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Assessment of microbial activity in LID facilities affecting nonpoint source pollutant treatment

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ABSTRACT

Human and automobile activities have discharged various types of pollutants into the water bodies through urban stormwater runoff, producing water contamination and damaging the aqua-ecosystem. In order to manage such nonpoint pollution sources, low impact development (LID) facilities are utilized; however, LID design incorporating ecological functions was difficult due to the lack of research on biological functions. Thus, the objective of this study was to design an effective environment for LID technology through long-term monitoring results of Horizontal subsurface flow wetland (HSSF) facilities with vegetation and an infiltration trench (IT) without vegetation and comparison of microorganism growth in the sediments captured in the filter media. For IT, the major mechanism was found to be the physicochemical mechanism in the pre-treatment tank (infiltration function) and media; the major mechanisms for HSSF were found to be the physical filtration through media and the biological function of the wetland plants. Pollutant-containing stormwater runoff passes through the media part, where the physical filtration in the media and the biological function of the wetland plant life alter the forms and types of the pollutants, resulting in changes in the phyla and count of microorganisms discovered. Using woodchips as the media and planting plants with deep roots were effective means of enhancing the biological processing capability of stormwater runoff with low organic content. Also, the habitat conditions of Gemmatimonadetes and Acidobacteria for a stormwater runoff with high concentrations of phosphorus, Nitrospirae for high nitrogen content, and Actinobacteria and Firmicutes for high metal concentrations were reflected in the LID facility design and this method was evaluated as effective. When processing stormwater runoff with high degradable organic content. By considering the nutrients (pollutants) and habitat conditions of each microorganism in the LID facility design, the biological removal efficiency can be improved.

Keywords: Low impact development; Non-point source pollution; Soil microorganism; Plants

1. Introduction

Generally, an ecosystem composed of organisms and the environment maintains its health and sustainability through pollutant cycling and energy flow. Various pollutants discharged into the ecosystem change in form and concentrations through physicochemical and biological mechanisms. Such natural purification capabilities are inherent to the ecosystem and are carried out through pollutant cycling and energy flow. Thus, biodegradation by living

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organisms including plants, microorganisms, and animals in the environment (soil, water, etc.) and physicochemical reactions with the soil particles are important mechanisms for natural purification. In general, thousands of microorganisms such as Proteobacteria (PTB), Acidobacteria (ACB), Actinobacteria (ATB), Chloroflexi (CF), and Planctomycetes (PTM) exist in the soil and water, and their type and count vary according to the influent pollutant type and physical environment conditions [1]. Growth of Proteobacteria is active in soil and water with a significant influx of nitrogen components, while the growth of ACB is active in acidic soil, where ACB was reported to exhibit good growth in harsh environments [2–5]. On the other hand, CF is known to decompose halogenated organics well, and PTM is an aquatic microorganism shown to have good growth at low temperatures [6]. Numerous microorganisms grow in the soil and water and contribute to the influent pollutant decomposition, converting organic pollutants into inorganic pollutants or absorbing inorganic pollutants thereby removing such pollutants [7,8].

The diverse developments and activities initiated by mankind produce pollutants that do not naturally exist in the ecosystem which are difficult to remove and that exist in quantities exceeding the removal capacity of the ecosystem; such pollutants deteriorate the health and sustainability of the ecosystem [9–11]. Human development and activities result in urbanization and industrialization induce various types of land development including commercial, residential, industrial, transportation, and public sectors. These land uses accumulate diverse pollutants during the dry season and discharge pollutants, due to high material imperviousness, in significant amounts during rainfall events. The polluted discharges were directed to water bodies and cause water pollution and aquatic ecosystem damage; such pollutants are called nonpoint pollutant sources. Nonpoint pollutant sources produced in urban areas include suspended solids, organics, inorganics, and various chemical substances. Each type of land use produces different pollutant forms and concentrations.

