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RESEARCH ARTICLE

BACTERIAL COMMUNITY PROFILE AND REMOVAL EFFICIENCY OF A HORIZONTAL SUBSURFACE FLOW CONSTRUCTED WETLAND TREATING FLORICULTURE WASTEWATER IN BISHOFTU TOWN, ETHIOPIA

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ABSTRACT: Bacterial communities associated with wetland plants are major partners in the removal of pollutants from constructed wetland (CWs) treatment plants. However, there is still a knowledge gap on the link among bacterial community structure of wetland, plant composition and treatment efficiency under tropical conditions. The objective of this study was to characterize the bacterial community structure of wetland plants in a horizontal subsurface flow constructed wetland (HSSFCW) in relation to their removal efficiency of nutrients. Sediment samples were collected from the rhizosphere of single planted (Cp), mixed planted (Mp) and unplanted (Up) CW units. The bacterial diversity was analyzed using high throughput sequencing of the 16S rDNA gene. A total of 2793, 2554, and 2282 operational taxonomic units (OTUS) with 26, 28, and 22 phyla were identified from samples of Cp, Mp and Up operational units, respectively. The data showed Firmicutes were dominant in planted CW units, contributing to 40.9% of the bacterial flora in single planted (Cp) CW units; whereas Proteobacteria contributed to 56.4% of the flora in unplanted (Up) units. The genera *Trichococcus*, *Chlorobium* and *Thermomonas* were dominant representing 18.1%, 13.5%, and 14.2% of the microflora in the Cp, Mp, and Up units, respectively. The analysis, in general, showed that higher abundance of the different taxonomic groups (phylum, class, genus, etc) was recorded in planted CW units than the unplanted control units. Species richness (OTUs and Chao) and Shannon diversity index (H') of the specific bacterial taxa were positively correlated with overall removal efficiencies of BOD_5, COD, NH_4^+, NO^{3-} and TP which were 72.4 ± 2.7%, 70.3 ± 1.9%, 96.2 ± 2.2%, 71.3 ± 3.8% and 52.7 ± 7.3%, respectively. In general, the data showed that sediment bacterial communities were influenced by CW planting which, in turn, affected the pollutant removal efficiency of the CWs.

Key words/phrases: Diversity indices, Firmicutes, Illumina sequencing, Pollutant removal, Proteobacteria.

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INTRODUCTION

The use of excess agrochemicals in agricultural and horticultural activities contribute to point and non-point source pollutions of aquatic environment which are implicated with health and environmental hazards (Berhan Teklu *et al*., 2016). The conventional stabilization pond technologies are considered less efficient in the treatment of most of the agrochemicals that enter surface waters through effluents (Miège *et al*., 2009). The use of most effective activated sludge pond systems is not affordable for they are energy and capital intensive (Rai *et al*., 2013). These days, ecological technologies are introduced as cheap alternatives for wastewater treatment compared to the expensive ones (Wu *et al*., 2015). Constructed wetland systems (CWs) are one of the ecological technologies used as cost effective and eco-friendly options.

CWs have gained attention for remediation of agricultural wastewater (Vymazal, 2011) and are successfully used to reduce a wide range of contaminants from various types of wastewaters through combined effects of wetland plants and associated microbial communities (Verlicchi and Zambello, 2014). Although active research on the importance of artificial CWs with different plant systems has begun since the 1950's, the recognition of the rhizosphere microbes as a "hotspot" for the degradation of pollutants is quite recent. Thus, the relationship amongst structures of wetland bacterial communities, wetland plants compositions and pollutants removal performance of CWs was largely unknown, particularly for remediation of agrochemical wasted water in floriculture industry.

Bacterial communities are distributed mainly on the surface of the roots (rhizosphere) of the macrophytes, biofilms surrounding the general media (sediment) and water column of the treatment system (Weber, 2016). These microbial communities offer suitable ecological niches to support a diversity of metabolic pathways to enhance rapid degradation of waste constituents in CWs (Hijosa-Valsero *et al*., 2010).

Wetland plants (macrophytes) in the constructed wetlands accommodate more microbial communities, and their taxonomic and functional diversity is influenced by design, and operational conditions of the CWs (Button *et al*., 2015; Weber, 2016).

In Ethiopia, Adey Feleke Desta *et al*. (2014) reported the microbial structure of a constructed wetland integrated with anaerobic-aerobic reactors treating tannery wastewater in Modjo town, Ethiopia. The authors showed diverse bacterial phyla and apparently associated with the removal of various

carbon-containing pollutants. However, there is still a need to show the relationship between microbial community structures of different CWs treating different wastewaters. The current study shows the diversity and compositions of the bacterial communities in a horizontal subsurface flow constructed wastewater (HSSFCW) system treating wastewater from a flower farm in Bishoftu Town, Ethiopia.

MATERIALS AND METHODS

Design and operation of constructed wetland

Three pilot-scale experimental HSSFCWs were established in a privately owned Yassin Flower Farm nearby Bishoftu Town, some 55 km south of the capital Addis Ababa, Ethiopia. A schematic representation of the experimental CWs is shown in Fig. 1. The surface area of each CW unit was 5.2 m^2 (4.0 m length and 1.3 m width) that is estimated base on the first-order design equation proposed by Kickuth for sizing of HSSFCWs (Vymazal, 2008).

