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Seasonal dynamics of cyanobacteria and eukaryotic phytoplankton in a multiple-reservoir recycling irrigation system

Ping Kong* , Patricia Richardson and Chuanxue Hong

Abstract

Background: Agricultural runoff recycling systems are manmade aquatic ecosystems of growing significance to global water sustainability, crop health, and production. This study investigated the seasonal dynamics of microbial community in a three-reservoir recycling irrigation system with a stepwise water flow and compared with that of an adjacent runoff-free stream. Runoff water from all production areas was captured in a sedimentation reservoir which overflowed to a transition reservoir then retention reservoir through a culvert. Stream water was pumped to replenish the reservoirs as needed during growing seasons.

Results: 16S rDNA PCR clone libraries of quarterly water samples from three reservoirs and one stream were sequenced, and 575 operational taxonomic units (OTUs) were identified and assigned to cyanobacteria, eukaryotic phytoplankton, and other bacteria. When compared to the stream, three reservoirs consistently had low microbial diversity. A distinct seasonal pattern of microbial community structure was observed for each reservoir and the stream. Stream was consistently dominated by other bacteria. Retention reservoir was dominated by cyanobacteria during the summer and fall and eukaryotic phytoplankton during the winter and spring. Sedimentation reservoir was dominated by cyanobacteria beginning in the spring but that dominance was altered when water was pumped from the stream from early spring to fall seasons. Transition reservoir had the greatest shift of microbial community structure, being dominated by other bacteria in the summer, cyanobacteria in the fall, and eukaryotic phytoplankton in the winter and spring. Water temperature and ammonium level were the two most important contributing factors to the seasonality of microbial community in these reservoirs.

Conclusions: The three recycling irrigation reservoirs consistently had lower microbial diversity and distinct community structure when compared to the stream. These reservoirs were typically dominated by cyanobacteria during warm seasons and eukaryotic phytoplankton during cool seasons. This seasonal pattern was altered when water was pumped from the stream. The cyanobacteria dominance was associated with rising water temperature and ammonium level. These results highlight the importance of preventing agricultural runoff from entering natural waterways and water resources and provide a useful framework for further investigations into the ecological processes of this emerging ecosystem.

Keywords: Bacteria, Cyanobacteria, Phytoplankton, Seasonal dynamics, Water recycling, Water quality

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Introduction

Agricultural runoff recycling systems are aquatic ecosystems of growing significance to global water and environmental sustainability, crop health, and production. Water scarcity and drought are among the most pressing sustainability issues in the twenty-first century (Brown and Ayres 1998). Agriculture uses 70% of the overall global consumptive water (Holdren 2008) and generates huge quantities of runoff, which is the leading source of impairments to rivers, lakes, and wetlands in the USA (http://water.epa.gov/pollution/nps/agriculture_facts.cfm) and elsewhere (Diaz and Rosenberg 2008; Ferber 2001; Guo 2007). The amount of irrigation runoff can be substantial with overhead irrigation systems which have water delivery efficiency as low as 20% (Haman et al. 2005). While capture and reuse of runoff for irrigation can tremendously increase agricultural water efficiency and reduce nonpoint source pollution (Hong et al. 2009), it also raises concerns about algal bloom, water quality deterioration, and plant health risk associated with pathogen recycling and redistribution (Hong and Moorman 2005; Hong et al. 2014; Kong and Hong 2014; Kong et al. 2009; Kong et al. 2012a, b).

Recent studies found that recycling irrigation system design can influence microbial diversity and population and water quality in associated reservoirs (Ghimire et al. 2011; Hong et al. 2014; Kong et al. 2017; Zhang et al. 2015a, b; Zhang et al. 2016). According to a study in the spring, cyanobacteria were predominant in sedimentation reservoirs but not in other downstream reservoirs of multiple-reservoir recycling irrigation systems (Kong et al. 2017). Another study on single-reservoir recycling irrigation system showed that populations of *Phytophthora* species, a group of important algae-like plant pathogens, decreased with increasing distance from runoff entrance (Ghimire et al. 2011). These studies suggest that multiple-reservoir system design may be effective for preventing pathogen dissemination by extending runoff turnover time and for control of algal bloom by sedimentation. However, it is not clear whether microbial community structure changes with time in these recycling irrigation reservoirs, especially during the summer and fall when water consumption and recycling dramatically increases.

Seasonal variations of phytoplankton in natural lakes and reservoirs have been well documented (Katsiapi et al. 2013; Marshall 2013; Rozon and Short 2013; Singh et al. 2014). Cyanobacterial blooms occur commonly in the summer and fall, while chlorophyte blooms occur at the beginning of winter in these aquatic environments (Katsiapi et al. 2013; Marshall 2013). In a single-reservoir recycling irrigation system in Virginia, USA, chlorophyll *a* level fluctuated over time (Hong et al. 2009). The objective of this study, focusing on phytoplankton, was to further investigate the seasonality of microbial diversity and

community structures in a multi-reservoir recycling irrigation system with a stepwise water flow and compare to those in an adjacent stream.

