soil systems

MDPI

Article

Comparison of Surface Water or Treated Municipal Wastewater Irrigation on Alfalfa Establishment, Soil Fertility, and Soil Microbial Conditions

Leonard M. Lauriault [1,*], Nicole Pietrasiak [2], Murali K. Darapuneni [1], Andrew J. Dominguez [2] and Gasper K. Martinez [1]

[1] Rex E. Kirksey Agricultural Science Center and Plant and Environmental Sciences Department, New Mexico State University, 6502 Quay Road AM.5, Tucumcari, NM 88401, USA
[2] Plant and Environmental Sciences Department, New Mexico State University, Las Cruces, NM 88003, USA
* Correspondence: lmlaur@nmsu.edu

Abstract: Water scarcity for agricultural irrigation is increasing globally while generation of treated municipal wastewater (TWW) is increasing due to urban expansion. Municipalities seek uses for their TWW, which is safe to apply to forage crops. Alfalfa (*Medicago sativa*) is the most important forage crop worldwide being adapted to a wide range of environmental factors, including irrigation with low quality water. A strip plot study with four replications at New Mexico State University's Rex E. Kirksey Agricultural Science Center at Tucumcari, NM USA, compared the effects of surface water (SW) and TWW on alfalfa establishment and soil fertility and microbial growth. Alfalfa established equally well when irrigated with equal amounts of TWW or SW. After one year, the application of TWW increased soil P and plant N and P more so than SW. Most microbial soil health indicators were positively increased by alfalfa establishment in virgin soil; however, the effect was greater with TWW compared with SW (1147, 1184, 1961, and 4991 nmol g^{-1} for total microbial biomass of soil irrigated with SW and TWW at seeding and after one year, respectively, $LSD_{0.05}$ = 710). Thus, TWW irrigation could reduce applied fertilizer P to meet alfalfa's requirement and increase soil health compared with SW.

Keywords: alfalfa; establishment; irrigation; microbial biomass; soil fertility; treated municipal wastewater

Citation: Lauriault, L.M.; Pietrasiak, N.; Darapuneni, M.K.; Dominguez, A.J.; Martinez, G.K. Comparison of Surface Water or Treated Municipal Wastewater Irrigation on Alfalfa Establishment, Soil Fertility, and Soil Microbial Conditions. *Soil Syst.* **2022**, *6*, 67. https://doi.org/10.3390/soilsystems6030067

Academic Editor: Yam Kanta Gaihre

Received: 24 June 2022
Accepted: 8 August 2022
Published: 10 August 2022

1. Introduction

Water scarcity is increasing globally, particularly for agricultural irrigation [1–4]. At the same time, treated municipal wastewater (TWW) is generated at an increasing rate due to ongoing urban expansion [3,5]. Municipalities seek uses for their TWW, which is generally safe to apply to animal feed and fiber crops while utilizing soils in their filtering function. Water purification via soil filtration minimizes the release of potential pollutants into surface and ground water bodies as well as saving fresh water for other uses [3,6–9].

Municipal TWW has multiple advantages compared with surface water (SW), the untreated form of freshwater from streams or stored in lakes and generally used for agricultural irrigation. These advantages include increasing and consistent availability of the water due to ongoing urban expansion [3,5], serving as a nutrient source [5,7], and improving soil health [2,5]. However, secondary-treated wastewater could pose environmental, crop safety, and livestock and human health concerns associated with a wide array of contaminants, also called compounds of concern [3,5–10], because the secondary treatment processes most commonly used do not remove the compounds of concern, including heavy metals [3,5,6,8] and organic compounds from pesticides and pharmaceuticals [3,4,8,11]; only solids and pathogens are removed or killed [11]. Additionally, treated municipal wastewater for irrigation is regulated by environmental guidelines [7,8,12], sometimes

limiting availability due to non-compliance (L. Lauriault, personal observation). While TWW in southern U.S. forage production poses an attractive alternative water source if managed appropriately, the effects of TWW on crop establishment, yield, and quality as well any potential interaction with regional that may soils arise, are still largely uncertain.

Alfalfa (*Medicago sativa*) is the most important forage crop worldwide being adapted to a wide range of environmental factors, including irrigation with low quality water [1,2,4,8], especially compared with forage grasses [3]. As a nitrogen-fixing legume [13], alfalfa also has greater crude protein (CP) and other nutritive value components for livestock feed than grasses [4,8,14,15].

Considerable research has been done on the effects of using various sources of wastewater on alfalfa production and soil health elsewhere. Adrover et al. [5] reported non-detrimental effects on alfalfa rotated with maize (*Zea mays*) and barley (*Hordeum vulgare*) after >20 years of flood irrigation with TWW, although soil pH was increased due to increased Na levels. Adrover et al. [2] found that soil salinity was increased with TWW irrigation compared with sea water-intruded groundwater, despite greater salinity in the groundwater, especially when soil clay and organic matter content were greater. Otherwise, Elfanssi et al. [4] reported a difference in the benefits of various irrigation water qualities, such that TWW increased agronomic productivity of alfalfa compared with fresh well water, but untreated (raw) municipal wastewater irrigation adversely influenced plant physiological measurements, such as stomatal conductance and chlorophyll inflorescence and content, leading to reduced yields. The effects of pharmaceutically-active compounds in TWW on crops are generally limited to prolonged germination periods, reduced seedling growth rates, and biochemical processes, namely, enzymatic activity, proline, sugars, and macro- and micronutrient contents [3,11]. Elfanssi et al. [4] deduced that TWW does not negatively influence the physiological state of alfalfa as did untreated (raw) wastewater, as indicated by proline content.

Determining the potential effects of irrigating alfalfa during establishment with TWW could assist producers in deciding whether to use that source of water for alfalfa irrigation. Consequently, the objectives of this study were to compare the effects of SW and TWW on establishment and the year after seeding of alfalfa, as well as soil fertility and microbial growth and diversity.

2. Materials and Methods

The test was conducted at New Mexico State University's Rex E. Kirksey Agricultural Science Center at Tucumcari, NM USA (35.20°, −103.69°; elev. 1246 m; Figure 1).

Strip plots were established on Redona fine sandy loam: fine-loamy, mixed, superactive, thermic ustic calciargids (https://soilseries.sc.egov.usda.gov/OSD_Docs/R/REDONA.html; accessed on 7 August 2022) in areas that had been center pivot irrigated by either SW or TWW. Wastewater treatment involved a Class 1B treatment [12] with mean 2 *E. coli* colony forming units/100 mL following 100% intensity UV irradiation as a secondary treatment. Water treatments were applied during the previous 18 months, during which time warm-season annual cereal forages were grown for pasture and hay production. Strip plots were separated by 90 m to allow for transition of the irrigation system between water sources. There were 4 replications within each irrigation water source strip plot and while this was part of a larger study, only results from the control plots from that study from each replicate are analyzed and reported here because the data for remaining treatments were proprietary. Figure 1 shows the study area with strip plots identified and location of the water source transition point. Unreplicated soil samples (16 2.5-cm diameter cores to 30 cm deep combined by strip plot) were collected immediately pre-planting for fertility [16]. A sample of each water source was collected for irrigation quality analysis [16] from an outlet at the irrigation system before and after the study period (August 2017 to October 2018). Weather data were collected from a station within 1 km of the study site (Table 1).