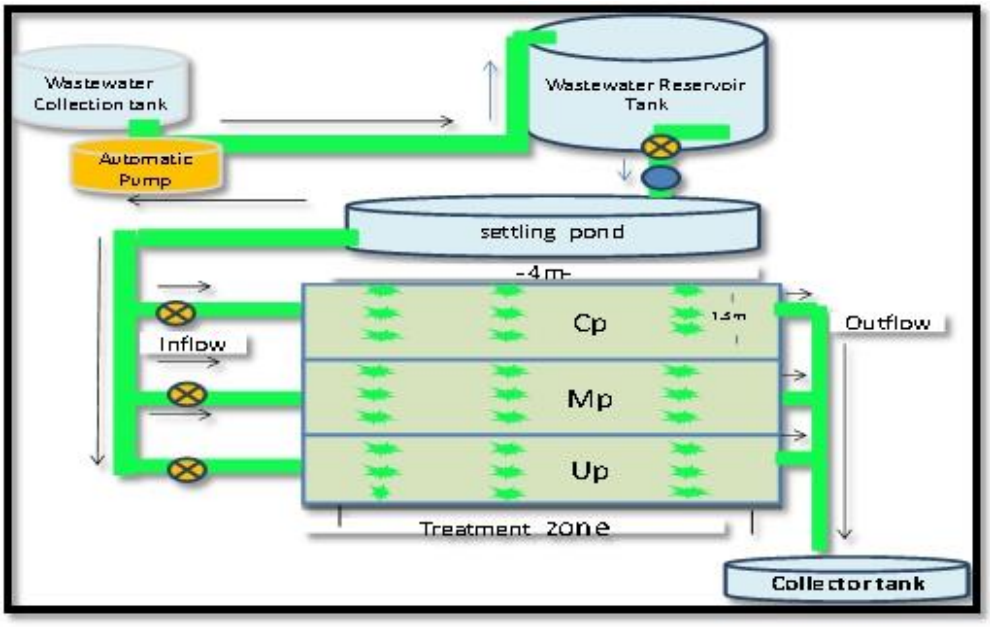

Fig. 1. Schematic view of CW units, flow control valves, electromagnetic flow meter, - Sampling site, ⟶ Flow direction.

The treatment zone of each CW unit was filled with gravels of a particle size between 2–4 mm. One of the CW units was single planted (Cp) with *Cyperus papyrus*, the second was mixed planted (Mp) with equal proportion

of *Cyperus papyrus*, *Pennisetum purpureum* and *Typha latifola* and the remaining was left unplanted (Up) and used as a control unit (Fig. 1). Each of the unit was receiving pre-polished wastewater in settling pond system and operating at 4 days of retention time through the experimental period.

Water sample collection and analysis

Inflow and outflow water samples (1 L) were collected using clean polyethylene bottles from each CW unit. Sediments samples (1 g) (fine gravels and accumulated organic matter) were also collected from the treatment zones of each CW unit using sterile 50 ml plastic tubes. The sediment was taken at a depth of 25–35 cm, from a distance of 1, 2 and 3 m intervals (in longitudinal direction of the 4 m length of CW) and at a distance of 0.3, 0.6, and 0.9 m (crosswise direction of the 1.3 m wide CW) (Fig. 1). Thereafter, equal proportion of the 9 sub-samples were mixed and homogenized in order to generate one composite representative sample. Collected water and sediment samples were kept in a cooler and transported to laboratory for analysis.

The water samples were analyzed for levels of biological oxygen demand after 5 days (BOD_5) and chemical oxygen demand (COD), ammonium (NH_4^+), nitrate (NO_3^-) and total phosphorus (TP) following standard methods (APHA, 2012). Then, percentage removal efficiency of these parameters was determined as follows:

$$R\% = \frac{(Ci - Co)100}{Ci}$$

where R% is pollutants removal percentage, C is the concentration of pollutants in mg/L, subscript *i* and *o* represent inflow and outflow samples, respectively.

The sediment samples were used for bacteriological analysis, Thus, total genomic DNA of each sediment sample was extracted using Power soil DNA extraction kit (Mo bio Laboratories) following the manufacturer's instructions at IGB, Germany. Three replicate DNA sample extracts of individual sediment samples were carried out and pooled together in equal proportions in order to obtain representative samples. The DNA was stored at –20 °C for subsequent Illumina Amplicon HiSeq sequencing using primers 27F and 519R (Lane, 1991). Library construction and sequencing were performed at MrDNA Molecular Research Shallowater, Texas.

Data analysis

Statistical analyses were carried out using PAST software (Hammer *et al*., 2001). Richness estimators (number of OTUs and Chao I) and diversity indices (Shannon-Wiener - H) were used for computing richness and diversity of the bacterial communities. Non-metric multidimensional scaling (NMDS) based on the Bray-Curtis similarity matrices and Simpson's dendrogram were used to represent the positions of the individual samples of the three differently planted CW units.