Materials and methods

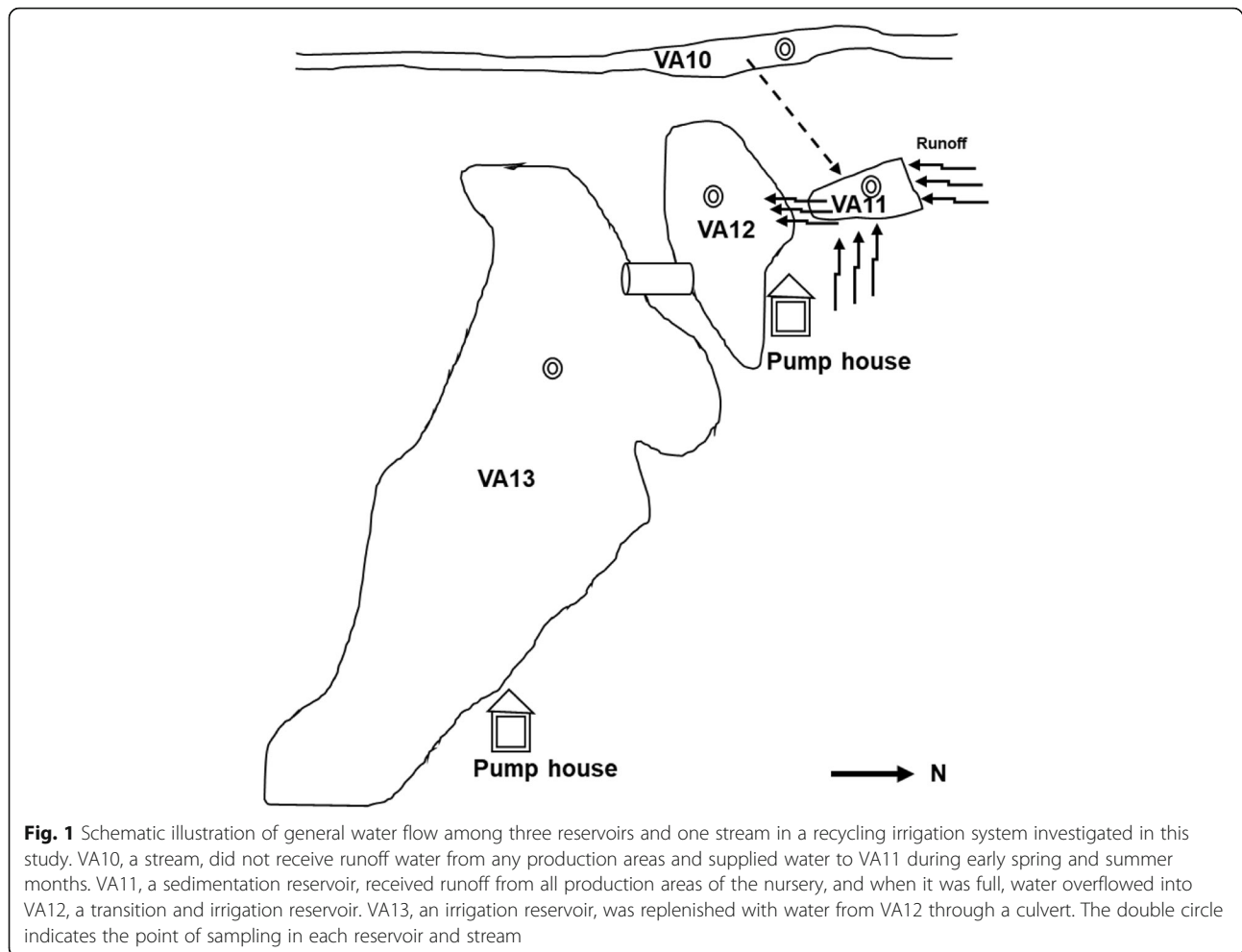
Reservoirs, water sampling, and metadata

Three reservoirs (VA11, VA12, and VA13) at an ornamental crop nursery and one adjacent stream (VA10) in eastern Virginia were included in this study. VA10 was on the property edge and did not receive runoff from any of the nursery production areas. VA11 was a small sedimentation reservoir, capturing runoff water from all production areas on the property. It also received water from VA10 from early spring to fall months to replenish the three reservoirs in the recycling irrigation system. When VA11 was full, water overflowed to VA12, a 0.8-hectare transition and irrigation reservoir, hereafter referred to as a transition reservoir. VA13, a 6.1-hectare spring-fed natural lake, is a retention and irrigation reservoir which was replenished with water from VA12 through a culvert. The general water flow among the three reservoirs and stream is illustrated in Fig. 1. The summer and fall of 2012 were pretty dry, the stream completely dried up in June. As a result, water was also pumped from VA13 to replenish VA12. There also was a significant storm event in August of 2012 during which stream water level surged and directly flowed to both VA11 and VA12.

