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Assessing Fungal Plant Pathogen Presence in Irrigation Water from the Rio Grande River in South Texas, USA

Miriam Calderon †, Chuanyu Yang † and Veronica Ancona *

Citrus Center, Texas A&M University-Kingsville, 312 N. International Blvd, Weslaco, TX 785799, USA; miriam.calderon@students.tamuk.edu (M.C.); chuanyu.yang@tamuk.edu (C.Y.)

* Correspondence: veronica.ancona-contreras@tamuk.edu

† These authors contributed equally to this work.

Abstract: Irrigation is important in many crop production systems. However, irrigation water can be a carrier of plant pathogens that can enter the system and spread to fields, resulting in crop damage and yield losses. The Lower Rio Grande Valley of South Texas is an important area for agricultural production which depends on the Rio Grande River as a source of water for irrigation. Thus, the presence of plant pathogens in the Rio Grande River could have important implications for crop productivity in the region. Cultured-based methods and molecular identification methods are used for monitoring plant pathogens in irrigation water. However, these methods are labor-intensive and just detect targeted pathogens. To overcome these limitations, in this study, the ITS2 amplicon metagenomic method was applied for evaluating the fungal diversity, composition, and presence of fungal plant pathogens in irrigation water from the Rio Grande River as it leaves the water reservoir (WR) and it arrives at an irrigation valve at a farm (FA). Results from the Shannon (WR = 4.6 ± 0.043, FA = 3.63 ± 0.13) and Simpson indices (WR = 4.6 ± 0.043, FA = 3.63 ± 0.13) showed that there are significant differences in the fungal diversity and community structure between the two locations and the PCA analysis showed a clear differentiation between both fungal communities. Several OTUs identified in both locations included potential plant pathogens from diverse genera including *Cladosporium*, *Exserohilum*, and *Nigrospora*, while others such as *Colletotrichum* and *Plectosphaerella* were found only in one of the two locations assessed. This work indicates that microbes, including plant pathogens, may enter or exit throughout the irrigation-water distribution system, thereby modifying the microbial community composition along the way. Understanding the dynamics of plant pathogen movement in irrigation water systems can help growers identify risk factors to develop measures to mitigate those risks. This study also shows the usefulness of the metagenomic approach for detecting and monitoring plant pathogen in irrigation water.

Keywords: metagenomics; irrigation water; ITS2 amplicon; Lower Rio Grande Valley; water quality

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1. Introduction

In many regions of the world, rivers are used as a source of irrigation water to enhance agricultural production, economic development, urbanization, etc. In South Texas, USA, limited water resources based on the Rio Grande River (RGR) support irrigation-dependent agriculture, rapid population growth and urbanization, and sensitive wildlife and marine habitats, while facing frequent periods of severe drought [1]. The suitability of river water for irrigation is affected by factors such as the amount of minerals, salts, heavy metals, or other contaminants in the water [2]. The RGR experiences water quality issues such as excessive loading of sodium, minerals, and nutrients which can vary during the year based on environmental events [3]. Although the water in the RGR along the Lower Rio Grande Valley (LRGV) is ranked as permissible for irrigation by several water quality indices [3], it may contain plant fungal pathogens that could be distributed through the irrigation system to crops.

Fungal plant diseases are major problems in agriculture, resulting in crop damage and yield loss. Many fungal pathogens are dispersed through water from irrigation, increasing the risks of crop production losses [4]. Important phytopathogens, such as the Oomycetes *Phytophthora* and *Pythium*, produce swimming zoospores and can cause substantial agricultural problems [4]. Other fungal phytopathogens, such as *Fusarium* and *Alternaria*, can also be found in irrigation water, although their long-term survival and infectivity have not been determined [4]. In greenhouses or systems where water is recycled for irrigation, the risk of pathogen contamination is amplified. Intensive care of horticultural crops is needed in closed and open systems to manage diseases, especially when plant pathogens are introduced from irrigation water. Pathogen distribution via irrigation water is very rapid; thus, the risks associated with pathogen presence and abundance in irrigation water have been recognized as a major crop health concern [4]. Contaminated irrigation water with fungal plant pathogens can be a major factor in disease distribution and spread in open fields [4,5]. Water from many sources, such as open reservoirs and ponds, can carry fungal plant pathogen propagules which are introduced by runoff water filled with crop debris from agricultural fields [6–10]. Irrigation water from the RGR is distributed to crops in South Texas from settling basins in the irrigation district's pump stations, and then transported through open or underground canals to farms [11,12]. Because the RGR, as well as its tributary rivers, Devils and Pecos, receive runoff water from agricultural fields, their water can have an increased level of plant pathogens that can be later distributed to new fields through irrigation water. Thus, it is important to detect the presence of fungal plant pathogens in RGR water used for irrigation.

