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도시 강우유출수 처리 인공습지의 토양특성 및 오염물질 저감에 따른 미생물 영향 평가

Chiny. C. Vispo* · Miguel Enrico L. Robles* · 오유경* · Haque Md Tashdedul* · 김이형**†

*공주대학교 건설환경공학과
**공주대학교 스마트인프라공학과

Microbial Influence on Soil Properties and Pollutant Reduction in a Horizontal Subsurface Flow Constructed Wetland Treating Urban Runoff

Chiny. C. Vispo* · Miguel Enrico L. Robles* · Yugyeong Oh* · Haque Md Tashdedul* · Lee Hyung Kim**†

*Department of Civil and Environmental Engineering, Kongju National University
*Department of Smart Infrastructure Engineering, Kongju National University

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요 약

인공습지는(CW)는 침투, 흡착, 저류, 식물과 미생물의 증발산 등과 같은 수문학적 및 생태학적 기작에 의하여 오염물질 제거, 탄소흡수 및 저장, 생물다양성 향상 등의 생태계서비스를 제공한다. 본 연구는 수평지하흐름 인공습지(HSSF CW)의 미생물 군집과 토양의 물리·화학적 특성 및 처리효율의 상관관계를 분석하기 위하여 수행되었다. 연구를 위한 모니터링은 강우시 수질특성, 토양특성, 미생물 분석이 수행되었다. 따뜻한 계절(>15° C) 에서 TSS, COD, TN, TP 및 중금속(Fe, Zn, Cd) 제거효율이33~74% 범위로 나타났다. 그러나 추운 계절(≤15° C)에서 TOC 35%로 가장 높은 제거 효율이 나타났다. 인공습지 내 토양은 인근에서 채취한 토양의 토양유기탄소(SOC) 함량보다 3.3배 더 높은 함량을 가지고 있는 것으로 나타났다. 유입부와 유출부의 탄소(C), 질소(N) 및 인(P)의 화학양론비(C:N:P)는 각각 120:1.5:1 및 135.2:0.4:1로 나타났으며, 탄소에 비해 질소와 인의 비율이 매우 낮아 미생물 성장에 문제가 발생될 수 있다. 미생물 분석에서는 생물다양성 지수를 통해 미생물 군집의 풍부도, 다양성, 균질성 및 균일성이 따뜻한 계절이 추운 계절에 비해 높게 나타났다. 인공습지의 강우유출수 오염물질 중 질소고정 미생물인 *Proteobacteria, Actinobacteria, Acidobacteria, Bacteroidetes*가 우점종으로 미생물 생장을 촉진하는 것으로 평가되었는데 이는 특정 토양특성 및 유입수 특성이 미생물 풍부도와 밀접한 관련이 있음을 의미한다.

핵심용어 : 생물다양성, 지하흐름 인공습지, 미생물, 토양유기탄소, 토양영양물질

Abstract

Constructed wetlands (CWs) deliver a range of ecosystem services, including the removal of contaminants, sequestration and storage of carbon, and enhancement of biodiversity. These services are facilitated through hydrological and ecological processes such as infiltration, adsorption, water retention, and evapotranspiration by plants and microorganisms. This study investigated the correlations between microbial populations, soil physicochemical properties, and treatment efficiency in a horizontal subsurface flow constructed wetland (HSSF CW) treating runoff from roads and parking lots. The methods employed included storm event monitoring, water quality analysis, soil sampling, soil quality parameter analysis, and microbial analysis. The facility achieved its highest pollutant removal efficiencies during the warm season (>15° C), with rates ranging from 33% to 74% for TSS, COD, TN, TP, and specific heavy metals including Fe, Zn, and Cd. Meanwhile, the highest removal efficiency was 35% for TOC during the cold season (≤15° C). These high removal rates can be attributed to sedimentation, adsorption, precipitation, plant uptake, and microbial transformations within the CW. Soil analysis revealed that the soil from HSSF CW had a soil organic carbon content 3.3 times higher than that of soil collected from a nearby landscape.

*To whom correspondence should be addressed.
Department of Civil and Environmental Engineering, Kongju National University, Cheonan, Korea
E-mail: leehyung@kongju.ac.kr

- **Chiny C. Vispo** Department of Civil and Environmental Engineering, Kongju National University / Graduate Student (ccvispo@smail.kongju.ac.kr)
- **Miguel Enrico** L. Robles Vispo Department of Civil and Environmental Engineering, Kongju National University / Graduate Student (roblesmiguelenrico@gmail.com)
- **Yugyeong Oh** Department of Civil and Environmental Engineering, Kongju National University / Graduate Student (yugyeong@smail.kongju.ac.kr)
- **Haque Md Tashdedul** Department of Civil and Environmental Engineering, Kongju National University / Graduate Student (tashdedulhaque@gmail.com)
- **Lee-Hyung Kim** Department of Smart Infrastructure Engineering, Kongju National University / Professor (leehyung@kongju.ac.kr)

Stoichiometric ratios of carbon (C), nitrogen (N), and phosphorus (P) in the inflow and outflow were recorded as C:N:P of 120:1.5:1 and 135.2:0.4:1, respectively, indicating an extremely low proportion of N and P compared to C, which may challenge microbial remediation efficiency. Additionally, microbial analyses indicated that the warm season was more conducive to microorganism growth, with higher abundance, richness, diversity, homogeneity, and evenness of the microbial community, as manifested in the biodiversity indices, compared to the cold season. Pollutants in stormwater runoff entering the HSSF CW fostered microbial growth, particularly for dominant phyla such as *Proteobacteria, Actinobacteria, Acidobacteria,* and *Bacteroidetes,* which have shown moderate to strong correlations with specific soil properties and changes in influent-effluent concentrations of water quality parameters.

Key words : biodiversity; horizontal subsurface flow constructed wetland; microorganism; soil organic carbon; soil nutrients

1. Introduction

The rapid urbanization of landscapes, characterized by the transformation of natural terrains into urban infrastructure like streets, roads, sidewalks, and parking lots, illustrates the impact of land use and land use changes (LULUCs) on natural ecosystems and the urban water cycle (Zhou et al., 2021). Urban areas, predominantly paved with impermeable materials, impede water infiltration, thereby disrupting natural hydrological processes and reducing groundwater recharge potential. These alterations contribute to increased stormwater runoff volume, pollution from non-point sources (NPS), and elevated risks of urban flooding, further degrading water quality (Forman and Alexander, 1998; Hansen et al., 2013; Lillesund et al., 2017). Moreover, as cities expand and alter land use patterns, the ability of natural landscapes to absorb carbon dioxide (CO2) has been limited. This exacerbates climate change by reducing the natural carbon sinks available to sequester CO2, a key factor in the global warming phenomenon (McGuire et al., 2001; Wang et al., 2015). Generally, the effects of climate change and LULUCs are expected to escalate economic, environmental, and social risks (Choi et al., 2021).

