Microbial Communities and Nitrogen Transformation in Constructed Wetlands Treating Stormwater Runoff

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Microbial communities play a vital role in nitrogen (N) removal in constructed wetlands (CWs). However, the lack of studies on microbial characteristics of wetland systems designed to treat stormwater demonstrates the importance of comprehensive investigation on microbial response to wetland fluctuations. Moreover, the observed inconsistency in N removal, and detected links between microbial shifts and wetland water level fluctuations is an area of research interest perculiar to stormwater applications. This study surveyed nearly 150 publications to provide a summary and evaluation of N removal efficiency in different types of CWs where microbial communities and their behavior have been correlated to regulating factors. Factors such as flow regime, plants, and physico-chemical properties (e.g., temperature, dissolved oxygen, pH, and nitrogen concentration) were found to significantly influence microbial diversity and composition. Although many studies have analyzed microbial N removal, a majority conducted their studies in bioretention systems. Accordingly, some of the microbial pathways in CWs designed for stormwater treatment have not been investigated. As such, it is suggested that pathways, such as dissimilatory nitrate reduction to ammonium (DNRA) and comammox activity and their changes over dry-wet cycles in stormwater constructed wetlands be investigated. This information could assist engineers to take advantage of the presence of other N transforming communities which could improve microbial diversity within wetland systems. Moreover, it is recommended to track microbial functional genes and their changes over wetland water fluctuation to develop an ecosystem with conditions favorable for microbial pathways with higher N removal potential. In conclusion, the findings of the current literature review reinforce the importance of stormwater runoff treatment and the implementation of new design strategies that are able to enhance microbial activity and diversity leading to a better treatment outcome.

Keywords: constructed wetlands, stormwater, microbial communities, nitrogen removal, regulating factors

INTRODUCTION

Excessive urbanization has led to increases in the volume and rate of stormwater runoff due to the increase in imperviousness (Janke et al., 2017; Ma et al., 2021). Moreover, excessive levels of nutrients contained in stormwater have caused hypoxia, eutrophication (Hobbie et al., 2017), and algal blooms in water bodies (Glibert and Burford, 2017; Paerl et al., 2018). Urban water

management approaches have therefore been designed for the harvesting and reusing stormwater via environmentally sustainable approaches (Prüss, 1998; Johnson et al., 2003; Hörman et al., 2004; Noble et al., 2006; USEPA, 2010; Converse et al., 2011). Thus, constructed stormwater wetlands as a green and cost-effective approach have been widely used to eliminate stormwater pollutants to safely discharge treated urban runoff into natural water bodies (Malaviya and Singh, 2012; Tippler et al., 2012; Fu et al., 2016; Hu et al., 2016).

Constructed wetlands time have the potential to reduce peak flow rates and eliminate stormwater pollutants (Converse et al., 2011; Garfí et al., 2012; Sani et al., 2013; Vymazal, 2014; Wu et al., 2015; Fu et al., 2016; Hu et al., 2016; Huang et al., 2018). The system mitigates the effects of added nitrogen via physical, chemical, and biological processes including filtration, sedimentation, volatilization, plant uptake, and microbial mediated mechanisms. While some of the CWs have been shown to exhibit high N removal rates, there is a wide variation in removal rates (**Table 1**). It has been demonstrated that N removal mechanisms are influenced by factors such as type of CW, wetland vegetation, wetland hydrology, physico-chemical and wetland biological properties (Malaviya and Singh, 2012; Tippler et al., 2012; Fu et al., 2016; Hu et al., 2016; Griffiths and Mitsch, 2020; Zhao and Piccone, 2020; Wei et al., 2021).

Microbial pathways play a primary role in N removal, and their diversity and community composition reflect the proper functioning and maintenance of CWs. Consequently, the inconsistency in N removal rates could be correlated to the variation in microbial community and their response to the wetland design factors and operational condition. Thus, a review of the literature was undertaken with the aim of identifying microbial communities present and their role in the treatment processes in CWs designed to capture stormwater. The objectives of this study were: (i) Provide a literature review where microbiological studies have been carried out for constructed wetlands, (ii) Understand the function of microbial communities and their role in the treatment process, and (iii) Identify research gaps and provide recommendations for future studies. The overall result of this review will assist water managers to improve urban water management to meet water quality standards.

TREATMENT OF STORMWATER RUNOFF

Previous studies have analyzed microbial pathway of N removal, communities present, and their response to wetland hydrology, hydraulic loading rates, nutrient concentrations, physico-chemical parameters, retention time, intermittent aeration, and plant biomass using advanced molecular techniques. However, a large portion of the literature deals with the treatment of domestic and industrial wastewater, while the current study focuses mainly on CWs that are designed to treat stormwater. The review therefore has been expanded to include these systems, highlighting features that are peculiar to stormwater as it is unique in many respects compared to other influents (Lucke et al., 2018; Wang et al., 2022). The total nitrogen concentration in stormwater is usually 10 times lower than in domestic

wastewater; this difference will be higher when compared against industrial wastewater and agricultural runoff as they are often linked to the specific land use of the catchment (Lucke et al., 2018). Another feature of stormwater that is unique is the intermittent nature since inflows occur only after rain events. A summary of studies investigating microbial communities responsible for N transformation in surface flow, subsurface flow, and vertical flow CWs is listed in **Table 1**.

The reported N removal rates for various types of CWs in the literature indicate the variation in wetland N removal efficiency. The reason(s) behind the inconsistency could be linked to the variations in microbial communities, suggesting that CWs are not in optimal performance, but also differences in application, which makes comparison difficult. As shown in **Table 1**, individual studies have focussed on different types of wetland systems and different microbial communities. The variations of microbial N removal are also linked to the types and quality of inflow source including raw or treated domestic wastewater, industrial effluent, and urban runoff.

A majority of studies on N transforming microbial community in CWs have been carried out for domestic (Bastviken et al., 2003; Criado and Bécares, 2005; Shipin et al., 2005; Truu et al., 2005; Dong and Sun, 2007; Sawaittayothin and Polprasert, 2007; Tietz et al., 2007; Wu et al., 2013; Fan et al., 2016; Li et al., 2020) or synthetic wastewater (Fan et al., 2013b; He et al., 2018; Liu et al., 2018; Sun et al., 2018; Xia et al., 2020). There are limited studies focussing on microbial community in CWs treating stormwater and the influence of wetland design features on N transforming microbes (Kyambadde et al., 2006; Domingos et al., 2011; Bledsoe et al., 2020). For example, Bledsoe et al. (2020) emphasized the importance of microbial controls on biogeochemical processes in stormwater constructed wetlands in order to improve water quality. They found that reduction of flooded areas and increasing shallow land area in a stormwater constructed wetland can enhance N removal while reducing greenhouse gas emissions. Changes in water chemistry were also examined to assess nutrient transformations following rewetting and recovery of microbial communities in CWs treating stormwater (Macek et al., 2020). Wang et al. (2022) have investigated N composition in stormwater runoff samples by studying the chemical properties before and after storm events in order to improve N pollution management. Another study conducted by Walaszek et al. (2018) considered the impact of dry/wet weather on variations in hydrologic conditions, physico-chemical parameters, and heavy metals loads.

