

## Article

# Soil Health Assessment of Three Semi-Arid Soil Textures in an Arizona Vineyard Irrigated with Reclaimed Municipal Water

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**Abstract:** The depletion of freshwater supply is occurring at a faster rate than it is being replenished. The agriculture sector is the largest consumer of freshwater for irrigation and production-related processes. The use of reclaimed municipal water for the irrigation of crops offers a sustainable alternative solution for reducing the dependence of agriculture on freshwater. However, the long-term and continuous use of reclaimed water may contribute to soil salinity and sodicity limitations in agriculture production. The chemical and microbial properties of three different soil textures (all Alluvial soil with 60% clay: pH 8.6; 30% clay: pH 8.2; and 20% clay: pH 7.9) were evaluated in a vineyard irrigated using reclaimed water (126 mg/L Na<sup>+</sup>, 154 mg/L Cl<sup>-</sup>, 7.6 water pH, and 1.2 dS/m EC<sub>w</sub>). The results indicate that the reclaimed irrigation water significantly ( $p < 0.05$ ) increased the pH (by 0.4 to 18%), nitrate-N (over 100%), electrical conductivity (EC) (over 100%), and sodium absorption ratio (SAR) in these arid soils. A significant decline in microbial respiration (48 to 80%) was also documented in the three different soil textures that received reclaimed water. Although using reclaimed water for crop irrigation may be a substitute for using limited freshwater resources and offer a partial solution to increasing water security for wine grape production, the development of innovative technologies is needed for the long-term use of reclaimed water to counter its undesirable effects on soil quality.

**Keywords:** recycled water; irrigation; soil health; wine grapes; wastewater



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## 1. Introduction

The United States Department of Agriculture Natural Resources Conservation Service [1] defines soil health as the continuous capacity of the soil to function as a vital living ecosystem that sustains plants, animals, and humans. Key to achieving this capacity is the availability of soil water, with the primary sources being groundwater from the soil water table, precipitation, and supplementation by irrigation. Water availability is critical to the production of agricultural food and fiber crops locally and globally, which, in turn, are crucial to human health and the secondary aspects associated with food safety, socioeconomic factors, and environmental protection [2]. Approximately 60 percent of the world's freshwater is needed to grow food and fiber crops in sufficient amounts to meet the needs of the global human population [3]. By 2050, global demand is expected to require a 70-percent increase in agricultural production compared to that of 2009. This projection is based on the World Food Program's (WFP) predictions that the global human population size will far exceed the potential for increased agricultural productivity per unit of land area [4,5]. The projected increases in production are expected to derive primarily from an even more intensive cultivation of existing drylands and irrigated lands [6]. However,

given the increasingly anticipated drought conditions with the compounded effects of desertification and water shortages in the U.S., particularly in the southwestern United States [7–9], alternative sources of water for agriculture such as reclaimed water must be considered, which is a prospect that presents many new challenges [10,11].

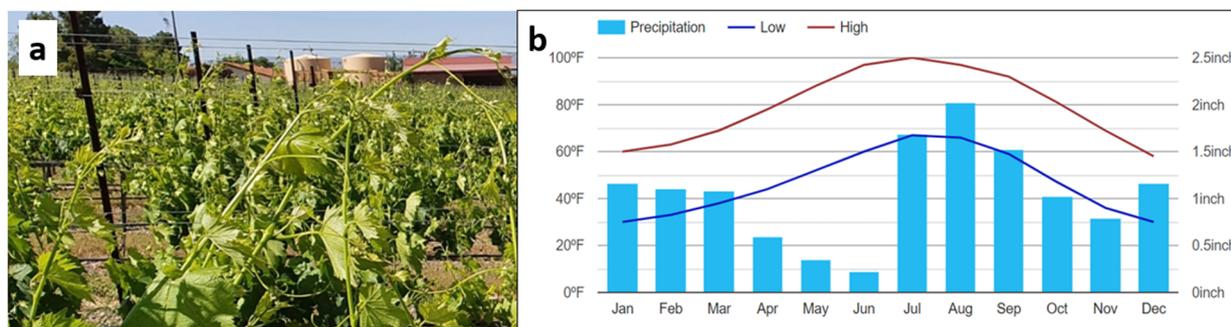
Reclaimed water use in agriculture is gaining traction as an alternative to freshwater use to meet agricultural demands. In 2012, the Environmental Protection Agency (EPA) revised the guidelines for wastewater reuse when the worldwide domestic wastewater use reached 500 to 1000 million cubic meters per day (compared to the capacity to treat advanced drinkable levels, which was only 4% (30 million cubic meters of water, globally, per day)) [12]. In 2020, North America and Europe alone produced approximately 146 billion cubic meters of wastewater per year [13], leading to the realization that city and municipal wastewater reuse could be expanded significantly [12]. In response, by 2010 in the U.S., approximately 50 of the 120 million cubic meters per day of wastewater was reclaimed and used for the agricultural production of food and fiber crops [12,14,15]. The majority of reuse occurs presently in California, Florida, Arizona, Texas, and Nevada [9,11,12,16].