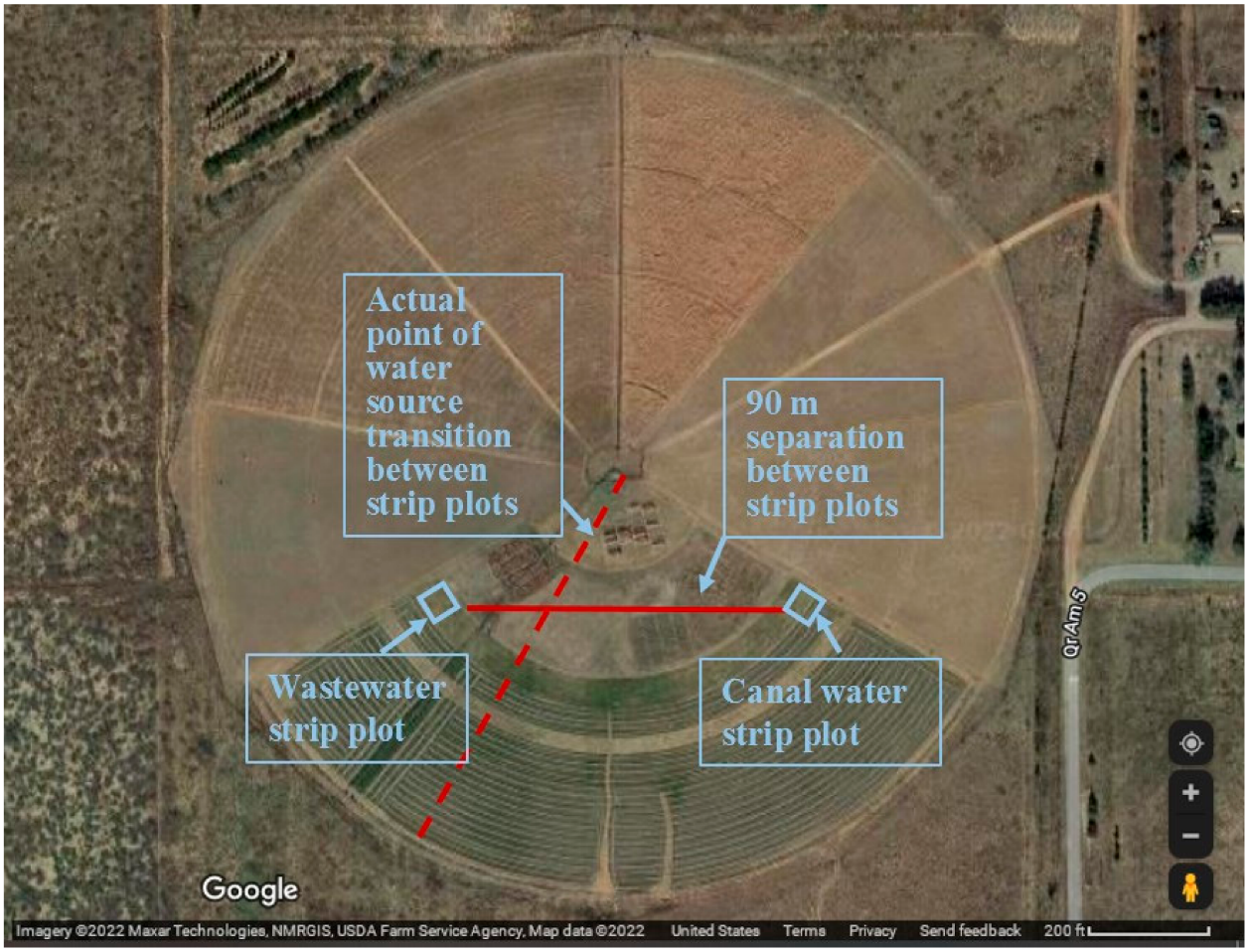

Figure 1. Map of the study site at Tucumcari, NM, USA, with irrigation water source strip plots identified.

Table 1. Monthly mean air temperatures and total precipitation and irrigation amounts at Tucumcari, NM, USA, during the study period in which alfalfa was planted on 18 August 2017 and sampled in late October of 2017 and 2018, and the long-term temperature means and precipitation totals (1905–2018).

Year	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.	Annual
Temperature, °C													
2017	3.9	10.0	6.7	14.4	17.8	25.6	27.8	23.9	21.1	15.6	11.9	4.8	15.3
2018	3.3	6.1	11.1	13.3	22.2	27.2	27.2	25.6	22.2	14.4	6.7	3.9	15.3
Long-term	3.3	5.6	9.4	14.4	18.9	24.4	26.1	25.0	21.7	15.6	8.9	3.9	14.7
Precipitation, mm													
2017	26	4	55	69	46	25	40	165	67	92	0	0	590
2018	0	1	4	13	46	14	29	85	20	108	14	16	351
Long-term	11	13	20	30	51	51	70	73	42	35	19	15	429
Irritation, mm, applied nearly equally to treatments													
2017	0	0	25	51	6	191	146	13	25	0	0	0	457
2018	0	0	0	0	32	89	85	49	44	0	0	0	299

Based on performance in New Mexico alfalfa variety tests [17,18], alfalfa variety, 6829R (NEXGROW Alfalfa, West Salem, WI, USA), was selected for use in the study. Prior to planting, a conventionally tilled, flat seedbed was prepared by chisel plowing to 60 cm followed by disking, leveling, and firming. Plots (1.5 m × 6.1 m) of multiple treatments including a control were sown under a pivot irrigation system on 18 August 2017, using a

disk drill fitted with a seed-metering cone at 22.45 kg inoculated seed ha^{-1} in a strip-plot design. The effective planting width was 1.22 m with a 15-cm row spacing. All irrigations, before and after seeding alfalfa, were applied at a minimum rate of 6 mm. The irrigation system failed in early October 2017 and was not repaired until late May 2018. No fertilizers or pesticides were applied in 2017. The irrigation system failure and another shutdown took place from 19 to30 August 2018 and again in late September 2018, the latter of which was not repaired until well after the growing season. Since the same irrigation system was used for both strip plot treatments, the effects of the irrigation system failures were equal, and the irrigation frequencies and application rates were nearly equal. To supplement 664 mm of precipitation during the study period, a total of 337 mm of irrigation was applied from August 2017 to October 2018 (Table 1), which is when the test was terminated. On 26 June 2018, 19 kg N and 63 kg P_2O_5 ha^{-1} were applied to the entire field in which both strip plots were located (Figure 1).

On 29 August and 6 September 2017, plants were counted within 1 m of row and averaged. On 25 October 2017 (68 days after planting (dap)), all plants in 0.37 m^{-2} from the end of each plot, including all rows, were hand-clipped to ground level weighed, dried at 60 °C for 48 h, and reweighed for calculation of dry matter proportion and dry weight. These samples were held for estimation of plant chemical constituents by near-infrared spectroscopy (NIRS; Ward Laboratories, Kearney, NE, USA [16]) using an equation developed for alfalfa. Forage nitrogen was calculated as the NIRS-generated estimate for CP/6.25 [14]. Immediately following plant sampling, the soil surrounding one plant (crown and root) from each of the two center rows within the plant sampled area of each plot was also sampled (2.5 cm) to 7.5 cm within the clipped area and the two cores from each plot were composited for soil fertility and phospholipid fatty acid (PLFA) analysis also by Ward Laboratories [16]. During the sampling, nodulation was verified on each seedling with intact root.

During 2018, whole plot standing forage was removed as growth permitted on 10 July and 15 September, but not measured. On 30 October 2018, regrowth forage from each plot was collected and handled as previously described. The area that had been sampled in 2017 was avoided for this sampling. On 20 November 2018, stand percentage was rated, as determined by seeded row fill, and two 2.5-cm diameter soil cores from within the area from which plants were sampled in each plot were collected to 30 cm and combined for soil fertility and PFLA analysis [16].