Given the escalating challenge of NPS pollution in urban environments, decentralized stormwater management employing nature-based solutions (NbS) has become a prevalent strategy to address these issues. This approach encompasses micro-scale facilities within a basin-wide framework to effectively manage peak flows, runoff volumes, and pollutant loads in stormwater runoff (Zhang et al., 2017). Constructed wetlands (CWs) have emerged as one of most commonly utilized NbS for stormwater management and pollution mitigation in urban areas, contributing to carbon neutrality, biodiversity conservation, and sustainable development (Vispo et al., 2023). Engineered to mimic the functions of natural wetlands, CWs consist of vegetation, soils, substrates, and microorganisms that work together through physicochemical and biological processes to remove pollutants and enhance the quality of water in receiving bodies (Saeed and Sun, 2012; Resende et al., 2019). CWs not only address the issue of stormwater runoff but also play an important role in the global carbon cycle. It is well recognized that the capacity of CW soils to sequester carbon (C) is strongly influenced by the availability of nitrogen (N) and phosphorus (P), which are vital for maintaining the stoichiometric balance necessary for biogeochemical cycles (Smith et al., 2015; Macdonald et al., 2018). The vegetation within CWs is integral to this process, sequestering atmospheric CO2 and contributing to climate change mitigation. However, the effectiveness of these systems is associated to the health and sustainability of the vegetation that populates them. The vitality of plants in CWs is largely dependent on the nutritional quality of the soil, particularly the availability of essential nutrients such as N and P (Reddy et al., 2022; Kadlec and Wallace, 2009).

Soil organic carbon (SOC) is a key determinant of soil health, influencing its physical, chemical, and biological properties, and providing a source of energy and nutrients for microbial populations (Lal, 2004). The interaction between these microorganisms and the plant roots facilitates cycling of nutrients, including N and P, which are essential for plant growth and the overall treatment efficiency of CWs (Vymazal, 2013; Faulwetter et al., 2009). The relationship between microorganisms and nutrients is further influenced by various soil properties such as temperature, pH, electrical conductivity, moisture content (MC), loss on ignition (LOI) and bulk density, which can greatly affect microbial metabolic processes and, consequently, nutrient cycling. For instance, microbial enzymatic activities, the rate of bacterial growth, the chemistry of pollutants, and the diversity of the microbial community are all temperature-sensitive and are considered important factors in biological remediation (Adams et al., 2015; Kebede et al., 2021). Therefore, understanding the behavior of the microorganisms in various soil properties becomes increasingly important for optimizing CW performance.

This study aimed to enhance the understanding of the components and interactions that drive treatment processes in CWs, thereby improving the efficiency and sustainability of future CW systems. Specifically, this study investigated the correlations between microbial populations, soil physicochemical properties, and treatment efficiency in a CW designed for urban runoff treatment, establishing how these interactions contribute to the overall functionality of the system. The research also focused on evaluating the CW's performance efficiency in removing various water pollutants during both the warm season (>15° C) and the cold season (≤15° C), examining how seasonal temperature fluctuations impact treatment outcomes. Additionally, this study assessed the chemical characteristics of soil, particularly TN, TP, and SOC, and determined the C, N, and P stoichiometric ratios in the soil to explore their roles in nutrient cycling and plant growth within the CW. Given that several biogeochemical processes that regulate the removal of nutrients in wetlands are affected by temperature (Kadlec and Reddy, 2001), this study also examined the growth and changes in microbial population in response to temperature fluctuations. Lastly, microbial biodiversity indices were developed to evaluate the diversity and abundance of microbial species in the wetland facility, aiming to enhance understanding of microbial community dynamics and their influence on treatment effectiveness.

2. Materials and Methods

2.1 Study area description and characteristics

A small horizontal subsurface flow constructed wetland (HSSF CW) was installed within the campus of Kongju National University, located in Cheonan City, South Korea (36° 51'01.1"N 127° 09'00.2"E), in 2010. The facility primarily functions to treat stormwater runoff from a 424 m^2 impermeable area comprised of a road and parking lot. Designed without an infiltration capability, the HSSF CW features an overflow channel and utilizes various filter media including sand, gravel, bio-ceramic materials, and woodchips to facilitate the treatment process. Iris ensatavar. spontanea,a native plant species in Korea known for its low maintenance, rapid growth, and effectiveness in wastewater treatment due to its high tolerance for water saturation and pollutant uptake efficiency (Choi et al., 2015; Choi et al., 2021), is the predominant vegetation in the HSSF CW. The dimensions of the system are 7 x 1 x 0.7 meters in length, width, and height, respectively, providing a pre-treatment volume of 0.67 m^3 . The overall storage volume of the wetland is 1.56 m^3 , with a surface area (SA) to catchment area (CA) ratio of 1.4%, and a storage volume (SV) to total volume (TV) ratio of 30.6%. These ratios indicate the system's designed capacity for managing water flow and retention. Additionally, the HSSF CW undergoes facility cleaning and maintenance twice a year, specifically after the winter and autumn seasons. These

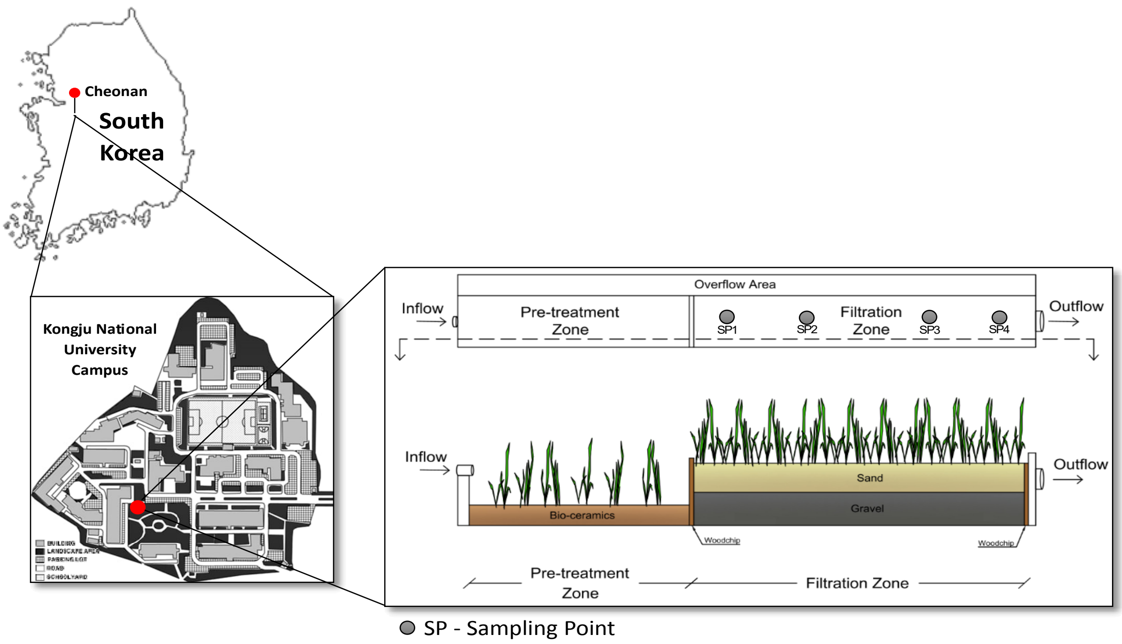

Fig. 1. Schematic diagram of HSSF CW located at Kongju National University.