The irrigation of agricultural fields accounts for the largest proportion of freshwater use in the U.S. [3,12]. This solution is threatened by climate extremes and other demands for water and increasing drought severity. Water demands in urban areas challenge the water quality and availability for agricultural use. Further irrigation constraints could impact sustainable and safe food production [17,18]. In Arizona, the impending reduction in the amount of water supplied from the Colorado River by 2022 will reduce the Central Arizona Project's agricultural water allocations by more than 50% [19]. This has led to a concerted effort to identify and harness other water sources for agricultural production, including reclaimed and recycled wastewater, toward the sustainable supply and use of available water. Sustainability is contingent upon research to better understand how reclaimed municipal water influences the physical, chemical, and biological soil properties to formulate best practices.

The objectives of this study were to evaluate the effects of reclaimed water on the chemical and biological properties associated with three different soil textural properties. The null hypothesis was that irrigating semi-arid soils with city-recycled water would not alter soil properties or the microbial composition or activity in the three soil textures.

## 2. Materials and Methods

*Location and climate:* The study was carried out in a vineyard established at the Southwest Wine Center, a thirteen-acre production area located at Yavapai College, Clarkdale, Arizona (34°44' N, 112°36' W) (Figure 1a). The average precipitation and temperature regimes recorded for the location from 1981 to 2010 are summarized in Figure 1b.



**Figure 1.** Southwest Wine Center vineyard showing the storage tank for the reclaimed water used for irrigating the vineyard (a). The monthly average climate in Clarkdale, Arizona from 1981 to 2010 (b) (the graphic was obtained from U.S. Climate Data 2022 (<https://www.usclimatedata.com/climate/clarkdale/arizona/united-states/usaz0309> (accessed on 17 August 2022))). The field picture in (a) was taken by Dr. Isaac K Mpanga.

History of the vineyard: The vineyard was planted in 2012 to support the Yavapai Community College Viticulture and Enology programs.

*Soil characteristics:* The field selected for planting the vineyard was found to contain three different soil textures. The formation of soil in the region has been aided by the erosion of the Great Colorado Plateau soils from streams entering the basin from the surrounding highlands, resulting in the three different soil textures and properties, summarized in Table 1.

**Table 1.** Soil physical and chemical properties of the three soil textures examined in this study.

Soil Properties	Field 1: 60% Clay Soil	Field 2: 30% Clay Soil	Field 3: 20% Clay Soil
	Physical soil description		
Soil classification	Alluvial	Alluvial	Alluvial
Clay	60%	30%	20%
Sand	Thick deposits of gravels	Thick deposits of gravels	Thick deposits of gravels
Rocks	Large rocks	Large rocks	Large rocks
Drainage	Low	Fair	Good
Organic matter	Low	Moderate	Moderate
	Chemical soil description		
pH	8.6	8.2	7.9
Nitrate	Very low	low	Medium
Phosphorus	Very low	Medium	High
Potassium	Very high	Very high	Very high
Calcium	Medium	Very high	High
Magnesium	Very high	Very high	Very high
Sodium	Very high	Medium	Low

*Irrigation water characteristics:* The reclaimed water used to irrigate the vineyard was obtained from the nearby city of Cottonwood. A reclaimed water line was installed near the vineyard and has been used to irrigate the Southwest Wine Center vineyard since 2014. The chemical characteristics of the reclaimed water are summarized in Table 2.