Statistical Analysis

Pre- and post-study water analyses from each water source were subjected to SAS MIXED [19] procedures for tests of significance to compare water source (surface water or treated wastewater) using year (2017 and 2018) as the replication factor. Forage dry matter m^{-2}, dry matter proportion, and plant chemical constituents and soil PLFA data from 2017 and 2018 were subjected to SAS MIXED [19] procedures for tests of significance to compare water source treatments (surface water or treated wastewater) and year (2017 and 2018) and their interaction. Plants m^{-2} from 2017 and stand percentage and soil fertility from 2018 were subjected to SAS GLM [19] procedures for tests of significance to compare water source treatments. For each analysis, replicates were defined as unique within water source and considered random and used as denominators for tests of significance [20]. All differences reported are significant at $p \leq 0.05$ as well as when a biologically significant trend ($0.05 < p < 0.10$ [21] is indicated. When a main effect or interaction was significant, a biologically significant trend ($0.05 < p < 0.10$ [21] was indicated, protected ($p \leq 0.05$) least significant differences were used to determine where differences occurred among treatment means using the PDMIX800 SAS macro [22].

Data variabilities, medians, and means of PLFA microbial biomass as well as community composition were graphed in R version 4.0.4 (The R Foundation, 2021; www.r-project.org; accessed on 7 August 2022). Changes in microbial community composition based on applied treatments were visualized with nonmetric multi-dimensional scaling (NMDS)

using the vegan package in R. PERMANOVA was applied to test for differences between sampling years and water source treatment. Further, due to mostly non-normal distribution, microbial PLFA groups were related to soil chemistry variables using Spearman Rank correlations with the 'rcorr' function in the Hmisc package and visualized as heatmaps with the 'corrplot' function in the R package, Corrplot.

3. Results and Discussion

3.1. Weather

Weather data for 2017 and 2018 are presented in Table 1. Temperatures were cooler than average in August 2017, but near average in September and October and warmer than average in November. The first autumn freeze occurred on 9 November 2017. Precipitation also was greater than average from August to October 2017 and irrigations totaling 38 mm were applied in August and September to supplement precipitation (Table 1). These conditions should have promoted growth for establishment well before the onset of the first temperature of −2.8 °C [23,24]. Additionally, Darapuneni et al. [25] reported that, for otherwise fully-irrigated alfalfa, irrigation termination after mid-September had little influence on overall alfalfa yield. In 2018, late spring and early summer temperatures were slighter warmer than average (Table 1). Irrigations totaling 299 mm were applied when possible, from May to October 2018 to supplement 328 mm of precipitation from November 2017 to October 2018.

3.2. Water Sources

Results of irrigation water quality analyses comparing SW and TWW are reported in Table 2. The lack of difference between water sources and values for water pH indicate a low concern for increasing Na problems [26] as also indicated by sodium absorption ratio (SAR) [27]. Differences between water sources existed for SAR, adjusted SAR, total dissolved solids (TDS), electrical conductivity (EC), cations, Na, K, Cl, and B, with trends ($0.05 < p < 0.10$) [21] for Mg and hardness, such that TWW had greater values that SW. These greater values represent relatively greater nutrient status of TWW over SW [4,5,7,8]. Other variables had numeric differences at $p < 0.20$. The combination of SAR and EC for both water sources (Table 2) indicates a low risk of soil infiltration problems with no reduction in alfalfa yield [26]. Regarding EC and TDS, SW presented a low salinity hazard (0.25–0.75 mmhos cm^{-1} EC and 160–480 ppm TDS; [26]), while TWW presented a medium salinity hazard, although barely (0.75–2.0 mmhos cm^{-1} EC and 480–1280 ppm TDS; [26]) (Table 2).

Based on these irrigation water quality analyses, SW, such as that used in the present study, should not be applied by overhead irrigation at rates < 5 mm [26]. Additionally, TWW should not be applied through overhead irrigation at all unless 3 kg H_2SO_4 ha-mm^{-1} are injected into the water to avoid lime deposits in crop leaves or fruit [26]. All irrigations were applied at >5 mm and no H_2SO_4 was used, although no lime residue was observed on the alfalfa. The analysis components for SW were somewhat consistent to those reported by Hopkins et al. [26] for canal water, which is the same as SW in the present study and are likely driven by the composition of the soil surrounding the streams, storage lakes, and canals that comprise each system. Alfalfa is relatively tolerant of low-quality irrigation water [2] and not considered a sensitive crop in regard to the levels of Cl and B in either water source (Table 2) [26]. Regarding plant nutrients, both water sources had low NO_3-N, Ca, and SO_4-S, while SW had low P and high Mg and TWW was high in both Mg and P (Table 2) [27]. Although statistically not significant, TWW had greater bicarbonates and alkalinity than SW.

Table 2. Means of irrigation water quality analysis and results of statistical analyses of canal water and Class 1B treated municipal wastewater used to irrigate alfalfa during establishment at Tucumcari, NM, USA. Values are the least squares means of samples (one from each water source) collected before and after the study period (2017–2018).

Variable	Canal Water	Wastewater	*p*-Value	SED
pH	8.05	7.85	0.6286	0.35
SAR	1.50	4.50	0.0194	0.42
AdjSAR	1.65	5.10	0.0182	0.47
TDS, ppm	416	694	0.0237	44
EC, mmho cm^{-1}	0.69	1.16	0.0250	0.08
Cations, me L^{-1}	8	13	0.0231	1
Anions, me L^{-1}	8	15	0.1418	3
Cations:anions	1.02	0.92	0.5863	0.13
Na, ppm	56	171	0.0229	18
K, ppm	7	22	0.0358	3
Ca, ppm	59	40	0.1273	8
Mg, ppm	26	42	0.0716	5
Hardness, ppm $CaCO_3$	253	271	0.0903	3
NO_3-N, ppm	0.1	0.6	0.5000	0.5
SO_4-S, ppm	66	73	0.7956	24
Cl, ppm	18	94	0.0321	14
CO_3, ppm	1.0	0.5	1.0000	1.0
HCO_3, ppm	177	478	0.1354	65
Alkalinity, ppm $CaCO_3$	147	408	0.1540	65
B, ppm	0.06	0.42	0.0274	0.06
Ortho P, ppm	0.06	3.35	0.1769	1.61
Total P, ppm	0.05	3.36	0.1614	1.52

SED, SAR, and TDS signify standard error of the difference between means, sodium absorption ratio, and total dissolved solids, respectively.

3.3. Soil Fertility

Results of soil fertility analysis in 2017 and 2018 with statistical analysis for 2018 are presented in Table 3. Preplant soil analysis revealed no apparent issues in regard to fertility (including toxicities) or potential salt problems for either strip plot, both of which had been irrigated with the respective test water source for at least 18 months before the alfalfa was planted.

Differences or trends ($0.05 < p < 0.10$) [21] between water sources existed in 2018 for all variables except for Fe, Mn, and Mg saturation (Table 3). The application of TWW led to an increase in soil salt content after one additional year of application. Soil P at planting in 2017 was below levels for optimum alfalfa growth [16], but at the end of study in 2018, the soil P content was statistically greater in the TWW-irrigated soil compared to SW-irrigated soil (Table 3), which is consistent with the findings of others who attributed to the high P content in the TWW [4,5,10], although it was not significantly greater in the present study than SW (Table 2). This may be due to greater bicarbonates in the TWW compared with SW. Bicarbonates tend to compete with the P in the soil to form carbonate complexes with Ca and Mg and hence increase the P availability in the soil [28]. In any case, while the P fertilizer application alone was not sufficient to increase the soil P above the critical level for alfalfa production of 25 ppm, possibly due to plant uptake, in TWW-irrigated soil, the soil P levels were elevated and could reduce the applied P fertilizer requirement, depending on the yield goal [16].