Microbial diversity and its effect on N removal have been investigated in different types of treatment systems including bioretention cells (Zuo et al., 2020; Biswal et al., 2021), stormwater detention basin (Morse et al., 2017). Other studies such as Sun et al. (2018) focused on microbial functional genes considering denitrifying bacteria, ammonium oxidizing bacteria (AOB), and their distribution to analyse N removal efficiency in a vertical flow CW. Bledsoe et al. (2020) studied denitrification rates and microbial community structure within permanently flooded and temporarily flooded areas of a surface flow constructed wetland treating stormwater runoff to examine N removal potential. A recently discovered microbial pathway,

TABLE 1 | Summary of studies investigating microbial communities responsible for N transformation in different types of CWs.

Wetland type	Microbial community	Focus of microbial analysis	Study objective	Scope of study	Nitrogen compound	Inflow source	Nitrogen removal (%)	References
SFCW	Sediment microbes (bacterial and archaeal)	Microbial community diversity, structure, and composition	Soil chemistry and greenhouse gas fluxes	Wetland hydrology, permanent pool, and shallow zones	TN NH$_4^+$ NO$_3^-$	Stormwater runoff	Denitrification rates: 24.45–20.29 ng N$_2$O-N hr^{-1} g^{-1} dry mass.	Bledsoe et al., 2020
Experimental VFCW	AOB community (amoA genes)	AOB community structure and phylogenetic tree	Nitrogen removal performance	Hydraulic loading rates and nutrient loading loads.	NH$_3$-N NO$_3$-N	Stormwater runoff and cooling tower blowdown	NH$_3$-N removal: 1.2 g/m^2/d	Domingos et al., 2011
SFCW	Autotrophic AOB	Microbial population, densities, and distribution	Spatial distribution and activity of AOB	Physico-chemical and biochemical water quality variables	TN NH$_4^+$ NO$_2^-$ NO$_3^-$	Stormwater runoff, raw and secondary domestic and industrial wastewater	TN removal: 13.5–71.2 mg/L	Kyambadde et al., 2006
SFCW	Denitrifying and nitrifying bacteria	Microbial community profiles, structures, abundance, diversity	Efficiency of the multi-stage SFCW	Temporal variation	TN NH$_4^+$ NO$_3^-$	Domestic wastewater	NH$_4^+$ removal: 91.35%	Li et al., 2020
VFCW	Gram-positive and Gram-negative bacteria	Microbial community structure and enzyme activities	Nitrate reductase activities and substrate enzyme activities	Retention time and treatment efficiency	TN NH$_4^+$ NO$_3^-$ NO$_2^-$	Domestic wastewater	TN removal: %51.66 NH$_4^+$-N: %42.50	Wu et al., 2013
Experimental VFCWs	AOB and nitrite-oxidizing bacteria (NOB) NOB	Microbial abundance and community composition	NH$_4^+$ and TN removal	Intermittent aeration	TN NH$_4^+$	Domestic wastewater	NH$_4^+$-N removal: 96% TN: 85%	Fan et al., 2016
VFCW + SFCW	AOB and Anammox bacteria	Functional genes and biomass	Coexistence of partial-nitrification and Anammox in CWs with feasible design	Pollutant removal, wetland design enhancement	TN, Total Kjeldahl Nitrogen (TKN), NH$_4^+$	Domestic wastewater	Total ammonium removal: 2.76 g m^{-3} day^{-1} Nitrite: 0.178 g m^{-3} day^{-1} TN: 54.83%	Dong and Sun, 2007
SSFCW	Not mentioned	Microbial processes (aerobic and anaerobic processes in the vegetated bed)	Biochemical processes (nitrification, C, N, and P mineralization, methanogenesis)	Plant biomass, nutrient concentrations	NH$_4^+$ NO$_2^-$ NO$_3^-$ TN	Domestic wastewater	TN removal: 53% NH$_4$: 78% NO$_3$: 40%	Edwards et al., 2006

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TABLE 1 | Continued

Wetland type	Microbial community	Focus of microbial analysis	Study objective	Scope of study	Nitrogen compound	Inflow source	Nitrogen removal (%)	References
Experimental SFCW	Nitrifying bacteria and Anammox bacteria	Bacterial species	Nitrogen mass balance	Plant uptake	TN	Municipal landfill leachate	TN removal: 78-96%.	Sawaittayothin and Polprasert, 2007
VFCW	AOB	Diversity of amoA	Spatial distribution of AOB pattern (season) and diversity of AOB in VFCW	Wetland filter substrate	NH_4^+ NO_3^- NO_2^-	Municipal wastewater	NH_4-N removal: 88.8 %	Tietz et al., 2007
Experimental SFCW	Rhizosphere microbial communities including bacteria, saprotrophic and arbuscular mycorrhizal fungi (AMF)	Microbial functional analysis (Enzyme specific activities represent a variety of soil processes) and microbial community structure and biomass	Nitrification potential assays in soils (indicators of microbial function)	Wetland hydrology	NO_3^-	Urban runoff	Nitrification potential: 0.28–1.8 µg nitrate hour^{-1} g soil^{-1}	Mentzer et al., 2006
SFCW and SSFCW	Nitrifying bacteria	Microbial composition and function	Nitrification	Biofilms	TN	Domestic wastewater	TKN removal: 80.64% NH_4^+: 72.5%	Criado and Bécares, 2005
SSFCW	Ammonia-oxidizing microbes	Microbial diversity and distribution	Spatial distribution of microbial community	Wetland hydrology	Not mentioned	Domestic wastewater	NH_4-N: 54.65%	Truu et al., 2005
SFCW	Aerobic and anaerobic ammonia oxidizers (Anammox), denitrifiers	Microbial community structure, abundance, seasonally fluctuating presence of the Anammox bacteria	N-removing capacity of microbial consortia	Treatment performance	NO_3^- NH_4^+ Total dissolved nitrogen (TDN), Organic N	Domestic and light industrial wastewater	TKN removal: 86.0 % NH_4^+: 84.6%	Shipin et al., 2005
SFCW	Nitrifying and denitrifying bacteria	Potential nitrification and denitrification	Examine nitrifying and denitrifying capacity of different surfaces in CWs	Water chemistry, water flow and different surfaces (filamentous macro-algae, submersed macrophytes, etc.)	NO_3^-	Municipal wastewater	TN removal: 44.4%	Bastviken et al., 2003
Experimental VFCW+SSFCW	Nitrifiers and denitrifiers	Functional genes (nirK gene)	Variations with depth	Water quality treatment performance	TN NH_4^+	Industrial wastewater	NH_4^+-N removal by VF: 79.2 % TN removal by HF: 63.5 %	Xu et al., 2016