There are several methods available for detecting, screening, surveilling, and identifying microorganisms in water samples. Cultured-based methods such as baiting are a common approach for monitoring plant fungal pathogens [13]. However, this method has many drawbacks, including the inability to detect slow-growing or unculturable microbes, which may cause inconclusive results. Molecular identification methods, including quantitative PCR (qPCR) and loop-mediated isothermal amplification (LAMP), which sometimes can overcome the limitations of cultured-based methods, are the most sensitive for detecting and quantifying microorganisms, but this method can detect only one or a few target microbes in a single reaction mixture [14,15]. High-throughput sequencing can overcome these drawbacks and has the potential to be an efficient method for the detection and monitoring of plant pathogens in water. It can be used for identifying potential harmful microorganisms, indicating the need for more specific analyses, while also providing comprehensive data on the microorganisms that are naturally occurring in an agricultural and environmental sample. Shortgun metagenomics is the untargeted high-throughput sequencing of the entire DNA of a sample. This technique has the significant advantage of detecting all microorganisms present in a sample, including both fungi and bacteria. In addition, shortgun metagenomics can also return genomes of the detected pathogens, making it ideal for sequencing unculturable ones [16]. To date, shortgun and amplicon-based metagenomics are widely applied for detecting and monitoring plant pathogens. Most studies have focused on surveillance of soilborne and airborne plant pathogens [17–20]; however, there are still few reports about applying the high-throughput sequencing approach for detecting plant pathogens in irrigation water [21]. Therefore, we hypothesized that a metagenomics approach may be useful for identifying potential pathogens in irrigation water.

With the goal of identifying if water from the RGR used for irrigation was carrying plant pathogens, and if those plant pathogens were transported to farms, in this study, an amplicon-based (ITS) metagenomics approach was used for detecting fungal plant pathogens in irrigation water collected from two locations, the water reservoir and the irrigation valve in a farm. The detection of fungal plant pathogens in the water reservoir and at the irrigation site were recorded and the populations of fungal plant pathogens from both locations were compared to determine the abundance, similarities, and differences between fungal populations.

2. Materials and Methods

2.1. Collection of Irrigation Water Samples

Two locations were selected for the sampling of irrigation water from the RGR: irrigation water that was emerging from an irrigation valve at a citrus orchard located at the Texas A&M University Kingsville Citrus Center South Research Farm (FA) (air temperature = 27 °C, precipitation = 0 (in), humidity = 70%), and from the water reservoir of the Mercedes irrigation district (WR) (air temperature = 29 °C, precipitation = 0 (in), humidity = 79%) from the Lower Rio Grande Valley region in South Texas. Water samples were collected from running water using two new, disinfected 5-gallon buckets during the early morning hours at 10:00 a.m. To avoid cross contamination, the buckets were washed with 70% ethanol and rinsed with sterile water before use. Water samples were collected the same day in October 2020 from both sites, transported to the laboratory, and processed within 1 h of collection following Redekar et al.'s method (2019) with minor modifications [21]. Briefly, four and a half liters of water samples were divided into three repeat groups for filtering through a 75-micron nylon screen into sterile flasks to remove large debris. The samples collected from the water reservoir were labelled as WR1, WR2, and WR3, and samples from the farm were labeled FA1, FA2, and FA3. Each filtered water sample was then vacuum-infiltrated onto 5 cellulose ester membranes with 5 µm pore size (Millipore-Sigma, Burlington, MA, USA). The membrane filters were cut into small pieces and placed in a 15 mL centrifuge tube. Then, 800 µL of extraction buffer (3% CTAB, 100 mM Tris-HCl, 20 mM EDTA, 1.4 M NaCl, 5% PVP, pH 8.0) was transferred to the tubes and stored in a −20 °C freezer before continuing with DNA extraction.