Table 3. Pre-planting soil analysis of irrigation water source strip plots (surface water or Class 1B, UV-irradiated treated municipal wastewater) in 2017 and results of by-plot sampling in 2018 one year after alfalfa establishment at Tucumcari, NM, USA. Soil testing in 2017 was not replicated and thus no statistical analysis was possible. Values for 2018 are the means of four replicates within each water source strip plot.

Variable	2017 Pre-Planting		2018			
	Surface Water	Wastewater	Surface Water	Wastewater	SED	*p*-Value
pH	8.3	8.3	8.2	8.3	0.35	0.6286
Salts, mmho cm^{-1}	0.29	0.24	0.23	0.36	0.05	0.0225
OM, %	1.3	1.1	1.3	1.7	0.4	0.0171
P, ppm	8.4	13.3	11.3	27.3	5.0	0.0001
K, ppm	253	332	458	720	209	0.0011
S, ppm	29	18	6	11	7	0.0689
Fe, ppm	4.8	5.8	7.1	9.1	1.1	0.1101
Mn, ppm	4.5	6.8	6.2	6.6	2.3	0.2257
Cu, ppm	0.36	0.35	0.38	0.49	0.02	0.0015
Ca, ppm	2230	1564	1787	2095	754	0.0608
Mg, ppm	256	287	372	434	32	0.0007
Na, ppm	134	167	48	131	71	0.0030
CEC	14.5	11.8	13.4	16.5	4.0	0.0112
K Saturation, %	4	7	9	11	1	0.0195
Ca Saturation, %	77	66	67	63	1	0.0117
Mg Saturation, %	15	20	23	22	5	0.4136
Na Saturation, %	4	6	1.5	3.3	3	0.0203

SED, OM, and CEC signify standard error of the difference between means, organic matter, and cation exchange capacity, respectively.

3.4. Soil Microbial Biomass and Community Composition

Microbial biomass, taxonomic composition, and diversity were different between years, water source treatments, and their interaction. Specifically, microbial biomass increased distinctly from 2017 to 2018 (Table 4; Figure 2A).

Table 4. Soil microbial biomass (nmol g^{-1}) and diversity index before and one year after alfalfa establishment when irrigated with surface water or treated municipal wastewater (Class 1B, UV-irradiated) at Tucumcari, NM, USA. Values are the means of four replicates within each water source strip plot each year.

Variable	2017				2018				SED	*p*-values		
	Surface Water		Waste-Water		Surface Water		Waste-Water			Year	Source	Year × Source
Total biomass	1147	C	1184	C	1961	B	4991	A	326	<0.0001	<0.0001	<0.0001
Total Bacteria	452	C	519	C	836	B	2225	A	125	<0.0001	<0.0001	<0.0001
Actinomycetes	67	C	81	C	178	B	385	A	19	<0.0001	<0.0001	0.0003
Gram-Negative	211	B	205	B	326	B	1102	A	90	<0.0001	<0.0001	<0.0001
Rhizobia	0		0		1		31		13	0.1270	0.1480	0.1480
Total Fungi	68	B	36	B	183	B	731	A	98	<0.0001	0.0030	0.0013
AM	14	C	0	C	66	B	174	A	13	<0.0001	0.0031	0.0006
Saprophytes	54	B	36	B	117	B	558	A	87	0.0031	0.0143	0.0096
Protozoa	0	B	0	B	5	B	33	A	7	0.0071	0.0280	0.0280
Gram-Positive	241	C	314	C	510	B	1123	A	64	<0.0001	<0.0001	<0.0001
Undifferentiated	627	B	630	B	937	B	2002	A	155	<0.0001	0.0004	0.0004
Diversity Index	1.28	B	1.18	B	1.47	A	1.53	A	0.04	<0.0001	0.5616	0.0662

SED and AM signify standard error of the difference between means and arbuscular mycorrhizae, respectively. Means within a row followed by similar letters are not significantly different at $p < 0.05$, even when a biologically significant trend ($0.05 < p < 0.10$) is indicated by the *p*-value for the interaction.

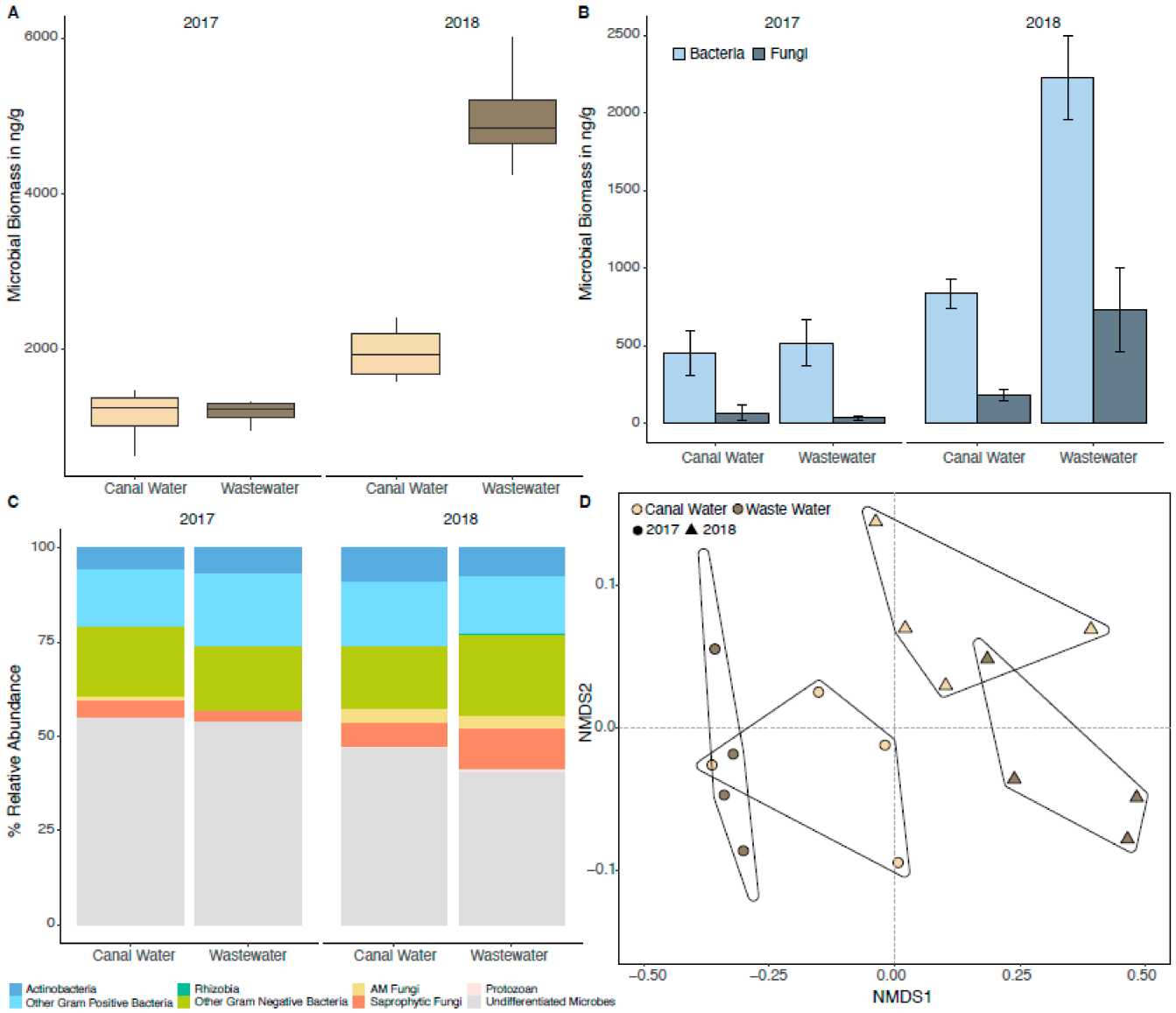

Figure 2. Soil microbial characteristics before and one year after alfalfa establishment when irrigated with surface water or treated municipal wastewater (Class 1B, UV-irradiated) at Tucumcari, NM, USA. (**A**) Total microbial biomass; (**B**) microbial biomass comparing bacteria and fungi; (**C**) relative abundance of microbial phospholipid fatty acid (PLFA) groups; (**D**) nonmetric multi-dimensional scaling (NMDS) ordination plot of the PLFA data.