**Table 2.** Chemical properties of the irrigation water, where EC<sub>w</sub> refers to the electrical conductivity of water.

Test	mg/L
Sodium	126
Calcium	38
Magnesium	27
Potassium	15
Carbonate	0
Bicarbonate	320
Chloride	154
Sulfate-S	12
Nitrate	1.8
Phosphate	<0.10
Boron	0.23
pH	7.6
EC <sub>w</sub>	1.2 dS/m

*Field layout and experimental design:* The field was divided into three experimental plots, based on soil textural type (Table 1), with two treatments—irrigated plots (within row–R) and non-irrigated plots (between rows–BR)—and with three replicated experiments per field.

*Planting dates and distance:* Field 1 was 60% clay soil, with Malvasia vines that were planted in 2013. Field 2 was 30% clay soil and was planted with Aglianico vines in 2014. Field 3 was 20% clay soil and was planted with Piguepoul Blanc in 2017. In all 3 fields,

the vines were planted at a spacing of 153 cm within rows and 244 cm between rows, culminating in approximately 1000 vines per acre in each field.

*Watering and Fertigation cycles:* Drip tape lines were established in the fields for drip irrigation, which also delivered fertilizer through fertigation. The drip irrigation was set up such that each vine was supplied by two emitters rated at 0.5 gallons per hour. The irrigation schedule was dependent on the physiological growth stages of the vines. During the growing season, irrigation/fertigation was run for an average of 12 h per week, supplying a total of 12 gallons to each vine. In the spring, to promote canopy development, the amount of irrigation was increased, while during the fall, when grape ripening and dormancy is desired, the irrigation amount was reduced. When natural rainfall was received (Figure 1), the irrigation schedule was adjusted accordingly. Fertigation was applied at a rate of 7.5 L per hectare, with the fertigation-delivered urea as the ammonium nitrate (UAN-32 or UN-32) fertilizer. The fertilizer UAN-32 (Simplot, Lathrop, CA, USA) weighs 5 kg per 4 L, 32% of which is nitrogen, and it therefore supplies 1.6 kg of nitrogen per 4 L of water [20].

*Soil sampling:* Soil samples were collected on 25 June 2021 from a depth of 10 cm within the rows where the drip irrigation water was applied and from between the rows (BR), which received no irrigation water. Ten random samples were collected from each replicated R and BR experimental plot and mixed thoroughly for composites sampled for laboratory analysis. The soil samples were held on ice in an ice chest in the field and immediately transferred to storage. There were stored at 4 °C for one week before being shipped on ice packs for the laboratory analysis of the soil's fertility, salt concentration, and microbial composition analyses.

*Soil pH, organic matter, fertility, and salt measurements:* The collected soil samples were analyzed by Ward laboratories Inc. (Kearney, NE, USA), with the analysis of the soil properties conducted according to standard laboratory methods. For soil testing, pH was measured in a 1: 1 soil: water suspension [21], organic matter was estimated by a loss-on-ignition method [22], and the exchangeable soil cations potassium (K), calcium (Ca), sodium (Na), and magnesium (Mg) were extracted using ammonium acetate and were analyzed using inductively coupled plasma-optical emission spectrometry (ICP-OES) [23]. The NO<sub>3</sub>-N concentration was determined using potassium chloride extraction [22], and the Olsen P concentration was determined following sodium bicarbonate extraction [24]. The elements iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu) were extracted from the soil using diethylenetriaminepentaacetic acid (DTPA) and the concentration was determined by inductively coupled plasma-optical emission spectrometry (ICP-OES) [25]. The soil's sodium adsorption ratio (SAR) was estimated based on the concentrations of Na, Mg, and Ca in the saturated soil paste extracts. Soil microbial respiration was analyzed by incubating 40 g soil samples for 24 h at 24 °C. The samples were wetted through capillary action by adding 20 mL of deionized water to a 236.6 mL glass jar and capping it. After 24 h incubation, CO<sub>2</sub>-C analysis was carried out using the infrared gas analyzer (IRGA) Li-Cor 840A (LI-COR Biosciences, Lincoln NE) [26]. The null hypothesis was tested using the student's t-test for  $p \leq 0.05$ , with a two-tailed distribution with an assumption of equal variance (homoscedasticity) in Excel. A comparison based on soil type could not be carried out because the vine planting and the applications of reclaimed water were not carried out at the same time.

