and accumulation in soils. [101,102] PAHs can persist in soils for years, making repeated use of insufficiently treated PW, with high concentrations of PAHs, for crop irrigation a potential concern. [103] The main mechanism for PAHs removal in soils is through microbial degradation, but chemical structure, concentration, and competition with other PAHs influence the degradation rate. [104] While the concentrations of PAHs in the conventional PW studied here are low, further studies into potential bioaccumulation in crops and biota are needed to better understand the implications of long term application of PW with these levels of PAHs for irrigation purposes. [105]

3.1.2. Toxicological Evaluation

While this study focused on a single timepoint, it is important to consider the long-term effects this PW might have with repeated field application. Based on the above analysis of the PW constituents, the water has a high potential for reuse for cattle irrigation or crop production after minimal treatment; however, chemical analysis alone cannot be used to evaluate the actual toxicity of the PW.

Standard 48 hour LC_{50} and EC_{50} tests for 3 wells sampled at the oil water separator and evaporation pond locations were completed with *Daphnia magna*, to provide information on the acute toxicity of the of the PW in an aquatic environment. [27] Oil-water separators (A) and evaporation pond (C) samples were tested to show potential changes in toxicity due to evaporation, biodegradation, and sorption. LC_{50} represents the concentration of PW (% v/v) that kills 50 % of the exposed

D. magna (Figure S6), while EC_{50} represents the concentration of PW (% v/v) that immobilizes or kills 50 % of the exposed *D. magna*. The trends of significantly reduced toxicity in the evaporation ponds (C) compared to the location of the oil water separators (A) are best illustrated when looking at immobility and lethality (EC_{50}) as an endpoint rather than simply lethality. While immobilized neonates may be technically alive based on the strictest definition, their inability to move will make them unable to feed or reproduce and will greatly increase their chances of dying due to exposure to unfavorable conditions or predation. These factors make immobilized *D. magna* ecologically dead, while still alive, since they are incapable of propagating the next generation of neonates and will die prematurely, albeit not during the 48 h observation window of the standardized assay. Similar to the lethality results, comparison of N4.A (north oil water separator) to N4.C (north evaporation pond) showed a significant reduction in toxicity in the C sample, with an EC_{50} of 88.9 %, compared to the EC_{50} in the N4.A sample of only 14.0 % (Fig. 4). These results indicate that the evaporation pond sample must be over 6 times more concentrated to elicit the same degree of immobilization as the oil water separator sample.

These results seem to indicate that the concentrations of certain toxicants were reduced, and those reductions had a significant impact on the adverse effects (immobility and mortality) at lower concentrations. The dose-response assessment on *D. magna* shows that processes, such as evaporation, degradation and adsorption to sediments play an important role in controlling the toxicity.

The PW, as seen in Figure S5, contained numerous surfactants which can create an emulsion that contribute to the immobilization of the *D. magna*. [106] Across the south sites and north sites collected at the oil water separator, the toxicity level for both EC_{50} and LC_{50} is relatively high and is consistent with other studies. [5] This was likely driven by the combination of organic compounds and high salt concentrations and the limited volatilization of toxic compounds within a closed system. In contrast, the toxicity level was drastically lower in the evaporation pond. EC_{50} values couldn't be calculated for the evaporation ponds within the south wells indicating that the PW did not affect the mobility of *D. magna*, while the north well that was tested still has a percentage that was calculated. The percentage was high (88.9 %) for the north well pond sample (N4.C), indicating that at high PW concentrations slight impacts on mobility were observed, possibly due to the presence of low concentrations of contaminants such as BTEX. On average, north well evaporation ponds were 2.5 times higher in BTEX than south wells (Table 1). The same trend was seen in LC_{50} (Figure S6), where the PW in north wells were generally more toxic than south wells, strengthening the hypothesis that BTEX was a likely contributor. Other organics, such as DRO, GRO, and PAH, followed the same decreasing trend from the oil water separator to evaporation pond, leading to the hypothesis that organic species are one of the main drivers towards toxicity, unlike other studies which have shown that salinity was the dominant driver.

While LC_{50} and EC_{50} toxicity tests provide valuable information regarding water quality, there has recently been a push for implementation of acute toxicity tests that are based on mammalian cells to better simulate potential toxicity towards humans. The AhR bioassay is a non-targeted method of detecting the presence of PAHs, dioxins, and dioxin-like chemicals, and have been used to assess the toxicity in produced water samples in other studies. [40,107–109] Water quality analysis performed on the PW samples indicated that each sample contained various PAHs at microgram per liter levels (Table S4). Bioactivity was detected in all produced water samples, with TCDD-BEQs ranging from 1.93 ng/L (S14) to 11.2 ng/L (S6), with an average value of 5.05 ng/L and standard deviation of 2.47 ng/L across the twelve sites (Figure S7).

Little to no reduction in bioactivity was observed downgradient of the oil water separators which indicates that there are toxic compounds binding to the receptors that are not being removed in the evaporation pond by volatilization or sorption (Fig. 5). Unlike the EC_{50} , which indicated a reduction in toxic compounds within the evaporation ponds,

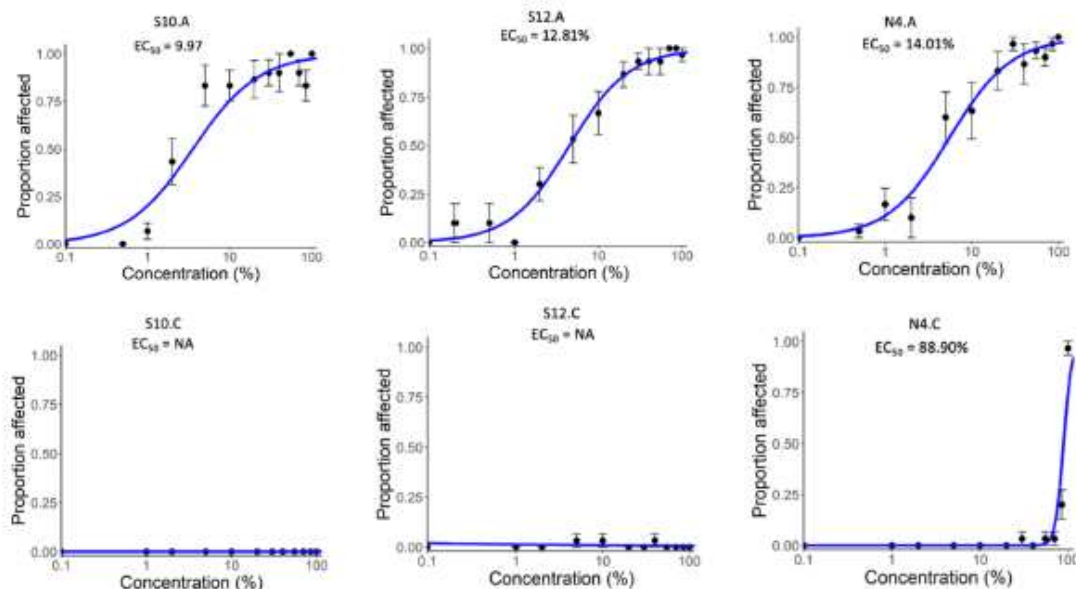


Fig. 4. Half maximal effective concentrations (EC_{50}) with *Daphnia magna* (*D. magna*) from two South wells (S10 and S12) and one North well (N4). Samples from both the oil water separator (A) and evaporation pond (C) were analyzed.

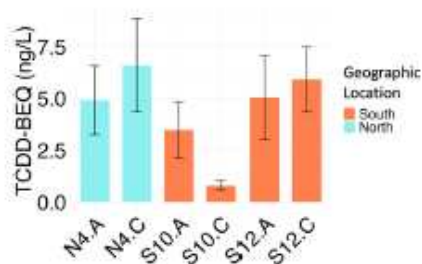


Fig. 5. Bar graph showing the toxicity of the produced water samples expressed as TCDD biological equivalents. "N" refers to north well sites. "S" refers to south well sites. A indicates sample was taken from the oil water separator, and C indicated the sample was taken from the evaporation pond. All numbers are specific wells, which can be seen in Fig. S1.

the AhR bioassay illustrate a level of toxicity not captured by the EC_{50} test. While there is no maximum contaminant level established for TCDD-BEQ, there are water recycling studies in which TCDD equivalents were used as a toxicity indicator. [110,111] The California State Water Board amended their Recycled Water Policy to include a monitoring trigger level (MTL) for TCDD-BEQ concentrations of 0.5 ng/L for recycled water intended for indirect potable reuse. [112]

Several studies have used AhR assays in PW, but few have used TCDD-BEQ representing a first for PW. Furthermore, Dagnino et al. (2010) noted the importance of similar incubation times when comparing TCDD-BEQs across different studies. [113] Longer incubation times (greater than 8 hours) have been shown to have reduced AhR activities in waters, as PAH-like compounds destabilize over longer incubation times, while dioxin like compounds stay stable up to 24 hours. [113,114] Macova et al. (2010) reported a value of 0.83 ng/L TCDD-BEQs in effluent from a sewage treatment plant, while Daniels

et al. (2018) reported values between 0.5 and 0.85 ng/L TCDD-BEQs in samples taken at municipal wastewater outfalls. [115,116] These results illustrate the ability of AhR bioassays to detect PAHs and dioxin-like compounds in wastewaters.