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TABLE 1 | Continued

Wetland type	Microbial community	Focus of microbial analysis	Study objective	Scope of study	Nitrogen compound	Inflow source	Nitrogen removal (%)	References
SFCW (Floodplain wetlands)	Denitrifier and archaeal ammonia oxidizer community	Distribution of microbes carrying nosZ and amoA	Soil moisture gradient	Moisture gradient and wetland soil	TN	Not mentioned	Not mentioned	Peralta et al., 2014
SFCW	*Nitrosospira* and *Nitrosovibrio*	Microbial community dynamics	Nitrogen transformation in sediment, microbial degradation of organic matter		TKN NH_4^+ NO_3^- NO_2^-	Treated effluent (secondary treatment)	TN: 65% TKN: 75% NH4+-N: 72%	Yeh et al., 2010
Experimental CWs	Polyphosphate accumulating bacteria (PAOs)	Microbial abundances distributions and function	Microbial condition, quantify PAOs	Wetland plants	TN NH_4^+ NO_2^- NO_3^-	Aquaculture wastewater	TN removal: 95%	Zhimiao et al., 2019
SFCW	Denitrifying bacteria and AOB community	Number and function of microbes and the relative presence of bacteria	N removal rates	Wetland design	NH_4^+ NO_2^- NO_3^-	Dairy farm wastewater	Ammonia-nitrogen: 99%, nitrate-nitrogen: 74%	Mustafa et al., 2009
SSFCW	Total bacterial community and AOB	Bacterial composition and function	Microbial function (amoA genes)	Soil and effluent	TN NO_3^-	Dairy wash water	TN: 25%	Ibekwe et al., 2003
Experimental SFCW	Bacterial community responsible for denitrification	Bacterial cell densities and benthic community structure	Environmental conditions and different denitrification potential (DNP) rates associated with bacterial community characteristics	Wetland hydrology	NO_3^-	River	Dissolved nitrate removal: 71.9–94%	Ishida et al., 2006
VFCW	Substrate microorganisms (bacteria, fungi and actinomyces)	Microbial abundance	Substrate microorganisms	Urease activities in the substrate	TKN	Lake water	TKN removal: 60-90%	Liang et al., 2003
SFCW	Microbial communities attached to plant shoots and in the water	Attached to plant shoots (periphyton) and in the water	Seasonal variation in denitrification rate in the periphyton associated with plant shoots and in sediments	Plants, sediments, and water	NO_3^-	Sewage treatment plant	NO3-N removal: 16.5–41.5%	Toet et al., 2003

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TABLE 1 | Continued

Wetland type	Microbial community	Focus of microbial analysis	Study objective	Scope of study	Nitrogen compound	Inflow source	Nitrogen removal (%)	References
SSFCW	Ammonia and denitrifying bacterial community	Functional gene nosZ and the corresponding composition of the bacterial community	Nitrification/denitrification activity	Depths in the wetland and sediment from a connected open pond	NH_4^+	Landfill leachate	Not mentioned	Sundberg et al., 2007b
SFCW	Nitrifying microorganisms	Bacterial characteristics associated with leachate, sequence data and Phylogenetic analysis	Molecular characterization of bacteria	N removal	NH_4^+	Landfill leachate	Not mentioned	Walsh et al., 2002
SFCW	*Nitrosomonas* spp.	Nitrosomonas presence in biofilm	Occurrence of effluent bacterial population of *Nitrosomonas europaea* and the biofilm populations	Physical and chemical parameters in the wetland	NH_4^+	Primary treated oxidation pond effluent	Ammonium removal: 20%	Silyn-Roberts and Lewis, 2001
SF wetlands (Natural and constructed wetlands)	Heterotrophic microbes	Microbial biomass and rates of denitrification	Relationships between process rates and soil properties.	wetland soils and regulators of microbial denitrification potentials in soils	TN	Not mentioned	Not mentioned	D'Angelo and Reddy, 1999
SFCW	Soil enzyme activities	Microbial activity	Electron transport system activity and soil enzyme activities	Sediment and hydrochemistry	NO_3^-	Field drainage tile water	Nitrate removal: 82–92.8% (Ohio wetlands) and 52% (Iowa wetland)	Kang et al., 1998
VFCW	Autotrophic and heterotrophic AOB, nitrite oxidizing bacteria, Anaerobic AOB	Bacterial function	Microbial community under different periods of drying and wetting	Wetland performance under unsaturated and saturated conditions	TIN NH_4^+ NO_3^- NO_2^-	Synthetic wastewater	Total inorganic nitrogen removal: 57.5–73.3% NH_4^+-N: 99.5%	Xia et al., 2020
Experimental VFCW	Denitrifying bacteria and AOB	Microbial diversity, abundance, and composition	Effects of C/N ratio on nitrification and denitrification rates	Hydraulic retention time (HRT), hydraulic loadings and nitrogen loadings	TN NH_4^+ NO_2^- NO_3^-	Synthetic wastewater	TN removal: 92.9% NH_4^+-N: 83.7% NO_3-N: 95.6%	He et al., 2018

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TABLE 1 | Continued

Wetland type	Microbial community	Focus of microbial analysis	Study objective	Scope of study	Nitrogen compound	Inflow source	Nitrogen removal (%)	References
Experimental SSFCW	Nitrifying bacteria (amoA and nxrA), denitrifying bacteria (narG, nirK, nirS, and nosZ), and Anammox bacteria	Abundance of nitrifying bacteria, and their functional genes	Simultaneous nitrification and denitrification process, N removal pathway	Limited-aeration and full-aeration phases and full-aeration phases and morphological characteristics of the substrate biofilm	TN NH_4^+ NO_3^- NO_2^-	Synthetic wastewater	TN removal: 72% (under limited-aeration) and 99% (under full-aeration phases)	Liu et al., 2018
VFCW	Denitrifying bacteria and AOB	Nitrogen functional genes, amoA, and denitrifying genes (nirS, nirK, nosZ)	Simultaneous nitrification and denitrification process, and distribution of functional genes	Nitrogen transformation along the flow direction	TN NH_4^+ NO_3^- NO_2^-	Synthetic wastewater	NH_4^+-N removal: 90.4% TN: 92.1%	Sun et al., 2018
SSFCW	Nitrifying bacteria (AOB and NOB)	Microbial population and activity of AOB and NOB	Nitrogen removal, anaerobic microbial denitrification	Anaerobic and aerobic conditions in SFCWs	TN NH_4^+ NO_3^- NO_2^-	Synthetic wastewater	NH_4^+-N removal: 95% TN: 80%	Fan et al., 2013b

SFCW, Surface Flow; SSFCW, Subsurface Flow; VFCW, Vertical Flow Constructed Wetland.

dissimilatory nitrate reduction to ammonium (DNRA) in CWs treating stormwater has also been proposed by Rahman et al. (2019a,b,c).