periods are critical for addressing accumulations of sediment and potential clogging in the system, ensuring optimal operational conditions. A schematic design of the HSSF CW facility is illustrated in Figure 1, detailing the configuration and components of the system.

2.2 Monitoring, sample collection and water quality analysis

South Korea, situated in the temperate climate zone, experiences four distinct seasons: spring, spanning from late March to May; summer, lasting from June to early September; autumn, from September to November; and winter, from December to mid-March. Each season is marked by unique climatic patterns that significantly influence environmental and hydrological dynamics. In this study, the warm season is specifically designated to include the months of June, July, and August, which received an average monthly rainfall ranging from 147 to 257 mm, alongside the highest mean temperature amounting from 27.4 to 28.9° C. On the other hand, the cold season, comprising March, October, and November, recorded an average monthly rainfall range of 43 to 51 mm and mean temperatures ranging from 4.9 to 14° C.During the study period spanning from 2010 to 2023, a total of 34 rainfall events were monitored, with 20 events occurring in the warm season and 14 in the cold season. These monitored events had an average ± standard deviation rainfall intensity of 3.5 ± 3.2 mm/hr. Most storm events, comprising 58.8% of the total sampling, were monitored during the warm season, while the remaining 41.2% were monitored during the cold season. In Cheonan City, rainfall volumes during the warm season were significantly higher than those in the cold season. Monitoring during rainfall events focused on the first flush phenomenon, typically observed in highly urbanized areas (Kim, 2010). Water samples were collected through manual grab sampling. The first sample was collected immediately upon observing inflow or outflow. Succeeding samples were collected after five, 10, 15, 30, 60 min and thereafter every hour until the runoff stops. Water samples were analyzed for various parameters, including total suspended solids (TSS), chemical oxygen demand (COD), total nitrogen (TN), total phosphorus (TP), and total organic carbon (TOC), based on the Standard Methods for the Examination of Water and Wastewater (APHA, AWWA and WEF, 1992). The concentrations of total heavy metals, including chromium (Cr), iron (Fe), nickel (Ni), copper (Cu), zinc (Zn), cadmium (Cd), and lead (Pb), were measured using inductively coupled plasma spectrometry. The calculation of event mean concentration (EMC) and pollutant removal efficiency are presented in Equations (1) and (2), respectively.

$$EMC = \sum \frac{(V_i C_i)}{C_i} \tag{1}$$

$$Removal Efficiency(\%)\left(\frac{EMC_{\infty low} - EMC_{outflow}}{EMC_{\infty low}}\right) \times 100 \tag{2}$$

where V_i is the volume of runoff for the i^{th} sample in liters, and C_i is the concentration of the pollutant in i^{th} sample in mg/L. The summation (Σ) extends over all samples collected during the event.

Table 1. Biodiversity indices used for analyzing bacterial distribution and abundance.

Diversity index	Formula	Description	Reference
Richness	R = number of categories, types, species, or classes.	Quantifies the number of types of species.	Jost (2006)
Abundance	Number of observations.	Identifies the number of individuals for each species.	Jost (2006)
Shannon Entropy/ Shannon-Weiner	$N = \sum_{i=1}^{R} P_i \ln P_i$ P_i: proportional abundance of the i^{-th} category.	Quantifies the uncertainty in predicting the species.	Shannon (1948)
Shannon Evenness/ Pielou's Index	$H_e = \frac{e^H}{R}$	Measure of the distribution of individuals among species.	Shannon (1997)
Simpson Dominance Index	$\lambda = -\sum_{i=1}^{R} P_i^2$	Provides more weight to dominant species.	Simpson (1949)
McIntosh Index	$D_{McIntosh} = \frac{N - \sqrt{\Sigma_{i=1}^{R} n_i^2}}{N - \sqrt{N}}$ n_i: number of observations of the i^{-th} category. N: number of observations	Expresses the heterogeneity of species in geometric terms	Thukral (2017)

2.3 Soil sampling for microbial and soil parameters analysis

Soil samples were collected from the inflow and outflow areas of the HSSF CW, designated as IS and OS, respectively. These samples, taken at depths of 0 to 10 cm and 10 to 20 cm, were gathered during each of the five sampling events conducted across different seasons and were analyzed for soil physicochemical properties and microorganism content. Additionally, soil samples from the nearby green spaces, referred to as SGS, were collected at a distance of at least 1 meter away from the HSSF CW. These samples were subjected to laboratory testing to determine the physicochemical characteristics of soil, including pH, electrical conductivity, moisture content, bulk density, TN, TP, and soil organic carbon (SOC). The TN and TP content of individual soils were quantified using the method proposed by Carter and Gregorich (2007), whereas the SOC content of the soil was determined using the Walkley-Black method. The Walkley and Black chromic acid wet oxidation method is widely utilized in quantifying the organic carbon content of soils (Reyes et al., 2024). The SOC content calculation using the Walkley-Black method is expressed in Equation (3):

$$SOC_w(\%) = \frac{(V_{blank} - V_{sample})\, x\, M_{Fe^{2+x0.003xfxmcf}}}{W} x f x mcf x 100 \qquad (3)$$

where SOC_w = Organic carbon content of soil obtained through the Walkley-Black Method, Vblank = volume of titrant in the blank (mL), Vsample = volume of titrant in the sample (mL), M_{Fe}^{2+} = concentration of standardized $(NH_4)_2\ Fe(SO_4)_2.6H_2O$ (molarity), 0.003 = carbon oxidized (constant), f = correction factor equivalent to 1.3, W= weight of soil (g), and mcf = moisture correction factor.