Principal component (PC) biplot analysis was performed to identify the bacterial taxa mainly responsible for dissimilarity between sediment bacterial assemblages in the three CW units. Pearson's correlation analysis was used to determine the links between sediment bacterial community composition and pollutant removal efficiency of the respective CW units. Data were analyzed by one-way ANOVA to detect the statistical significance ($p \leq 0.05$) of differences between mean values of parameters.

RESULTS AND DISCUSSION

Performance of wetland systems

In most cases, the removal findings >40% in both planted and unplanted CW units for BOD_5, COD, NH_4^+, NO_3^- and TP might indicate involvement of plant independent removal processes such as media adsorption and microbial degradation (Table 1). However, enhanced removal ($p<0.05$) in planted (Cp and Mp) CW units compared to the unplanted (Up) except for NH_4^+ and NO_3^- could suggest substantial contribution of the plant species on performance of the CWs. This is consistent with the findings of previous study (Zhang *et al*., 2016) which showed higher overall removal for water quality parameters (COD, NH_4^+, NO_3^- and PO_4^{3-} by 6.4%, 15.5 %, 30.6 %, and 12.2%, respectively) from wastewater in *Typha* planted HSSFCWs compared to the unplanted control. These findings also support the result obtained from mass balance analysis and revealing as plant up-taking to account for 10–15% of the nutrients removal in CWs (Zhu *et al*., 2017).

Moreover, highest removals obtained in the mixed (Mp) planted CW unit might be functional complementarity among *Typha latifola*, *Cyperus papyrus* and *Pennisetum purpureum* in this experiment. It could be attributed to the temporal and spatial compensations in plant activity as well a root affinity for different microorganisms (Zhu *et al*., 2017). In general, the results demonstrate that plant presence and plant combination in CWs are key factors influencing removal of organic matter and nutrients from

floriculture wastewater. Accordingly, the combination of the selected species of *Typha*, *Cyperus* and *Pennisetum* (Mp) in HSSFCWs could offer a good approach for improved wastewater treatment in floriculture industry as observed from removal of organic matter and nutrients in this study.

Table 1. Removal efficiencies of BOD_5, COD, NH_4^+, NO_{3-} and TP in the CW units, n=3.

Parameters	**Removal % in**		
	Cp	**Mp**	**Up**
BOD_5	75.9 ± 3.0a	77.8 ± 1.9a	69.2 ± 3.0b
COD	72.5 ± 3.6°	77.3 ± 0.9a	64.1 ± 0.7b
NH_4^+	92.3 ± 1.2°	99.0 ± 1.0a	97.0 ± 4.4a
NO_{3-}	64.5 ± 4.5b	82.7 ± 1.2a	65.8 ± 5.8b
TP	57.0 ± 8.5°	60.3 ± 9.5a	40.7 ± 4.0b

There is a significant ($p \leq 0.05$) difference between a vs. b among the CW units

Bacterial taxonomic richness and diversity

In this study, the OTU and Chao1 data indicated higher species richness in planted CW units i.e., in Cp (single planting) and Mp (mixed planting) compared to unplanted units suggesting that the plant rhizosphere offered supporting habitats and niches for the microbes (Table 2). The result was comparable to species richness of higher OTUs of 2232 and Chao of 2564 detected in *Cyprus* planted SSFCW units treating a secondary effluent compared to OTUs of 1941 and Chao of 2380 recorded from unplanted control units in Yan *et al*. (2017).

The Shannon diversity index (H) was relatively higher in planted CW units (4.93, 5.22) than unplanted ones (4.7) (Table 2) which was comparable to the one recorded (4.9–5.3) from a constructed wetland treating tannery wastewater (Adey Feleke Desta *et al*., 2014). The result was much higher than the expected high Shannon (H) index of 2.0 recorded from CWs and other aquatic systems (Garrido *et al*., 2014) could suggest better establishment of the bacterial community regardless of inhibitory properties of residual agrochemicals in the wastewater.

Table 2. Bacterial communities' richness and diversity indices.

CW units	**Read**	**OTU**	**Chao1**	**Evenness**	**Shannon(H)**
Single (Cp)	99039	2793	3423	0.04	4.93
Mixed (Mp)	63982	2554	3106	0.07	5.22
Unplanted (Up)	76392	2228	2718	0.06	4.72

Bacterial community composition

The diversity of the bacterial communities existing at various CW units was catalogued at the phylum, class and genus levels. The relative sequence abundance of the bacterial communities at a phylum level is shown in Fig.

2. A total of 28 bacterial phyla were identified and number of phyla detected from Cp, Mp and Up were 26, 28 and 22 phyla, respectively. The relative higher abundance in plants could be the effects of plants, where plants roots provide exudates for microbial growth as well as surface area for attachment of diverse microbes (Yan *et al*., 2017; Ma *et al*., 2018). The number of phyla obtained in this study was slightly lower than the number of phyla (31) reported from a constructed wetland planted with *Phragmites australis* (Cav.) for treating tannery wastewater (Adey Feleke Desta *et al*., 2014).