2.2. DNA Extraction

Tubes containing the membranes in extraction buffer were incubated at 65 °C in a water bath for 30 min. After incubation, the filter papers and extraction buffer from each sample were divided and placed into two 2 mL tubes. 400 µL of 5M potassium acetate (KoAc)/acetic acid were added to each tube, mixed, and incubated for 5 min on ice. The tubes were then removed from the ice and centrifuged at 13,000 rpm for 5 min. The supernatant was removed from each tube and the two supernatants corresponding to each water sample were combined and transferred into a new 2 mL tube. Next, the samples were centrifuged for 5 min at maximum speed. An amount of 600 µL of supernatant was transferred to a new 2.0 mL tube, and 900 µL of ice-cold isopropanol was added and mixed gently for DNA precipitation. Then, 700 µL was pipetted onto a spin column (Genessee Scientific, San Diego, CA, USA), followed by centrifugation at 8000 rpm for 1 min. These steps were repeated until all the supernatant was processed via centrifugation. Once the last centrifugation was complete, the spin column was placed in a clean 2 mL tube with the cap open and all the flowthrough was discarded. To wash the column, 500 µL of 70% ethanol was added to the column and centrifuged at 10,000 rpm for 1 min, and the flowthrough was discarded. This was performed twice, and an extra spin in the centrifuge was performed to completely remove the ethanol from the column. The column was placed into new sterile 1.5 mL tubes and the DNA was eluted through adding 50 µL of AE buffer and centrifuging at 8000 rpm for 2 min. This step was repeated with 20 µL of AE buffer. The DNA was kept at −20 °C.

2.3. High-Throughput Amplicon Sequencing

Genomic DNA concentration and purity were determined on a NanoDrop ND-2000 (NanoDrop Technologies, Wilmington, DE, USA) spectrophotometer. For fungal community analysis, the internal Transcribed Spacer (ITS1-2) sequence was amplified with primer set ITS1 (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS-2 (5′-GCTGCGTTCTTCATCGATGC-3′) [22,23]. For multiplex sequencing, the primers were barcoded. PCR amplifications were performed in a 25 µL reaction with 5 µL 5× reaction buffer, 5 µL 5× GC buffer, 2.5 mM dNTPs, 10 µM of forward and reverse primers, 2 µL DNA template, 8.75 µL ddH_2O, and 0.25 µL Q5 high-fidelity DNA polymerase (NEB, Ipswich, MA, USA). The PCR protocol

was initiated with a denaturation step at 98 °C for 2 min, followed by 30 cycles at 98 °C for 15 s, 55 °C for 30 s, and 72 °C for 30 s, as well as a final extension at 72 °C for 5 min. To minimize bias, three PCR amplifications were pooled for analysis. PCR products of 350 bp were purified after gel electrophoresis with a DNA Gel Extraction Kit (Axygen, Union City, CA, USA). The sequencing of PCR amplicons of fungal DNA was carried out on the Illumina MiSeq sequencer from BGI Co., Ltd. (San Jose, CA, USA).

2.4. Data Processing

Raw sequences were assembled for each sample based on the unique barcode via the QIIME pipeline (Quantitative Insights Into Microbial Ecology, v1.8.0 qiime.org) [24]. Low-quality sequences were filtered following Gill's method [25]. High-quality sequences were assembled into operational taxonomic units (OTUs) at 97% sequence identity via UCLUST [26]. Representative sequences from each OTU were used to establish the taxonomic classification through performing a search against the UNITE (Release 5.0, https://unite.ut.ee/ accessed on 4 November 2020) Database for fungal sequences [27].