The TN, TP, and SOC content of samples from the inflow and outflow areas of the HSSF CW, as well as the OS, were compared to assess chemical properties and to determine the C, N, and P stoichiometric ratios in the soil. Moreover, soil microbial analysis was performed through 16S rRNA gene sequencing, utilizing Roche 454 pyrosequencing technology in accordance with the analytical methods outlined in the study of D'Argenio and Francesco (2015). This study employed various biodiversity indices to analyze the distribution and abundance of bacteria within the samples. Table 1 summarized the indices used, including their formulas and specific applications. The relationships of microbial populations

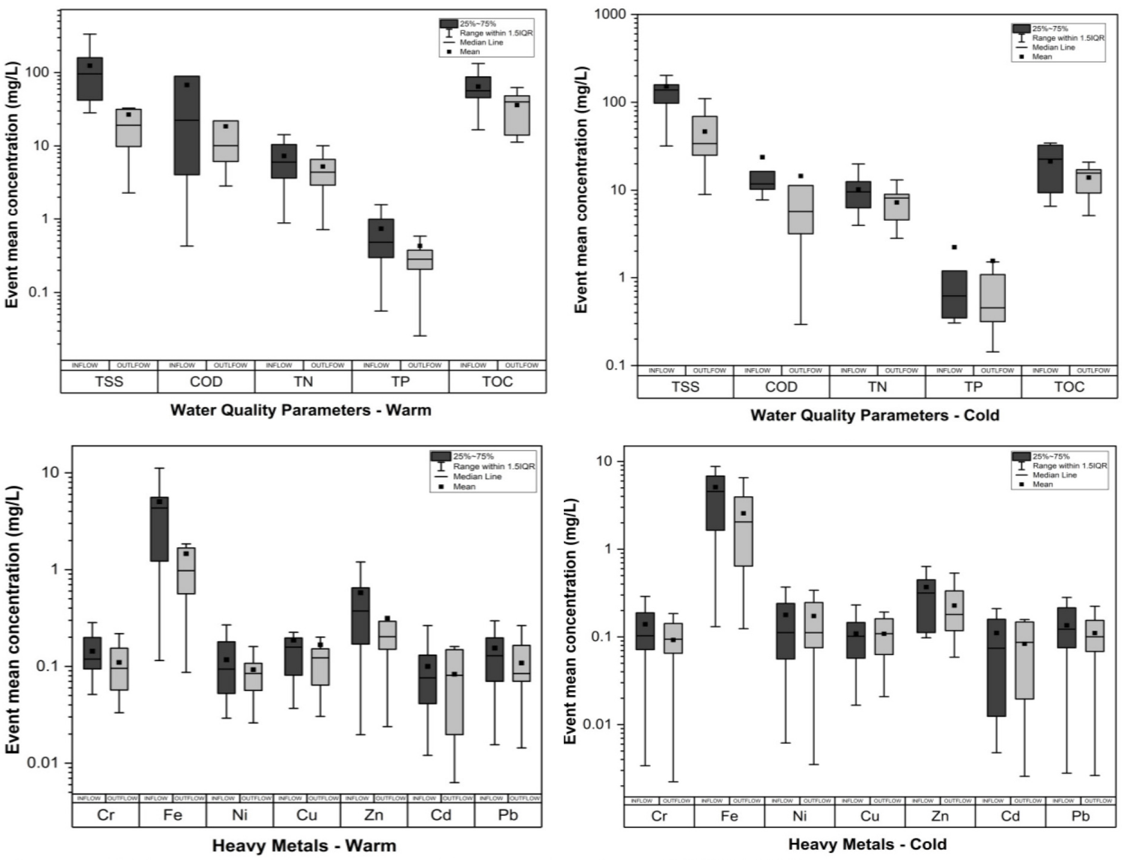

Fig. 2. Inflow and outflow water quality concentrations of HSSF CW.

within each phylum with various soil parameters were analyzed using the Spearman correlation coefficient (r). Prior to this analysis, the Shapiro-Wilk Test was employed to assess the normality of the dataset. However, the results of the Shapiro-Wilk Test revealed that the data were not normally distributed. As a result, non-parametric methods were considered appropriate for subsequent analyses. Significant differences between parameters were identified at a 95% confidence level, indicating a p-value of less than 0.05.

3. Results and Discussions

3.1 Water Quality Analysis and Pollutant Removal Efficiency

Parking lots and roads, as major sources of water pollution in urban areas, contribute a range of pollutants, including TSS, metals, anthropogenic organic compounds, nutrients, and microbial contaminants (Revitt et al., 2014). Figure 2 presented the EMC inflow (EMC_{in}) and EMC outflow (EMC_{out}) of stormwater samples during the warm and cold season for various pollutants. The observed differences in average TSS reduction (EMC_{in} − EMC_{out}) were 98 mg/L for warm season and 106 mg/L for the cold season. During the warm season, average EMC_{in} values for COD, TOC, Cr, Cu, Zn, and Pb, amounting to 67.8±102.6 mg/L, 64.5±36.9 mg/L, 0.14±0.08 mg/L, 0.19±0.20 mg/L, 0.58±0.63 mg/L, and 0.16±0.12 mg/L respectively, were higher compared to the EMC_{in} values for the same parameters recorded during the cold season. In the study area, the warm season is typically associated with more intense and frequent rainfall events, leading to increased runoff which leads to the transport of more organic materials from various sources, including roads, parking lots, and landscapes. Moreover, atmospheric deposition of pollutants is generally higher in the warm season due to increased volatilization and atmospheric chemical reactions, leading to greater deposition of organic compounds and heavy metals on urban surfaces that are subsequently washed off by stormwater (Davis et al., 2001; Sabin et al., 2006). Conversely, during the cold season, nutrient concentrations, specifically TN and TP, were found to be higher, with mean EMC_{in} values of 10.2±4.4 mg/L and 2.2±3.2 mg/L, respectively. The higher concentrations of TN and TP observed in this season could be attributed to reduced biological activity, leading to less nutrient uptake by microorganisms and plants. Normally, microorganisms and plants absorb these nutrients, helping reduce nutrient loads in the environment. However, diminished activity in the lower temperature leads to decreased nutrient uptake, resulting in the accumulation of nutrients in runoff water (Miller, 2014; Mesquita et al., 2017).