The dominant phyla were Bacteroidetes, Firmicutes and Proteobacteria contributing to 67.2%–86.7% of the total phyla recorded (Fig. 3a). The phyla Proteobacteria and Bacteridoites were more dominant in the unplanted CW unit than the planted units; whereas the phylum Firmicutes was much higher in the CW planted with Mp (32.1%) and Cp unit (40.9%), compared to the unplanted CW units (4.0%). Therefore, it implies a remarkable difference in relative abundance of the dominant phyla amongst the experimental CW units (Fig. 3a).

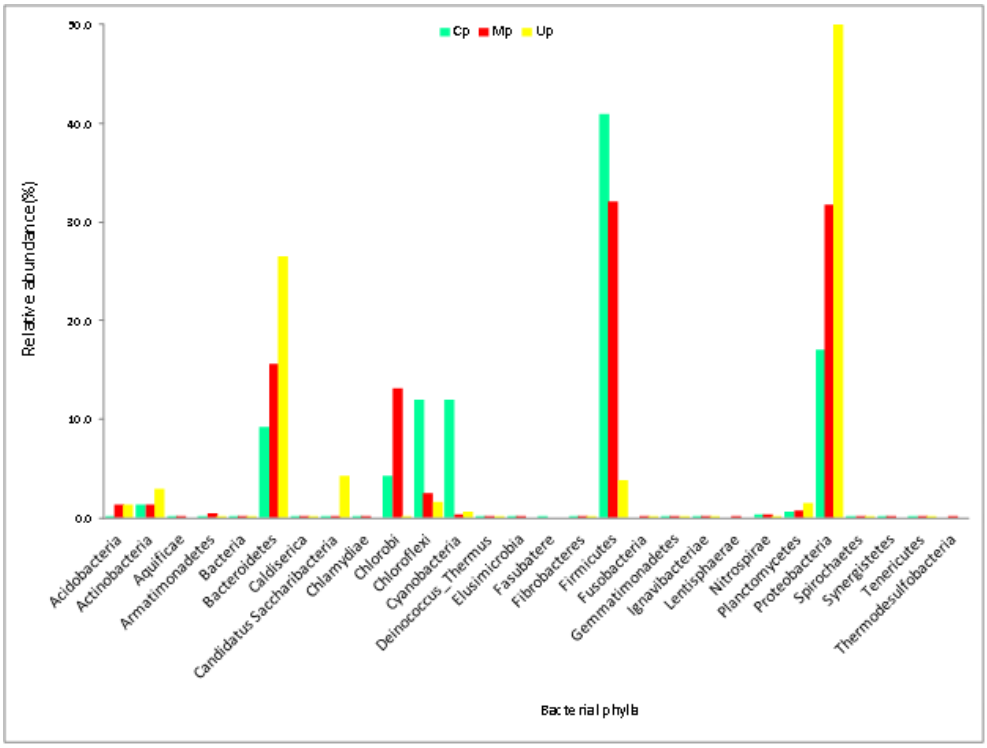

Fig. 2. Bacterial community composition at the phylum level in the three CWs units.

Although the number of phyla (28 phyla) recorded in this study was closer to the number of phyla (31 phyla) recorded from a CWs treating tannery wastewater in the nearby area (Adey Feleke Desta *et al*., 2014), the overall distribution pattern of the dominant bacterial phyla was different. They reported that the Firmicutes contained 40% of all the sequences analyzed, followed by Proteobacteria and Bacteroidetes, representing 30% and 15% of the sequences, respectively. The difference between their and our studies might be the variation of operational factors of CWs (e.g., design, type of plant species), wastewater characteristics and sequencing methods employed within the bio-informatics pipeline (Deng *et al*., 2014).

The bacterial profile also corresponded to a number of 50, 50 and 46 classes in Cp, Mp and Up units, respectively (Fig. 3b) which was slightly, but not significantly different from the 52, 42 and 50 classes observed from three different types of HSSFCWs treating secondary sewage effluent (Wang *et al*., 2016). The most dominant classes were Flavobacteria (Bacteroidetes), Alpha-proteobacteria and Gamma-proteobacteria (Protobacteria) Clostridia and Bacilli (Firmicutes) and Chlorobia (Chlorobi) of which Flavobacteria existed in Cp (37.4%) and Clostridia in Mp (20.9%), respectively. The result was considerably different from Adey Feleke Desta *et al*. (2014) who reported the class Bacteroidea and Beta-proteobacteria were the second most dominant group in the constructed wetland sites.

Likewise, a total of 544, 503 and 456 bacterial genera were identified in the Cp, Mp and Up units, respectively, of which 37 genera were the most frequently detected ones with a relative abundance of greater than 0.01 (Table 3). This result was nearly 2 times higher than the number of bacterial genera identified from sediment samples of *Typha* planted HSSFCWs receiving caffeine-enriched wastewater (Zhang *et al*., 2016). Wang *et al*. (2016) showed that this difference is attributed to the type and load of wastewater and type of methods used to estimate diversity.

The genus *Thermomonas* in the class Gamma-proteobacteria (14.2%) and *Flavobacterium* from the class Flavobacteriia (13%) were dominant in the Up sample; whereas the genera *Chlorobium* of the class Chlorobia (13.5%) and *Oscillatoria* (Cyanobacteria) (11.5%) were abundant in Mp and Cp units. In addition, the genus *Trichococcus* in the class Bacilli was abundant in both planted units; CP (18%) and MP (9.0%). All taken together, the bacterial composition analysis showed that the three CW units did not share 28.6% of the phyla, 12.0% of the classes and 32.0% of the genera.