The relationship between the concentrations of the analyzed PAHs and TCDD BEQs was also explored. The AhR activity was plotted against various PAHs and summed combinations of the studied PAHs, with their R-Squared being calculated (Table S5). These R-squared values were all less than 0.5, suggesting little to no correlation between these PAHs and the TCDD-BEQ values. This indicates that other chemicals are driving AhR activities, as these 18 PAHs do not explain the AhR activities observed. Halogenated aromatic hydrocarbons, including benzyl chloride and 1,4-dichlorobenzene have been found in PW from other sites and may contribute to the results reported here. [117] These compounds were not targeted in our PAHs methods but could be investigated in future research to understand the chemicals eliciting this AhR response. Additionally, there could be other chemicals, such as dioxins or other aryl based compounds, that could be responsible for these AhR responses. [118]

While the toxicological and chemical results provide some confidence towards reuse, it is critical to conduct a treatment assessment and a TEA to fully understand the feasibility of the water's reuse potential for cattle and crop irrigation, and surface water discharge.

3.2. Techno-Economic Analysis (TEA)

The PW contained low concentrations of inorganic and organic compounds, but still resulted in toxicity levels of concern and thus must be treated before the water can be reused. Past research has shown negative effects with higher salinity water when simply diluted, indicating that treatment is essential to ensure sustainable reuse. [105,119,120] Nanofiltration (NF) is a potential treatment option that removes salts and organic to help meet the requirements for reuse. [5,121–123] In past studies, NF removed 20–40 % of chloride and 50–90 % of various organic compound groups, [5] which would bring salt and organic

concentrations, for all wells tested in this study, below a level of concern for reuse based on current water reuse guidelines as listed in Table 1. In order to determine whether treating the low salinity PW is economically feasible for livestock and crop irrigation, a techno-economic analysis was run using a NF treatment system and solar panels for energy production.

In the base scenario, the system required 236 kWh/day of energy, 121 m² of solar collector area, and a solar system capacity of 122 kW (Table 2). This configuration produced 103 m³ of clean water per day. The Levelized Cost of Water (LCOW) was 5.38 USD/m³ (\$1.42/barrel), with 43 % attributed to equipment costs, 50 % to operations and maintenance (O&M) costs, and 7 % to taxes. The lithium-ion battery constituted 61 % of the equipment costs and 33 % of the operating costs for the base scenario. The flat plate PV panel represented 9 % of the equipment costs and 6 % of the O&M costs. The NF unit accounted for 4 % of the equipment costs and 5 % of the O&M costs in the base scenario, with the membrane cost making up 49 % of the NF module's equipment costs.

In a future scenario, where the wells are optimized to generate more PW by increasing the pumping capacity, the system would require 5903 kWh/day of energy, 296 m² of solar collector area, and a solar system capacity of 3038 kW. This configuration would produce 2850 m³ of clean water per day. The LCOW was 3.83 USD/m³ (\$0.60/barrel) for this scenario, with 55 % attributed to equipment costs, 36 % to O&M costs, and 9 % to taxes. The lithium-ion battery constituted 74 % of the equipment costs and 70 % of the operating costs for the base scenario. The flat plate PV panel represented 11 % of the equipment costs and 13 % of the O&M costs. The NF unit accounted for 5 % of the equipment costs and 11 % of the O&M costs in the base scenario, with the membrane cost making up 49 % of the NF module's equipment costs.

Previous studies on PW treated via membrane filtration identified the lowest possible cost for LCOW as 1.19 USD/m³ (\$0.19/barrel). [124] Farmers who have access to freshwater typically pay between 0.05 and 0.25 USD/m³, placing the results of this study on the higher end of the cost spectrum. [125] However, our study sites are located within an area of dryland agriculture due to limited freshwater access and therefore access to treated PW has the potential for providing an alternative water source that can support crop and livestock production. [126] Other states in the US, like Oklahoma, have produced water treatment or disposal costs ranging from \$3.59/m³ (\$0.57/barrel) to \$44/m³ (\$7/barrel). [7] Overall, treatment costs range from \$2.90 to \$13.30/m³ (\$0.46 to \$2.11/barrel), depending on the TDS concentrations. [127] Our assessment indicates that treatment and reuse of the produced water is cheaper than current water management options. Thus, oil and gas companies will most likely be able to sell the treated water to local farmers at a competitive price point. Dryland agriculture is common in Eastern Colorado due to lack of irrigation water sources. Consequently, treated produced water could serve not only as a water source but also a way to increase crop yields and income for farmers. A study completed in Kern County, CA shows that purely by blending water, they've expanded the availability of irrigation water. [10]

The 23 wells investigated in this study currently produce billions of

liters of wastewater every year, or around 7 million barrels (Table 3). Our findings clearly showed that the PW has the potential to be reused if minimal treatment is applied. The amount of water produced from the wells investigated here is not sufficient to support enough irrigation water for crop production at an average sized Colorado farm (Table 3). However, livestock irrigation requires less water, and thus (establishment of) dairies or confined animal feeding operations would likely benefit more from this non-traditional water source. [128] With 23 wells, at the current production level, almost 23,000 cows could be irrigated per year. In addition, it is possible to maximize production by installing bigger downhole pumps and larger separation vessels, which would increase the water production almost 50 times. This amount of water would support irrigation of 572,000 cows per year (Table 3). This amount of water would allow farms to be built or expanded in areas that have no or limited access to freshwater sources.

4. Conclusions

The goal of this study was to provide a case study showing the potential for treating and reusing low salinity PW from conventional wells in eastern Colorado located within the DJ Basin for agriculture. Based on detailed chemical and toxicological assessment of the PW we conclude that nanofiltration treatment by is likely a viable and economically feasible pathway for producing water of a high enough quality for cattle or crop irrigation, or discharge into rivers and streams. The reuse potential of PW is not only promising but may become essential as wastewater management continues to pose significant challenges.

A techno-economic analysis was also completed considering the scenario of the treated water being used for cattle production. The price of water would be \$5.38/m³ (\$1.42/barrel), which at first glance appears high. However, as water prices increase or freshwater sources become scarcer, access to non-traditional water sources will become more attractive. Along with water scarcity, current management practices are not sustainable due to the high volumes and adverse environmental impacts, making treatment and reuse of PW an attractive alternative.

This case study serves as a valuable foundation for developing new regulations and guidelines for PW treatment and reuse. Multilateral partnerships such as the Colorado Produced Water Consortium (CPWC), the Texas Produced Water Consortium (TxPWC), and the New Mexico Produced Water Research Consortium (NMPWRC) aim to address the scientific, technical, economic, legislative, and regulatory needs associated with PW management and reuse. These partnerships have produced numerous scientific studies, guidance documents, and project reports to inform feasibility evaluations, science-based decision-making processes, and promote sustainable management strategies. [129] A key focus of their efforts is determining the achievable water quality levels for treated PW at non-prohibitive costs. The findings of this study provide critical insights into the water quality of PW effluents in Colorado and the anticipated costs of achieving acceptable quality levels for applications such as surface water flow augmentation and irrigation of salt-tolerant crops.

No research has evaluated beneficial reuse of low salinity PW from a multifaceted perspective including the potential chemical, toxicological, and economical constraints. Thus, our findings can assist regulators and water managers by providing a roadmap for comprehensive PW analysis for various end users. While our study provides a foundation for reuse, future studies should expand further on understanding the PW reuse potential for developing wetland areas, and discharge into surface water as additional management options. Studies should also focus on crop irrigation impacts, emphasizing soil and plant health, while also conducting comprehensive assessments of chronic toxicity risks to both mammals and aquatic organisms. Such efforts are crucial for improving risk evaluation and preventing adverse outcomes in the implementation of beneficial reuse of PW.

Table 2
Values of the base and future scenarios for a solar-powered nanofiltration treatment system of produced water.