The summary of the pollutant removal efficiencies of HSSF CW facility during warm and cold seasons was depicted in Figure 3. Among the assessed water quality parameters, the facility demonstrated highest TSS removal efficiencies of approximately 75% during warm season and 70% in the cold season, indicating a relatively stable performance with a slight reduction in efficiency during colder months. The warm season exhibited higher removal efficiencies for COD, recorded at 70%, whereas the cold season showed a decrease in the treatment efficiency for this contaminant, dropping to 40%. Similarly, heavy metals, including Fe, Zn, Cd, and Pb, all exhibited higher removal efficiencies during the warm season, recorded at 71%, 46%, 33%, and

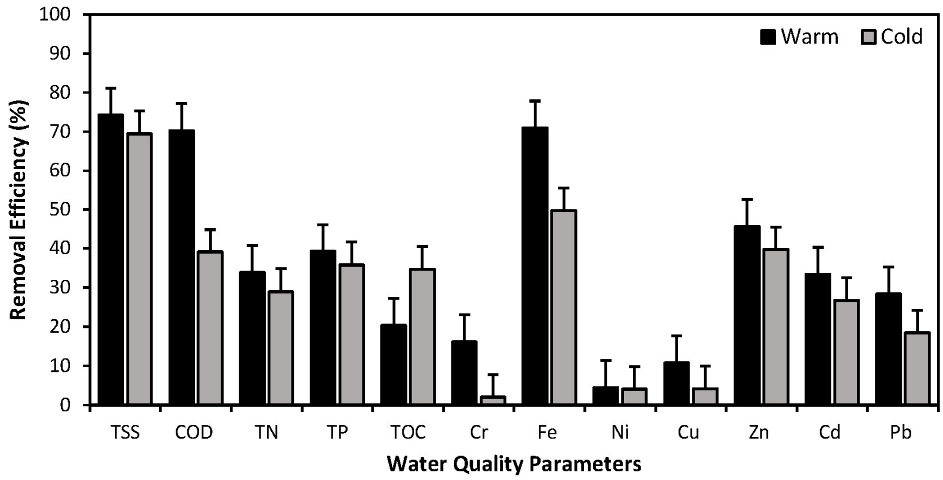

Fig. 3. Pollutant removal efficiency of HSSF CW in treating various pollutants.

28%, respectively, compared to the cold season, which recorded removal rates ranging from 18% to 50%. This reduction in efficiency during cold season might be attributed to slower microbial degradation processes at lower temperatures. Microbial processes are temperature-dependent, and the metabolic rates of the microbial community decrease as temperatures drop, leading to lower degradation rates of organic matter and reduced bioavailability of heavy metals for microbial uptake or adsorption (Boscolo-Galazzo et al., 2018). For both TN and TP, removal efficiencies where within the 29% to 40% range, exhibiting a slightly higher removal rate during the warm season. The high TP removal efficiency within the system could be due to the combined process of settling sediment-bound phosphorus and plant uptake. On the other hand, the slight reduction in TN removal rates during the cold season may be linked to a slowdown in nitrification and denitrification activities due to lower temperatures. Miller (2014) emphasized that microbial activities related to the nitrogen cycle are influenced by temperature, indicating that nitrogen removal processes are susceptible to seasonal temperature fluctuations. Overall, the high removal efficiencies of TSS, COD, and heavy metals were attributed to the combined physical, chemical, and biological processes within the HSSF CW, including settlement, adsorption, and microbial degradation (Schulz et al., 2003). The presence of a pre-treatment zone or sedimentation basin played a crucial role in these processes, where particulate-bound pollutants are settled out before reaching the main wetland body (Choi et al., 2021). This not only reduced the pollutant load but also prevented clogging and maintained the permeability of the wetland substrate, which is essential for effective stormwater treatment.

3.2 Soil characteristics in HSSF CW

Soils have the capacity to store carbon, with SOC serving as a key indicator of soil quality and productivity. SOC plays a vital role in nutrient cycling, soil structure, microbial activity, and water retention (Wiesmeier et al., 2019). In the analysis of soil characteristics within HSSF CW, the concentrations of SOC, TN, and TP were compared between the IS and OS of the facility, and the SGS, as illustrated in Figure 4. The SGS exhibited a lower SOC mean concentration of 13,340 mg/kg, compared to 44,685 mg/kg and 44,860 mg/kg in the IS and OS, respectively. The increased amount in SOC within the facility could be due to a higher rate of organic matter inputs and conditions conducive to its accumulation, influenced by the presence of bioceramics and woodchip filter media within the system. The higher SOC content in HSSF CW suggested a substantial carbon storage potential, which is approximately 3.3 times higher than that of the SGS. The average TN concentration in sediments collected from inflow part of HSSF CW amounted to 553 mg/kg, which was found to be 2.5 times greater than SGS. However, the TN concentration in the outflow part of the facility was reduced to approximately 23% of the IS level and 59% of the SGS level. This reduction in TN from the IS to OS may reflect the system's role in nitrogen management, primarily through mechanisms such as denitrification, plant uptake, and microbial assimilation. These processes convert nitrogen from its soluble forms in the inflow sediments to less soluble forms or gaseous nitrogen, thereby reducing its concentration by the time water passes through to the outflow (Vymazal, 2007). TP concentrations were relatively stable across the different

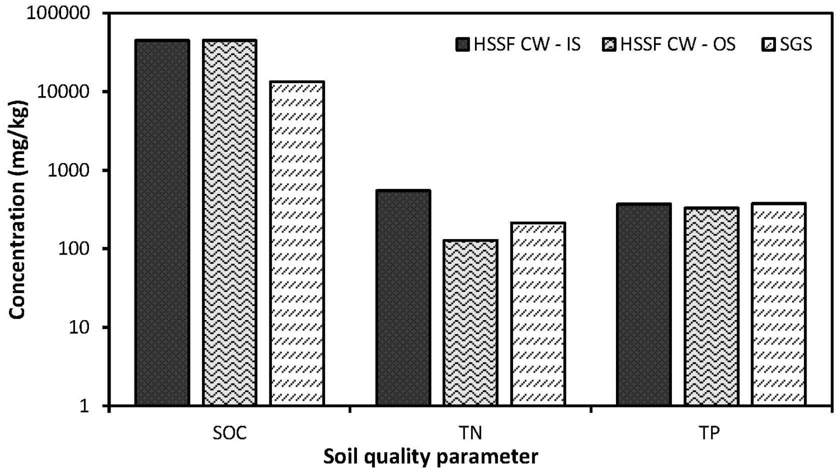

Fig. 4. Comparison of selected chemical parameters between HSSF CW soils and soils from nearby green spaces.

soil samples, with rates ranging from 331 to 380 mg/kg. The consistency of TP levels suggested a potential equilibrium within the system, where phosphorus is tightly bound to soil or sediment particles (Geronimo et al., 2021) and is thus less affected by the wetland's filtering process.