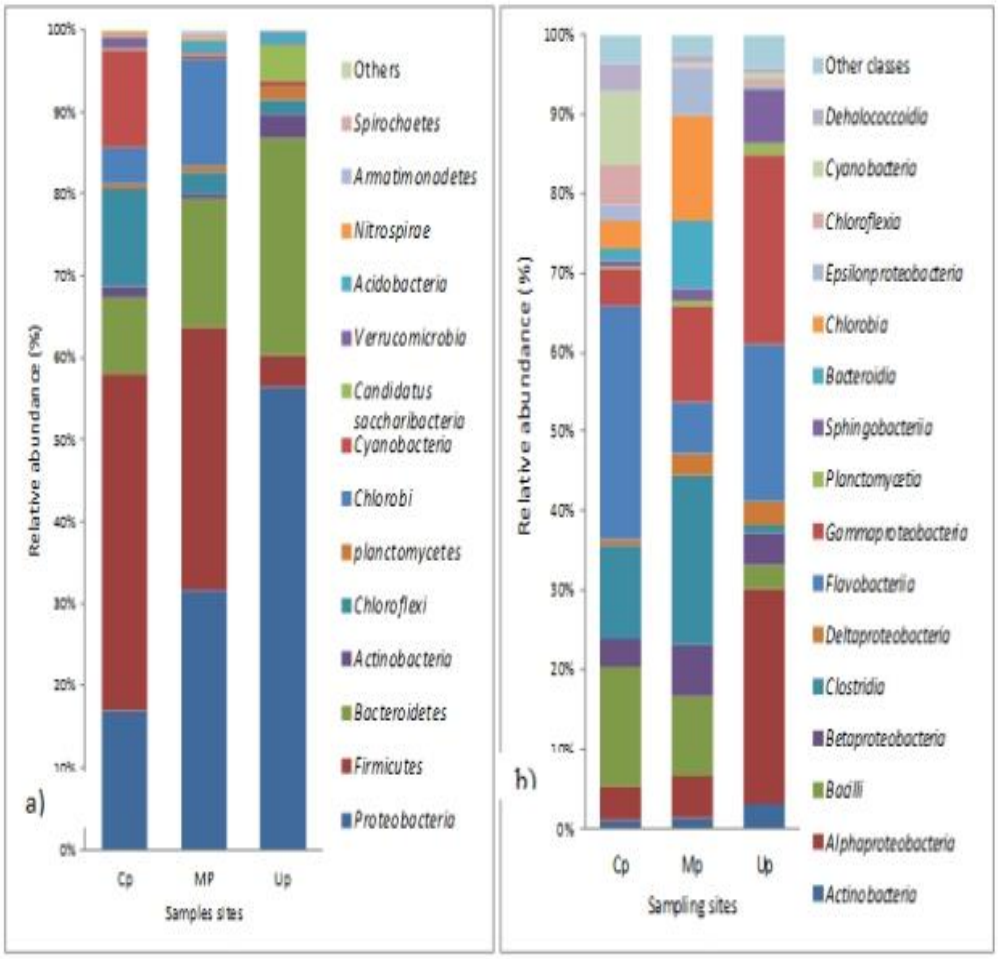

Fig. 3. Comparison of quantitative contribution of sequences affiliated with different phyla (a) and classes (b) from total sequences in samples of the CW units, 'Others'' indicate all rare taxa combined.

Table 3. Relative abundance (RA) of the bacterial general at a cut off >0.01.