### 3. Results and Discussion

#### 3.1. Recycled Water's Effects on Soil pH, Organic Matter, and Elements

The effects of reclaimed water irrigation on soil pH, organic matter, and elements for the three different textured soils are summarized in Table 3. The soil pH was significantly affected by irrigating with reclaimed water ( $p < 0.05$ ) in that the soil pH was significantly higher within the rows (R) than between the rows (BR) (not irrigated) for the three soil textures. For the 60% clay soil, the mean pH was 8.3 in the irrigated R soil, compared with 8.0 for non-irrigated BR soil. For the 30- and 2-percent clay soils, the mean pH levels of

the R soil were 8.1 and 8.4, respectively, whereas the mean pH levels of the BR soils were 7.3 and 7.1, respectively (Table 3). These observations are consistent with those reported in a previous study in which appreciable increases in soil pH were associated with long-term irrigation with reclaimed water [27]. The higher pH levels associated with irrigated soils are most likely attributable to the alkaline pH (7.6) of the reclaimed water used for irrigation. Reclaimed water characteristically contains bicarbonate ions, salts, and plant nutrients that can result in the increased pH of soil when used for long-term irrigation (Table 2; [28,29]).

**Table 3.** Recycled water effects on soil pH, organic matter, and elements in varied soil textures in vineyard under drip irrigation. \* = significant t-test at  $p \leq 5\%$  ( $n = 3$ ).

	Field 1: 60% Clay Soil		Field 2: 30% Clay Soil		Field 3: 20% Clay Soil	
	Irrigated	Non-Irrigated	Irrigated	Non-Irrigated	Irrigated	Non-Irrigated
Soil pH (1: n1)	8.3 *	8	8.1 *	7.3	8.4 *	7.1
CEC (me/100 g)	38.9	40.7	29.2 *	24.6	18.1 *	14.9
Organic matter (%)	4.8	5.2 *	3.3	3.5	3.1	3.1
Nitrate-N (mg/g)	13.7 *	5.1	23.8 *	10.3	10.1 *	2.9
Olsen P (mg/g)	13.4	12.9	28.8	44.0 *	8.9	23.5
Potassium (mg/g)	628.3	534	653	764.0 *	423.7	778.3 *
Sulfate (mg/g)	126.4	100.8	145.5	115.8	47.5	51.4
Calcium (mg/g)	4947.3	6419.7 *	3698.3	3872.7	2129	2056.7
Magnesium (mg/g)	957.3	833	718.7 *	380.3	606.3 *	304.3
Sodium (mg/g)	1052.3 *	65.7	697.0 *	23	318.0 *	18.3
Zinc (mg/g)	8.4	11.2 *	9.8 *	8.3	8.5	13.5 *
Iron (mg/g)	6.3	11.7 *	8.7	14.6 *	6.6	17.8 *
Manganese (mg/g)	9.9	9.6	24.4	57.6 *	10.8	64.0 *
Copper (mg/g)	32.7	45.9 *	14.5	20.9 *	15.1	34.8 *
Recycled water irrigation start year	2012		2014		2017	

The recycled water for irrigation resulted in a significant increase in the nitrate-N content of the three soil textures. For the 60%, 30%, and 20% clay soils, the mean nitrate-N content within the rows was 13.7, 23.8, and 10.1 mg/g, respectively, whereas the mean concentration between the rows was significantly lower at 5.1, 10.3, and 2.9 mg/g, respectively (Table 3; [10]). Nitrate-N accumulation was associated with the addition of nitrogen derived from the reclaimed water. Another study reported an average increase of 5.2–40.4% in the mineral nitrogen content of the rhizosphere [30]. Consequently, a positive outcome is that the presence of nutrients such as nitrate-N in reclaimed water reduces the need for additional nitrogen fertilization in agricultural fields irrigated with recycled water.