The C:N:P stoichiometry in soils greatly determines nutrient availability for plants and soil microorganisms (Vitousek and Farrington, 1997) and functions of the terrestrial ecosystem (Zheng et al., 2021). The C:N ratio is particularly indicative of the quality of organic matter and the nitrogen availability for plants and microorganisms. Ratios above 20 suggest nitrogen limitation for microbial activity, while ratios below 20 suggest a carbon limitation in the environment (Bengtsson et al., 2003). In this study, all C:N ratios exceeded 20, indicating a lack of N inputs in soil. The C:N ratio in both the IS (81:1) and OS (352:1) was considerably higher than that in the SGS (62:1) suggesting a relative abundance of carbon in the CW system. This abundance could lead to nitrogen being a limiting nutrient for microbial processes, such as decomposition and denitrification (Xu et al., 2012). The C:N:P ratio is a comprehensive measure for evaluating nutrient dynamics and microbial activities in soils. This ratio not only affects nutrient cycling but also contributes to the heterogeneity of SOC stocks (Huang et al., 2021). Studies from Abou-Shanab (2011), Ying et al. (2019), Ouriache et al. (2020) and Kebede et al., (2021) suggested that a C:N:P ratio of 120:10:1 is optimal for maximizing the bioremediation potential of microorganisms. However, in this study, the C:N:P ratios for the IS (120:1.5:1) and OS (135.2:0.4:1) indicated a skewed balance, particularly with an extremely low proportion of N and P compared to C, which may challenge microbial remediation efficiency. The SGS had a C:N:P ratio of 35:0.6:1, which is closer to balanced conditions but still suggested potential limitations in N and P. The ratio of N and P to C was relatively low, especially when considering the ideal C:N:P ratio (120:10:1), implying that there may not be enough N and P to meet the needs of plants and soil microbes. In general, the analysis suggested that both HSSF CW and SGS are lacking in sufficient nutrient inputs. Therefore, supplementing these soils with nutrients could be essential to achieve a balanced nutrient ratio, hence improving the biogeochemical processes within urban soils.

3.3 Behavior and interactions of microbes in HSSF CW

3.3.1 Changes in microbial populations

A fundamental characteristic of CWs is that their functions are largely governed by the interactions of microorganisms and their metabolic activities. These interactions are particularly sensitive to environmental conditions such as temperature. Figure 5 presented a comparative analysis of the microbial counts in the soil samples collected from the inflow and outflow sections of a HSSF CW during the warm season (>15° C) and the cold season (≤15° C). The warm season observed the highest total microbial populations for both inflow (47,356 counts) and outflow soil (43,565 counts). These findings suggested that warm temperatures favor higher bacterial growth rates, which is consistent with the understanding that microbial enzymatic activities and nutrient uptake tend to increase with temperature (Singh and Chandra, 2014). Conversely, during the cold season, there was a noticeable decline in total bacterial populations,

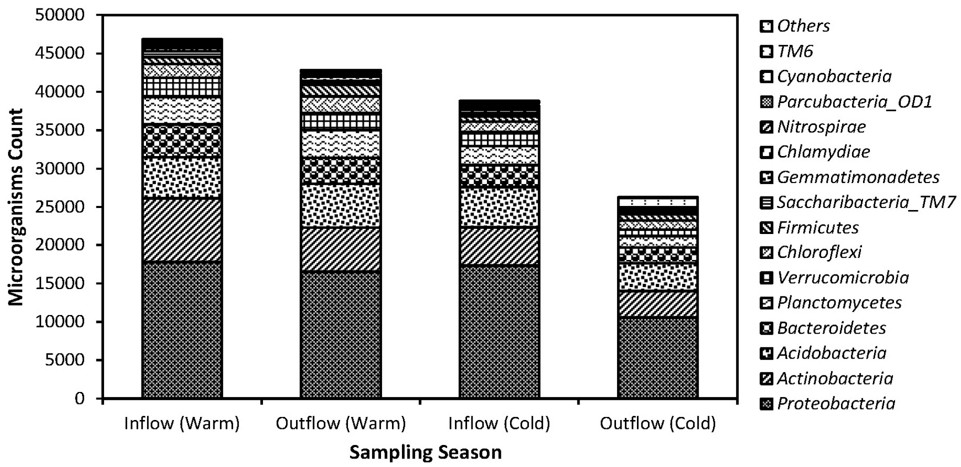

Figure 5. Changes in microbial population with respect to temperature.

with a 17% decrease in the inflow and a 39% decrease in the outflow. The reduction during the cold season in the study area, typically associated with the transitional periods of autumn and spring, may be influenced by several factors. In spring, the growth of plants during this time may lead to increased competition for nutrients, as plants actively absorb N and P for new growth, potentially leaving less available for soil microorganisms (Shigyo et al., 20198; Wu et al., 2020). The autumn season is marked by litterfall, which alters the composition of soil organic matter. While this newly added organic material can provide a fresh source of C for soil microbes, the breakdown of complex litter compounds is a slower process (Giweta, 2020). Although there has been a slight decrease in the presence of microorganisms from warm to cold season, it was previously observed that CW soils contained considerable amounts of SOC, thus promoting efficient bacterial growth despite seasonal variations.

Proteobacteria is the most dominant microorganism phylum in both seasons, comprising 38% of the total bacterial population during the warm season. In cold temperatures, the fraction of *Proteobacteria* in inflow and outflow soil, with respect to the total bacterial population, increased to 44% and 68%. This is in line with previous studies reported phylum *Proteobacteria* to be among the most abundant in terrestrial soil (Miyashita 2015; Montecchia et al., 2015; Kim et al., 2021). *Proteobacteria* are a diverse group of Gram-negative bacteria that play various roles in ecological processes, including nutrient cycling and contributing to soil fertility, plant growth, and development (Mhete et al., 2020). *Actinobacteria*, known for their role in organic matter decomposition, also recorded a relative contribution to the total bacterial population in both seasons ranging from 13% to 21%. The fraction of *Acidobacteria*, *Bacteroidetes* and *Planctomycetes* with respect to the total microbial population, were recorded as 11% to 21%, 7% to 12%, and 6% to 13%, respectively. *Acidobacteria* are known for their ability to thrive in low-pH environments and contribute to soil nutrient cycling (Barns et al., 1999). *Bacteroidetes* are involved in the degradation of complex organic compounds (Pinnell et al., 2022), whereas *Planctomycetes* are known for their unique cellular structures and roles in nitrogen cycling and carbon metabolism in various environments (Buckley et al., 2006).

3.3.2. Correlations between soil properties, water quality, and microbial community

Spearman's rho correlation was used to analyze non-linear relationships between the abundance of dominant bacterial phyla and water quality parameters, with results summarized in Table 2. *Proteobacteria*, the most dominant bacterial phylum, showed a positive strong correlation only with TP (r = 0.7), indicating a potential involvement in nutrient cycling. Similarly, *Acidobacteria* and *Actinobacteria* showed similar strong positive correlations exclusively with TN (r = 0.7). *Bacteroidetes* were negatively correlated with pH (r = −0.8) and COD (r = −0.9), implying a decrease in their abundance with increasing pH and organic load. However, the same bacteria exhibited high positive correlations (r values:

Table 2. Correlation between water quality parameters and abundant bacterial phyla.