Three 1-l water samples were collected from the center of each reservoir and the stream quarterly from spring of 2012 to early 2013. They were taken at approximately 20 cm below the surface near a 6600V2-4 Multiparameter DataSonde deployed in each reservoir and the stream (Fig. 1). The collected water samples were kept in a cooler and transported to the laboratory for processing. To determine the nutrient level, 10-ml aliquots of each sample were immediately filtered through a 0.22- μ m membrane, frozen at -20°C , and shipped to Brookside Labs (New Bremen, OH, USA) for analysis. To extract DNA, the remaining water samples were centrifuged at $12,230\times g$ for 50 min in an Avanti J-26XP (Beckman Coulter, Atlanta, GA, USA). The sampling dates, water quality data, and nutrient levels of each sample are listed in Table 1.

DNA extraction, PCR clone library construction, and sequencing

Immediately after water samples were centrifuged, supernatants of individual samples were filtered through 0.22- μ m membranes and pellets were collected. DNA was extracted from the filters and pellets, then quantified as described previously (Kong et al. 2017). Twenty nanograms of DNA from both filter and pellet of each sample were combined and used as template in PCR. To amplify



the 16S rDNA genes in cyanobacteria and in chloroplasts of eukaryotic phytoplankton, the primers CYA359F and CYA781R (Nübel et al. 1997) were used. PCR and construction of clone libraries of sample PCR amplicons were conducted as previously described (Kong et al. 2017). More than 100 clones were randomly selected from the library of each sample and amplified with vector T3 and T7 primers for sequencing.

Sequence process and analysis

Poor-quality sequences of samples were removed based on the quality control report from the sequencing facility. Remaining sequences were edited using Mega 6 (Tamura et al. 2013) and Mothur software package V.1.33.3 as previously described (Kong et al. 2017). Clustering, classification, and phylogenetic analysis of the edited sequences was performed using Mothur. Operational taxonomic units (OTUs) were used for building clusters which were determined by a distance matrix of the sequences from all water samples at a pairwise distance cutoff of 0.15 (a percent similarity threshold of 85%). OTUs were then classified based on known

sequences in a constructed cyanobacteria reference library or through Arb-Silva-SINA against Silva and Greengenes 16S gene libraries at the default setting of 70% sequence identity. OTUs were clustered through a neighbor-joining tree at distance cutoffs of 0.03 for phylogeny analysis. "Unclassified" OTUs were grouped based on their closest positions to knowns in the phylogenetic tree (Additional file 2: Figure S1). A taxon was assigned with a name of knowns in the closest cluster or assigned as unknown if there were no knowns nearby.

Microbial diversity analysis

Alpha diversity as an indicator of complexity of the samples was measured by inverse Simpson index. Beta diversity or the membership and microbial community composition as well as their relation to environmental variables were measured using OTU- and taxon-based approaches. Both alpha and beta diversity were determined through Mothur (Schloss et al. 2011). In OTU-based analysis, a heat map was generated through R version 3.1.0 (<http://www.R-project.org/>) (R Core Team 2013) with a shared file of OTUs from individual reservoirs at cutoffs

Table 1 Water quality and nutrient levels in a multiple-reservoir water recycling system at the time of sampling

Season (date)	Reservoir ^y	Water quality ^z						Nutrient level			
		Chla (µg/ L)	DO (mg/ L)	EC (µS/ cm)	pH	Temp (°C)	Turb (NTU)	NH ₄ (mg/L)	NO ₃	NO ₂	PO ₄
Spring ^x (4/11/12)	VA10	0.55	8.58	243.00	5.40	12.28	3.90	0.29	7.42	0.09	< 0.01
	VA11	24.30	9.07	437.33	6.32	16.42	52.10	5.18	12.07	0.09	1.76
	VA12	32.90	9.49	252.00	6.65	18.08	7.7	2.54	5.17	0.09	0.69
	VA13	2.5	9.85	192.93	7.46	17.60	45.3	0.29	0.46	0.09	0.01
Summer (7/16/12)	VA10	0.70	6.79	241.67	5.87	20.75	3.60	0.29	25.78	0.11	0.09
	VA11	23.60	6.20	741.33	6.12	28.95	17.00	0.65	165.52	0.81	18.92
	VA12	31.40	10.40	328.33	7.75	29.85	2.17	0.48	50.31	1.79	6.56
	VA13	3.71	10.39	311.00	10.3	29.66	17.02	0.29	0.15	0.09	0.09
Fall (10/1/12)	VA10	0	8.11	244.00	6.02	17.32	2.60	0.29	39.42	0.09	0.09
	VA11	26.73	5.83	343.67	6.65	19.92	6.20	1.16	86.82	1.72	15.64
	VA12	51.07	7.38	230.00	7.22	22.35	14.57	0.29	41.8	0.66	8.14
	VA13	24.87	7.06	181.33	9.42	21.63	76.07	0.29	0.09	0.09	0.09
Winter (1/22/13)	VA10	1.33	9.75	236.00	5.57	8.39	1.27	0.09	21.94	0.16	0.09
	VA11	11.60	11.88	71.00	6.23	6.59	37.17	0.45	6.81	0.19	2.25
	VA12	13.60	11.4	79.67	6.79	7.47	33.30	0.21	4.01	0.28	2.52
	VA13	45.03	8.93	240.00	5.79	12.86	6.13	0.09	2.42	0.09	0.09

^xData has been published (Kong et al. 2017)

^yRunoff from production areas was channeled into VA11 which overflowed into VA12 then to VA13. VA10 was an independent small stream

^zWater quality reading at the time of sampling: *Chla* chlorophyll *a*, *DO* dissolved oxygen, *EC* electrical conductivity, *Temp* temperature, *Turb* turbidity

of 0.03. The map included the relative abundance of dominant OTUs in each reservoir which contained ten or more clones and a dendrogram showing relationships between the reservoirs. Variations of taxon composition in the reservoirs were investigated using non-metric multidimensional scaling (NMDS) by the vegan “metaMDS” function in R (Oksanen et al. 2015). To analyze the correlation between community composition and environmental variables that included water quality and nutrient Metadata (Table 1), the vegan “envfit” function was used to fit the variables into the NMDS ordination.