In the vineyard soils, a significantly higher soil cation exchange capacity (CEC) was documented for the within-row, compared to between-row, values for the 30% and 20% clay soils. However, the R and BR soils with 60% clay showed no significant difference in CEC (Table 3). In previously reported studies, irrigation with wastewater resulted in an increase in CEC in the range of 33 to 42% in corn fields [31] and 15% in rice fields [32]. In this study, the within-row sodium level was significantly higher than that of the between-rows for the three different clay soil types. For the 60%, 30%, and 20% clay soils, while the Na concentrations in the BR soil were 65.7, 23.0, and 18.3 mg/g, respectively, the respective concentrations in the R soils were much higher at 1052.3, 697, and 318 mg/g, respectively (Table 3). The recycled irrigation water applied to the vineyard had a high sodium content (126 mg/L) compared to the other elements evaluated (Table 2). The elevated within-row sodium content was most likely due to the placement of the reclaimed drip irrigation emitters above the vines (within-row). This is similar to the results reported by other studies in which sodium levels were increased when soils were irrigated with reclaimed water [10,29].

Significantly lower soil potassium was observed in the vineyard R soils that received reclaimed water compared to the non-irrigated BR soil in fields 2 and 3. This result

is most likely due to the exchangeable sodium that promotes the loss of exchangeable potassium through leaching into the soil, given that potassium can be replaced by sodium in CEC complexes [33]. There was no significant difference in the potassium concentrations between the R and BR soil in field 1. This can be attributed to the high clay content of field 1's soil texture. Clay-rich soils have low saturated hydraulic conductivity, are rated as a very slow permeability class, and are high in water holding capacity [34,35]. Collectively, these properties afford resilience against leaching at any given water potential, minimizing the loss of exchangeable potassium.

### 3.2. Recycled Water Effects on Soil Salt (Saturated Paste)

The results of the analysis of the saturated soil paste extracts for cations ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , and  $\text{Na}^+$ ) and anions ( $\text{Cl}^-$  and  $\text{SO}_4^{2-}$ ), electrical conductivity (EC), and SAR are summarized in Table 3, while the effects of the reclaimed water irrigation on these parameters are shown in Table 4.

**Table 4.** Recycled water's effects on soil salt (saturated paste) in varied soil textures in a vineyard under drip irrigation, where EC = electrical conductivity. \* = significant t-test at  $p = 5\%$  ( $n = 3$ ).

	Field 1: 60% Clay Soil		Field 2: 30% Clay Soil		Field 3: 20% Clay Soil	
	Irrigated	Non-Irrigated	Irrigated	Non-Irrigated	Irrigated	Non-Irrigated
EC (mmho/cm)	5.3 *	1.7	6.2 *	1.9	2.4 *	1.4
Chlorine (mg/g)	1242.7 *	162.3	1399.3 *	48.7	369.7 *	39.7
Calcium (mg/g)	295	290.3	413	370.3	124.7	231.0 *
Magnesium (mg/g)	91.0 *	56.3	166.7 *	62.3	58.3 *	51.7
Sodium (mg/g)	904.7 *	52	897.3 *	20.3	329.0 *	12.3
Sulfur (mg/g)	237.2 *	170.4	337.0 *	281.3	142.8	164.4 *
Sodium absorption ratio	11.8 *	0.7	9.4 *	0.2	6.1 *	0.2
Recycled water irrigation start year	2012		2014		2017	

The results showed that the concentrations of chlorine, magnesium, and sodium in the irrigated fields were significantly higher compared to the non-irrigated fields among all three soil textural types. Further, soil in the rows receiving irrigation water showed significantly higher EC and SAR levels (Table 4). Soil salinity is one of the primary concerns associated with reclaimed water used for irrigation because salt accumulation can deteriorate soil quality [36]. Soil salinity measurements are characteristically based on EC values [37].

In this study, plant mortality was associated with high soil salinity, a result that is consistent with a higher EC, compared to the freshwater control. This is consistent with the results of a previous study that reported a higher EC in soil planted with alfalfa and irrigated with municipal wastewater [38]. Additionally, a study conducted on reclaimed wastewater found that the average soil salinity in the wastewater-irrigated plots was 19.2% higher than in the plots irrigated with freshwater [39]. However, there was no measurable negative affect on the growth of bluegrass and buffalo grass in this experiment, likely because the latter grasses exhibit some degree of salt tolerance. Finally, [40] reported a gradual increase in EC values in topsoil after each irrigation period, with values of 51.6, 78.6, 113.2, and 122.7  $\mu\text{S}/\text{cm}$  at 0-, 3-, 8-, and 20-years post-irrigation, respectively.