Methods of managing the various pollutants produced from nonpoint pollutant sources include low impact development (LID), sustainable urban drainage system, water sensitive urban design, and green infrastructure; different nomenclatures are employed in different countries. All these methods decrease imperviousness, increase green space and permeability, and increase evapotranspiration and water storage to recover natural water circulation as well as remove nonpoint pollutant sources. LID includes various technologies including rain gardens, bioretention, infiltration trenches (IT), tree box filters, infiltration planters, constructed wetlands, permeable pavement, and green roofs [12]. Common components of all LID methods include plants, microorganisms, and filter media (including the soil); the water circulation and pollutant removal capacity are expressed through the physicochemical and biological interaction of the components. Thus, LID technology refers to sites selectively composed of ecosystem components to maximize the natural purification of the ecosystem. However, existing LID technology guidelines only require the consideration of physical mechanisms in the technology design. For effective implementation of natural purification mechanisms, LID technology design integrating organisms (microorganisms, plants, etc.) and the environment (soil, media, habitat environment, etc.) is necessary. When such an integrated design is carried out, LID technology that reacts efficiently to the influent composition can be designed. However, research on biological mechanisms occurring within LID facilities and ecological aspects for LID design was still lacking and therefore needs to be improved. Therefore, in this study, an optimized, efficient environment design solution for LID technologies is proposed by performing long-term monitoring of LID technologies of different configurations to compare the obtained influent compositions, influent pollutant removal capabilities, and microorganism activities in the sediments captured in the filter media.

2. Materials and methods

2.1. Site description

In order to analyze the removal efficiency of the LID facility in terms of microorganism activity, two different LID facilities were selected for this study. Particularly, the IT and horizontal subsurface flow wetland (HSSF) were selected for monitoring to analyze different influent compositions and microorganism growth considering the presence of vegetation. Fig. 1 shows the monitored facility installed in a university campus located in Cheonan city. IT was established in 2009 while HSSF was established in 2010; together, more than 6 y of monitoring data has been collected. Urban stormwater runoff from the road flows into the IT; this is a facility composed of a sedimentation tank and a media part without plants (Table 1). On the other hand, HSSF received stormwater runoff from a combined parking lot and road catchment; the facility was designed to have a sedimentation tank and media part with plants.

2.2. Runoff monitoring and analyses of LID technologies

Long-term monitoring was conducted to assess the water quality improvement and flow characteristics and relate it to microorganism activity in IT and HSSF facilities. A total of 44 storm events were monitored for IT starting from May 2009 while 31 storm events were monitored for HSSF starting from July 2010. The runoff before entering the facility and eventually discharged by the facility was collected to assess the efficiency of the facilities. Water samples were analyzed for total suspended solids, organics in the form of chemical oxygen demand (COD), nutrients including total nitrogen (TN) and total phosphorus (TP), and total heavy metals including chromium (Cr), iron (Fe), nickel (Ni), copper (Cu), zinc (Zn), cadmium (Cd) and lead (Pb). Water samples were initially collected in short intervals of 5, 10, 15, and 30 min, followed by collection at 1 h intervals until stormwater event termination. This water quality monitoring method was applied to consider the first flush phenomenon of land use with high imperviousness. Using the water quality characteristics and flow data collected through the monitoring, the event means concentration (EMC) was calculated using Eq. (1) and the pollutant removal efficiency was calculated using Eq. (2) [13].