2.5. Bioinformatics Analysis

Analysis of the sequences were conducted using QIIME (v1.8.0) and R packages (v3.2.0). OTU tables were separated according to WR and FA type and normalized using total sum scaling (TTS). Diversity and richness indices including alpha, Chao1, ACE, Shannon, and Simpson were calculated using the OTU table in QIIME (v1.8.0). Because the data did not follow a normal distribution, statistical differences were calculated using nonparametric Kruskal–Wallis one-way ANOVA followed by Dunn's multiple-comparisons post hoc test ($p < 0.05$). To determine the variation amongst the fungal communities and structures from WR and FA samples, a weighted UniFrac distance was calculated in Mothur (v.1.25.1). PCoA (Principal Coordinate Analysis) was conducted on distance metrics, and coordinates were used to produce a 2D graph [28]. The Venn diagram was constructed using R packages (v3.2.0). Relative abundances of fungal OTUs between WR and FA samples were analyzed using one-way ANOVA ($p < 0.05$) using SPSS Version 16.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Diversity of Fungal Communities in Irrigation Water from Two Locations: Mercedes Irrigation District (WR) and South Farm (FA)

High-quality sequences of 899,335 reads (average connect ratio = 96.30 ± 2.36%) for ITS2 were obtained for an average of 940,496 fungal ITS2 reads (Table S1). In total, 1793 OTUs of fungi from six samples in both locations were acquired after discarding OTUs less than 0.001% of total sequences cross all samples. In total, 1286 and 1265 OTUs were detected in three samples from WR and FA, respectively. In addition, there were no statistically significant differences in the number of OTUs, or in the richness indices Chao-1 and ACE, in fungal communities between WR and FA. However, significant differences were detected in the Shannon and Simpson diversity indices between locations (Table 1). The Shannon index was statistically higher in WR samples than the FA samples ($p < 0.05$). In contrast, the Simpson index was lower in WR than the FA samples ($p < 0.05$).

Table 1. Fungal diversity and richness indices of irrigation water collected at the water reservoir (WR) and at the farm (FA). Different letters represent significant differences. All data were analyzed with Duncan's multiple range tests using SPSS software.

Sample Name	OTU Number	Chao-1	ACE	Shannon	Simpson
WR	940.67 ± 166.54 a	976.86 ± 189.91 a	975.5 ± 191.78 a	4.6 ± 0.043 a	0.03 ± 0.003 b
FA	955 ± 50.03 a	1049.99 ± 52.37 a	1049.99 ± 53.79 a	3.63 ± 0.13 b	0.11 ± 0.03 a

3.2. Differences in Composition and Structure between WR and FA

The similarity of the fungal communities in both two locations (WR and FA) was measured using weighted Unifrac PCoA. The results indicated that the fungal communities associated with the water from the water reservoir (WR) and the water collected at the farm (FA) were clearly separated (Figure 1) and statistically different from each other (analysis of similarity, R = 1, $p = 0.014$). The principal coordinates PC1 accounted for 88.63% of the variance, and PC2 represented 5.27% of the variance (Figure 1). The cumulative contribution of the variances represents 93.90% of the variance.