	Base Scenario	Future Scenario	Unit
Nanofiltration Energy Requirement	236	5903	kWh/day
Collector Area	121	296	m ²
Solar System Size	122	3038	kW
Lithium-Ion Battery Size	1181	29,515	kWh
Clean Water Produced	103	2850	m ³ /day
Capital Cost	1140,688	23,530,384	USD
Operations & Maintenance Cost	64,086	746,134	USD/year
Levelized Cost of Water	5.38	3.83	USD/m ³

Table 3

Water production based on the 23 tested wells and the corresponding number of cows and irrigable acres that could be supported.

	PW (L/year)	PW (barrels/year)	Water Use (L/cow/y)	Cows Supported	Irrigable Area (acres)
Current Production (N = 23)	1154,968,715	7263,954.18	50,461	22,888	191.90
Maximized Production	28,874,217,881	181,598,854.6	50,461	572,209	4797.39

Environmental Implications

Oil and gas PW is a waste product primarily managed by evaporation ponds and deep well injections. It has been shown to result in increased seismicity, ground water contamination, and volatile contaminants released into the atmosphere. This study is the first to examine not only conventional PW quality, but the wastewater's toxicity, cost of reuse, and impact on various end users. This includes recommendations for expansion of guidelines to cover common contaminants in PW for safe beneficial reuse. Our findings demonstrate a potential for reuse of PW, which can alleviate environmental stress caused by current management practices.

CRedit authorship contribution statement

Roth Holly: Writing – review & editing, Formal analysis, Conceptualization. Wadler Claire: Writing – review & editing, Formal analysis. Blewett Tamzin: Writing – review & editing, Investigation, Formal analysis, Conceptualization. Borch Thomas: Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. Tarazona Yennifer: Writing – review & editing, Writing – original draft. Xu Pei: Writing – review & editing, Writing – original draft. Malik Huma Tariq: Writing – review & editing, Investigation. Coupannee Maelle: Writing – review & editing, Investigation, Formal analysis. Stewart Connor: Writing – review & editing, Investigation, Formal analysis. Rosenblum James: Writing – review & editing, Investigation, Formal analysis. Ballenger Brooke: Writing – review & editing, Formal analysis. Quinn Jason: Writing – review & editing. Wiltse Marin: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. Sudowe Ralf: Writing – review & editing. Zhang Yanyan: Writing – review & editing, Writing – original draft.

Declaration of Generative AI and AI-assisted technologies in the writing process

Biorender.com was used to create the graphical abstract.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Thomas Borch reports financial support was provided by The USDA National Institute of Food and Agriculture through AFRI Grant No. 2021-67019-33726. If there are other authors, they 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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Supplementary Information

Additional methodologies, ICPMS measurements, surfactant identification, individual compound concentrations, and radium

concentrations.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jhazmat.2025.138581](https://doi.org/10.1016/j.jhazmat.2025.138581).

Data availability

Data will be made available on request.

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APPENDIX E: CO-AUTHOR CONTRIBUTIONS TO SAFETY AND TECHNICAL
FEASIBILITY OF SUSTAINABLE REUSE OF SHALE GAS

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According to CRediT criteria, my co-author contributions to this work included writing – review & editing.

Safety and Technical Feasibility of Sustainable Reuse of Shale Gas Flowback and Produced Water after Advanced Treatment Aimed at Wheat Irrigation

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ABSTRACT: Treatment and reuse of flowback and produced water (FPW) from shale gas extraction for agricultural irrigation has often been proposed as a sustainable alternative to disposal via deep-well injection. Here, we investigate the effects of FPW on the germination period, macroscopic growth, element enrichment, and grain gene expression of wheat upon dilution and advanced membrane treatment of the liquid stream. Compared to tap water, irrigation with treated FPW shortened the germination time, slightly improved the seed vigor index, and ensured a similar germination rate. On the other hand, the biomass and grain yield of mature wheat irrigated with treated FPW and with FPW diluted to 5% groups decreased compared to tests using tap water. After a whole growth cycle of wheat, higher concentrations of nutrients, such as K, Ca, and Mg were enriched in mature wheat tissue irrigated with treated FPW. However, the Pb and Cr contents of mature wheat grains treated with three types of irrigation waters exceeded the standard to varying degrees. A total of 1973 differentially expressed genes were mainly related to binding, catalytic activity, cellular process, metabolic process, and cell part, more than half of which were upregulated and induced by irrigation with treated FPW. These findings provide critical guidance for the reuse of treated shale gas FPW for agricultural application from the perspective of plant uptake of toxic elements, as well as crop and human health risks.

KEYWORDS: flowback and produced water, agricultural irrigation, wheat, heavy metals, transcriptomic analysis



■ INTRODUCTION

The rapid increase in global production of tight hydrocarbons, particularly shale gas, comes with risks of local environmental pollution and health-related problems.^{1–3} The expansion of energy production toward the utilization of shale or unconventional plays results in the consumption of high volumes of water (20,000–60,000 m³/well in major shale gas plays) for hydraulic fracturing and in the co-production and potentially improper disposal of large volumes of high-salinity flowback and produced water (FPW).^{4–6} This trend has brought about environmental and economic challenges, exacerbated by water competition between different water-intensive sectors, and has caused health problems, especially in densely populated areas and where water resources are scarce.^{7–9} The main management option for shale gas FPW has been so far direct deep-well injection, which may however result in increased subsurface water pressure and stimulate tight shale formations, potentially inducing earthquakes.^{4,10,11} Indirect external use strategies for managing FPW have been facing challenges; such strategies

include dust suppression, discharge of treated FPW (TFPW) to surface water, and agricultural irrigation.¹²

After the completion of the hydraulic fracturing stage in shale gas operations, wastewater mainly returns to the surface as flowback water (the initial water that is recovered with shale gas) and produced water mostly comprising the natural deep formation water.^{13–15} Therefore, FPW usually contains high concentrations of total dissolved solids (TDS), organic matter (dissolved organic carbon; DOC), metals, hydrocarbons and other volatile compounds, some synthetic organic chemical additives, as well as some naturally occurring radioactive materials that may be present in the formation.^{16–21} In recent years, the option of applying FPW directly or indirectly for

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agricultural irrigation has become a focus of growing discussion.^{22,23} Small amounts of organic matter and micro-nutrient elements in municipal sewage and industrial wastewaters, at times also including rare earth elements (e.g., Ce), have been shown to promote the growth of some algae strains or higher plants under certain circumstances.^{24–25} On the other hand, the salinity, alkalinity, and ionic composition of oilfield-produced water may significantly hinder its reuse in agriculture since these characteristics can cause nutrient imbalances, as well as osmotic and specific ion stress, in plant cells.^{28,29} Methods of reusing produced water with minimal TDS treatment or upon dilution with freshwater for farmland irrigation are currently being applied in Kern County (California), but the complexity of FPW components can result in detrimental effects on humans and ecosystems.³⁰

Salt and organic compound tolerance differs among plants. The concentrations of TDS and DOC of irrigation water are generally lower than 3500 and 5 mg/L, respectively, to maintain normal dryland plant growth and biomass accumulation, and the use of water with substance contents above the current guidelines to irrigate crops may lead to yield reduction and germination problems.^{31,32} Previous studies on the reuse of oil and gas wastewater for crop irrigation without treatment or upon simple dilution have shown that this approach has potentially significant side effects on plant growth, yield, and soil health.^{22,28,31–33} Applying different percentages of produced water (PW) to irrigate wheat has been observed to lead to a decline in yield and a negative effect on physiological parameters, even when the wastewater is diluted by as much as 90% with tap water (TW), also inducing an adverse impact on soil health and microbial diversity.^{22,29,31} In addition, irrigating wheat with diluted PW has been shown to compromise the plant's immune response.³⁴ The other study reported that simulated produced water used to irrigate nonfood biofuel crops resulted in significantly lower growth and worse physiological characteristics of the crops due to high PW salinity and excess organic carbon.²⁸ However, a reverse osmosis (RO) effluent and a diluted forward osmosis (FO) draw solution were used for the short-term cultivation of some leafy vegetables (e.g., lettuce and Chinese cabbage) without significant adverse effects under certain circumstances; the FO draw solution was found to promote plant growth at different concentrations of salts.^{35,36}

In this work, we present the physiological and biochemical effects of irrigating wheat with TFPW. Also, differences in wheat grain gene expression as well as accumulation levels of various nutrients and toxic elements are evaluated. This study is the first one to analyze the effect of FPW on wheat gene expression using transcriptomics. It aims at providing a better understanding of the potential side effects of FPW reuse for irrigation purposes to support improvements in this reuse strategy and to promote water-saving strategies.