Regarding stormwater control measures, it has been found that there is insufficient studies focused on constructed wetlands to understand how the types of microbial community present and their behavior over different cycles of wet and dry conditions affect the N removal rates in these systems (Gold et al., 2019). The majority of studies investigate nitrogen removal from stormwater in systems such as bioretention cells (Chen et al., 2013; Norton et al., 2017; Waller et al., 2018; Biswal et al., 2021), wet ponds (Blaszczak et al., 2018; Gold et al., 2021), wet/dry detention basins (McPhillips and Walter, 2015; Morse et al., 2017) or filtration/infiltration basins (Bettez and Groffman, 2012) (**Table 1**). Stormwater control measures such as bioretention cells have been assessed for the impact of operational conditions, climatic conditions, wet-dry alternation, influent loads, N concentration, and hydraulic residence time on wetland microbial community. The measures or strategies for increasing N removal are then proposed from the perspectives of structural improvement of the bioretention system, optimization of media composition, and enhancement of the N removal reaction processes. Moreover, while different types of CWs have been analyzed for microbial N removal from wastewaters and correlating behavior with wetland design factors and operational condition, there is an apparent lack of studies focused on constructed wetlands designed for stormwater treatment.

NITROGEN TRANSFORMATION IN CWS

Constructed wetlands contain diverse groups of microorganisms which are critical for the proper functioning and maintenance of the system (Sims et al., 2012; Adrados et al., 2014; Ligi et al., 2014; Cao et al., 2017; Zhang et al., 2020). However, the extent of treatment depend upon microbial N transforming processes (Fernandes et al., 2015; Ibekwe et al., 2016; Cao et al., 2017; Fu et al., 2017; Gold et al., 2019; Zhang et al., 2020). Microbial communities and their responses to the wetland types, hydrologic dynamics and physico-chemical variables have been investigated in previous studies to understand their role in N removal. The presence of diverse microbial groups enables CWs to better cope with environmental changes and by the proliferation of organisms which are better adapted to the new conditions (Ibekwe et al., 2003).

One of the major N treatment mechanisms in CWs occurs via microbial interactions with different forms of nitrogen. A significant proportion of pollutants containing nitrogen are firstly converted to inorganic ammonia nitrogen (NH_4^+-N) during ammonification process in both aerobic and anaerobic conditions. Thereafter, NH_4^+-N is mainly removed by nitrification-denitrification processes in CWs under sequential oxidative stages. Nitrification implies a chemolithoautotrophic oxidation of ammonia to nitrate (NO_3^-) (ammonia oxidation) performed by microbes such as *Nitrosomonas* under strict aerobic conditions. Then, denitrifying bacteria (denitrifiers) such as

Psuedomonas, *Micrococcus*, *Achromobactor* and *Bacillus* reduce nitrite and nitrate into nitrogen gas under anaerobic or anoxic conditions (Lee et al., 2009). The comammox bacteria, members of the genus *Nitrospira* are also involved in N transformation (Daims et al., 2016). This genus contains full genetic complement for both ammonia and nitrite oxidation which could directly convert ammonium and nitrite to nitrate without nitrous oxide production. However, there are limited studies investigating the presence of comammox pathway in CWs (van Kessel et al., 2015; Pelissari et al., 2018; Sun et al., 2020; Zhang et al., 2021) and further research is needed to understand the relative importance of comammox process in N removal in CWs in comparison to other removal mechanisms (Zhang et al., 2021). Microbial N removal via denitrification accounts for as much as 90% of overall N removal (Lin et al., 2002; Faulwetter et al., 2009; Li et al., 2018a). Anammox (anaerobic ammonium oxidation) process has also been identified as an alternative processes in total nitrogen removal (Mulder et al., 1995). Anammox bacteria perform denitrification and transform NH_4^+-N into nitrogen gas without the need for aeration and addition of an external carbon source (Ward, 2003). Relatively high TN removals via this process have been reported (Dong and Sun, 2007; Sun and Austin, 2007). Stormwater does not contain sufficient biodegradable carbon or an external organic source to carry out denitrification. The co-function of different groups of N transforming microbes in CWs along with optimal operating parameters could potentially enhance the N removal performance of CWs (Third et al., 2001).

Among the environmental variables, pH, DO, and nitrogen concentration could potentially change the spatial distribution of microbial community structures, contributing to a change in microbial composition (Wang et al., 2020a). Wetland characteristics and components such as plants, organic carbon content (Sun et al., 2012; Liao et al., 2019), DO (Jianlong and Ning, 2004), redox condition (Faulwetter et al., 2009), temperature and pH have significant influences on microbial N removal (Kozub and Liehr, 1999; Sirivedhin and Gray, 2006). For example, denitrification rates strongly depend on NO_3^- concentration, type and quality of organic carbon sources, periods of wetting and drying, plant residues, redox potential, soil type, the presence of overlying water, temperature, and pH (Vymazal, 1995; Bastviken et al., 2005; Sirivedhin and Gray, 2006). Denitrifying bacteria have been identified as the main bacteria group at elevated levels of organic carbon in CWs while ammonium oxidizing bacteria (AOB) are the main bacteria group in the absence of organic carbon (Sun et al., 2012). Nutrient and organic carbon concentrations have been considered as one of the main drivers of diversity of microbial communities. The predominant anoxic–anaerobic condition within CWs increase the level of denitrification which is a key process of N removal (Vymazal, 2007). Observed variations in microbial communities have also been correlated with dry-weather flow or additional inter-wetland pollution sources (Staley et al., 2015; Huang et al., 2018) as the function, diversity and abundance of N transforming microbes depend on type of CWs, their design and operation conditions (Andersson et al., 2002; Kadlec, 2009; Peralta et al., 2010; Pelissari et al., 2017).