Results

A total of 1600 clones were generated from the water samples of the three-reservoir recycling irrigation system and the associated stream. Among them, 1421 high-quality partial 16S sequences were selected and classified into 575 OTUs (Additional file 1: Table S1). Representative sequences of the identified OTUs were deposited into the NCBI GenBank (504 in Accession #KT 270949–271452 and 71 within KP 769594–769768).

Seasonal patterns of microbial diversity

There were distinct seasonal patterns in the number of OTUs detected among the three reservoirs and the stream (Fig. 2a). Consistently high number of OTUs was detected in the stream, VA10, across all four sampling

times. The OTU number detected from VA11 was also high during the spring, but it dropped during the summer and fall, and then increased slightly during the winter. Comparatively, the number of OTUs in the transition and retention reservoirs (VA12 and VA13) was lower and varied considerably, peaking in the summer and bottoming in the fall.

The inverse Simpson index was consistently low in all three reservoirs (VA11, VA12, and VA13) when compared to that of the stream, VA10 (Fig. 2b). The index climbed after the spring, peaked in the fall, then slightly declined in the winter. On the other hand, VA11 had index values and dynamics similar to VA12 and VA13 although it appeared slightly more complex in the fall and winter seasons (Fig. 2b).

Cyanobacteria, eukaryotic phytoplankton, and other bacteria detected are summarized in Table 2. The bacteria group had the greatest diversity with 414 OTUs. This was particularly obvious with 158 OTUs detected in the summer and 113 OTUs in the fall. Comparatively, the other two groups were not as diverse with 121 OTUs for cyanobacteria and 127 for eukaryotic phytoplankton.

The greatest cyanobacterial diversity was detected during the summer (51 OTUs) and the least during the winter (19 OTUs) with the spring and fall in between. Specifically, some cyanobacterial OTUs such as those of *Aphanizomenon*, *Limnothrix*, *Microcystis*, and *Synechococcus*

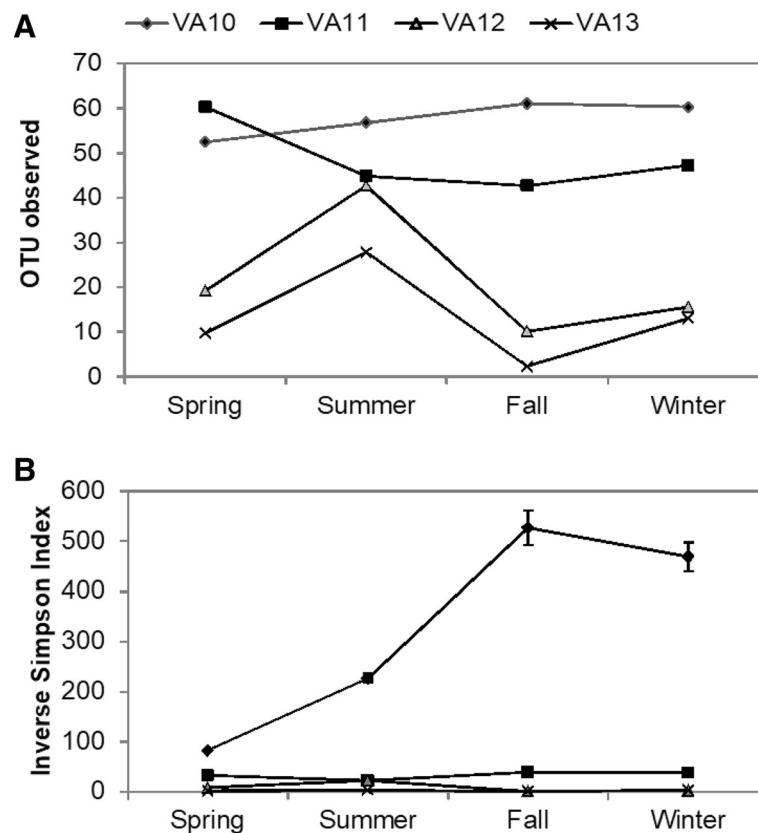


Fig. 2 Seasonal alpha diversity of microbes in three reservoirs and one stream. **a** Number of operational taxonomic units (OTUs). **b** Inverse Simpson index. Bars in the lines are standard errors of 65 clone sequences in individual samples

were predominant or only present in the summer. There were four classified subgroups of eukaryotic phytoplankton. As shown by most dominant subgroups: *Chlorophyta* (33 OTUs) and *Stramenopila* (49 OTUs), they were more diverse in cool than warm seasons (106 vs. 21 OTUs). This seasonal pattern was opposite to that of the bacteria group. Overall, more diverse cyanobacteria and other bacteria were detected during the summer while more phytoplankton during the cool seasons (winter and spring).