	Bacterial taxa			RA in CW units		
	Class	**Family**	**Genus**	**Cp**	**Mp**	**Up**
1	Alphaproteobacteria	Erythrobacteraceae	*Erythromicrobium*	0.309	0.019	0.976
2	Alphaproteobacteria	Sphingomonadaceae	*Novosphingobium*	0.282	0.272	1.782
3	Alphaproteobacteria	Rhizobiaceae	*Rhizobium*	0.222	0.321	3.045
4	Alphaproteobacteria	Rhodobacteraceae	*Rhodobacter*	0.399	0.298	1.681
5	Alphaproteobacteria	Acetobacteraceae	*Saccharibacter*	0.182	0.469	2.815
6	Alphaproteobacteria	Sphingomonadaceae	*Sphingomonas*	0.724	0.623	2.841
7	Alphaproteobacteria	Sphingomonadaceae	*Sphingopyxis*	0.077	0.199	3.406
8	Bacilli	Bacillales	*Exiguobacterium*	0.687	0.473	1.364
9	Bacilli	Carnobacteriaceae	*Trichococcus*	18.065	9.345	0.092
10	Bacteroidia	Bacteroidaceae	*Bacteroides*	1.173	2.018	0.037
11	Bacteroidia	Porphyromonadaceae	*Macellibacteroides*	1.446	2.430	0.012
12	Bacteroidia	Porphyromonadaceae	*Paludibacter*	0.938	2.826	0.037
13	Betaproteobacteria	Rhodocyclaceae	*Rhodocyclus*	0.646	2.106	0.020
14	Candidatus saccharibacteria	Candidatus saccharibacteria	*Candidatus saccharimonas*	0.093	0.159	4.289
15	Chlorobia	Chlorobiaceae	*Chlorobium*	4.248	13.470	0.046
16	Chloroflexia	Chloroflexaceae	*Chloroflexus*	6.085	0.352	0.587
17	Clostridia	Eubacteriaceae	*Acetobacterium*	1.344	1.522	0.041
18	Clostridia	Clostridiales Family Xiii. IncertaeSedis	*Anaerovorax*	3.016	4.749	0.029
19	Clostridia	Clostridiaceae	*Clostridium*	6.570	7.576	0.325
20	Clostridia	Clostridiales Family Xii. IncertaeSedis	*Fusibacter*	0.073	3.126	0.135
21	Clostridia	Ruminococcaceae	*Ruminococcus*	0.840	1.021	0.009
22	Cyanobacteria	Oscillatoriales	*Oscillatoria*	11.541	0.109	0.038
23	Dehalococcoidia	Dehalococcoidaceae	*Dehalococcoides*	4.414	0.877	0.041
24	Deltaproteobacteria	Desulfovibrionaceae	*Desulfocurvus*	0.009	0.019	1.820
25	Deltaproteobacteria	Geobacteraceae	*Geobacter*	0.319	2.010	0.043
26	Epsilonproteobacteria	Campylobacteraceae	*Sulfurospirillum*	0.408	1.119	0.039
27	Epsilonproteobacteria	Helicobacteraceae	*Wolinella*	2.087	1.195	0.017
28	Erysipelotrichia	Erysipelotrichaceae	*Erysipelothrix*	6.301	0.793	0.018
29	Flavobacteriia	Flavobacteriaceae	*Cloacibacterium*	2.132	4.643	13.068
30	Flavobacteriia	Flavobacteriaceae	*Flavobacterium*	1.611	1.253	4.626
31	Gammaproteobacteria	Moraxellaceae	*Acinetobacter*	0.475	7.065	2.351
32	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas*	0.078	0.475	1.829
33	Gammaproteobacteria	Xanthomonadaceae	*Pseudoxanthomonas*	0.046	1.414	3.556
34	Gammaproteobacteria	Xanthomonadaceae	*Thermomonas*	1.150	0.653	14.164
35	Gammaproteobacteria	Chromatiaceae	*Thiocapsa*	2.062	0.199	0.050
36	Sphingobacteriia	Chitinophagaceae	*Flavihumibacter*	0.215	0.563	2.913
37	Sphingobacteriia	Chitinophagaceae	*Parasegetibacter*	0.016	0.015	1.776

Similarity of bacterial composition

Based on Non-metric Multi-Dimensional Scaling (NMDS) of OTU data, the two planted CWs were plotted together in the same ordination plot (Fig. 4a). The same ordination plot indicate the presence of highly similar group of bacterial communities in the in the two planted CWS. In addition,

hierarchical clustering of OTUs indicated that there was a remarkable difference (>45%) in the community of bacteria between the planted vs. unplanted CW units while the differences between planted CWs were less than 30% (Fig. 4b). This showed that the presence of plants in CWs has a strong influence on bacterial composition. Zhang *et al*. (2016) showed a 60% distinct clustering difference in bacterial composition between planted CWs and unplanted units. The dissimilarities between CWs is mainly associated with rhizosphere effects, where plant roots modify their surrounding environment by releasing a variety of root exudates and providing surface area for attachment (Yan *et al*., 2017; Ma *et al*., 2018).