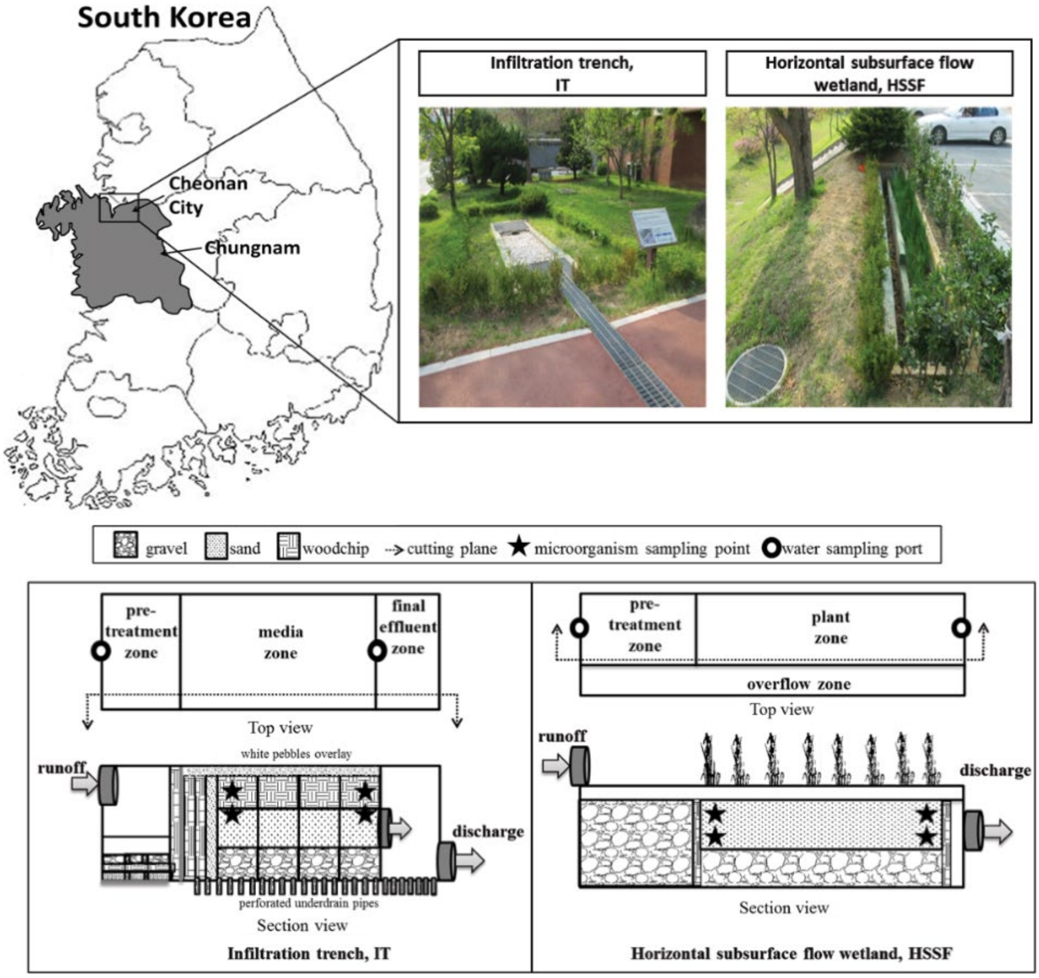


Fig. 1. Location and schematic diagram of the LID facility.

Table 1
Summary of the characteristics of the LID facilities

	Infiltration trench (IT)	Horizontal subsurface flow wetland (HSSF)
Year constructed	2009	2010
Infiltration capability	Yes	No
Vegetation	No	Iris
Media	Sand, gravel, woodchip	Sand, gravel, woodchip
Runoff source	Road	Road and parking lot
Catchment area, m^2	371	323
SA/CA, facility surface area to catchment area ratio %	1.3	1.6
SV/TV, facility storage volume to total volume ratio %	45.4	33.9

Here, $C(t)$ refers to the pollutant concentration considering rainfall duration, $Q_{\text{TRu}}(t)$ refers to the runoff rate according to the duration time t, $C_{\text{in}}(t)$ refers to the inflow water quality concentration according to the rainfall duration time t, $q_{\text{in}}(t)$ refers to the inflow rate according to the rainfall duration time t, $C_{\text{out}}(t)$ refers to the outflow water quality concentration according to the rainfall duration time t, and $q_{\text{out}}(t)$ refers to the outflow rate according to the rainfall duration time t.