Seasonal patterns of microbial community structure

There were four distinct seasonal patterns of microbial community structure as measured by the relative percentage of OTUs (Fig. 3) and representative clone among three groups (Fig. 3b). First, other bacteria consistently dominated in the stream, VA10, irrespective of the season. This is likely attributed to the nonpolluted nature of stream water. Second, other bacteria also dominated in VA11 during the summer then gradually declined through the fall and winter. These bacteria were carried over from VA10, when stream water was pumped to replenish VA11. Eukaryotic phytoplankton became dominant in the winter while cyanobacteria co-dominant in the spring. Third, VA12, the transition

reservoir, had the greatest shift of microbial community structure, being dominated by other bacteria in the summer, cyanobacteria in the fall, and eukaryotic phytoplankton in the winter and spring, respectively. Fourth, VA13, the retention reservoir, was dominated by cyanobacteria during the warm seasons (summer and fall) and eukaryotic phytoplankton during the cool season (winter and spring). As shown in the heatmap (Fig. 3c), there were clear associations in the dominant group and subgroups between connected reservoirs and consecutive seasons. For example, OTU1 or *Microcystis* sp. dominated in two connected reservoirs (VA12 and VA13) during two consecutive seasons (summer and fall).

Overall, cyanobacteria as represented by *Microcystis* spp. typically dominated these nutrient-rich reservoirs during warm seasons while eukaryotic phytoplankton dominated during cool seasons as shown in VA13 (Fig. 3a, b). However, cyanobacteria as the dominant group in VA11 during warm seasons were replaced by other bacteria from the stream. During the hot summer, substantial quantity of stream water was pumped to replenish VA11 then VA12 and VA13. As a result, other bacteria were carried over from the stream and dominated VA11 almost exclusively and to a lesser extent in the

Table 2 Diversity and populations of cyanobacteria and phytoplankton identified from a multiple-reservoir water recycling system over four seasons

Group	Taxon ^x	No. of OTUs				Representative OTU# ^z	NCBI accession #
		Spring ^y	Summer	Fall	Winter		
Cyanobacteria (CYA)	Anabaena		1			S67	KT271014
	Aphanizomenon		3			S272	KT271215
	Arthronema	1				S57, R51	KP769657
	Chamaesiphon	1				S405, R155	KP769620
	Cyanobacteria	3	15	12	4	S12	KT270960
	Cylindrospermopsis		1			S269	KT271212
	Leptolyngbya	6		1		S73, R50	KP769666
	Limnothrix		2			S100	KT271044
	Microcoleus	1			1	S72	KT271019
	Microcystis	1	10	3	1	S1	KT270949
	ML635J			1	1	S53	KT271000
	MLE	2	2	1	5	S111	KT271055
	Nostoc		2			S237	KT271180
	Nostocaceae	1	1			S502, R162	KP769641
	Phormidium	2		1		S83, R37	KP769675
	Planktothrix		1			S371	KT271314
	Pseudanabaena	1			1	S10	KT270958
	Pseudanabaenaceae	1				S506, R168	KP769648
	Synechococcus		5			S281	KT271224
Unclassified CYA	5	9	6	6	S88, R175	KP769598	
Xenococcaceae	1				S514, R206	KP769652	
Σ		26	51	25	19		
Eukaryotic phytoplankton (EP)	Chlorophyta	11	2	3	17	S2	KT270950
	Cryptophyta	2	4	1	7	S3	KT270951
	Stramenopila	14	2	8	25	S6	KT270954
	Streptophyta	1				S68	KT271015
	Unclassified EP	12		1	17	S4	KT270952
Σ		40	8	13	66		
Other bacteria (OB)	Acidobacteria			2		S325	KT271268
	Candidate	15	31	18	15	S19	KT270967
	Chloroflexi	2			1	S407, R140	KP769621
	Deinococcus	1	1			S217	KT271160
	Firmicutes		2			S215	KT271158
	Nitrospirae			2	1	S256	KT271199
	Pelotomaculum		1			S322	KT271265
	Unclassified OB	44	123	91	62	S7	KT270955
WD272				2	S460	KT271374	
Σ		62	158	113	81		

^xTaxa were determined through SINA (v1.2.11) with the least common ancestor (LCA) method based on Greengenes and Silva at ARB-Silva. Those identified to genus level are in bold text while those that are identified to family, class or order, phylum or division are in regular text

^yData has been published (Kong et al. 2017)

^zOTUs grouped in previous and this study are prefixed with R (reservoir) and S (season), respectively