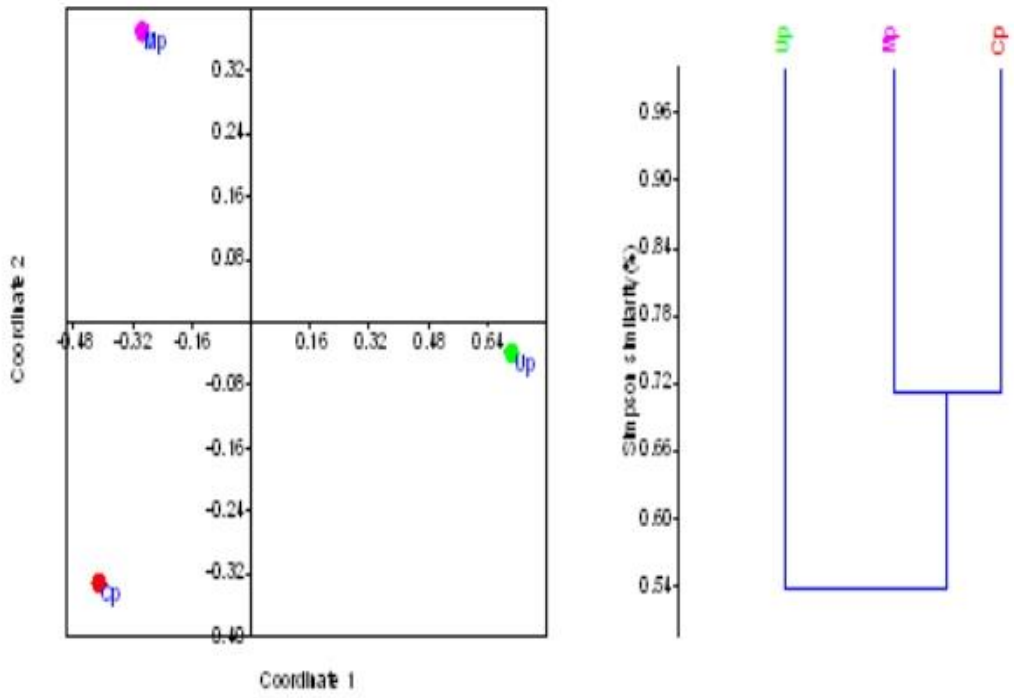

Fig. 4. NMDS (a) and Cluster (b) analysis of the relative abundance of bacterial OTU.

Moreover, the biplot principal component analysis (PCA) of the OTU data illustrated the degree of the bacterial richness towards planted CW units (Fig. 5). PC1 showed 74.8% variation between the planted CW units (Cp and Mp) and unplanted units (Up). The PC1 variation obtained in this study was higher than the PC1 values (59.4%) obtained between rhizosphere and sediment bacterial community structure in pilot-scale CWs (Ma *et al*., 2018). This implied that the sediment collected from unplanted CW have higher dispersion than the planted CW. Zhang *et al*. (2015) also showed

strong correlation of sediment microbial community with plant diversity.

The PCA identified seven taxa (OTU-9, OTU-10, OTU-11, OTU-12, OTU-22, OTU-37 and OTU-278) as important factors in relation to the ordination of the three CW units (Fig. 5). This showed a major difference of bacterial community assemblage between planted (CP and Mp) and unplanted (Up) CW units. The taxa with high positive (OTU-10, OTU-11, OTU-12, OTU-22 and OTU-37) PCI loading were more abundant in planted CW units; whereas taxa with high negative PCI loading (OTU-9 and OTU-278) were more abundant in unplanted CW units (Fig. 5).

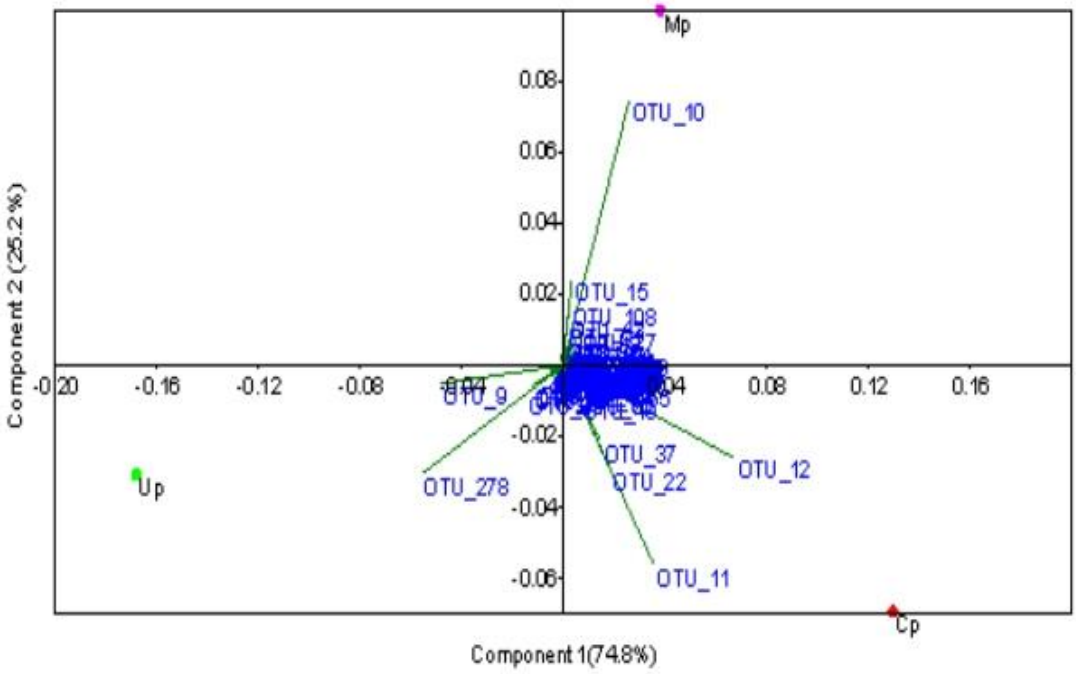

Fig. 5. Principal component (PC) analysis of the OTU abundance between CW units.

Taxa with positive loadings on PC1 (OTU-10, OTU-11, OTU-12, OTU-22 and OTU-37) were belonging to *Chlorobium* (Chlorobi), *Oscillatoria* (Cyanobacteria), *Trichococcus* (Firmicutes), *Chloroflexus* (Chloroflexi) and *Erysipelothrix* (Firmicutes), respectively. Taxa with negative loading on PC1 (OTU-9 and OTU-278) were belonged to the genera *Cloacibacterium* (Bacteroidetes) and *Thermomonas* (Proteobacteria), respectively. Overall, this PCA result based on individual OTUs provided insight on the variation of bacterial richness and diversity in the CW units, which is predictive for removal performance of the CW units (Zhang *et al*., 2015).