FACTORS REGULATING MICROBIAL COMMUNITIES IN CWS

Type of Constructed Wetland

Constructed wetlands have traditionally been categorized based on their hydrological regime and the resultant flow characteristic (Vymazal, 2007). The type and magnitude of N removal processes can vary based on the type of CWs as microbial growth and activity depend on wetland design and operational condition (Vymazal, 2013b). For example, N removal rates can vary from 40 to 50% with removed loads ranging between 250 and 630 g N m^{-2} yr^{-1} (Vymazal, 2007). The influence of wetland flow characteristics on microbial structure and function have been previously investigated by Peralta et al. (2010) who found that natural wetlands had significantly different assemblages of denitrifiers in comparison to restored wetlands. The comparison of denitrifying genes (*narG, nirS, nosZ*) in sediments within two different types of wetlands, estuarine vs. wastewater effluent-fed CWs, indicated that nitrite-reducing gene *nirS* was dominant over *narG* and *nosZ* in the effluent-fed wetland (Chon et al., 2011). Moreover, the abundance of specific microbial communities such as *Nitrosomonas* spp. as the N transforming bacteria can vary based on the type of CWs (Flood et al., 1999; Silyn-Roberts and Lewis, 2001). The population and composition of AOB were found to be different in vertical and horizontal CWs, with a higher percentage of *Nitrosospira*-like sequences (Ibekwe et al., 2003; Gorra et al., 2007; Tietz et al., 2007) in comparison to an overland flow system which did not show any changes in AOB composition (Sundberg et al., 2007a).

Specific design features of conventional wetland systems can bring about changes to N removal and alter the main removal mechanisms in wetlands. Vertical subsurface flow CWs (VSSF-CWs) with partially saturated conditions operating under low organic loads favored simultaneous nitrification and denitrification processes (Pelissari et al., 2017). Moreover, VFCWs with high redox potentials favor aerobic microbial processes such as ammonification and nitrification (Kadlec et al., 2000). The comparison between VF, SF and HFCWs showed a significantly higher biochemical oxygen demand removal and nitrification but relatively lower denitrification in VF-CWs than the SF and HFCWs (Vymazal, 2007). Nitrification and denitrification processes have been shown to be the major N removal mechanisms in HSSF-CWs rather than volatilization, adsorption, and plant uptake (Cooper et al., 1997; Vymazal, 2007).

Plants

A wide range of technologies from biofiltration systems (Shirdashtzadeh et al., 2017; Wu et al., 2017) to CWs (Wu et al., 2016) have employed plants to treat wastewater because of their complex interactions with pollutants and microorganisms (Harris et al., 1996; Abal et al., 2002; Brix, 2020). Indeed, plants are one of the most ubiquitous components of CWs, and can directly uptake and assimilate nutrients (Vymazal, 2007, 2013a; López et al., 2016), stabilize the bed surface, serve as substrate for microbial attachment, release oxygen (Peng et al., 2014) and stimulate the growth of N transforming microbes (Li et al., 2018b; Han et al., 2020). Plants also regulate hydraulic

conditions and reduce wind stress, which supports sedimentation and prevents re-suspension (Dieter, 1990; Vymazal, 2013a). Nitrogen attenuation by plants is a process encompassing several steps including uptake, assimilation, translocation and as the plant is aging, recycling and remobilization while the main process is the absorbance of nitrogen from the soil in the form of nitrate (NO_3^-) and ammonium (NH_4^+-N). However, the studies on N take up by plants and microorganisms reveal that microbes may take up different N forms than plants which may lead to less competition (Schimel and Chapin, 1996; Kaye and Hart, 1997; Hodge et al., 2000; Lipson and Näsholm, 2001).

Biofilms created by submerged plants (or a part of plants) provide large surfaces for microcolonies which are composed of different bacterial communities such as denitrifiers (Zhang et al., 2016). In this regard, microbial communities of planted wetland systems have been found to have higher number of soil microbes and higher activities of soil enzymes (Salvato et al., 2012) accompanied by higher N removal rates (Lin et al., 2002; Yousefi and Mohseni-Bandpei, 2010), compared to unplanted wetlands (Lai et al., 2011; Huang et al., 2012; Boog et al., 2014). The enhanced microbial density, activity, and diversity in the plant rhizosphere create an effective N transformation zone (Picard et al., 2005; Kadlec and Wallace, 2008; Faulwetter et al., 2009; Truu et al., 2009) due to the abundance of organic matter (Gagnon et al., 2007; Truu et al., 2009; Xu et al., 2017) and the presence of the oxygenated root zone (Lai et al., 2012; Peng et al., 2014). The oxygenated root zone supports aerobic processes even under flooded conditions with a bulk of influent NO_3^- reduced in the vicinity of plant roots (Han et al., 2020).

Microbial functional genes involved in NO_3^- reduction processes such as denitrification, Anammox, dissimilatory nitrate reduction to ammonium (DNRA), and denitrifying anaerobic methane oxidation (DAMO)] (Wang et al., 2020b) and nitrification (Stottmeister et al., 2003) have been found in the plant rhizosphere. The rhizosphere mosaic characteristic of aerobic and anaerobic zones assist the process of nitrification and denitrification (Zhu et al., 2010). For example, greater numbers of nitrifying bacteria and higher bacterial activity were detected on plant roots compared to the bulk matrix (Kyambadde et al., 2006). The rhizosphere denitrifying bacteria had different community structure from bulk sediment revealed by PCR-denaturing gradient gel electrophoresis (DGGE) of nosZ. The results demonstrate the effect of vegetation on the functioning and structure of bacterial communities involved in N removal in CWs (Ruiz-Rueda et al., 2009).

Plants also play a significant role in the removal of NH_4^+ via nitrification-denitrification pathways (Coban et al., 2015). However, the rates of N removal such as denitrification can vary significantly among vegetation communities especially in less aerated systems (Vymazal, 1995; Toet et al., 2003; Bastviken et al., 2005; Sirivedhin and Gray, 2006; Hernandez and Mitsch, 2007). Wetland vegetation density has been found to control microbial community composition (Toscano et al., 2015; Zheng et al., 2016; Han et al., 2020), diversity (Ibekwe et al., 2007) and distribution (e.g., ammonia oxidizers and denitrifiers) (Zhang et al., 2017) via enhancing wetland retention time (Jadhav and Buchberger, 1995; Vymazal, 2013a).