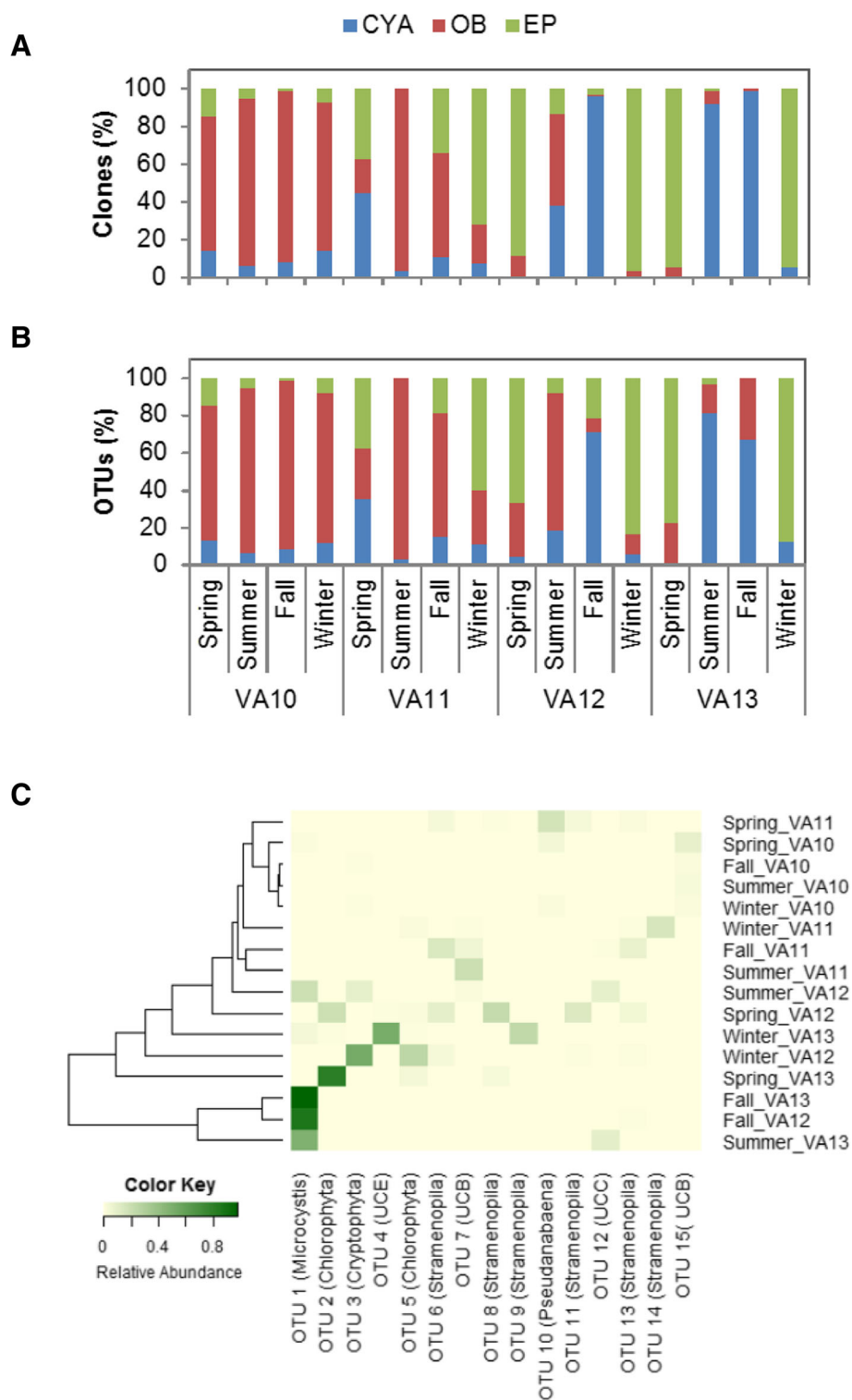


Fig. 3 Seasonal beta diversity of microbes in three reservoirs and one stream. **a** Community structure of three groups—cyanobacteria (CYA), eukaryotic phytoplankton (EP), and other bacteria (OB) by clone sequence. **b** Community structure by OTU. **c** Heatmap of OTUs with 10 or more clone sequences in the communities with identified taxa in parentheses. Association of samples (row) is depicted with a dendrogram

downstream reservoir VA12. Likewise, when movement of stream water to VA11 was reduced in the fall, the dominance level of other bacteria also decreased.

Correlation of seasonal microbial diversity and water quality

The correlation between seasonal microbial diversity and water quality is presented with non-metric multidimensional scaling (NMDS) (Fig. 4). Eight taxa fit best with water quality variables although only the fitness of *Microcystis*, *Limnothrix*, *Candidata*, *Chloroflexi*, and some unclassified other bacteria was significant (Additional file 1: Table S2). Among them, cyanobacteria and other bacteria were common taxa in the summer and fall while *Stramenopila* phytoplankton and *Chloroflexi* bacteria were common spring and winter taxa (Fig. 4).