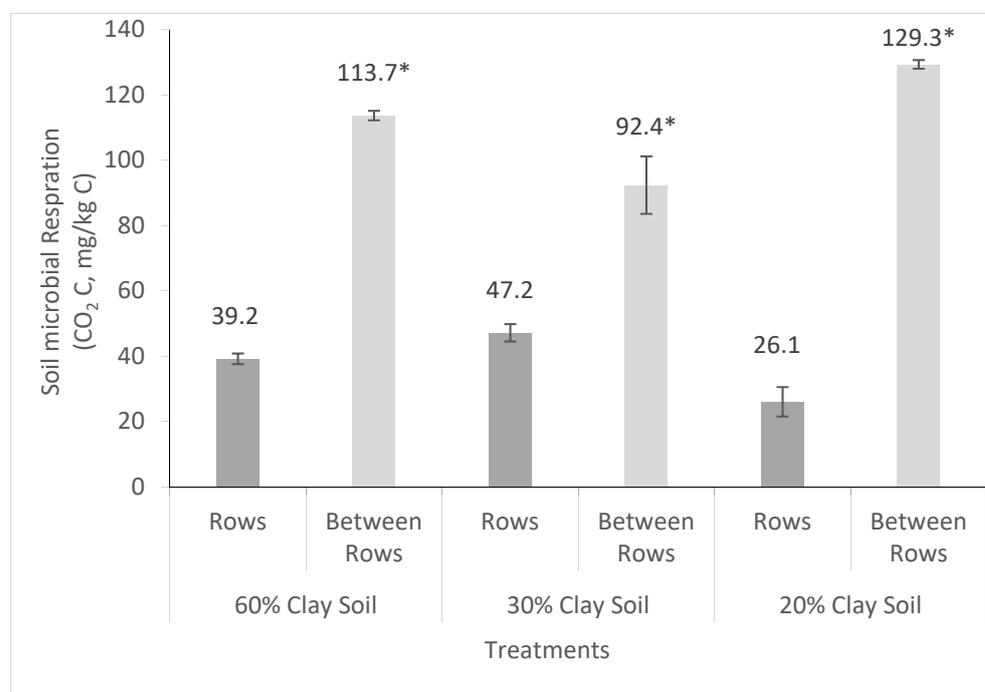
In this study, the sodium and chloride concentrations in the reclaimed water was 126 mg/L and 154 mg/L, respectively (Table 2). Greater soil salinity with respect to EC values contributed by  $\text{Na}^+$  and  $\text{Cl}^-$  has been reported in previous studies [41–44], collectively, which supports the conclusion that sodium and chlorine accumulate in soils irrigated with reclaimed water.

Soil SAR is an index measuring the proportion of sodium ( $\text{Na}^+$ ) to calcium ( $\text{Ca}^{2+}$ ) and magnesium ( $\text{Mg}^{2+}$ ), and it is calculated from cation concentrations in the water extract from saturated soil paste [45]. When SAR values are greater than 13 (US salinity lab staff, 1954),

the potential for soil sodicity increases. Though the SAR values within the vineyard rows were less than 13, the long-term and continuous use of reclaimed water for crop irrigation will likely increase the risk of developing a sodic soil.

### 3.3. Recycled Water Effects on Soil Microbial Activities (Respiration)

The use of reclaimed water significantly reduced soil microbial respiration (Figure 2). For field 1 with 60% clay soil, the mean soil microbial respiration was 39.2 mg CO<sub>2</sub>-C/kg for the R soil and 113.2 mg CO<sub>2</sub>-C/kg for the BR soil (Figure 2). Similarly, fields 2 and 3 had significantly lower soil microbial respiration rates for the in-row soil (Field 2, R: 47.2 mg CO<sub>2</sub>-C/kg and Field 3, R: 26.1 mg CO<sub>2</sub>-C/kg) compared to the soil sampled from between the rows (Field 2, BR: 92.4 mg CO<sub>2</sub>-C/kg and Field 3, BR: 129.3 mg CO<sub>2</sub>-C/kg) (Figure 2). The higher EC and concentrations of the soluble cations Na<sup>+</sup>, Mg<sup>2+</sup>, and Cl<sup>-</sup> were observed within the rows in the present study. A lower rate of microbial respiration in rows where recycled irrigation water has been applied is consistent with high salt concentrations, as was previously reported by [46].