Comparing across water sources, no microbial biomass differences were observed between TWW and SW water source plots in 2017. However, in 2018, TWW had significantly greater microbial biomass compared with SW. Lack of irrigation from October 2017 to May 2018 may have reduced microbial biomass; however, the event was equal to both water source treatments and likely not a factor in treatment differences. Furthermore, greater bacterial and fungal biomasses also were observed when TWW was the irrigation source (Figure 2B, Table 4). Water and nutrients are the strongest limiting factors for microbial and plant growth in semi-arid regions [1,29]. Thus, a temporal modification of the soil microclimate and nutrient availability (Tables 2 and 3) may explain the observed results (Table 4; Figure 2). For example, an increase in microbial biomass could be linked to interannual changes in the soil climate during alfalfa crop establishment in semi-arid agroecosystems [29]. While alfalfa is in the early growth stages a large area of the soil is still exposed to the climatic elements of semi-arid regions with high radiation, air and soil

temperature, water loss, and erosive forces that feed back into limiting microbial growth and activity [29]. Later on, a denser stand with established alfalfa plants in 2018 may have buffered against these harsh climatic conditions and moderate temperature and soil moisture and, consequently, the soil microbiome can proliferate [29]. The greater nutrient status of TWW (Table 2) may have additionally stimulated microbial growth [5,29]. The combination of plant soil feedbacks and greater nutrient availability in the TWW-irrigated soil (Tables 2 and 3) may have driven the proliferation of soil microbes observed (Table 4).

Aside from undifferentiated microbes (not identifiable by PLFA), the most abundant microbes in our samples were bacteria, with gram-negative bacteria, gram-positive bacteria, and actinobacteria in decreasing abundances (Figure 2C, Table 4). Fungi were the next most abundant group, while rhizobia and protozoans were rare and only detected in 2018. Only in 2018 in the TWW plots, fungi were the fourth most abundant microbial group followed by actinobacteria (Figure 2C). Saprophytic fungi were greater in abundance than arbuscular mycorrhizal (AM) fungi, which is typical for agroecosystems [15].

The absolute biomass values of individual microbial groups exhibited year × water source interactions except for Rhizobia (Table 4). However, this difference was not apparent when comparing the relative abundances of the microbes where only percent total fungi and gram-negative bacteria showed a significant year × water source interaction. Aside these interactions, the sample year comparison detected the most differences in relative abundances of soil microbial groups (Table 5) with 2018 having the highest values for Actinomycetes, Saprophytic and AM Fungi, and Protozoans, while undifferentiated microbes were comparable less abundant, declining by 10% from 2017 to 2018 (Table 5).

Table 5. Relative abundance values (%) of soil microbial biomass before and one year after alfalfa establishment when irrigated with surface water or treated municipal wastewater (Class 1B, UV-irradiated). Values are the means of four replicates within each water source strip plot.

Variable	2017				2018				SED	*p*-Values		
	Surface Water		Waste-Water		Surface Water		Waste-Water			Year	Source	Year × Source
Total Bacteria	43								2	0.2312	0.3076	0.5921
Actinomycetes	6.36 B				8.52 A				0.51	0.0209	0.6740	0.1374
Gram-Negative	18	AB	17	B	17	B	22	A	2	0.2434	0.2424	0.0462
Rhizobia	0.00		0.00		0.06		0.59		0.25	0.1180	0.1915	0.1915
Total Fungi	6	BC	3	C	10	AB	14	A	2	0.0004	0.5709	0.0415
AM	1.12	B	0	C	3.41	A	3.47	A	0.38	<0.0001	0.1121	0.0553
Saprophytes	5	B	3	B	6	B	11	A	2	0.0048	0.3231	0.0528
Protozoa	0.00 B				0.48 A				0.16	0.0225	0.3653	0.3653
Gram-Positive	21	A	26	A	26	A	23	A	3	0.6408	0.7772	0.0518
Undifferentiated	54 A				44 B				3	0.0031	0.2233	0.3692
Fungi:Bacteria	0.14	BC	0.07	C	0.22	AB	0.32	A	0.05	0.0007	0.7547	0.0372
GramPos:GramNeg	1.18	A	1.57	A	1.60	A	1.05	A	0.27	0.7842	0.7048	0.0339
Saturated:Unsaturated	3.05	AB	3.50	A	2.35	BC	1.40	C	0.50	0.0018	0.4991	0.0701

UV, SED, and AM signify ultraviolet standard error of the difference between means and arbuscular mycorrhizae, respectively. Test mean and SE for total bacteria are given due to lack of any significant differences at $p < 0.20$. Year means are given when only the year effect was significant. Means within a row followed by similar letters are not significantly different at $p < 0.05$, even when a biologically significant trend ($0.05 < p < 0.10$) is indicated by the *p*-value for the interaction.

Furthermore, the increase in relative abundance of these microbial groups was the greatest in TWW-irrigated soil, which had the greatest relative abundance of all identifiable microbial groups (Table 4, Figure 2C). The increase in microbial abundances of multiple groups seems to indicate that within one year TWW does not preferentially select for the proliferation of one specific microbial group but a suite of diverse microbes including symbionts (AM), decomposers (saprophytes), and predators (protozoans), which could stimulate nutrient cycling and soil health. Further, significant interactions for total microbial biomass, total bacteria, Actinomycetes, AM, and gram-positive microbes all indicate an increase from 2017 to 2018 also when SW was the irrigation source (Table 4). This suggests that irrigated alfalfa production alone can stimulate the growth of a diverse soil microbial

community with several functional groups similar to the report by Bhandari et al. [15] who compared monoculture grass with an alfalfa–grass mixture.

Rhizobia were present in 2018, but not detected in the PLFA samples in 2017 (Table 4), although the alfalfa roots were nodulated. While the soil samples were collected to include plant roots, most plant parts were excluded from the soil samples submitted for fertility and PLFA analysis. It is not surprising that Rhizobia was not detected in 2017, as alfalfa had not been grown in the field for at least 25 years, if ever [13,30]. Additionally, perhaps there was a lack of detectable Rhizobia (Table 4) because either nodules had not been shed to release nitrogen into the soil, which also would have released the Rhizobia [30], or the population sizes were too low to be detected with the PLFA analysis. In 2018, alfalfa was cut and removed twice before the regrowth allowing nodule shedding and Rhizobia release into the soil [30].

In addition to effects to microbial biomass, abundance, and taxonomic composition sampling year and water source impacted microbial diversity. Soil microbial alpha diversity index increased from 2017 to 2018 with a trend [21] toward a year × treatment interaction because diversity increased more when TWW was the irrigation source (Table 4). The increase of diversity was mostly attributed to the detectable amounts of Protozoans and Rhizobia, which were not observed in 2017. The NMDS ordination visualizing patterns of microbial turnover (beta diversity) data revealed distinct similarities of samples by year with 2017 samples grouping on the left of the origin and 2018 samples grouping on the right (Figure 2D). Results of the PERMANOVA indicated a significant ($p < 0.01$) difference of microbial community beta diversity by year. This result supported our findings of an increase in microbial abundance and suggests similar factors may be the driving force for a diversity change. Although not significant, there was a trend of samples grouping together by water source (Figure 2D).