Temperature, chlorophyll *a*, and ammonium (NH_4^+) levels were associated with microbial seasonal predominance (Fig. 4). Specifically, temperature was significantly

fitted in the microbial seasonal variation. Cyanobacteria only predominated when water temperatures were over 20 °C in VA12 and VA13 (Fig. 2, Table 1). This suggests that temperatures at 20 °C or higher in the reservoir may trigger a bloom of cyanobacteria such as *Microcystis* and *Limnothrix*. Interestingly, chlorophyll *a* level appeared to correlate well with predominance of *Microcystis*, suggesting that this parameter is a reliable measurement for monitoring *Microcystis* blooms. On the other hand, among the four investigated nutrients, only ammonium was associated with the presence of predominant taxa during cool seasons (Fig. 3, Additional file 1: Table S3). *Chloroflexi* was detected in VA10 in the spring and winter and in VA11 in the spring. However, the ammonium levels in these reservoirs varied between 0.29 and 5.18 mg/L. Similarly, *Stramenopila* was detected in all the reservoirs with ammonium levels that varied from winter to spring. Therefore, the correlation between these taxa and ammonium levels requires further investigation.

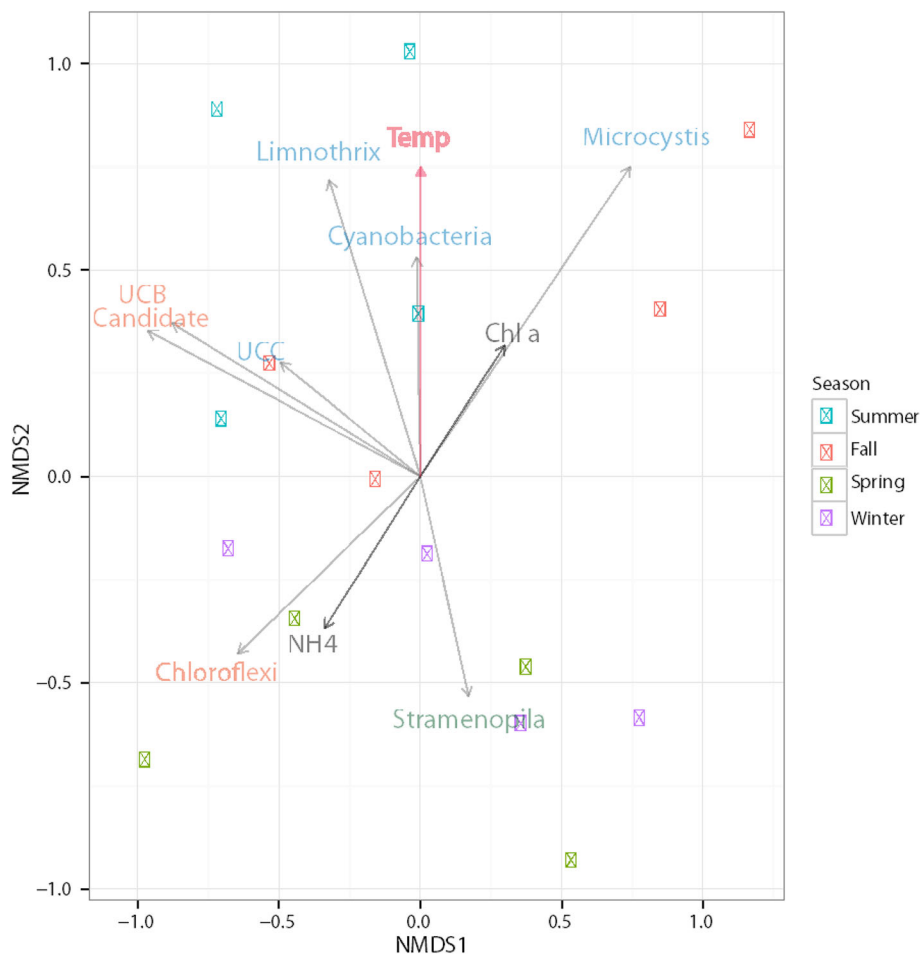


Fig. 4 Two-dimensional non-metric multidimensional scaling (NMDS) ordination plots of the best sets of water quality parameters (dark arrow lines) with maximum correlation with community dissimilarities against the best sets of taxa responsive to seasonal variation. The arrow length and direction depict the strength and nature of the relationships

Discussion

This study revealed the seasonality of microbial community structure in multiple-reservoir agricultural runoff recycling systems with a stepwise water flow while providing additional evidence demonstrating nutrient-rich agricultural runoff's potential negative impacts on aquatic microbial diversity. These findings have several important implications.

All three reservoirs (VA11, VA12, and VA13) consistently had much lower levels of microbial diversity throughout the year when compared to that of the adjacent stream (VA10). These results are in agreement with our previous study that investigated spring microbial diversity of several water recycling systems (Kong et al. 2017). Similar negative impacts of agricultural runoff on microbial diversity were also reported in Canada (van Rossum et al. 2015). In addition to nutrients, fungicide residue in agricultural runoff also impacted microbial diversity and activities as reported in Sweden (Zubrod et al. 2015). The present study again highlights the importance of preventing agricultural runoff water from entering natural waterways and water resources.