**Figure 2.** The effects of reclaimed water use on soil microbial activity, measured as respiration, in the three different soil textures from our study's vineyard, using drip irrigation. \* = significant *t*-test at  $p = 5\%$  ( $n = 3$ ). Rows = irrigated with reclaimed water and between rows = non-irrigated.

Several studies have evaluated the impact of salt on the composition of soil microorganisms. Some have shown that soil microorganisms adapt to or tolerate osmotic stress resulting from increased salinity [47,48]. In other instances, the accumulation of higher salt concentrations has been shown to negatively affect soil microbial composition and activity [49]. Soil biological processes, including microbial respiration, have been shown to be adversely affected by the increased soil salinity levels associated with reclaimed water use [50]. In another study, soil respiration was reduced by more than 50% in saline soil, with EC values of greater than 5.0 dS/m [51]. A metagenomic analysis of the soil microbiome concluded that the higher salinity level of reclaimed water can promote more specialized, less-diverse microbial communities [52], while a functional genomics study showed an altered expression of proteins, particularly those involved in cellular metabolism [53]. Thus, the higher salinity characteristically associated with the use of reclaimed water for agricultural crops influences the soil EC and soluble cation availability and the composition

and cellular metabolism of the soil-inhabiting microbial community, resulting in reduced microbial respiration.

### 3.4. Approaches to Recycled Water Use with Reduced Undesirable Effects on Soil Health

To address the challenges associated with the undesirable effects of reclaimed water use on soil and microbial health, one proposed solution is to focus on water recovery instead of nutrient supply to ensure the delivery of higher-grade reclaimed water free of high concentrations of minerals and other substances, such as the salts and heavy metals that are toxic to both the soil microbial community [10] and, often, to the crop plant, as well. This may require policy changes and additional research to achieve relevant innovations and circularity, especially in the U.S. southwest, and in other arid land locales, globally. In addition, heavy rainfall events (Figure 1) can aid in flushing salts from the soil, reducing the overall accumulation. Wood and Blankinship [54] advocated for a clear link to management among the criteria pertinent to practical soil health management and the interpretation of the respective soil health indicators. Other best management practices are recommended, such as regular cover-cropping, mulching, and planting salt-tolerant crop varieties, as well as avoiding or alternating the use of reclaimed and fresh water in salt-stressed soils.

## 4. Conclusions

The interpretation of soil health indicators is complicated by differences in land use practices, cropping systems, ecosystems (natural or otherwise), governing policies, and cultural/management practices, among other factors, necessitating tailor-made definitions of “soil health” at scales equivalent to multiple or an individual farms. This is evident among small-scale farms in Arizona, and it is exemplified by the vineyard studied here. The results reported here demonstrate that reclaimed water has a direct effect on the physical and mineral compositions of the soil, which are useful indicators of soil health. The use of reclaimed water to irrigate the vineyard studied here resulted in increases in the soil’s electrical conductivity, sodium adsorption ratio, and concentrations of  $\text{Na}^+$ ,  $\text{Cl}^-$ , and  $\text{Mg}^{2+}$  ions, respectively. Soil microbial respiration was lower in the soils irrigated with reclaimed compared to fresh water. The use of reclaimed water can contribute to the increased availability of water for irrigation in arid regions; however, prolonged and continuous irrigation solely with reclaimed water will require innovative technologies and modified practices to counter the negative effects on soil health due to salt accumulation and the resultant increased soil salinity. This information is expected to lead to more informed decision-making when using reclaimed water for irrigating arid farmlands.

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**Data Availability Statement:** The data are available and can be provided by the authors when formally request in writing.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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