Bacteria	pH	Cond	TSS	COD	TOC	TN	TP
Proteobacteria	−0.3	0.4	0.3	0.4	0.3	0.6	0.7
Actinobacteria	−0.4	0.3	0.5	0.3	0.4	0.7	0.4
Acidobacteria	−0.4	0.3	0.5	0.3	0.4	0.7	0.4
Bacteroidetes	−0.8	0.1	0.7	−0.9	0.8	0.9	0.8
Planctomycetes	−0.7	0.1	0.8	0.5	0.7	0.9	0.3
Verrucomicrobia	−0.8	−0.4	1	0.6	0.8	0.9	0.3
Chloroflexi	0	0.1	0.3	−0.1	0	0.4	0.3
Firmicutes	−1	0.1	0.8	−0.9	1	0.9	0.3
Saccharibacteria_TM7	−0.9	0.2	0.6	1	0.9	0.8	0.6
Gemmatimonadetes	−0.8	0.1	0.7	−0.9	0.8	0.9	0.8
Chlamydiae	0.1	−0.2	0.1	0.2	−0.1	0.2	0.9
Nitrospirae	−0.3	0	0.3	0.6	0.3	0.5	1
Parcubacteria_OD1	−0.1	0.3	−0.1	0.5	0.1	0.5	0.9
Cyanobacteria	0.2	−0.9	0.2	−0.1	−0.2	−0.1	0.3
TM6	0.1	−0.2	0.1	0.2	−0.1	0.2	0.9

NOTE: Values in dark gray cells indicate a strong correlation; values in light gray cells denote a moderate correlation; and values in white cells suggest weak to no correlation.

0.7 to 0.9) with other water quality parameters except for conductivity. *Verrucomicrobia* displayed a perfect positive correlation with TSS (r = 1), indicating a possible preference for, or resilience in, turbid conditions. This could be due to their role in cellulose degradation, where turbidity often results from organic particulates (Bao et al., 2019). *Firmicutes* were strongly negatively correlated with pH (r = −1) and COD (r = −0.9) yet demonstrated a strong positive correlation with TN (r = 0.9) and TOC (r = 1). The negative correlation with pH may indicate an optimal acidic environment for *Firmicutes*, while their positive correlation with TOC and TN indicates an active role in the decomposition of organic matter and nutrient cycling in the soil (Tian et al., 2021). *Saccharibacteria_TM7*, revealed strong positive correlations with COD, TOC, TN, Cr, Cd, and Pb with correlation coefficients ranging from 0.8 to 1. Similarly, *Gemmatimonadetes* displayed a positive correlation with TOC, TN, TP, Cr, and Cd, with r values between 0.7 and 0.9. Additionally, strong positive correlations (r values: 0.7 to 0.9) were observed between Ni and *Cyanobacteria*, as well as between Cr and the phyla *Bacteroidetes* and *Firmicutes*, while Cd demonstrated a perfect positive correlation with *Firmicutes*. Among the microbial phyla, *Chlamydiae, Nitrospirae*, *Parcubacteria_OD1* and *TM6* exhibited very strong correlations with TP (r values: 0.9 to 1). In particular, *Nitrospirae* showed a perfect positive correlation with TP, which may be associated with their enrichment in the inner layers of biofilms where nitrification conditions are favorable, characterized by lower oxygen levels and the presence of suitable substrates (Zhou et al., 2023).

Table 3 presented the correlation analysis between the dominant microbial populations and soil quality parameters. Only a few microorganisms exhibited high positive and negative correlations with soil parameters, specifically pH, conductivity, TN and TP. *Proteobacteria* were found to have a strong negative correlation with TP (r = −0.83), while no correlation was found with TN. *Proteobacteria* accounts for 34.2% in the wetland rhizosphere, indicating their abundance despite low phosphorus levels (Cao et al., 2018). Moreover, Warren (2020) found that *Proteobacteria* populations increase in less phosphorus environments due to soil microbial populations substituting phospholipids with betaine lipids allowing them to maintain growth and cellular functions where phosphorus is limited. *Verrucomicrobia* exhibited extremely strong positive correlations with pH (r = 0.89) and conductivity (r = 0.94), indicating their preference for specific soil pH conditions and ionic environments. Similarly, *Bacteroidetes and Saccharibacteria_TM7* showed high positive correlation with pH (r values: 0.73 to 0.94), whereas *Acidobacteria, Firmicutes* and *Gemmatimonadetes* were found to be positively correlated with conductivity (r values: 0.71 to 0.94). Among the microorganisms, only *Acidobacteria* and *Chlamydiae* revealed a strong positive correlation with moisture content, with r values of 0.77 and 0.74, respectively, while only *Planctomycetes* exhibited strong correlation with LOI (r = 0.71). A strong negative correlation was observed between *Cyanobacteria* abundance and SOC (r = −0.77). Vijayakumar et al.,

Table 3. Correlation between soil physicochemical properties and phylum bacterial populations.

Bacteria	pH	Cond	Temp	MC	LOI	SOC	TN	TP
Proteobacteria	0.49	0.60	−0.14	0.31	0.49	0.43	0.00	−0.83
Actinobacteria	0.49	0.43	−0.43	0.03	0.49	0.09	−0.40	−1.00
Acidobacteria	0.14	0.71	0.09	0.77	0.60	0.54	−0.20	−0.26
Bacteroidetes	0.83	0.43	−0.71	−0.60	0.26	−0.60	−0.40	−1.00
Planctomycetes	0.20	0.60	−0.14	0.66	0.71	0.54	−0.20	−0.26
Verrucomicrobia	0.89	0.94	−0.49	−0.03	0.66	−0.09	−0.40	−0.77
Chloroflexi	−0.14	0.20	−0.09	0.26	0.26	−0.09	−0.80	−0.31
Firmicutes	0.49	0.83	−0.26	−0.03	0.26	−0.43	−0.80	−1.00
Saccharibacteria_TM7	0.94	0.66	−0.66	−0.43	0.37	−0.37	−0.40	−1.00
Gemmatimonadetes	0.66	0.94	−0.43	0.03	0.49	−0.26	−0.80	−1.00
Chlamydiae	−0.06	0.34	0.17	0.74	0.46	0.57	0.40	0.14
Nitrospirae	−0.26	0.31	0.09	0.66	0.43	0.14	−0.40	0.14
Parcubacteria_OD1	−0.14	0.03	−0.03	−0.03	−0.03	−0.54	−0.80	−0.09
Cyanobacteria	−0.03	−0.09	0.14	−0.43	−0.49	−0.77	−0.40	−0.37
TM6	0.43	0.14	−0.03	0.20	0.31	0.49	0.80	0.09

NOTE: Values in dark gray cells indicate a strong correlation; values in light gray cells denote a moderate correlation; and values in white cells suggest weak to no correlation.