MATERIALS AND METHODS

Preparation of Water, Soil, and Seeds. The untreated shale gas FPW was obtained from a shale gas mining station in the Sichuan Basin. The primary components of FPW in this study are fracturing fluid and formation water, representative of the typical FPW in the Sichuan Basin. The TFPW was collected from the shale gas FPW treatment plant located in Fuling, Chongqing. As shown in Figure S1, the treatment plant consists of a multistage pretreatment coupled with two-stage RO filtration, for a daily processing capacity of 2400 m³. The treated effluent meets the first-level standard of the integrated wastewater discharge standard (GB8978-1996).³⁷ The soil was

excavated from the campus land of Sichuan University (Chengdu, China) and then thoroughly mixed and crushed into smaller particles before the experiments. Wheat was selected as the crop of interest for this study.³⁸ The spring wheat (*Triticum aestivum* L., Jinchun 6) seeds were purchased from Fu Yi Chun Seed Co., Ltd. (Hejian, China) and stored in the dark and under dry conditions until use.

Germination Test of Wheat Seeds. Wheat seeds were cultivated with three different kinds of irrigation water sources: a TW control, TFPW, and diluted untreated FPW (FPW5, equivalent to 5% FPW + 95% TW). Dilutions of 10 and 50% for wheat irrigation have been reported to lead to adverse effects, such as stunted growth and reduction in wheat biomass.³¹ On the other hand, it should be considered that a large amount of TW would be consumed to treat the same volume of FPW with a dilution of 0.1 or 1%. Each treatment group contained 30 wheat seeds, individually weighing 0.045 ± 0.005 g, and was equally divided and transferred to five sterile Petri dishes comprising a qualitative filter paper and 15 mL of corresponding water, which were both replaced every 24 h. All Petri dishes were exposed to natural light at room temperature (~ 20 °C) for 6 days,³⁵ and the number of wheat roots and sprouts was recorded daily to compare the germination rates between the control and the experimental groups. Seedling height and root length were measured using a millimeter ruler. The seed vigor index (SVI) was calculated using the following equation³⁹

$$\begin{aligned} \text{Seed vigor index (SVI)} \\ = \text{Germination \%} \times \text{seedling length (Root + Shoot)} \quad (1) \end{aligned}$$

Greenhouse Trial and Harvesting. After the 6 day incubation in Petri dishes described above, six wheat seedlings were picked for each group and then transplanted into an open plastic container, six-sevenths of which were filled with the soil. The internal length, width, and height of the rectangle plastic container were 59, 20, and 14 cm, respectively. The transplantation depth of all seedlings was about 3 to 4 cm, and the above-ground height was between 4 and 5 cm. All wheat plants were irrigated with corresponding water every 2 days for a total of 65 days. Each irrigation event consumed about 300 mL of corresponding water. Approximately 10 L of irrigation water was used for each wheat cultivation group during the whole experiment. The above-ground height from the soil surface to the top of the wheat in each treatment group was recorded every 6 days. Upon harvesting, all wheat plants were rinsed with TW, washed with ultrapure water (ULUPURE, Chengdu, China) three times, and then dried in an electric thermostatic drying oven (DHG-9070A, Shanghai) at 60 °C until a constant weight was achieved. The above-ground biomass, below-ground biomass, and grain yield of each plant were then determined.

Analysis of the Irrigation Water Quality. The TDS and conductivity were determined using an Ultrameter II 6PFC portable multifunctional meter (Myron I. Company, Carlsbad, CA, USA). The turbidity and UV absorbance were measured using a turbidimeter (2100Q, Hach Company, Loveland, CO, U.S.A.) and a UV–Vis spectrophotometer (Orion AquaMate 8000, Thermo Fisher Scientific, Inc., MA, USA), respectively. Samples were filtered through 0.45 μm polyethersulfone membrane filters and analyzed for total organic carbon (TOC) and total nitrogen (TN) using a TOC analyzer (TOC-L CPH, Shimadzu, Kyoto, Japan). In addition, quantitative analyses of ions and elements were performed using ion chromatography (Dionex ICS-1100, Thermo Fisher Scientific, Inc., MA, USA) and an inductively coupled plasma mass spectrometer (NexION 1000G PerkinElmer, Inc., MA, USA).

Soil and Wheat Tissue Digestion and Quantitative Analysis. Samples of bulk soil (not used to cultivate plants) and rhizosphere soil (extracted from the vicinity of mature wheat roots in each treatment group) were collected and classified as raw soil, TFPW soil, TW soil, and FPW5 soil depending on the irrigation source. These samples were crushed, then passed through a 100-mesh nylon sieve, and finally placed in an electric thermostatic drying oven until a constant weight was reached. A total of 100 mg of dry soil samples was then placed in a polytetrafluoroethylene (PTFE) tube, mixed with 6 mL of nitric acid

Table 1. Water Quality of Three Irrigation Waters (TFPW, TW, and FPWS) and Typical FPW in the Sichuan Basin; National and International Guidelines for Irrigation^a

indicator	TFPW	TW	FPWS	FPW in the Sichuan Basin ^{1,3,45}	water quality for irrigation (China) ^{45,46}	water quality for irrigation (UN) ⁴⁷
turbidity	0.20	0.23	0.67	37.1–455		
conductivity ($\mu\text{S}/\text{cm}$)	49	336	3224	11290–23600		3000
DOC (mg/L)	2.90	0.85	1.18	78–1975		
TN (mg/L)	5.136	0.854	3.456	NA		
UV ₂₅₄ (cm^{-1})	0.006	0.010	0.013	NA		
TDS (mg/L)	22	160	1660	6906–30200	1000	2000
Na (mg/L)	6.22	4.92	534.79	2109–22266		920
Ca (mg/L)	0.464	50.293	105.204	70.9–1823.1		400
Mg (mg/L)	0.029	11.486	17.301	6.25–499		60
K (mg/L)	0.31	3.09	12.63	93.09–499		
Ba (mg/L)	0.091	0.252	4.573	0.179–907.91		
Sr (mg/L)	0.097	0.295	7.687	4.9–542		0.2
Fe ($\mu\text{g}/\text{L}$)	21.91	494.86	756.93	13.67–72219		5000
Mn ($\mu\text{g}/\text{L}$)	0.053	0.704	37.705	479–3760	300	200
Cu ($\mu\text{g}/\text{L}$)	0.609	0.663	9.493	0.65–6500	500	200
Zn ($\mu\text{g}/\text{L}$)	57.6	404.2	393.7	30–398	2000	2000
Mo ($\mu\text{g}/\text{L}$)	0.5	1.3	1.7	NA	500	10
Ni ($\mu\text{g}/\text{L}$)	0.09	4.75	6.10	11–103.69	100	200
Cr ($\mu\text{g}/\text{L}$)	10.45	11.05	11.15	1.31–49	100	100
Se ($\mu\text{g}/\text{L}$)	0.15	0.52	5.26	NA	20	20
As ($\mu\text{g}/\text{L}$)	0.009	0.396	2.705	NA	50	100
Pb ($\mu\text{g}/\text{L}$)	0.01	0.15	0.20	0.034–1.11	200	5000
Cd ($\mu\text{g}/\text{L}$)	NA	NA	0.005	0.044–9	10	10
Ag ($\mu\text{g}/\text{L}$)	0.008	0.004	0.005	NA		
Al ($\mu\text{g}/\text{L}$)	7.17	101.50	109.04	3100		5000
Co ($\mu\text{g}/\text{L}$)	NA	0.061	0.089	NA	1000	50
Sb ($\mu\text{g}/\text{L}$)	0.40	0.50	2.75	NA		
Sn ($\mu\text{g}/\text{L}$)	0.125	0.039	0.085	NA		
V ($\mu\text{g}/\text{L}$)	0.04	0.41	16.08	NA	100	100
F ⁻ (mg/L)	0.039	0.172	0.444	0.875–17.6	2	1
Cl ⁻ (mg/L)	8.501	30.871	870.265	4019–41158	350	350
Br ⁻ (mg/L)	0.11	3.48	5.67	21–257		
NO ₃ ⁻ (mg/L)	0.15	5.33	7.16	5.641–217		10
SO ₄ ²⁻ (mg/L)	0.584	44.803	48.209	0.441–72.9		960

^aNote: NA, not available; TFPW, treated FPW; TW, tap water; FPWS, equivalent to 5% FPW + 95% TW.

and 1 mL of hydrogen peroxide, and digested using a microwave dissolver (MDS-6G, SINEO, Shanghai, China). Then, samples of the resulting solutions were diluted to a volume of 50 mL using ultrapure water. Similarly, mature wheat tissues (root, stem, leaf, and grain) intended for quantitative analysis of macro- and trace elements were also washed, dried, digested, and diluted. After digestion and dilution, all samples were filtered using a 0.22 μm PTFE filter and quantitatively assayed for elemental composition. For homogeneity comparison, the element content in soil and plant tissue samples is presented as a mass ratio (mg/kg).