A suite of soil chemical variables was related to the abundances of PLFA microbial groups (Figure 3). Gram-negative bacteria correlated positively with organic matter and potassium while this relationship was negative for gram-positive bacteria. Rhizobia were strongly influenced by phosphorus and sulfate (Figure 3). Phosphorus availability was also associated with a higher abundance of protozoans. We further observed strong positive correlations of saprophytic fungi biomass with soil cation exchange capacity, P, and K (Figure 3). Sodium, Cu, and Fe were also associated with greater abundances of saprophytic fungi. Correlations of soil chemical variables with AM fungi were much weaker compared with saprophytes with phosphorus being the strongest factor (Figure 3). Although our correlation analysis does not permit us to deduce causal relationships, complex links can exist between soil chemical variables and the soil microbiome. For example, increased microbial activity and population sizes due to the application of TWW (Table 4) can lead to higher rates of nutrient cycling and decomposition, which release greater amounts of plant and microbial available nutrients [2,5,15] while plant soil feedbacks derived by planting perennial crops, such as greater soil moisture availability and increased organic matter, can feed and stimulate the growth of the soil microbiome and vice versa [15]. Future manipulative studies should investigate these interesting multilevel relationships.

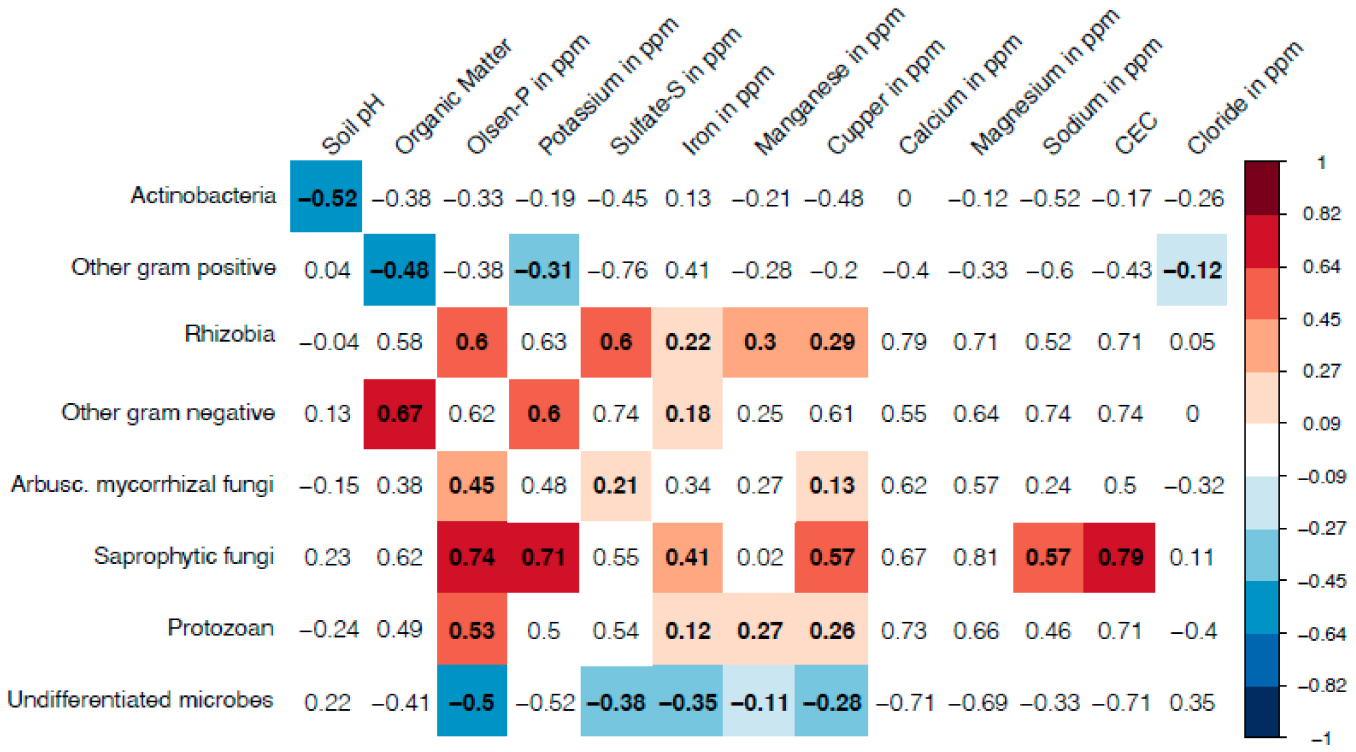

Figure 3. Correlation heat map relating soil chemistry data to PLFA soil microbial groups for the 2018 sampling year. Scale shows the Spearman rank correlation coefficient. Only significant ($p < 0.05$) relationships are shown in colored tiles.

3.5. Forage Dry Matter and Plant Chemical Constituents

Considerable variation in plant vigor was observed between the water source strip plots at plant sampling time in 2017 (68 dap) (data not shown), suggesting a degree of variation over time in germination and/or emergence of the alfalfa associated with irrigation with TWW, similar to that reported by Rekik et al. [3]. Data and results of statistical analysis for forage components are presented in Table 6. While there was no difference between irrigation sources in the number of plants m^{-2} in 2017, there was a difference in stand percentage in 2018, which may indicate more rapid stand failure due to irrigation with the treated wastewater. Adrover et al. [2,5] reported no concerns for alfalfa persistence after 20 years of irrigation with secondary TWW in Spain. Dry matter proportion was greater for seedling alfalfa irrigated for establishment using SW compared with TWW, but for 1-year-old alfalfa, the dry matter proportion was greater when TWW-irrigated (Table 6). However, there were no differences between water source treatments in plant dry matter g m^{-2} and no year × water source interaction, which is consistent to the findings of Elfanssi et al. [4]. The lack of interaction is indicative of alfalfa's resilience in remaining productive until there is a significant stand decline. Unpublished data from the location of this study (Lauriault unpublished data) suggests that individual harvest yields and season-long yields will not be different between stand percentages of 95 and 88% for surface water-irrigated alfalfa in the first production year after late summer seeding.

Table 6. Alfalfa plant counts, stand percentage, dry matter production, and selected plant chemical components after planting and one year after alfalfa establishment when irrigated with surface water or treated municipal wastewater (Class 1B, UV-irradiated). Values are the means of four replicates within each water source strip plot each year.

Variable	2017		2018		SED	*p*-Values		
	Surface Water	Waste-Water	Surface Water	Waste-Water		Year	Source	Year × Source
Plants m^{-2}, 2017	324	330	———	———	51	———	0.9151	———
Stand %, 2018	———	———	94 A	87 B	2	———	0.0086	———
Dry matter, g kg^{-1}	267 A	260 B	232 D	240 C	3	<0.0001	0.7907	0.0043
Dry matter, g m^{-2}	10.29 B		26.06 A		2.81	0.0011	0.2604	0.5251
aNDF, g kg^{-1}	283 aA	236 aB	199 bA	172 bB	7	<0.0001	0.0013	0.1821
ADF, g kg^{-1}	211 A	181 B	168 BC	156 C	67	0.0002	0.0064	0.0665
Lignin, g kg^{-1}	53.7 A	44.4 B	37.4 C	34.2 C	1.6	<0.0001	0.0044	0.0070
Nitrogen, g kg^{-1}	37.9 C	38.7 C	44.8 B	50.2 A	0.9	<0.0001	0.0005	0.0042
Phosphorus, g kg^{-1}	2.38 C	2.73 B	2.93 B	3.53 A	0.10	<0.0001	<0.0001	0.1089

SED, aNDF, and ADF signify standard error of the difference between means, amylase-treated neutral detergent fiber and acid detergent fiber, respectively. Data for plants m^{-2} and stand % are presented within a year because data were only collected that year. Means within a row followed by similar letters are not significantly different at $p < 0.05$ for the highlighted effect, even when a biologically significant trend ($0.05 < p < 0.10$) is indicated by the *p*-value. aNDF means followed by lower case letters are not significantly different at $p < 0.05$ for the main effect of year and when followed by similar upper-case letters they are not significantly different for the main effect of water source.