$$\text{EMC} = \frac{\int_0^T C(t)\cdot Q_{\text{TRu}}(t)dt}{\int_0^T Q_{\text{TRu}}(t)dt} \tag{1}$$

$$\text{Pollutant removal efficiency}(\%) = \frac{\sum_{t=1}^{t=T} C_{\text{in}}(t) q_{\text{in}}(t) - \sum_{t=1}^{t=T} C_{\text{out}}(t) q_{\text{out}}}{\sum_{t=1}^{t=T} C_{\text{in}}(t) q_{\text{in}}} \times 100 \quad (2)$$

2.3. Soil microorganism sampling of the LID facilities

Since the microorganism biological pollutant processing mechanism at IT and HSSF occurs in the media part, samples for microorganism analysis were collected at the initial 10 cm of the media part after the inlet and 10 cm of the media part before the outlet. The flow of influent water into the media part varies with the rainfall depth, but generally, it flows horizontally and vertically from the inlet to the outlet. Therefore, the top and bottom layers of the media part have different properties including water content, temperature, and pollutant concentration. As such, sample collections for microorganism analysis were conducted at the top and bottom layers of the sample points near the inlet and outlet of the media part. The top layer of the IT facility was composed of woodchips and sand. In order to compare the microorganisms within the soil of the LID facilities, and in the landscape area distant from direct runoff, microorganism analysis was performed by collecting in situ soil 1 m away from each facility. 16S rRNA gene sequence was used in the Roche 454 pyrosequencing technology for the sequencing and handling of soil and media samples. The polymerase chain reaction method was employed to analyze the microorganism type and frequency. Table 2 shows the microorganism types and acronyms used in this study.

3. Results and discussion

3.1. Runoff monitoring results

Fig. 2 shows the characteristics of the stormwater runoff entering IT and HSSF, along with the pollutant removal efficiency of each facility. Comparing the inflow EMC (EMCin) of IT and HSSF, suspended solids, organics, and heavy metals in the stormwater runoff discharged from the road were found to be higher by 1.3 to 2.8 times compared to parking lot due to the frequency of vehicular activity on the road. For both IT and HSSF, it was observed that the outflow EMC was relatively less than EMCin. The pollutant removal in IT was higher than the HSSF facility caused by the various components employed in IT including the sedimentation tank, media capabilities, infiltration mechanism, and microorganism activity. Major pollutant removal mechanisms in IT were sedimentation, infiltration, physical absorption and filtration by the sand and woodchip media, and biological pollutant removal through microorganism respiration within the media. Major pollutant removal mechanisms in HSSF were sedimentation, physical filtration by the sand media, phytoremediation through plants, and biological pollutant removal through microorganism respiration in the media. Among the pollutant removal mechanisms employed in IT and HSSF, sedimentation, adsorption, and filtration in the sedimentation tank and by the media part were found to be dominant among the various pollutant removal mechanisms. However, the higher TN removal efficiency of HSSF compared to IT was determined to be influenced by the phytoremediation potential of wetland plants, appropriate media water content, and microorganisms distributed in the rhizosphere soil of plants.

3.2. Comparison of microorganisms in media layers

The stormwater runoff entering the LID facility flows horizontally and vertically through the media part where pollutant forms and amount change. Fig. 3 provides a comparison of the microorganism types and quantities measured from the inlet and outlet of the media parts for the IT and HSSF facilities and nearby soil samples. Results showed that microorganism phylum and count differed at the media part inlet and outlet due to the water quality. Approximately 30 identified microorganism species and hundreds of unidentifiable microorganism species existed in the in situ soil near the LID facilities and the filter media employed in the LID facility.