Transcriptome Sequencing of Wheat Grains. Before wheat matured completely, a weight of slightly more than 0.1 g of fresh grains (eight wheat grains) in each group was taken out, washed with DEPC water (RNase-free), frozen directly in liquid nitrogen for 30 min, and stored at $-80\text{ }^{\circ}\text{C}$ for RNA extraction. The total RNA was extracted from wheat grains using the TRIzol reagent. Afterward, the transcriptome sequencing process was executed using the Illumina NovaSeq 6000 sequencer (Majorbio Bio-pharm Technology Co., Ltd, Shanghai, China). Details including RNA extraction, sequencing, read mapping, differentially expressed genes (DEGs), and functional enrichment are summarized in Text S1 (Supporting Information). Raw sequencing data were stored in the NCBI database under bioproject number PRJNA813217, with biosample numbers SAMN26455859, SAMN26455866, and SAMN26455867.

Statistical Analysis. Data analyses and graphic drawings were performed using Microsoft Excel 2021 and Origin 2021 (OriginLab, Hampton, MA), respectively. The results are presented as mean $\pm$ standard deviation (SD) using one-way analysis of variance (ANOVA) for statistical significance analysis with a significance level (p -value) set at 0.05.

RESULTS AND DISCUSSION

Characterization of Irrigation Water. Detailed water quality indicators for the three irrigation water sources and for the typical FPW in the Sichuan Basin are summarized in Table 1. Even when diluted with TW, the dominant ionic composition of FPWS remained similar to that of typical FPW, mainly including sodium and chloride ions. It can also be inferred that the results of the main indicators of the untreated FPW used in this study are within the interval of typical FPW quality data reported for the Sichuan Basin. TFPW inevitably contains some chemical components, but their contents were within the limits set by China and by FAO irrigation standards with respect to maximum salinity and element toxicity, while the other two types of water did not fully comply with the standards.^{13,40} In addition, it has been reported that FPW treated by ozonation–ultrafiltration (UF)–RO process could

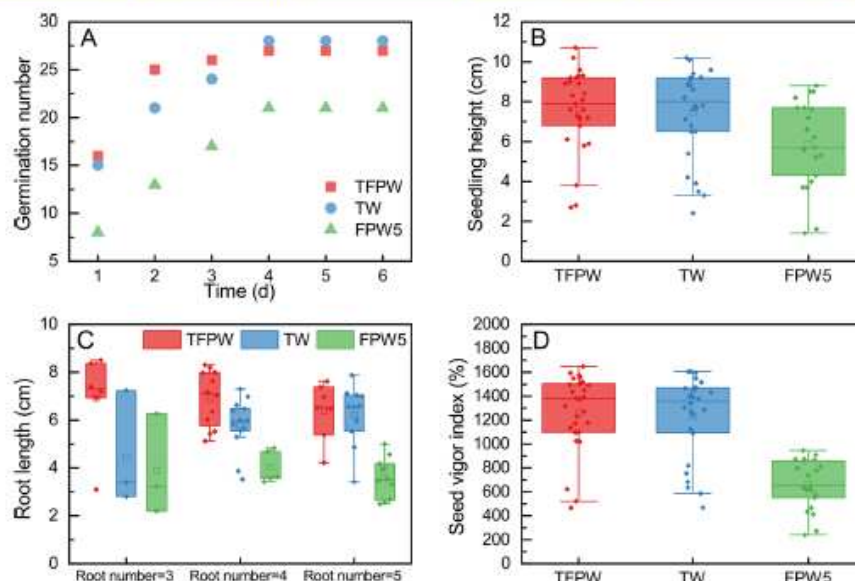


Figure 1. Germination performance of wheat seeds in different irrigation groups: TFPW (red), TW control (blue), diluted FPW (FPW5, green). (A) Germination number; (B) seedling height on the sixth day; (C) root length for samples in which the number of seedling roots was 3, 4, and 5; (D) SVI.

meet the water quality standards of irrigation and discharge.⁵ Therefore, TFPW from various combined processes can also be considered for reuse aimed at crop irrigation. In this study, only the content of strontium (Sr) in TW (0.295 mg/L) exceeded the value recommended by FAO guidelines (0.2 mg/L), and only the concentration of Zn was higher, if slightly, in TW compared to FPW5. With respect to FPW5, several parameters, including TDS and arsenic (As), were significantly higher compared to those in TFPW and TW. The contents of various detected components in TFPW were lower than those of corresponding substances in TW, apart from the DOC, TN, sodium (Na), silver (Ag), and tin (Sn). The relatively low concentration of heavy metals in TFPW should minimize the potential for the accumulation of these elements in plants and soil. However, the contents of DOC, TN, Ag, and Sn in TFPW were the highest among the three types of irrigation water. It could be attributed to the dilution with TW resulting in the presence of higher concentrations of TFPW components than FPW5. In addition to the primary inorganic components in shale gas FPW, such as salts and metals, the toxic organic compounds, such as benzenes and polycyclic aromatic hydrocarbons, may be detrimental to crop development and metabolic activity, a topic that requires further research.^{41,42}

Effects on Seed Germination and Phenotypic Analysis. The results on the physiological conditions of wheat in different treatment groups during germination stages are summarized in Figure 1, including the germination number, seedling height, root length, and SVI. After 6 day germination culture, the germination number of wheat seeds exposed to TFPW was comparable to that of the TW control group, whereas the germination number was lower for FPW5 irrigation (Figure 1A). Specifically, the germination rates of the TFPW and TW groups exceeded 85%, while the FPW5 was only 70%. As a result, irrigation with TFPW slightly shortened

the germination period. Application of TFPW practically maintained the same seedling height and seed vigor observed for TW. On the contrary, the application of FPW5 remarkably decreased ($p < 0.05$) both the seedling height and SVI by roughly 20 and 45% (Figure 1B,D). Figure 1C presents the results obtained for the root length related to root numbers in the seed germination test. Even within the same irrigation group, the number and length of taproots varied. When considering samples with the same number of roots (i.e., 3, 4), the relationship between the average root length of the three groups was: TFPW > TW > FPW5. Seed germination involves multiple complex factors such as water relationship, environmental factors, and hormone regulation. The differences in germination performance may be mainly related to different salinity and heavy metal levels.⁴³ In summary, the use of treated shale gas FPW was suitable in the germination stage of wheat seeds, consistent with previous reports related to leafy vegetable cultivation.^{35,36}

Effects on Wheat Growth and Harvesting. After being transplanted, the wheat plants were cultivated for 65 days and then harvested (Figures S2 and 2A). There was no significant difference in the above-ground height of wheat among the three groups (Figure 2B). Irrigation with FPW5 slightly increased the average above-ground height of wheat in the first 20 days compared with the other two groups, which may be attributed to the high amount of nutrients. The wheat treated with TW grew faster at a later stage, and the average above-ground height of wheat in the TW group was the highest at harvest. The results suggest that the growth of wheat in soil followed an opposite trend compared to germination in the Petri dishes with respect to the use of the three irrigation water types, which may be related to the degree to which salinity, and nutrients are decisive in different culture environments.