The irrigation system failure, coupled with little precipitation from November 2017 to May 2018 delayed growth in spring 2018 and the alfalfa in both water source strip plots struggled to recover, especially in light of warmer spring and early summer temperatures (Table 1). Darapuneni et al. [25] reported that total annual yields of alfalfa would be reduced by about 3 Mg ha^{-1} when irrigation was withheld until May, from early July to mid-August, and again after mid-September at this location (designated treatment 2-3-5 in Darapuneni et al. [25]. The amount of irrigation applied in that scenario was approximately the same as that applied in 2018. The late October/early November yield of that 2-3-5 treatment with the same regrowth period averaged 0.41 Mg ha^{-1} [25], which is only slightly more than the 0.26 Mg ha^{-1} measured in the present study (calculated from Table 6).

Year × water source treatment interactions or trends [21] also existed for all plant chemical constituents, except amylase-treated neutral detergent fiber and P (Table 6). Greater fiber values in the seedling stage may be related to a longer growth period from August to late October in 2017 than between mid-September and late October in 2018. The greater reduction for acid detergent fiber and the trend [21] toward that for lignin, as indicated by the year × source interaction (Table 6), both to the point of no difference in 2018, suggest that the surface water-irrigated alfalfa was more mature when sampled in 2017. This could indicate that water source was a factor in the rapidity of establishment that was overcome within a year after seeding and did not influence dry matter production that year.

Plant N and P contents of the alfalfa measured in the present study were similar to or greater than those measured elsewhere [8] and sufficient to high for whole plant levels [16]. This could be due to stage of maturity at harvest as plants were completely vegetative in the present study while the alfalfa is generally in at least an early stage of reproduction when evaluated for forage production. The increase in plant nitrogen across years, even for surface water-irrigated alfalfa (Table 6) could be related to nitrogen fixation by Rhizobia in low organic matter soils (Tables 3–5) coupled with the N applied in the fertilizer in 2018; however, the interaction shows that the increase for wastewater-irrigated alfalfa was greater than for surface water-irrigated alfalfa (Table 6), which is not explained by a year × source interaction for Rhizobia biomass (Table 4).

The fertilizer application also could be a factor in the increase in P across years in the alfalfa (Table 6), but the trend [21] toward a year × source interaction with a greater change for wastewater-irrigated alfalfa having greater plant P is likely due to additional P added

through the wastewater (Table 2) bringing soil test levels from the medium range for the Olsen P test of 9–16 ppm in 2017 to the high range in 2018 (Table 3) [10,16]. Greater soil P availability (Table 3) in the ionic form increases the P uptake by plants (Table 6) [31,32]. Elfanssi et al. [4] also observed greater alfalfa plant P when irrigated with TWW than with well water. The increase in AM biomass (Table 4) also may have been a factor as AM is known to increase P availability for plant uptake [33]. Both P and water availability can have the effect of reduced acid detergent fiber, which, in turn, influences digestibility [34]. While whole plant P was sufficient to no limit growth [16], soil P when SW-irrigated (Table 2) suggests that annual application would be necessary to replace P removed by the alfalfa crop.

4. Conclusions

Alfalfa established equally well when irrigated with TWW compared with SW, despite the less than optimum quality of both water sources. Over the course of alfalfa establishment and the first production year, the application of TWW increased soil P and plant P more so than SW, in addition to increasing plant N, which is the basis for crude protein that is critical for livestock production. Thus, irrigation with TWW could reduce the amount of fertilizer P necessary to meet alfalfa's requirement and increase crude protein content in the alfalfa. Nearly all microbial indicators of soil health were positively increased by alfalfa establishment where it had not been previously grown; however, the effect was greater when TWW was used as the water source for establishment.

Author Contributions: Conceptualization, L.M.L.; methodology, L.M.L., N.P., M.K.D., A.J.D. and G.K.M.; validation, L.M.L., N.P., M.K.D. and A.J.D.; formal analysis, L.M.L., N.P., M.K.D. and A.J.D.; investigation, L.M.L. and G.K.M.; resources, L.M.L., N.P. and M.K.D.; data curation, L.M.L.; writing—original draft preparation, L.M.L.; writing—review and editing, L.M.L., N.P., M.K.D. and A.J.D.; visualization, L.M.L., N.P., M.K.D. and A.J.D.; supervision, L.M.L.; project administration, L.M.L.; funding acquisition, L.M.L. All authors have read and agreed to the published version of the manuscript.

Funding: Salaries and research support were provided by state and federal funds appropriated to the New Mexico Agricultural Experiment Station, including USDA National Institute of Food and Agriculture Hatch projects 1004803 and 1021538.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are available upon reasonable request from the authors.

Acknowledgments: Appreciation is expressed for secretarial and field assistance to Patricia Cooksey, Jason Box, Jared Jennings, and Shane Jennings; as well as NMSU College of Agricultural, Consumer and Environmental Sciences Information Technology; and other University support services, such as the Library Document Delivery Service.

Conflicts of Interest: The authors declare no conflict of interest and the funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Shomar, B.; El-Madhou, F.; Yahya, A. Wastewater reuse for alfalfa production in Gaza Strip. *Water Air Soil Pollut.* **2010**, *213*, 105–119. [CrossRef]
2. Adrover, M.; Moya, G.; Vadell, J. Seasonal and depth variation of soil chemical and biological properties in alfalfa crops irrigated with treated wastewater and saline groundwater. *Geoderma* **2017**, *286*, 54–63. [CrossRef]
3. Rekik, I.; Chaabane, Z.; Missaoui, A.; Bouket, A.C.; Luptakova, L.; Elleuch, A.; Belbahri, L. Effects of untreated and treated wastewater at the morphological, physiological and biochemical levels on seed germination and development of sorghum (*Sorghum bicolor* (L.) Moench), alfalfa (*Medicago sativa* L.) and fescue (*Festuca arundinacea* Schreb.). *J. Hazard. Mater.* **2017**, *326*, 165–176. [CrossRef] [PubMed]
4. Elfanssi, S.; Ouazzani, N.; Mandi, L. Soil properties and gro-physiological responses of alfalfa (*Medicago sativa* L.) irrigated by treated domestic wastewater. *Agric. Water Manag.* **2018**, *202*, 231–240. [CrossRef]