Among the 30 microorganism phylum found in IT, five phyla such as Bacillariophyta (BRP), Deinococcus-Thermus (DT), Lentisphaerae (LP), Thaumarchaeota (TC),

Table 2
List of the microorganism name acronyms

Name	Acronym	Name	Acronym	Name	Acronym
Proteobacteria	PTB	Chlamydiae	CL	Acidobacteria	ACB
Cyanobacteria	CB	Actinobacteria	ATB	Elusimicrobia	EM
Chloroflexi	CF	Chlorobi	CRB	Planctomycetes	PTM
Armatimonadetes	ATD	Bacteroidetes	BTD	Euryarchaeota	ECT
Verrucomicrobia	VCM	Streptophyta	SPT	Gemmatimonadetes	GMD
Chlorophyta	CRP	Nitrospirae	NS	Bacillariophyta	BRP
Firmicutes	FM	Crenarchaeota	CC	Spirochaetes	SCT
Xanthophyceae	XTP	Fibrobacteres	FBB	Tenericutes	TRC
Deinococcus-Thermus	DT	Lentisphaerae	LP	Thaumarchaeota	TC
Fusobacteria	FSB	Loukozoa	LKZ	Phaeophyceae	PPC
Deferribacteres	DFB				

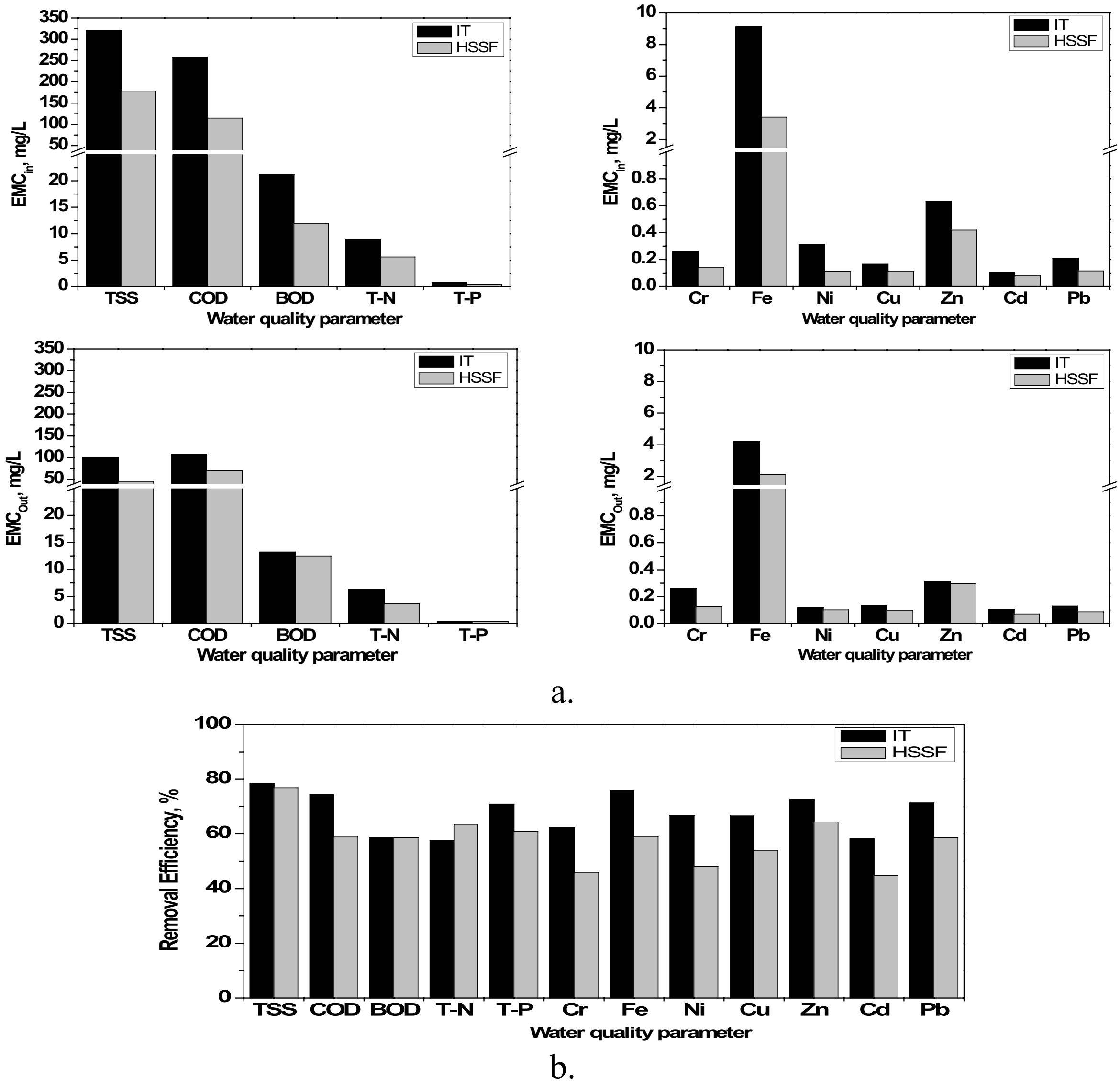


Fig. 2. Plots of (a) event mean concentration (EMC) and (b) pollutant removal efficiency of LID facilities.

and Deferribacteres (DFB) inhabited the inlet but were not found in the top media layer of the outlet. Meanwhile, Tenericutes (TRC) was found in the inlet but not observed in the outlet for the bottom media layer. For HSSF, DT was retained only in the bottom layer media layer of the outlet. Although these microorganisms were not dominant species found in each facility and media, they were observed or removed as a result of the pollutant characteristics and growth environment of the inlet. Among the observed 30 microorganism