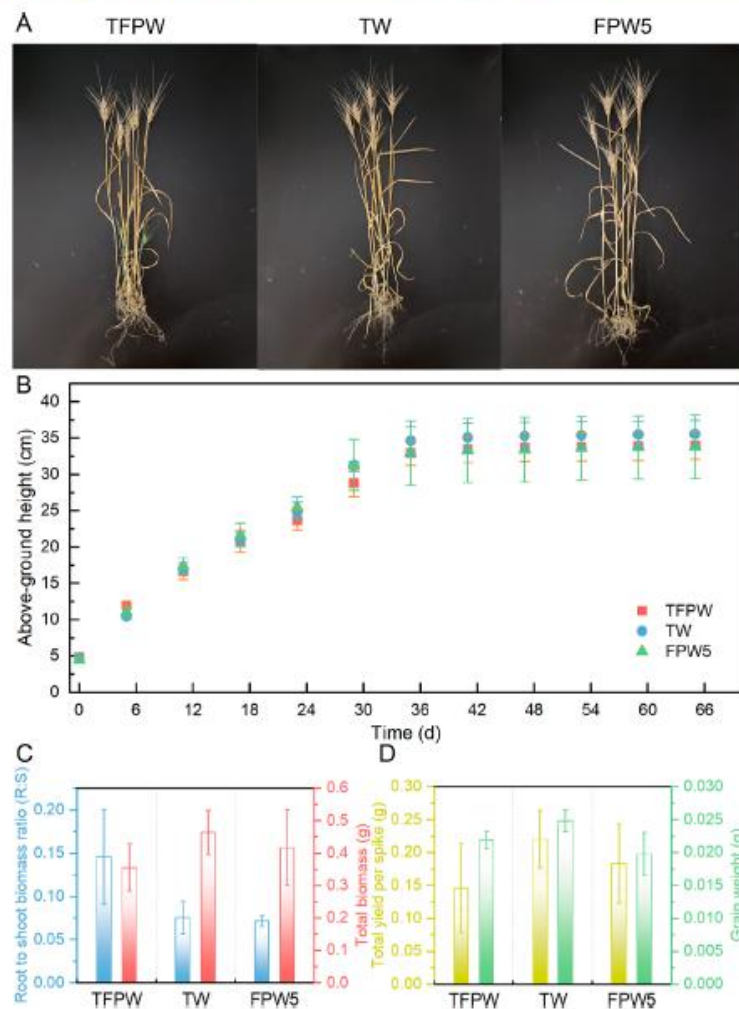


Figure 2. (A) Representative images of mature wheat irrigated with different waters. (B) Above-ground height of wheat irrigated with different waters in soil. (C) Wheat R/S ratio (blue) and total biomass (red). (D) Total wheat yield per spike (yellow) and average grain weight (green).

Irrigation with reused wastewater showed some stress effects. Compared to the TW, TFPW irrigation significantly ($p < 0.05$) increased the root-to-shoot biomass ratio (R/S ratio), while the R/S ratio changed little ($p > 0.05$) under the FPW5 treatment. Additionally, irrigation with TFPW and FPW5 significantly ($p < 0.05$) decreased wheat total biomass by approximately 23 and 10%, respectively (Figure 2C). The results were in line with previous reports, suggesting that a higher concentration of DOC in the irrigation water may lead to smaller biomass.²⁸ Relative to the control (i.e., TW), the total yield per spike in TFPW and FPW5 groups decreased markedly ($p < 0.05$) by 34 and 17%; the grain weight reduced by 12% for TFPW irrigation and 20% for FPW5 irrigation ($p < 0.05$, Figure 2D). Among the three irrigation water types, the DOC concentration of TFPW was the highest and equal to 2.9

mg/L, which was however lower than the maximum value of 5 mg/L suggested in the literature.²⁸

Effect on Element Accumulation in Soil. As displayed in Figure S3, 15 elements in the soil, including crop nutrient elements, heavy metal elements, and metalloid elements, were measured at the beginning and at the end of the irrigation experiment, with the relative standard deviations (RSDs) presented in Table S1. Compared to the raw soil, the contents of most elements in TFPW-irrigated, TW-irrigated, and FPW5-irrigated soils increased to a certain extent. However, the content changes of selenium (Se) and silver (Ag), rarely existing in soil, had different trends. The accumulation of zinc (Zn), copper (Cu), manganese (Mn), cobalt (Co), nickel (Ni), strontium (Sr), and vanadium (V) in FPW5 soil was much higher than that of the other two groups. On the contrary, molybdenum (Mo) and barium (Ba) seemed to

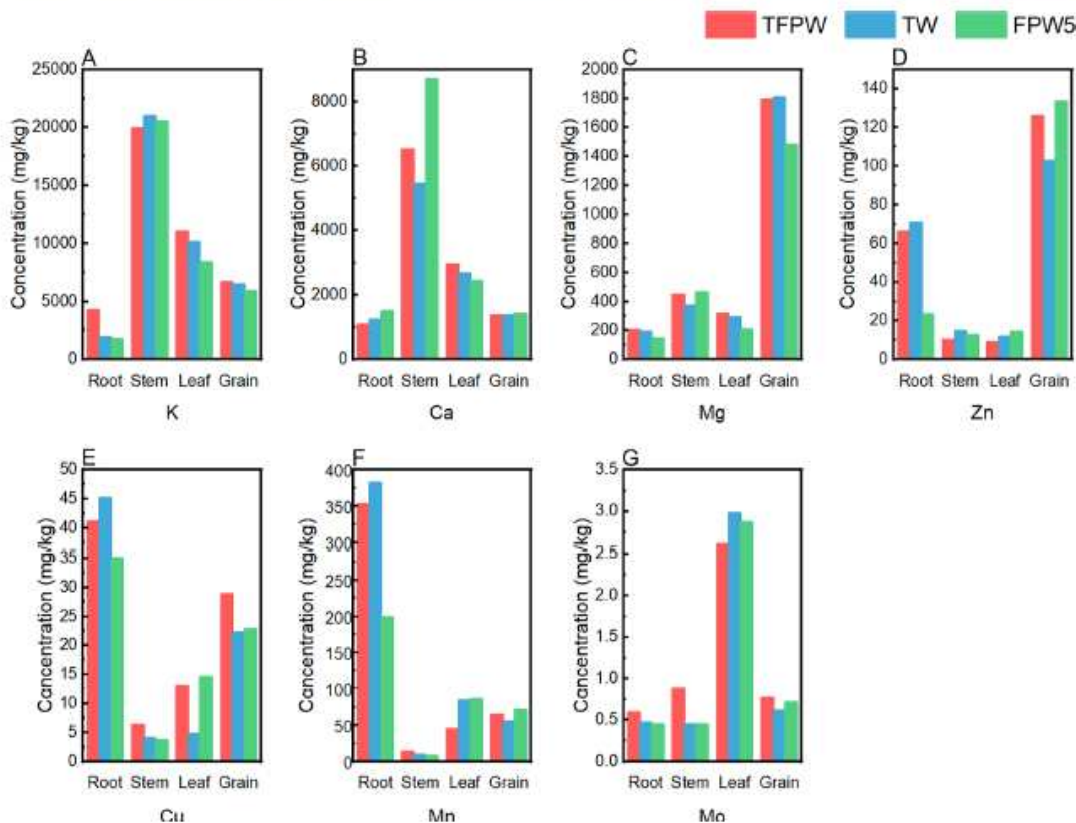


Figure 3. Concentrations of K (A), Ca (B), Mg (C), Zn (D), Cu (E), Mn (F), and Mo (G) in wheat tissues, including root, stem, leaf, and grain in wheat cultivated for 65 days applying different kinds of irrigation water.

accumulate more in TFPW- and TW-irrigated soil, which may be partly attributed to slow uptake or inhibition in wheat when Ba concentration in irrigation water was low.

The accumulation of heavy metals in soil may not only adversely affect soil biota through microbial processes and soil-microbe interactions but also harm human health through the food chain.^{48,49} Compared with soil environmental quality standards summarized in Table S3, the content of several heavy metals in the soil of each irrigation group was lower than the recommended risk control value, suggesting that the irrigation-related reuse of properly diluted or treated shale gas FPW within a wheat life cycle might not cause adverse effects on the soil environment. Nevertheless, diluted and treated effluents contributed to the addition of chemical components to the soil, which may accumulate over time.

Effect on Element Accumulation in Wheat Tissues.

Nutrient elements and toxic heavy metals in plant tissues were measured to provide insights into the translocation and accumulation effects.^{49–51} The contents of seven nutrient elements and eight toxic elements in the wheat root, stem, leaf, and grain are displayed in Figures 3 and 4, respectively, with the RSDs presented in Table S2. The accumulation of these elements in wheat leaves was higher than in grains (except for Mg, Zn, Cu, and Mn), indicating that the transport of these

elements in wheat was sequestered in leaf tissues rather than continuing to reach grains, regardless of the type of irrigation water.^{29,52} Except for Mn and Mo, the concentration trends of each element in different tissues of wheat were consistent among the three treatment groups. Interestingly, although FPW5 contained a higher concentration of substances and salinity, the contents of several elements within specific tissues of wheat cultivated with FPW5 were not the highest among the three groups. The high ionic strength may prevent the transport of toxic elements, thus thwarting plant uptake.^{31,53}

In detail, compared with the TW group, the wheat in the other two groups absorbed and stored more Ca, which could be a major messenger molecule under various stress and facilitate plant resistance to osmotic stress.⁵⁴ The K content in the wheat irrigated with TFPW increased significantly, and even the content in the roots of the wheat was twice that of the other groups, suggesting that TFPW promoted wheat uptake of K. Mechanisms for K uptake to the plant root are related to an affinity transport system with feedback regulation. With similar ionic radius and ion hydration energy, Na competes with K for major uptake sites including high-affinity transporters (HKT) and low affinity (nonselective cation channels) at the plasma membrane.⁵⁵ Therefore, the negligible Na content in TFPW may be the main reason for the elevated K