Organic Carbon and Nutrient Concentration

Nutrient concentration can affect diversity, composition, and structure of microbial communities (Hu et al., 2014). It has been reported that nutrient availability can increase the richness of microorganisms, as water samples from natural sites contain higher bacterial richness and diversity than sediment and surface water sampled from urban runoff (Logue et al., 2012). However, other studies reported that nutrient availability can reduce the diversity of microbial communities (Van Horn et al., 2011). Among the different types of nutrients, total phosphorus (TP) and NO_3^- have been recognized as the main factors affecting the composition of microbial communities in urban aquatic systems (Wang et al., 2018). A higher concentration of NO_3^- has been correlated with increased occurrence of denitrifying genes (Zhang et al., 2016), higher denitrification rates in sediments (Vymazal, 1995; Bastviken et al., 2005; Sirivedhin and Gray, 2006) and more vigorous and robust populations of denitrifiers within wetland sediments (Vymazal, 1995; Bastviken et al., 2005; Sirivedhin and Gray, 2006).

Microbial denitrification is considered as the dominant N removal process at high NO_3^- loading rates (Spieles and Mitsch, 1999; Xu et al., 2017) while low concentration of NO_3^- results in coupled nitrification-denitrification as the major removal process in CWs. The rate of DNRA in CWs has been related to NO_3^- loss (Kendall et al., 2007), while the presence of both NH_4^+ and NO_3^- can inhibit NO_3^- uptake by microorganisms (Coban et al., 2015). *Nitrosospira* spp. has been detected as the prevalent group in low-ammonia (NH_4^+) environments (Kowalchuk et al., 2000; Bäckman et al., 2003), while *Nitrosomonas* species is the prevalent bacterial group at high NH_4^+ concentrations (Schramm et al., 1996; Juretschko et al., 1998; Okabe et al., 1999; Purkhold et al., 2003; Sundberg et al., 2007a; Tietz et al., 2007).

The abundance of N transforming microbes has a significant correlation with organic matter content (Vymazal, 1995; Hernandez and Mitsch, 2007; Sundberg et al., 2007b; Kim et al., 2016), their availability and degradability potential (Kozub and Liehr, 1999; Sirivedhin and Gray, 2006). Dissolved organic matter (DOM) has been found as one of the major contributing factor to the microbial variation, composition and structure in surface waters (Logue and Lindström, 2008; Pang et al., 2014). The variation in loading rates of organic carbon has been found to influence microbial diversity (e.g., nitrifiers) and increase the competition among different microbial groups (Thompson et al., 1995; Schramm et al., 1996; Grunditz et al., 1998; Kowalchuk et al., 2000; Webster et al., 2002; Truu et al., 2005), with an uneven distribution of available organic matter known to cause a sparse and heterogeneous distribution of microbes in CWs. The chemical oxygen demand to nitrogen ratio (COD/N) has been used as an assessment method to represent the efficiency of wetland N removal (Wu et al., 2009; Rodziewicz et al., 2019).

Higher levels of diversity and abundance of AOB have been detected at higher COD (Prosser, 1990; Van Niel et al., 1993; Hunt et al., 2003; Zhao et al., 2011; Fan et al., 2013a; Si et al., 2019). Heterotrophic denitrification is negatively influenced by low

carbon-to-nitrogen ratios (mol/mol) (Lee et al., 2009; Wu et al., 2009), while denitrification in some CWs have been significantly enhanced with increased COD/N because of enrichment by denitrifiers in CWs (Xu et al., 2017). The limitation of COD and the presence of NH_4^+ favor Anammox bacteria, where the abundance of *amoA* has been correlated with N removal efficiency (Zhang et al., 2017). Microbial N transforming processes in wetland bed sediments such as ammonification, nitrification and denitrification depend on characteristics of bed sediment such as nutrient availability (Vymazal, 1995; Bastviken et al., 2005; Sirivedhin and Gray, 2006; Truu et al., 2009; Repert et al., 2014). Soil nutrient concentration can significantly impact N removal, for example, wetlands with mineral soils showed more rapid N removal rates compared to those with organic soils (Gale et al., 1993). Moreover, some types of soil can provide better sources of carbon to promote microbial processes in CWs (Dordio and Carvalho, 2013).

Temperature

Temperature correlates strongly with N removal in CWs by regulating microbial processes as metabolic rates at higher temperatures generally result in higher microbial growth and activity (Zhang et al., 2008; Faulwetter et al., 2009). Different functional groups of microbes have different growth temperature optima, for example, ammonia-oxidizing bacteria grow faster than nitrite oxidizing bacteria at temperatures above 15°C while nitrite oxidizing bacteria growth can be inhibited at 25°C (Paredes et al., 2007). *Nitrosomonas* and *Nitrobacter* require temperatures above 5°C for their growth (Cooper et al., 1996) while temperatures lower than 10°C inhibit nitrification (Herskowitz et al., 1987; Xie et al., 2003). Studies have reported different optimal temperatures for nitrification (Vymazal, 1995; Paul, 2014). Truu et al. (2009) reported a range of 28–36°C for nitrification while other studies reported optimal temperature to be as low as 0 to 5°C (Sundblad and Wittgren, 1991; Sundberg et al., 2007a). However, nitrifying communities can adapt to temperature variations and may maintain their activity at lower temperatures via their metabolic adaptation (Cookson et al., 2002).

Seasonal temperature increases of more than 6°C have been found to have a significant impact on NH_4^+ and total inorganic N removal via simultaneous partial nitrification and Anammox process (He et al., 2012). It has been reported that bacteria conducting nitrification and denitrification are sensitive to lower temperatures. However, bacteria in the upper layers were more sensitive to the temperature changes than bacteria in the lower layers of vertical flow filter systems (VSSF-CW) (Truu et al., 2009). Denitrification can only occur above 5°C in CWs (Herskowitz et al., 1987; Brodrick et al., 1988; Werker et al., 2002) with complete denitrification accomplished at 52°C (Liao et al., 2018). The increase of temperature from 4 to 25°C was found to have the largest positive effect on the potential rate of denitrification (increasing from 0.0021 to 0.8100 kg N_2O/kg sediment per day) (Sirivedhin and Gray, 2006). Although low temperatures

($<5°C$) can suppress denitrification in CWs (Werker et al., 2002; Burchell et al., 2007), no seasonal or spatial influences were observed on bacterial abundance or diversity, and lower temperatures did not change the N removal rates during winter period (Kern, 2003).