species, BRP, which is a phytoplankton variant, and DT, which is a microorganism that can survive in harsh environments like sunlight, were found in the inlet top media layer of IT but were found to have been removed from the outlet due to lack of nutrients. The two phyla were ranked 19th and 24th in terms of count among the 30 discovered microorganisms. Also, the microorganism phylum LP, which was commonly parasitic in the intestines of mammals, exhibited active growth in the inlet media part influenced by the stormwater runoff characteristics. TC, affected by the nitrogen and carbon compounds in the inlet, plays a key role in biogeochemical cycles such as the nitrogen and carbon cycles. DFB is an obligate thermophile and has been reported to be a microorganism that exhibits heavy metal removal capabilities and utilizes nitrates as electron acceptors [14].

Microorganism phyla including BRP, Fusobacteria (FSB), Loukozoa (LKZ), and Phaeophyceae (PPC) were found in the inlet bottom media layer of IT and HSSF facilities but were not found in the outlet. In HSSF, there were four phyla [Spirochaetes (SCT), LP, Crenarchaeota (CC), FSB] in the top media layer and five phyla [Fibrobacteres (FBB), TRC, LP, CC, and LKZ] in the bottom media layer.

For the bottom layer of IT, the microorganisms FSB, and that grows well in anaerobic conditions, LKZ, a heterotrophic microorganism that grows well in various environmental conditions, and PPC, a brown algae variant, were determined