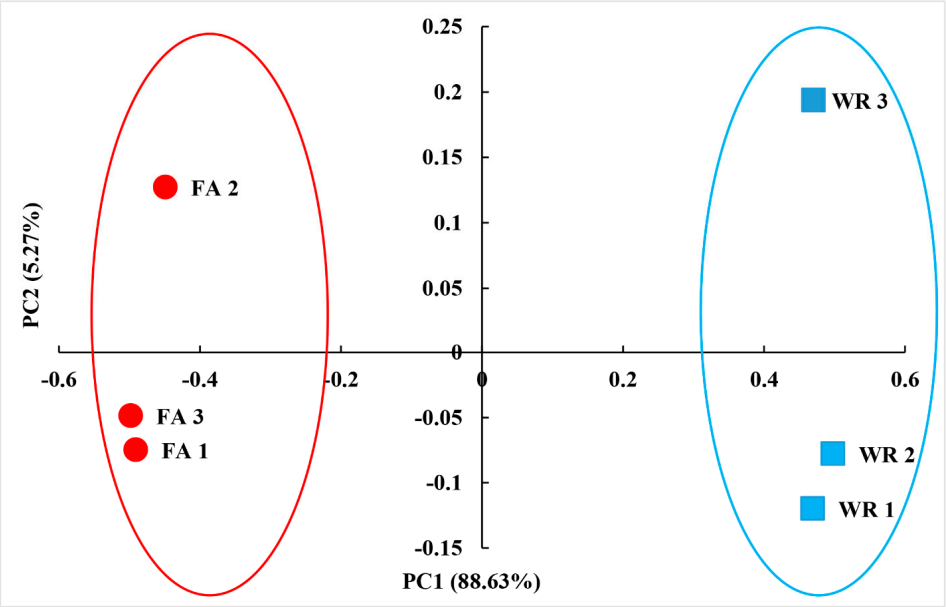

Figure 1. UnFrac-weighted principal coordinate (PC) analysis of fungal community structures in irrigation water from water reservoirs (WR) and irrigation valve (FA) in South Texas, USA. Analysis of similarities was conducted to evaluate the significant differences in microbial community using QIIME (Version 1.8.0) ($p < 0.05$).

In this work, 608 and 670 OTUs were detected in WR and FA samples, respectively (Figure 2A,B). In total, 284 OTUs were shared between WR and FA samples, while 324 and 386 OTUs were just present in WR or FA samples, respectively (Figure 2C). Of the 284 OTUs that were present in both WR and FA samples, 8 were identified at the species level (OTU672, OTU456, OTU34, OTU199, OTU213, OTU251, OTU112, OTU589), 2 at the genus level (OTU381, OTU162), 1 at the order level (OTU45), and 2 at the phylum level (OTU294, OTU125) (Table S2). The relative abundance of six OTUs (including OTU456, OTU294, OTU125, OTU672, OTU45, and OTU381) in FA samples was significantly higher than in WR samples (Table S2). In WR samples, 30 of the 324 special OTUs were identified at least at the phylum level, and 50 of the 386 OTUs in FA samples were confirmed at least at the phylum level (Tables S3 and S4).

The phyla that were more abundantly represented in both locations were *Chytridiomycota* followed by *Ascomycota*, *Basidiomycota*, and *Monoblepharomycota*. The *Chytridiomycota* phylum showed large relative abundances in both locations, with 4.4% in WR and 1.9% in FA. Relative abundance between both locations showed that there was greater relative abundance of *Basidiomycota* in the FA as opposed to the WR location, which shows a minimal representation from the *Basidiomycota* at less than 0.1%. The classes with relative abundance higher than 0.01% are listed on Table 2 for both locations. Two classes belonging to *Chytridiomycota*, GS13 ($p = 0.0161$) and *Rhizophydiomycetes* ($p = 0.0373$) in WR were significantly higher than in FA (Table 2). Moreover, the classes *Cladochytriomycetes* and *Tremellomycetes* were only present in WR and FA, respectively (Table 2).