Liao et al. (2018) investigated denitrifying genes (*nirK*, *nirS*, *narG*, and *nosZ*) in CWs and found that temperature changes can cause shifts in microbial structure, diversity, and abundance. It has also been reported that sensitivity of microbial processes in CWs to temperature variations are higher in dry conditions compared to flooded conditions (Nurk et al., 2005). Low temperature was found to have a negative effect on the number of nitrifying bacteria and nitrification in a HFCW during the winter period (Kern, 2003). However, the impact of temperature on nitrification can be limited because low temperatures can enhance other environmental factors which are beneficial to the nitrifiers (e.g., increased redox potential). Heterotrophic activity of microorganisms is not likely to be impacted by temperature variation probably due to the mixed populations contributing to activities at different temperatures (Tao et al., 2007). Lastly, the sensitivity of N removal processes to temperature may depend on wastewater properties (Truu et al., 2009).

Dissolved Oxygen and pH

Microbial function and diversity depend on presence or absence of oxygen in CWs. Dissolved Oxygen (DO) is negatively correlated with the relative abundances of denitrifying genes (Zhang et al., 2016) and plays a significant role in N availability as it determines the oxidized and reduced forms of nitrogen. High DO levels was reported to have led to the increase in abundance of nitrifying bacteria, denitrifying bacteria, and anammox bacteria (Liu et al., 2018). DO is necessary for ammonification in the micro-gradient of aerobic and anaerobic zones of CWs, and also affects the steep redox gradients in wetlands leading to changes in the spatial distribution of microbial biomass in wetlands (Reddy and D'angelo, 1997; Scholz and Lee, 2005; Truu et al., 2009). Furthermore, the microsites with steep oxygen gradients within wetlands allow denitrification and nitrification to occur in sequence in very close proximity to each other (Kadlec and Wallace, 2008).

Aerobic nitrifying bacteria perform complete nitrification at the upper layers of a water body (Robertson and Kuenen, 1984; Kern, 2003; Nurk et al., 2005) or the oxygenated zones of rhizosphere (Zhu and Sikora, 1995; Martin et al., 1999; Münch et al., 2005) because of the presence of adequate amount of oxygen (Hammer, 1986; Tchobanoglous et al., 1991; Vymazal, 1995). At the water-sediment interface, which is characterized by an oxygen gradient, aerobic reactions can take place near the surface while anaerobic reactions take place at the deeper layer of bottom sediments (Reddy et al., 1984). Thus, the depth that microbial communities occupy in the bed sediments of CWs plays a significant role in defining the aerobic/anaerobic processes (Wei et al., 2021).

pH plays a vital role in development of microbial communities (Paredes et al., 2007; Vymazal, 2007; Mayes et al., 2009; Saeed and Sun, 2012), as ammonification, nitrification, denitrification and Anammox are all pH dependent processes (Vymazal, 1995; Bastviken et al., 2005; Sirivedhin and Gray, 2006; Wang et al., 2017). The optimal pH for ammonification was found to be 6.5 to 8.5 (Reddy et al., 1984; Vymazal, 1995; Saeed and Sun, 2012). The ideal pH for nitrifying bacteria ranges between 7.2 and 9.0 (Tchobanoglous et al., 1991; Cooper et al., 1996; Paredes et al., 2007; Vymazal, 2007), while pH between 7.5 and 7.8 can result in partial nitrification over nitrite oxidation (He et al., 2012). The optimal pH of denitrification ranges from 6 to 8, while it reduces at pH 5, and is negligible below pH 4 (Vymazal, 2007). Although pH can affect nitrification rates, its influence is reported to be negligible under neutral pH conditions (Reddy et al., 1984). Microbial mediated processes can also change the pH, as denitrification can increase pH by leading to higher wetland alkalinity.

Hydrology and Hydraulics of Constructed Wetlands

Constructed wetlands experience different hydrologic and hydraulic behavior as well as different degrees of drying and re-wetting conditions. The impact of dry-weather condition and storm events on N transforming microbes in CWs have been studied by Converse et al. (2011), Vymazal (2014), Wu et al. (2015), Huang et al. (2018). Variations in wetland hydrology and hydraulics can account for the observed inconsistency in treatment rates and shifts in microbial communities (Chen et al., 2013; Bernardin-Souibgui et al., 2018; Huang et al., 2018; Kan, 2018; Rose et al., 2018).

Foulquier et al. (2013) reported that the structure of bacterial communities under dry–wet cycles differ from those found under permanently inundated conditions. Moreover, a series of dry–wet stress cycles and the reduction in soil respiration rates have been linked to microbial community composition (Fierer et al., 2003). Thus, effective N removal requires stability in hydraulic response which influences retention, high contact between water and wetland components and flow distribution (Schimel et al., 2007; Wallenstein and Hall, 2012). The retention time, a key parameter is CW design, is a measure of the average amount of time that inflow to the wetland is retained before final discharge and has been shown to affect microbial community structure, composition, and abundance (Toet et al., 2005; Faulwetter et al., 2009).

Water level fluctuations is one of the key characteristics of CWs that can cause shifts in microbial communities as microbial composition, structure, and function are sensitive to the depth, frequency, and duration of fluctuations. The dynamics of wetland hydrology not only alter the response of wetland systems, but also alter the microbial genes responsible for N removal pathways (Bambauer et al., 1998; Fritsche et al., 1999; Kämpfer et al., 1999; Doronina et al., 2010; Drury et al., 2013; Eichmiller et al., 2013; Liu et al., 2013; Chen et al., 2014; Mo et al., 2016; Isabwe et al., 2018). Systems with high water levels contained significantly higher DO concentrations in their bed sediment which can change the microbial community structure and composition (Han et al., 2020; Huang et al., 2020). In addition, during periods

of drawdown, enhanced diffusion of oxygen can lead to the promotion of nitrification process over denitrification (Baldwin and Mitchell, 2000; Knorr et al., 2008). The rates of microbial functions can be continually affected in seasonally dewatered zones compared to the permanently inundated areas. Saturated conditions limit the diffusion of oxygen in sediment and promote anaerobic processes such as denitrification over aerobic processes (e.g., nitrification) as the hydrological regime can regulate gas exchange between the atmosphere and sediments (Hefting et al., 2004; Reddy and DeLaune, 2008; Siljanen et al., 2011). Moreover, hydraulic fluctuations affect the steep redox gradients in CWs which control microbial mediated mechanisms (Reddy and D'angelo, 1997; Scholz and Lee, 2005). Periodic drying and wetting conditions can cause changes in microbial community dynamics (Huang et al., 2018; Kan, 2018; Rose et al., 2018). Previous studies have investigated the processes of microbial assembly and dynamics under wet and dry condition within CWs, creek, and watershed (Bambauer et al., 1998; Fritsche et al., 1999; Kämpfer et al., 1999; Doronina et al., 2010; Drury et al., 2013; Eichmiller et al., 2013; Liu et al., 2013; Chen et al., 2014; Mo et al., 2016; Huang et al., 2018; Isabwe et al., 2018; Kan, 2018; Rose et al., 2018).