5. Adrover, M.; Farrus, E.; Moya, G.; Vadell, J. Chemical properties and biological activity in soils of Mallorca following twenty years of treated wastewater irrigation. *J. Environ. Manag.* **2012**, *95*, 5188–5192. [CrossRef] [PubMed]
6. Gharaibeh, M.A.; Marschner, B.; Heinze, S. Metal uptake by tomato and alfalfa plants as affected by water source, salinity, and Cd and Zn levels under greenhouse conditions. *Environ. Sci. Pollut. Res.* **2015**, *22*, 18894–18905. [CrossRef]
7. El Moussaoui, T.; Mandi, L.; Wahbi, S.; Masi, S.; Ouazzani, N. Soil properties and alfalfa (*Medicago sativa* L.) responses to sustainable treated urban wastewater reuse. *Arch. Agron. Soil Sci.* **2019**, *65*, 1900–1912. [CrossRef]
8. Soufan, W.; Okla, M.K.; Al-Ghamdi, A.A. Effects of irrigation with treated wastewater or well water on the nutrient contents of two alfalfa (*Medicago sativa* L.) cultivars in Riyadh, Saudi Arabia. *Agronomy* **2019**, *9*, 729. [CrossRef]
9. Shigei, M.; Ahrens, L.; Hazaymeh, A.; Dalahmeh, S.S. Per- and polyfluoroalkyl substances in water and soil in wastewater-irrigated farmland in Jordan. *Sci. Total Environ.* **2020**, *716*, 137057. [CrossRef]
10. Carrillo, G.R.; Cajuste, L.J. Heavy metals in soils and alfalfa (*Medicago sativa* L.) irrigated with three sources of wastewater. *J. Environ. Sci. Health Part A* **1992**, *27*, 1771–1783.
11. Christou, A.; Antoniou, C.; Cristodoulou, C.; Hapeshi, E.; Stavrou, I.; Michael, C.; Fatta-Kassinos, D.; Fotopoulos, V. Stress-related phenomena and detoxification mechanisms induced by common pharmaceuticals in alfalfa (*Medicago sativa* L.). *Sci. Total Environ.* **2016**, *557–558*, 652–664. [CrossRef] [PubMed]
12. NMED. *NMED Ground Water Quality Bureau Guidance: Above Ground Use of Reclaimed Domestic Wastewater*; New Mexico Environment Department: Santa Fe, NM, USA, 2007. Available online: https://cloud.env.nm.gov/water/?r=5582&k=cdcde6cbdf (accessed on 26 May 2021).
13. Bringhurst, R.M.; Cardon, Z.G.; Gage, D.J. Galactosides in the rhizosphere: Utilization by *Sinorhizobium meliloti* and development of a biosensor. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 4540–4545. [CrossRef] [PubMed]
14. Kizekova, M.; Tomaskin, J.; Cunderlik, J.; Jancova, L.; Martincova, J. The yield stability and quality of legumes during two consecutive, extremely dry years. *Agriculture* **2013**, *59*, 167–177. [CrossRef]
15. Bhandari, K.B.; West, C.P.; Acosta-Martinez, V. Assessing the role of interseeding alfalfa into grass on improving pasture soil health in semi-arid Texas High Plains. *Appl. Soil Biol.* **2020**, *147*, 103399. [CrossRef]
16. Ward Laboratories. *Wardguide*; Ward Laboratories: Kearney, NE, USA, 2020. Available online: https://www.wardlab.com/resources/ward-guide/ (accessed on 21 June 2022).
17. Lauriault, L.M.; Ray, I.; Pierce, C.; Burney, O.; Flynn, R.P.; Marsalis, M.A.; O'Neill, M.K.; West, M. *The 2015 New Mexico Alfalfa Variety Test Report*; Agricultural Experiment Station, College of Agricultural, Consumer and Environmental Sciences: Las Cruces, NM, USA, 2015. Available online: https://pubs.nmsu.edu/variety_trials/alfalfa_2015.pdf (accessed on 21 June 2022).
18. Lauriault, L.M.; Ray, I.; Pierce, C.; Burney, O.; Flynn, R.P.; Marsalis, M.A.; O'Neill, M.K.; Cunningham, A.; Havlik, C.; West, M. *The 2016 New Mexico Alfalfa Variety Test Report*; Agricultural Experiment Station, College of Agricultural, Consumer and Environmental Sciences: Las Cruces, NM, USA, 2016. Available online: https://pubs.nmsu.edu/variety_trials/alfalfa_2016.pdf (accessed on 21 June 2022).
19. SAS Institute. *The SAS 9.4 for Windows*; SAS Inst., Inc.: Cary, NC, USA, 2013.
20. Littell, R.C.; Milliken, G.A.; Stroup, W.W.; Wolfinger, R.D. *SAS System for Mixed Models*; SAS Institute Inc.: Cary, NC, USA, 1996.
21. Ramsey, F.L.; Schafer, D.W. *The Statistical Sleuth: A Course in Methods of Data Analysis*, 2nd ed.; Duxbury: Pacific Grove, CA, USA, 2002; p. 42.
22. Saxton, A.M. A macro for converting mean separation output to letter groupings in Proc Mixed. In Proceedings of the 23rd SAS Users Group International, Nashville, TN, USA, 22–25 March 1998; pp. 1243–1246.
23. Fribourg, H.A.; Strand, R.H. Influence of seeding dates and methods on establishment of small-seeded legumes. *Agron. J.* **1973**, *65*, 804–807. [CrossRef]
24. Hall, M.H. Plant vigor and yield of perennial cool-season forage crops when summer planting is delayed. *J. Prod. Agric.* **1995**, *8*, 233–238. [CrossRef]
25. Darapuneni, M.K.; Lauriault, L.M.; VanLeeuwen, D.M.; Angadi, S.V. Influence of irrigation regimes on alfalfa dry matter yield and water productivity in a semiarid subtropical environment. *Irrig. Drain.* **2020**, *69*, 1063–1071. [CrossRef]
26. Hopkins, B.G.; Horneck, D.A.; Stevens, R.G.; Elllsworth, J.W.; Sullivan, D.M. *PNW-597-E, Managing Irrigation Water Quality*; Oregon State University, University of Idaho, and Washington University Cooperative Extension Service and USDA: Corvallis, OR, USA, 2007. Available online: https://catalog.extension.oregonstate.edu/sites/catalog/files/project/pdf/pnw597.pdf (accessed on 21 June 2022).
27. Ayers, R.S.; Westcot, D.W. *Water Quality for Agriculture. FAO Irrigation and Drainage Paper 29*; FAO: Rome, Italy, 1985. Available online: http://www.fao.org/3/a-t0234e.pdf (accessed on 21 June 2022).
28. Olsen, S.R.; Cole, C.V.; Watanabe, F.S.; Dean, L.A. *Circular 939, Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate*; United States Department of Agriculture: Washington, DC, USA, 1954.
29. Zong, N.; Shi, P. Soil properties rather than production strongly impact soil bacterial community diversity along a desertification gradient on the Tibetan Plateau. *Grassl. Sci.* **2020**, *66*, 197–206. [CrossRef]
30. Philipp, D.; Jennings, J. *Forage Legume Inoculation*; University of Arkansas Cooperative Extension State Office: Little Rock, AR, USA, 2012. Available online: https://www.uaex.uada.edu/publications/pdf/FSA-2035.pdf (accessed on 21 June 2022).
31. Schachtman, D.P.; Reid, R.J.; Ayling, S.M. Phosphorus uptake by plants: From soil to cell. *Plant Physiol.* **1998**, *116*, 447–453. [CrossRef]

32. Raghothama, K.G.; Karthikeyan, A.S. Phosphate Acquisition. *Plant Soil* **2005**, *27*, 37–49. [CrossRef]
33. Miller, M.H. *Arbuscular mycorrhizae* and the phosphorus nutrition of maize: A review of Guelph studies. *Can. J. Plant Sci.* **2000**, *80*, 47–52. [CrossRef]
34. Auge, R.M. *Arbuscular mycorrhizae* and soil/plant water relations. *Can. J. Soil Sci.* **2004**, *84*, 373–381. [CrossRef]