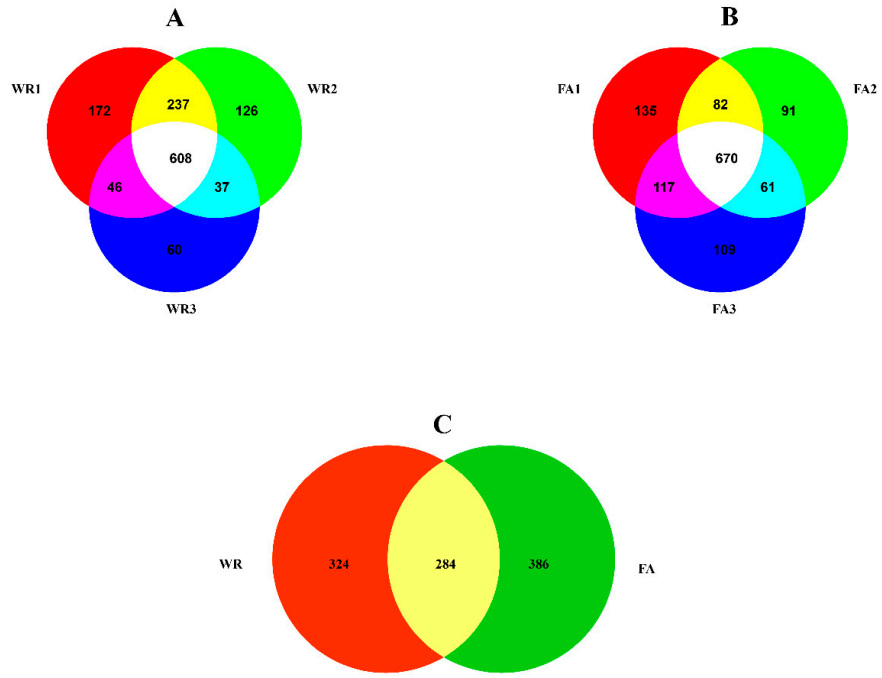

Figure 2. Venn diagrams indicating the number of OTUs obtained from water samples. (**A**): Number of OTUs obtained in samples from the water reservoir (WR). (**B**): Number of OTUs obtained in water samples collected at the irrigation valve at the farm (FA). (**C**): Number of OTUs co-present in irrigation water samples collected at the water reservoir (WR) and irrigation valve at the farm (FA).

Table 2. Dominant classes (relative abundance more than 0.01%) in irrigation water collected at the water reservoir (WR) and at the farm (FA).

Phylum	Class	Relative Abundance (%)		*p* Value
		WR	FA	
Ascomycota	*Dothideomycetes*	0.2740%	0.4626%	0.3083
	Saccharomycetes	0.0383%	0.1041%	0.3542
Basidiomycota	*Tremellomycetes*		0.0408%	
Chytridiomycota	*Cladochytriomycetes*	0.0230%		
	Chytridiomycetes	1.4648%	1.7089%	0.5811
	GS13	0.0696%	0.0192%	0.0161 *
	Rhizophydiomycetes	0.5661%	0.1422%	0.0373 *
Monoblepharomycota	*Sanchytriomycetes*	0.0348%	0.0456%	0.3009
	Sordariomycetes	0.1054%	0.1118%	0.9255

* indicates significant differences between WR and FA at 0.05 level.

At the family level, five dominant families (relative abundance of more than 0.01%), including *Chytridiaceae*, *Cladosporiaceae*, *Pleosporaceae*, *Sanchytriaceae*, and *Stachybotryaceae*, were present in both WR and FA, without significant differences between locations (Table 3). In WR samples, *Aquamycetaceae*, *Lentitheciaceae*, *Nowakowskiellaceae*, *Plectosphaerellaceae*, and *Trichosphaeriaceae* were the dominant families, while in FA samples, *Alphamycetaceae*, *Aureobasidiaceae*, *Bulleribasidiaceae*, *Metschnikowiaceae*, and *Microdochiaceae* were the dominant families (Table 3).

Based on the 48 OTUs identified at the species level, the most abundant species was the *Chytridiomycota Zygophlyctis melosirae*, which was associated with 17 OTUs (Tables S2–S4). Moreover, eight OTUs were associated with plant pathogenic fungi representing six species (Table 4). Three of these OTUs were present in both locations, while the other five were present either at WR or FA (Table 4). The relative abundance of OTU456 was significantly different between the WR and FA samples (Table 4).

Table 3. Dominant families (relative abundance more than 0.01%) in irrigation water collected at the water reservoirs (WR) and at the farm (FA).

Phylum	Class	Family	Relative Abundance (%)		*p* Value
			WR	FA	
Ascomycota	*Dothideomycetes*	*Cladosporiaceae*	0.1541%	0.2111%	0.3989
	Dothideomycetes	*Pleosporaceae*	0.0470%	0.0948%	0.4757
	Sordariomycetes	*Stachybotryaceae*	0.0155%	0.0531%	0.4837
	Dothideomycetes	*Lentitheciaceae*	0.0215%		
	Dothideomycetes	*Plectosphaerellaceae*	0.0216%		
	Saccharomycetales	*Saccharomycetales*	0.0256%		
	Sordariomycetes	*Trichosphaeriaceae*	0.0288%		
	Dothideomycetes	*Aureobasidiaceae*		0.0343%	
	Saccharomycetes	*Metschnikowiaceae*		0.0929%	
	Sordariomycetes	*Microdochiaceae*		0.0108%	
Basidiomycota	*Tremellomycetes*	*Bulleribasidiaceae*		0.0272%	
Chytridiomycota	*Chytridiomycetes*	*Chytridiaceae*	1.3873%	1.6499%	0.5338
	Cladochytriomycetes	*Nowakowskiellaceae*	0.0230%		
	Rhizophydiomycetes	*Aquamycetaceae*	0.0735%		
	Rhizophydiomycetes	*Alphamycetaceae*		0.0186%	
Monoblepharomycota	*Sanchytriomycetes*	*Sanchytriaceae*	0.0348%	0.0456%	0.3011

Table 4. OTUs associated with plant pathogenic fungi in irrigation water collected at the water reservoir (WR) and at the farm (FA).