Bacterial taxa and pollutant removal efficiency

In general, bacterial community richness (OTUs and Chao1) as well as diversity (Shannon-H) index indicated a positive correlation with mean removal efficiencies of BOD_5, COD, NH_4^+, NO_{3-} and TP (Table 4). These results were similar to the study of Zhang *et al*. (2015) where the microbial biomass carbon (MBC) and the Shannon diversity index (H) were positively correlated with the removal of BOD_5 (r = 0.74; r = 0.92), COD (r = 0.65; r = 0.41), NH_4-N (r = 0.71; r = 0.83) and NO_3-N (r = 0.71; r = 0.80) in different planted CWs.

Oopkaup *et al*. (2016) also showed strong positive correlations between species richness (number of OTUs) and removal efficiency of NH_4^+ (r = 0.61) and NO_{3-} (r = 0.83) as well as between bacterial diversity and removal efficiency of NH_4^+ (r = 0.82), NO_{3-} (r = 0.52) and BOD_5 (r = 0.65) in HSSFCW mecosomes treating municipal wastewater. The positive correlation between removal efficiency and bacterial diversity often considered as predictor of removal efficiencies that are mainly driven by bacterial processes (Button *et al*., 2015).

In this study, majority of the dominant bacterial taxa also showed positive correlation ($r>0.50$) with removal of BOD_5, COD, NH_4^+, NO_{3-} and TP. Specifically, relative abundance of Bacteroidetes and Firmicutes showed strong positive correlation ($r\geq0.97$) with the removal efficiencies of nutrients (Table 4) which related to degradation of organic matter and nutrients in different treatment plants (Zhang *et al*., 2015). The positive relation between the phyla Chloroflexi and Cyanobacteria with organic matter and nutrients removal (Table 4) also reinforced the results obtained through the use of these bacteria for bioremediation contaminants from wastewaters in Dubey *et al*. (2011) and Wang *et al*. (2016).

Table 4. Pearson's correlation analysis of bacterial community variables and pollutant removal efficiency of the CW units.

Taxa	**BOD_5**	**COD**	**NH_4+**	**NO_{3-}**	**TP**
Number of OTU	0.870	0.810	0.640	0.830	0.850
Shannon (H)	0.856	0.909	0.985	0.893	0.880
Bacteroidetes	0.938	0.971	0.996*	0.961	0.953
Firmicutes	0.954	0.915	0.782	0.929	0.939
Chloroflexi	0.500	0.400	0.155	0.433	0.458
Cyanobacteria	0.409	0.304	0.053	0.338	0.364
Bacilli	0.890	0.830	0.660	0.850	0.870
Chlorobia	0.796	0.859	0.961	0.840	0.824
Clostridia	0.910	0.950	0.940	0.940	0.930
Flavobacteria	0.025	-0.087	-0.337	-0.051	-0.023
Gammaproteobacteria	-0.914	-0.863	-0.707	-0.881	-0.894
Chlorobium	0.790	0.850	0.960	0.830	0.820
Chloroflexus	0.736	0.807	0.971	0.785	0.767
Erysipelothrix	0.641	0.712	0.875	0.690	0.672
Oscillatoria	0.710	0.781	0.944	0.758	0.741
Trichococcus	0.369	0.440	0.603	0.417	0.400

Notes: * $p<0.05$, ** $p<0.01$

The correlation analysis for the dominant class also showed a positive relation ($r\geq0.65$) between relative abundance of *Bacilli*, *Chlorobia* and *Clostridia* and the removal efficiency of the CW system (Table 5). Other studies revealed the association of these bacterial genera with the removal efficiency of CW integrated with other wastewater treatment components (Adey Feleke Desta *et al*., 2014; Zhao *et al*., 2017).

Moreover, the majority of the important bacterial genera in relation to ordination of the CW units including *Chlorobium*, *Chloroflexus*, *Oscillatoria*, *Trichoccus* and *Erysipelothrix* had a positive correlation with the removal efficiency of the CW (Table 4) that implies relative abundance was closely linked to mineralization of organic matters in the CW units. The strong correlation of Chlorobium with removal of NH_4^+ ($r = 0.96$), was comparable to the results in Edberg *et al*. (2012) and gave an insight of the role of this genus in the transformation of NH_4^+ in the CW units. Vijayakumar (2012) and Dai *et al*. (2018) also showed the strong correlation of *Oscillatoria* and *Trichoccus* with the removal efficiency of the nutrients from biological treatment systems and suggested the potential application of these groups in the bio-augmentation and bioremediation of wastewater in biological treatment systems.

CONCLUSION

In this study, 16S rDNA Illumina Miseq sequencing analysis revealed the bacterial community diversity of HSSFCW treating floriculture wastewater planted with different macrophyte species. The wetland plants maintained higher bacterial community composition compared to unplanted units. It was shown that species richness, diversity as well as relative abundance of some bacterial groups have a positive influence on removal efficiency of organic matter and nutrients from the CW units. This research provides information on bacterial communities among HSSFCWs established with different planting, suggesting the importance of multiple planting of HSSFCW to best support a diverse group of wetland microbial communities for better remediation of pollutants.

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