During storm events, overland flow introduce new groups of microbes to the wetland, which can change microbial abundance and composition (Sundberg et al., 2007a). While drying and wetting conditions cause immediate physiological stresses to microorganisms, repeated dry–wet cycles can cause long-term effects at community level through the selection of taxa (Fierer et al., 2003; Mikha et al., 2005; Schimel et al., 2007; Xiang et al., 2008). Thus, storm events can change the microbial function, with the potential for alteration of subsequent biogeochemical transformations. Kan (2018) who tracked changes of bacterial community before, during, and after storm events found that the diversity of bacterial community significantly increased during the peak discharge, with the starting bacterial community being very different from the storm community. There are also differences in the distribution patterns of bacterial community, which are event specific, with each bacterial phylum showing distinct response to the event (Kan, 2018). During large storm events, functional genes changed notably and distinct bacterial groups (e.g., nitrifying and denitrifying genes) became the dominant groups (Merbt et al., 2015). During dry weather, the microbial communities have been shown to transform from heterotrophic at the inlet to predominantly autotrophic at the outlet (Huang et al., 2018) indicating a high spatial variation in composition of bacterial communities during dry weather (Petersen et al., 2005; Sercu et al., 2008; Parks and VanBriesen, 2009). Moreover, dry period water samples have been found with differences in the abundance of distinct bacterial families (Isabwe et al., 2018) with less diversity in comparison to the wet weather samples (Huang et al., 2018). The inconsistency of denitrification rates between the sampling locations have been best described by concentration of nitrogen in sediments and hydrological processes, including water residence times (Kjellin et al., 2007). The fluctuation of water regime reduces microbial mediated processes due to the negative impact on wetland vegetation (Greenway et al., 2007;

Raulings et al., 2010; Vanderbosch and Galatowitsch, 2011; Webb et al., 2012).

CONCLUSIONS AND RECOMMENDATIONS

There is a large body of literature investigating treatment efficiencies in CWs with less attention paid to associating microbial processes with treatment processes in constructed wetlands. The conclusions that can be stated from this review are:

(i)   There is a disproportionate number of studies understanding microbial community dynamics in constructed wetlands designed to treat stormwater as there are for wastewater. Of the 34 studies of functioning constructed wetlands including surface, vertical and subsurface flow configurations, only four studies dealt specifically with microbial analysis and constructed wetlands treating stormwater.

(ii) The hydrology and hydrologic behavior of stormwater wetlands are complicated with frequent emptying and drying and fluctuations of the water surface. Such perturbations can cause significant changes in the oxic state in the wetland which can alter the microbial genes responsible for the N removal pathways and may ultimately be responsible for the variability in treatment effectiveness reported.

(iii) The influence of other physico-chemical parameters such as organic carbon, nutrients, temperature (seasonal), dissolved oxygen and pH do not vary by as large an extent and are less likely to cause large shifts in microbial communities as compared to wetland hydraulics and hydrology. Nevertheless, as nutrient concentrations in stormwater are comparatively low compared to wastewater, the response of microbial communities under low nutrient and organic carbon condition requires further assessment.

This review has also highlighted pertinent questions that require further investigation:

(i)   How does N transforming microbes respond to the wetland physico-chemical features?
      Previous studies have investigated microbial response to CWs' physico-chemical variables in different types of CWs. The environmental variables explained microbial shifts, however their impact on N removal could be insignificant depending on wetland condition. They could potentially change the spatial distribution of the microbial community and microbial compositions; thus, we observe different rates of N removal. Studies of N removal performance and mechanisms under physico-chemical variations have identified main pathway(s) of N removal in CWs. These findings could assist to determine the optimal conditions where different pathways could co-exist while different groups of microbes perfume their role at different levels of N removal.

(ii) How do microbial communities respond to the changes in wetland hydrology?

Microbial shifts were associated with variations in wetland hydrology and hydraulics which can account for the observed wetland performance inconsistency. The microbial responses to wetland hydrology such as water level fluctuations caused shifts in community composition, structure, and function. The dynamics of wetland hydrology alter the microbial genes; thus, the effectiveness of N removal depends on stability in wetland hydrology.

(iii) How do microbial communities differ under wet and dry weather conditions?

Investigation of microbial assembly and dynamics under wet and dry condition showed that during storm events, new groups of microbes could be introduced to CWs which can change the microbial abundance and composition. Periodic drying and rewetting conditions could cause long-term effects at the community level through the selection of taxa with immediate physiological stresses to microorganisms. Tracking bacterial dynamic before, during, and after storm events demonstrated the differences in distribution patterns of bacterial community at each storm event where each bacterial phylum representing distinct response to the event. As the fluctuation in water regime could inhibit microbial mediated processes, diversity and abundance of N transforming microbes could be used to assess the CWs treatment performance.

(iv) Can microbial shifts be used to assess the CWs' N removal performance?

It has been revealed that microbial characterization, their structure, diversity, and composition could indicate wetland health and its ability to function as intended. The abundance of genes that are involved in N transformation were positively or negatively correlated with total N removal following rain events. Moreover, the presence of top microbial phyla with the highest degree of relative richness have been used as reliable alternative indicators of wetland treatment performance. Indeed, previous works have strongly supported the implication of new designs in CWs to promote bacterial diversity which is essential for N removal while the microbial shifts could be used as ecological indicators.

Although some of these questions have been raised by others in previous research studies, the general lack of information about the microbial shifts in CWs that treat stormwater needs to be addressed. Many studies have analyzed microbial N removal, and bacterial community but a majority have conducted their studies in bioretention systems. It is therefore recommended, in the context of stormwater constructed wetlands to:

(i) Study less investigated N transformation pathways such as DNRA and comammox processes and focus on microbial community involved in these processes and their changes over dry-wet cycles. This information could assist engineers to take advantage of the presence of other N transforming communities leading to higher microbial diversity within CWs treating stormwater.

(ii) Track microbial recovery in terms of their structure, composition, and distribution in CWs to reveal the impact of the flow regime in CWs. The results should be carefully analyzed and interpreted because the extent of microbial reaction to fluctuations depends on a wide range of factors.

(iii) Employ advanced molecular techniques to link microbial properties of stormwater wetlands to the transport and sources of nitrogen. Tracking the microbial functional genes assists scientists to develop ecosystems that are favorable to the establishment of pathways with higher N removal potential in stormwater treatment.

Lastly, the findings of the current literature review reinforce the importance of stormwater runoff treatments and implementation of new design into CWs to enhance microbial activity and diversity leading to better treatment outcomes.

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