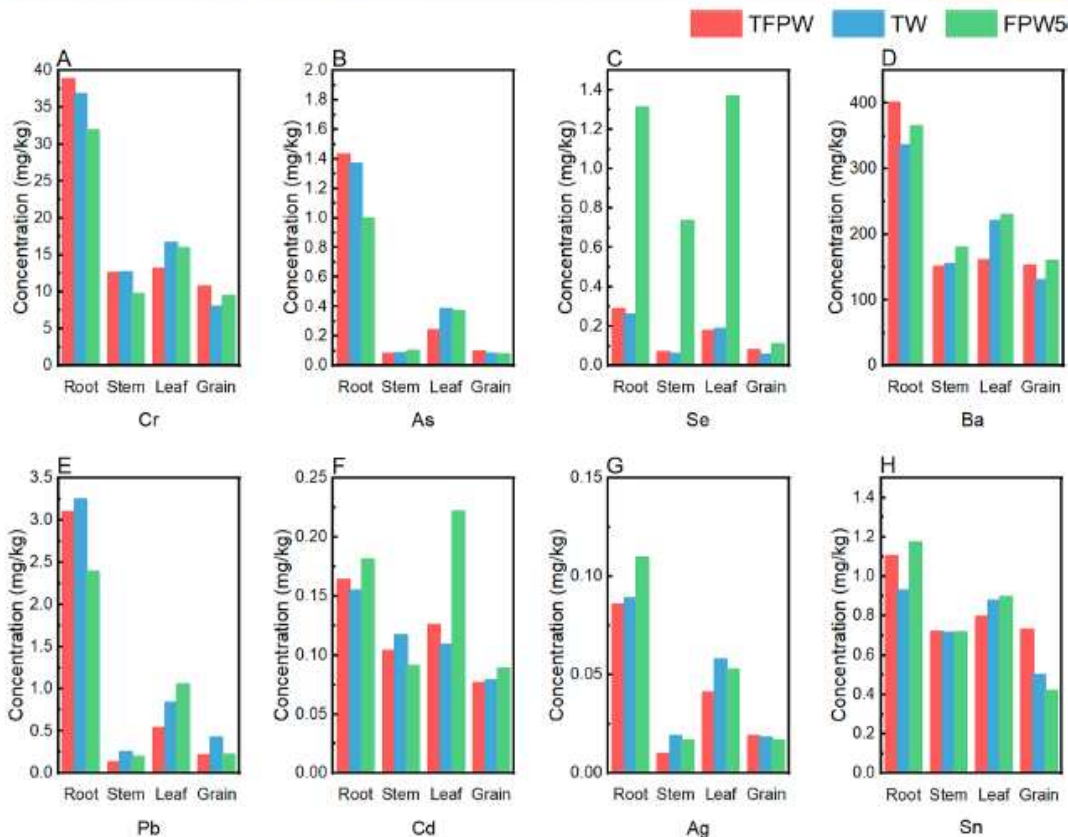


Figure 4. Concentrations of Cr (A), As (B), Se (C), Ba (D), Pb (E), Cd (F), Ag (G), and Sn (H) in wheat tissue including the root, stem, leaf, and grain in wheat cultivated for 65 days applying different kinds of irrigation water.

uptake in wheat. As shown in Figure 3C, compared with TW and FPW5, the Mg content associated with photosynthesis and carbohydrate synthesis was relatively higher in wheat leaves irrigated by TFPW. K, Ca, and Mg are present in significant amounts in various tissues of plants and play a wide range of roles, including but not limited to regulating cell permeability, activating enzymes, regulating product transport, and participating in cell structure composition. The results in Figure 3 also indicate that the content of Zn, Cu, Mn, and Mo in various tissues of wheat was smaller than that of K, Ca, and Mg by several orders of magnitude. Their presence in trace amounts is essential for plant growth and grain yield but toxic when exceeding certain threshold levels.^{29,56} For instance, the presence of high concentrations of Zn and Cu in plants can reduce metabolic activity, generate reactive oxygen species, and induce oxidative damage.⁵⁶ Similarly, several toxic heavy metals and metalloids listed in Figure 4 were classified as nonessential elements for plant growth and might cause serious acute and chronic health hazards to plants and humans. Pb and Cr are also toxic and/or carcinogenic to humans and can cause damage to the nervous system and to the immune system, causing a variety of diseases similar to excessive As.^{49,57,58}

The actual content of these toxic elements in the grains of wheat is a concern for food safety. As shown in Figure 4B, the accumulation of As in the roots of wheat was much higher than that in the shoots (stem, leaf, and grain), which was consistent with what was described in a previous study.⁵⁹ No significant differences were observed between the content of As in wheat grains irrigated by different waters. Irrigation with FPW5 significantly increased the Se content in various tissues of wheat, which was attributed to the higher Se concentration in FPW5 (Figure 4C). This study found that only Cr and Pb exceeded the maximum values of 1 mg/kg for Cr and 0.2 mg/kg for Pb established by the Chinese Food Safety Standard, while the other metals met the safety requirements.⁶⁰ Specifically, the Pb contents in wheat grains irrigated with TFPW and FPW5 were 0.217 and 0.226 mg/kg, respectively, slightly above the limit and were approximately half that of the TW group (Figure 4E). The concentrations of Cr in the three groups of wheat grains were not significantly different (7–10 mg/kg) but were all considerably higher than the limit set by the Chinese Food Safety Standard. Based on these results, it can be concluded that the type of irrigation water is not the main contributor to the grain element contents exceeding the limits. The generally increased uptake of some heavy metals by

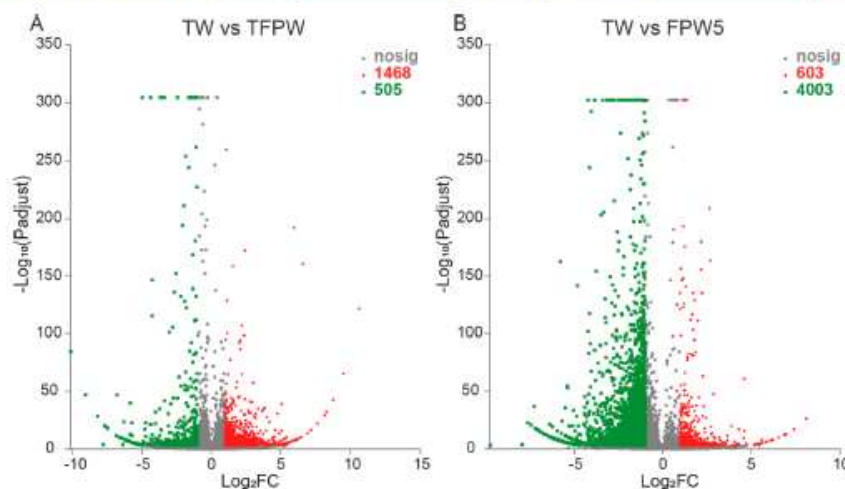


Figure 5. Volcano plots of differences in gene expression among (A) TW vs TFPW and (B) TW vs FPW5 analysis. Gray dots were not considered as significantly differentially expressed. Red (upregulation) and green (downregulation) dots indicate DEGs ($|\log_2(\text{fold change})| > 1$, $P_{\text{adj}} < 0.05$).

wheat in this experiment may be related to the magnitude and intensity of their solubility, mobility, and bioavailability in soil.⁶¹ In addition, the transport of many heavy metals in plants was regulated by the same transporter, which led to a competitive relationship in heavy metal accumulation. For example, the translocation and redistribution of Cd and Zn were regulated by plant cadmium resistance proteins.⁶² The differences in the content of elements absorbed by plant tissues in each group not only reflect the competition and complexation between the components of irrigation water but also the comprehensive results of plants responding to various stress conditions.

Effect on Expressed Genes in Wheat Grains. To better understand the effect of irrigation using shale gas FPW on wheat grain genetic and physiological basis traits, grains at the grain filling stage were used for transcriptome analysis based on the RNA-seq technology.^{38,63} The RNA bands were clear and free of impurities, such as pigments, proteins, and sugars, based on the results reported in Table S4. High-quality mapped reads for transcript assembly and expression calculation were obtained, and their derivation distribution and detailed sequencing data are presented in Tables S5 and S6, respectively. In this experiment, the total number of known expressed genes in wheat grains irrigated with TFPW, TW, and FPW5 was 71570, 70278, and 67836, respectively, with a large number of coexpressed genes (62788) identified in all detected samples (Figure S4).