(2007) reported that *Cyanobacteria* can thrive with less organic content due to favorable conditions including oxidizable organic matter, high calcium, abundant nutrients, and low dissolved oxygen levels. The abundances of *Chloroflexi, Firmicutes, Gemmatimonadetes* and *Parcubacteria_OD1* were negatively correlated with TN (r = −0.80). Among the microbial phyla, *Actinobacteria, Bacteroidetes, Firmicutes, Saccharibacteria_TM7* and *Gemmatimonadetes* were found to have perfect negative correlation (r = −1) with TP. Although *Bacteroidetes* and *Saccharibacteria_TM7*, demonstrated a high positive correlation to pH, a negative correlation with TP suggested that while these bacteria thrive in more alkaline conditions, their populations may decrease in soils with high phosphorus content. The phyla's response to soil pH and TP could be related to their metabolic processes, as some studies, such as Shimizu (2016), suggested that certain members of this group are sensitive to soil nutrient levels. Generally, these correlations highlighted the relationships between bacterial populations and soil physicochemical properties, revealing how microorganisms adapt to and influence their habitat.

3.3.3. Microbial biodiversityin HSSF CW

Microbial biodiversity maintains the ecological balance in the soil ecosystem which helps in the sustainability of constructed wetlands. Interactions between microorganisms, soil components, and vegetation facilitate the cycle of nutrients which enhance the soil ecosystem and food web in CWs, thus, monitoring of microbial biodiversity is essential to having a sustainable and healthy wetland ecosystem (Wang et al., 2022). Biodiversity indices are quantitative measures or statistical representations of biodiversity which exhibits the variety of life in a community such as richness, abundance, spread or evenness, as well as dominance of species. Considering the abundance and richness components of diversity, four different biodiversity indices were used in this study to calculate the microbial phyla diversity and indicate the different aspects of the functional diversity of microorganisms living in the HSSF CW, as depicted in Figure 6.

Shannon Index, often referred to as the Shannon-Weiner Index, is used to quantify the diversity within an ecological community by considering the richness or number of phyla living in the HSSF CW and the evenness or how evenly the individuals are distributed among these different phyla (Zhao et al., 2012). Simpson Index manifests the most common phyla and estimates the dominance of these microorganisms in the community. On the other hand, McIntosh Index measures the homogeneity of phyla, whereas Pielou's Index measures the evenness of the microbial community in the HSSF CW. For Shannon Index, a higher diversity was observed during warm season (1.981 ± 0.100) than the cold season (1.825 ± 0.279) due to higher richness and abundance of microbial community. On the other hand, Simpson Index manifested a higher mean in the cold season (0.820 ± 0.035) than in the warm season (0.793 ± 0.065) due to the dominance of certain microbial phyla in the HSSF CW. McIntosh Index, which measures the homogeneity of microbial phyla in the community depicted a higher value in the warm season (0.448 ± 0.073) than in the cold season (0.413 ± 0.054) which implied a more homogenous microbial community. Finally, for Pielou's Evenness Index, warm season (0.518 ± 0.039) exhibited a more even microbial community than cold season (0.495 ± 0.054) due to higher index value.

4. Conclusion

The interactions between microorganisms, nutrients, soil properties, and vegetation influence the effectiveness of HSSF CW in managing urban stormwater runoff.

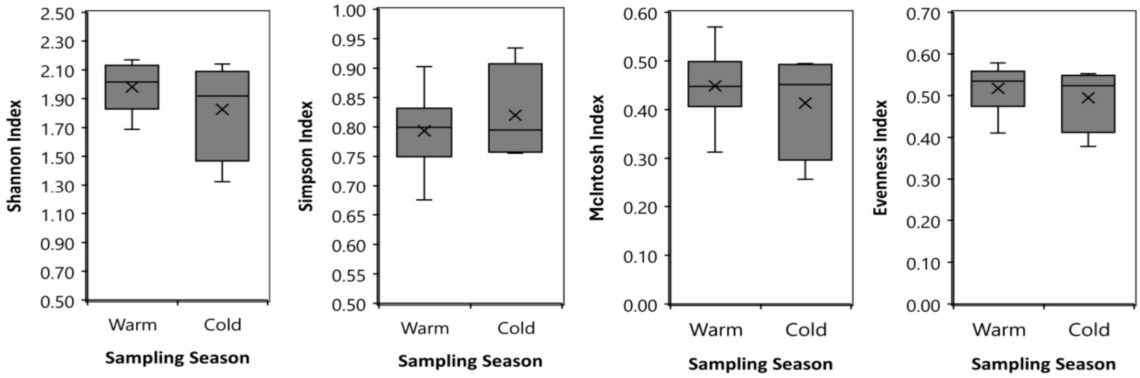

Fig. 6. Microbial biodiversity indices for warm and cold season sampling in HSSF CW.

Seasonal variations impact microbial activities, population growth, biodiversity, and nutrient cycling, all of which affect pollutant removal efficiency within the wetland ecosystem. The facility demonstrated higher removal efficiencies during the warm season, achieving 74% for TSS, 70% for COD, 34% for TN, 39% for TP, and 28% to 71% for specific heavy metals, including Fe, Zn, Cd, and Pb. Conversely, the removal efficiency for TOC was higher during the cold season. These high removal rates were attributed to the combined physical, chemical, and biological processes within the HSSF CW that includes settlement, adsorption, and microbial degradation. Moreover, the HSSF CW soil displayed a higher SOC content of approximately 3.3 times that of the OS. This increase in SOC content can be attributed to organic matter inputs from stormwater runoff and the presence of organic filter media such as bioceramics and woodchips. The stoichiometry ratios of C:N:P within HSSF CW were recorded as 120:1.5:1 and 135.2:0.4, indicating a low ratio of N and P relative to C. This suggested potential nutrient imbalances, with N and P possibly insufficient to meet the demands of plants and soil microbes, especially when compared to the ideal ratio of 120:10:1. Such imbalances may affect microbial remediation efficiency and overall system sustainability. *Proteobacteria* was the most dominant microbial phylum in both seasons, comprising of 38% to 68% of the total bacterial population. The fractions of *Actinobacteria*, *Acidobacteria*, and *Bacteroidetes* also made significant contributions to the total bacterial population, ranging from 13% to 21%, 11% to 21%, and 7% to 12%, respectively. These bacteria phyla exhibited moderate to strong correlations with specific soil physicochemical properties and changes in influent-effluent concentrations of water quality parameters. The biodiversity of the microbial community within the HSSF CW was also established to be temperature-sensitive as it exhibited higher abundance, richness, diversity, homogeneity, and evenness during the warm season, as manifested from biodiversity components and indices. These findings provide a deeper understanding of the design considerations and treatment mechanisms to enhance the efficiency and sustainability of HSSF CW systems for urban runoff treatment.

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