ID	Plant Pathogen	Host	WR	FA
OTU112	*Cladosporium cladosporioides*	*Strawberry and grapevine*	√	√
OTU213	*Exserohilum rostratum*	*Tiger Grass and pineapple leaf*	√	√
OTU456	*Nigrospora oryzae*	*Rice, wheat, sorghum, barley, maize and cotton*	√ *	√
OTU295, OTU229	*Curvularia_verruculosa*	*Bean, cotton, and grape*		√
OTU609, OTU730	*Colletotrichum sublineola*	*Sorghum*		√
OTU316	*Plectosphaerella cucumerina*	*Melon, pepper and tomato*	√	

* indicates significant differences in relative abundance of OTUs between WR and FA at 0.05 level.

4. Discussion

To assess the risks that fungal phytopathogens in river water pose to agriculture, we focused our work on the irrigation water that is supplied by the Rio Grande River to the LRGV agricultural system. In this study, we used ITS2 amplicon metagenomic analysis to evaluate the fungal diversity and composition of the irrigation water from the Rio Grande River as it leaves the water reservoir and it arrives at an irrigation valve at the farm level. This is the first report that utilizes a metagenomic approach to assess fungal microorganisms in river water, with the aim to identify fungal phytopathogens that could pose a risk to agricultural production. This work shows that the communities and structure of the fungal microbiome found in the water reservoir (WR) change substantially from the microbiome found at the irrigation valve at the farm (FA). This study showed a significant difference in the Shannon and Simpson indices between locations, indicating differences in the diversity of species present. The Shannon index of the water reservoir (WR) had a higher value than the water collected at the farm (FA). This could indicate that WR water has lower diversity than FA water. The Simpson index showed opposite results. The WR had lower Simpson diversity values than that of the FA, indicating that there are more species identified at the WR (higher richness), but that higher species evenness was present in the water collected at the farm. The PCA analysis showed a clear differentiation of the fungal communities between the locations at the WR and the FA, indicating loss or gain of species during the distribution of water from the source (WR) to the farm (FA). These results suggest that microbes may enter water systems at several points along the distribution path and change the water microbial communities and structures. Any irrigation method in which the water

comes in contact with soil or plant debris has an in an increased potential to acquire new microbes into its water path [4]. Furrow flooding and runoff from other irrigation methods can carry microbes from soil throughout the water area [4]. Therefore, several fungal or bacterial microbes may enter irrigation water from WR to FA through these pathways, which resulted in the change of the communities and structure of the fungal microbiome from the WR to the FA. Moreover, the water from the WR traveled 10 miles before reaching the irrigation valve at the farm (FA), being in contact with lines with plants and debris that could trap or release microbes into the water.