Among the three major groups of microbes investigated in this study, cyanobacteria typically dominated the recycling irrigation reservoirs during the summer and fall while eukaryotic phytoplankton during the winter and spring as shown in VA13. These observations generally accord with the previous studies of lakes and reservoirs in Virginia (Marshall 2013), urban lake in Greece (Katsiapi et al. 2013), Bay of Quinte, Canada (Rozon and Short 2013), and high altitude lakes of India (Singh et al. 2014). Associated with the cyanobacterial dominance during the warm seasons are dramatic diurnal and seasonal fluctuations of water quality in these reservoirs (Hong et al. 2009; Zhang et al. 2015a, b; Zhang et al. 2016). The diurnal fluctuation is mostly due to photosynthesis process in which algae remove carbon dioxide from water during day time while releasing into water during night time. Likewise, the seasonal fluctuation is attributed mainly to algal bloom and subsequent decomposing process. These fluctuations of water quality have profound impacts on plant pathogens in the same reservoirs (Kong 2013; Kong and Hong 2014; Kong et al. 2009; Kong et al. 2012a, b; Zhang and Hong 2017) and crop production (Hong 2016; Hong 2017a, b; Hong et al. 2009; Zhang and Hong 2017). The cyanobacterial dominance was associated with rising temperature and nutrient level in particular of ammonium. This dominance may be affected by the introduction of exogenous microbes in an extent proportional to the quantity of water introduced from outside sources—other bacteria carried through water from the stream in the present study. This was most obvious in the sedimentation reservoir (VA11) and to a lesser degree in the transition reservoir (VA12).

Microcystis species were identified as the most abundant cyanobacteria in these reservoirs although their implications for aquatic life, crop health, and production are yet to be elucidated. *Microcystis* species are known to produce harmful toxins (Feng et al. 2019; Gu et al. 2019; Lin et al. 2019; Qian et al. 2019; Ross et al. 2019; Takaara et al. 2019; Xie et al. 2019). They may also produce microcystins that could be algicidal, larvicidal, or herbicidal (Berry et al. 2008). Further investigations into their potential impacts on plant pathogens in the same reservoirs and also on crop health and productions are warranted.

It should be pointed out that the data presented here were collected from three recycling irrigation reservoirs and an associated stream at the same site. This selection of one site over four sites with a single reservoir was intended for collecting the most meaningful data. First, this recycling irrigation system had a stepwise water flow with runoff from all production areas captured in the sedimentation reservoir then overflowed to transition and retention reservoirs. As a result, the sedimentation reservoir had the most nutrient load while the retention reservoir had the least at all sampling dates except for the winter (Table 1). Second, the stream was runoff-free, so providing an ideal reference. Third, transfer of water from the stream to the sedimentation reservoir from early spring to fall months provided a window to see how exogenous microbes may change the dynamics of microbial diversity and community structure in the water recycling system. Nevertheless, the results of the present study must be interpreted with caution. Although each reservoir had a distinct seasonal pattern of microbial diversity and community structure, these seasonal patterns are yet to be validated through sampling additional sites, ideally with reservoirs of different holding capacities and water turnover times.

Conclusions

This study revealed for the first time the seasonality of microbial diversity and community structure in multiple-reservoir agricultural runoff recycling systems with a stepwise water flow. First, all three reservoirs consistently had much lower levels of microbial diversity throughout the year when compared to an adjacent runoff-free stream, highlighting the importance of preventing agricultural runoff water from entering natural waterways and water resources. Second, among the three major groups of microbes investigated in this study, cyanobacteria typically dominated the reservoirs during the summer and fall while eukaryotic phytoplankton during the winter and spring as shown in VA13. Third, the cyanobacterial dominance was associated with rising temperature and nutrient level in particular of ammonium and it was replaced by other bacteria to an extent

proportional to the quantity of water transferred from the stream. Fourth, *Microcystis* species were identified as the most abundant cyanobacteria in these reservoirs although their implications for aquatic life, crop health, and production are yet to be elucidated. These findings provide a useful framework and tools for further investigations into the ecological processes in this emerging aquatic system and for better understanding, monitoring, and management of recycled water quality.

Additional files

Additional file 1: Table S1. OTU, taxon and group of individual clones in a recycled irrigation system across seasons. **Table S2.** Non-metric multidimensional scaling (NMDS) ordination of the best taxa responsive to seasonal variation. **Table S3.** Non-metric multidimensional scaling (NMDS) ordination of water quality parameters best fitting in seasonal dynamics of microbes. (DOCX 266 kb)

Additional file 2: Figure S1. Neighbor joining phylogenetic tree of OTUs based on 16S rDNA sequences of microbial communities in the recycled irrigation system across seasons (PDF 34 kb)

Abbreviations

Chla: Chlorophyll *a*; CYA: Cyanobacteria; DO: Dissolved oxygen; EC: Electrical conductivity; EP: Eukaryotic phytoplankton; OB: Other bacteria; OTU: Operational taxonomic units; Temp: Temperature; Turb: Turbidity

Acknowledgements

Water samples were collected and DNA extracted by G Cafá to characterize general bacterial diversity as part of the SCRI project (www.irrigation-pathogens.info).

Authors' contributions

PK conceived, designed, and performed the experiments. PK and CH analyzed the data and wrote the paper. CH contributed reagents/materials/analysis tools. PR helped in the water sample collection, measured the water quality at the time of sampling, and reviewed the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data are included in this manuscript.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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