Notably, the number of DEGs between TW versus TFPW and TW versus FPW5 is significantly different and lower in TW versus TFPW. The results indicate that a total of 1973 genes were differentially expressed when comparing the use of TW and TFPW, of which the expression of 1468 genes was upregulated and that of 505 was downregulated when the irrigation water was TFPW (Figure 5A). Instead, irrigation with FPW5 led to 4606 genes differentially expressed compared with TW control. Among these DEGs, 4003 genes were downregulated and 603 were upregulated in the TW

versus FPW5 group (Figure 5B). The emergence of upregulated and downregulated genes positively and negatively affect the physiological and developmental characteristics of wheat at the grain filling stage, respectively, which in turn determined the size and number of mature grains.⁶⁴

The aforementioned DEGs in TW versus TFPW and TW versus FPW5 were analyzed for functional and biological information using Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) annotation, respectively. GO annotation analysis showed that the DEGs could be annotated into three categories, namely, biological process, molecular function, and cellular component, with the top 20 enriched GO subcategories terms displayed in Figure 6. A high percentage of intersection DEGs related to binding (46.53%), catalytic activity (36.85%), cellular process (33.65%), metabolic process (33.10%), and cell part (33.55%) were induced by TFPW irrigation water in wheat grains (Figures 6A). However, in the intersection group of TW versus FPW5, cell part (50.80%), binding (47.81%), cellular process (43.36%), metabolic process (39.77%), organelle (34.65%), and catalytic activity (33.78%) apparently ranked as the top six terms (Figures 6B). The detailed regulated genes annotated by the KEGG database were classified into five pathways: metabolism, genetic information processing, environmental information processing, cellular processes, and organismal systems. In the intersection group of TW versus TFPW, only the number of upregulated DEGs annotated to carbohydrate metabolism pathways was not higher than that of downregulated DEGs (Table S7). Interestingly, the number of downregulated DEGs related to each KEGG pathway was separately higher than that of upregulated DEGs of the corresponding pathway in TW versus FPW5 (Table S8).

Furthermore, combined with enrichment analysis, we explored the effect of TFPW and FPW5 on major gene functions and metabolic pathways in wheat grains compared to irrigation with TW. Figure 6C shows that, apart from the highest enrichment degree of functional genes that determined

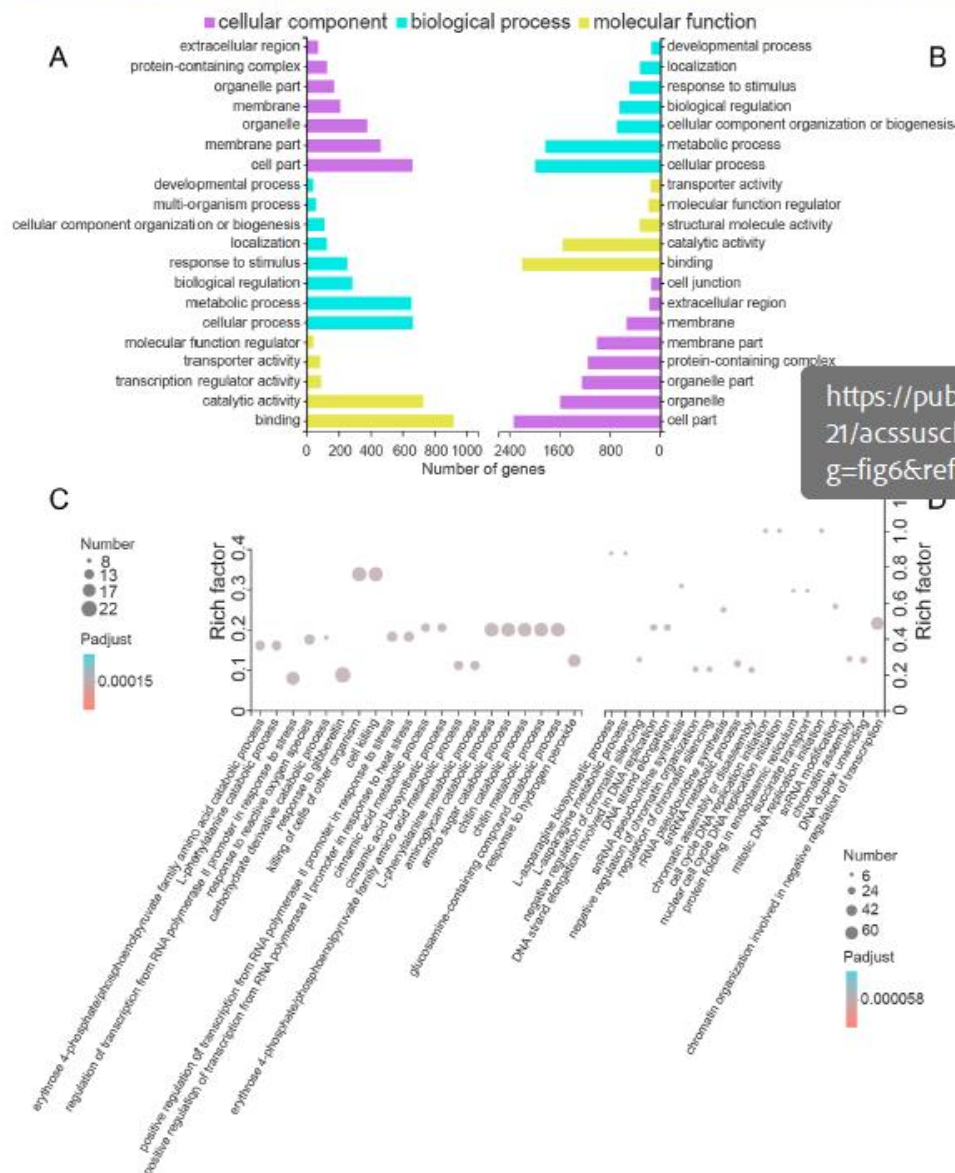


Figure 6. Function annotation and enrichment analysis of the intersection DEGs of wheat grains based on GO databases. (A,B): Subcategories of GO terms of the DEGs in TW vs TFPW and TW vs FPWS analysis, respectively. (C,D): Bubble diagram of top 20 ranked GO terms of DEGs in TW vs TFPW and TW vs FPWS analysis, respectively.

cells death (0.34), the genes related to glucosamine-containing compound catabolic process, chitin catabolic process, amino-glycan catabolic process, and cinnamic acid biosynthetic and metabolic process presented a high rich factor (0.2–0.22) among the top 20 ranked GO terms of DEGs. In addition, the carbohydrate derivative catabolic process, cell killing, glucosamine-containing compound catabolic process, and response to reactive oxygen species were more abundant functional

groups in comparison. Figure S5A presents the top 10 ranked KEGG pathways of enrichment, of which the highest enrichment was the MAPK signaling pathway-plant, followed by amino sugar and nucleotide sugar metabolism, and protein processing in the endoplasmic reticulum. Moreover, a large number of DEGs were involved in the phenylpropanoid biosynthesis (40), phenylalanine metabolism pathways (13), and starch and sucrose metabolism (24), which play an

important role in plant growth, development, and response to stress. As presented in Figures 6D and S5B, unlike the TW versus TFPW analysis, a large number of DEGs related to DNA replication and ribosome pathways appeared in TW versus FPW5 analysis.

CONCLUSIONS

This study focused on the toxic and biochemical effects of using diluted and treated shale gas FPW for the irrigation of wheat. Compared with TW, the total biomass and yield of wheat irrigated with TFPW decreased by about 20–25%, but nutrient elements accumulated more, including K, Ca, and Mg. In addition, more than half of the DEGs were induced upregulated, mainly involved in binding, catalytic activity, the cellular process, and the metabolic process. With the continuous use of irrigation water, the content of many elements in the soil increased. Notably, irrigation with TFPW resulted in some accumulation of heavy metals, namely, lead and chromium in wheat grains slightly above legislative limits, which might pose potential health risks to humans. However, accumulation was also observed when wheat was irrigated using TW, suggesting that this phenomenon is not necessarily related to the use of FPW and that it should be further investigated under field conditions. This study provides valuable and deep insights into the effects of irrigation water quality on wheat growth, toxicological characteristics, and gene transcriptional expression differences. Such findings are expected to support the sustainable reuse of shale gas FPW for farmland irrigation in terms of safety and technical feasibility.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acssuschemeng.2c02170>.

RNA extraction and sequencing; RSDs; risk control values; quality of RNA samples; mapped reads and sequenced data; KEGG annotation analysis; wastewater treatment processes; image of the wheat growth process; element concentration of soil; Venn diagram; and KEGG pathway enrichment analysis (PDF)

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Notes

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