Several OTUs associated with fungal plant pathogens were detected and identified in the water reservoir (WR) and the irrigation valve at the farm (FA). In both sampling locations, OTU112, OTU213, and OTU456 were present (Table 4). OTU112 was identified as *Cladosporium cladosporioides*, which is associated with strawberry blossom blight [29] and Cladosporium rot of grapevine disease [30]. OTU213 was identified as *Exserohilum rostratum*, causing Exserohilum leaf spot on tiger grass [31] and pineapple leaf spot disease [32]. OTU456 was identified as *Nigrospora oryzae*, which causes leaf spots and rot diseases in many important crops including rice, wheat, sorghum, barley, maize, and cotton [33–37]. OUT316 was only detected in the WR samples and was associated with *Plectosphaerella cucumerina*, which causes root and collar rots in melon, pepper, and tomato [38,39]. Other potential fungal plant pathogens were only detected in FA samples, including *Curvularia verruculosa* and *Colletotrichum sublineola*. *Curvularia verruculosa* is a common plant pathogen that can cause leaf spot disease in many plants including bean, cotton, and grape [40–42]. Anthracnose caused by *Colletotrichum sublineola* is an important sorghum disease worldwide [43,44]. Therefore, in this study, the amplicon-based metagenomics method was efficient for detecting fungal plant pathogens, compared with culture-based methods [13] and molecular identification methods [14,45,46]. However, metagenomics approaches still have limitations that should be taken into account in future research. For instance, the presence of DNA from dead cells can detect the presence of specific genera, but that does not mean viability. A novel DNA-binding dye, propidium monoazide (PMA), can differentiate living and dead cells, and PMA coupled with metagenomics approaches has been applied for investigating microbiomes to exclude nonviable microorganisms [47,48]. Moreover, two different versions of the database, UNITE Version 5.0 and UNITE Version 8.0, were used for analyzing metagenomics data in this study. In total, 1793 OTUs were detected in all water samples using Version 5.0, while just 639 OTUs were found in Version 8.0. Meanwhile, the number of OTUs in the WR (OTUs = 515.33 $\pm$ 39.1) and FA (OTUs = 500 $\pm$ 13.61) samples were also smaller using Version 8.0, relative to the 940.67 $\pm$ 166.54 and 955 $\pm$ 50.03 OTUs obtained for WR and FA, respectively, using Version 5.0. Although UNITE Version 8.0 comprises more fungal ITS sequences than UNITE Version 5.0, they may be incomplete or yield inconsistent results due to taxonomic reclassifications [49]. This may be reason why more OTUs were detected using Version 5.0 compared with Version 8.0. In addition, the objective of this study was to identify potential fungal plant pathogens in irrigation water, and if fewer OTUs are obtained from the metagenomics data, several OTUs related to fungal pathogens may be lost. Acquiring more OTUs and more information from metagenomics data was beneficial for identifying plant fungal pathogens in irrigation water. Therefore, UNITE Version 5.0 was selected for analyzing data in this study. For metagenomics approaches, the taxonomic attribution of each sequence depends upon alignment to a database; thus, the most comprehensive database is Genbank Uniprot, which is not revised and not always reliable [50]. Therefore, users must ensure the accuracy of sequences regarding the target plant pathogens in databases. In addition, the application of metagenomics approaches is also limited by the expertise required to properly manage sequencing data. Even if much software, such as QIME and Mothur [24,51], can be used remotely and with a user-friendly interface through the Galaxy web platform [52], an in-depth knowledge of the commands and operations necessary to perform the analyses remains necessary to choose the best methods and avoid mistakes.

5. Conclusions

To overcome the limitations of traditional methods for identifying plant pathogens in water, the metagenomic approach was useful for detecting fungal pathogens in river water used for irrigation. This study showed that there is a high diversity of fungal species present in the irrigation water coming from the Rio Grande River, including plant pathogens, which could also hold true for other rivers that supply water for irrigation. Many of the plant pathogen genera that were found in the water can be of economic importance in the region, as they can be pathogenic to the crops planted in the area. However, the threshold for their abundance in irrigation water is unknown. Moreover, it is important to acknowledge that the water source used for irrigation is very dynamic and the presence of plant pathogens that can affect agricultural crops can fluctuate between seasons and increase without warning. Further studies that assess the abundance of plant pathogens in the water in different seasons would be helpful to growers of the region so that they can assess the risks and continue producing important crops in the LRGV.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture13071401/s1, Table S1: Statistics of Illumina sequencing of internal transcribed spacer (ITS) amplicons for irrigation water collected at the water reservoir (WR) and at the farm (FA). Table S2: The abundance and classification of OTUs that co-presented in irrigation water from water reservoirs (WR) and irrigation valve (FA). Table S3: The abundance and classification of OTUs that presented in irrigation water from water reservoirs (WR). Table S4: The abundance and classification of OTUs that presented in irrigation water from irrigation valve (FA).

Author Contributions: Conceived and designed the experiment: V.A. Performed the experiments: M.C. and C.Y. Analyzed the data: M.C. and C.Y. Contributed reagents/materials/analysis tools: V.A. Wrote and revised the manuscript: C.Y. and V.A. All authors have read and agreed to the published version of the manuscript.

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