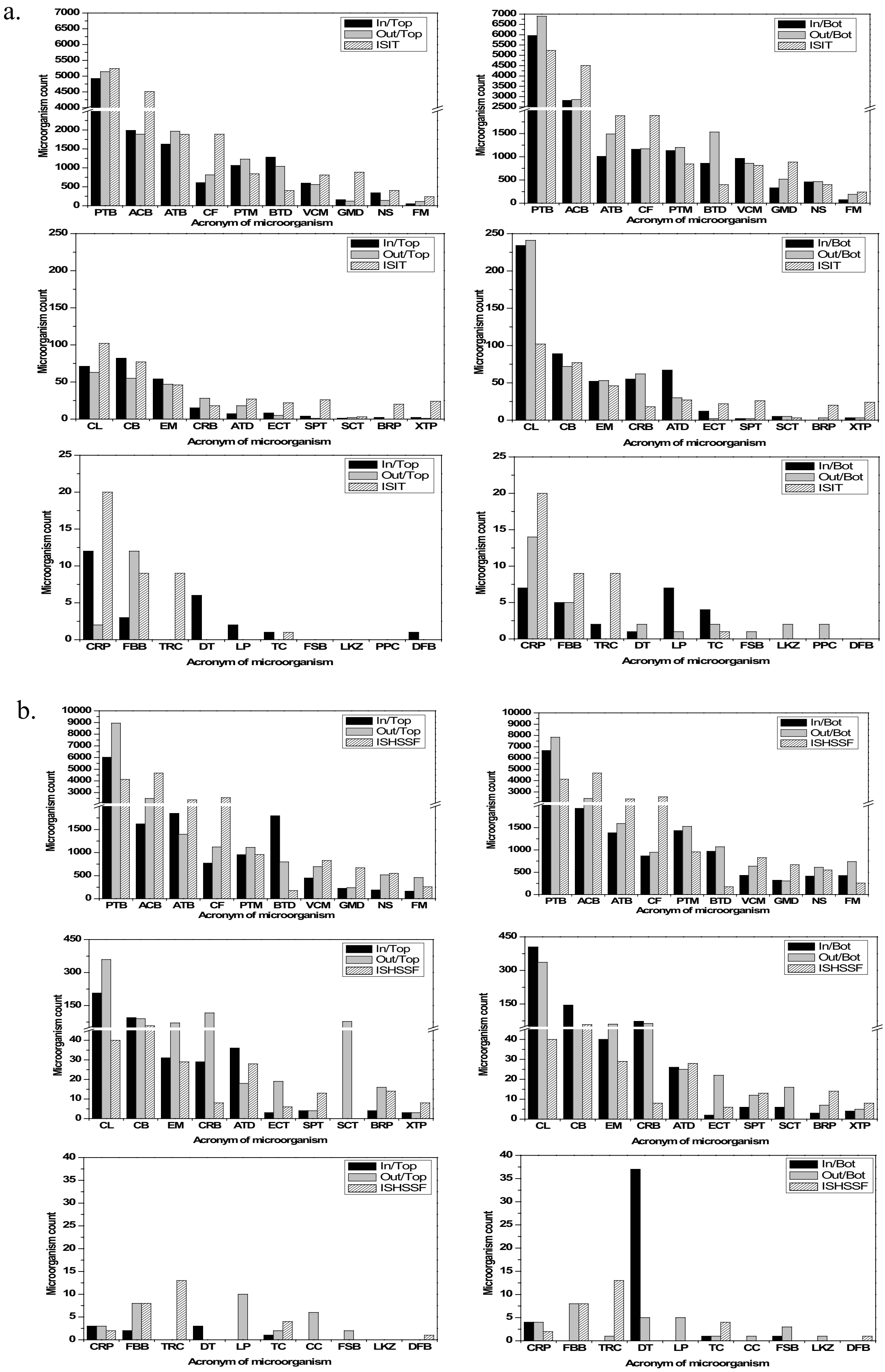


Fig. 3. Changes in the microorganism count at inlet and outlet at (a) IT and (b) HSSF.

to exist due to the anaerobic conditions and harsh environment of the media part end [15,16]. SCT, known as a chemical heterotrophic microorganism, was found in the HSSF top layer, as was CC, which has been reported to be a microorganism that grows well in harsh environments like high temperatures and performs a vital role in the nitrogen and carbon cycles [17,18]. LP and CC were commonly found in HSSF, while microorganisms in the HSSF bottom media layer were FBB, which contributes to the decomposition of plant-based cellulose, and TRC, which is an avian and mammalian pathogen with no cell wall. These microorganisms were not the dominant species in the facilities but were determined to be present a new habitat environment established by the varying count and concentration of the pollutant as it passed through the media.

For both IT and HSSF, larger quantities of microorganisms were found in the bottom media layer than compared to the top layer and in the outlet than in the inlet. For IT, the woodchip filling in the media part top layer decomposed over time and the released organics COD and nutrients affecting the microorganism growth in the media part outlet. For HSSF, the microorganism count showed a similar trend to IT. The roots of the plants in HSSF underwent aging and become dislocated, acting as new organic material and nutrients and affecting the microorganism growth in the latter part of the media. The greater microorganism count in the media part bottom layer compared to the top layer was determined to be a result of the provision of organic materials and nutrients, along with environmental factors such as appropriate water content, pH, and dissolved oxygen.

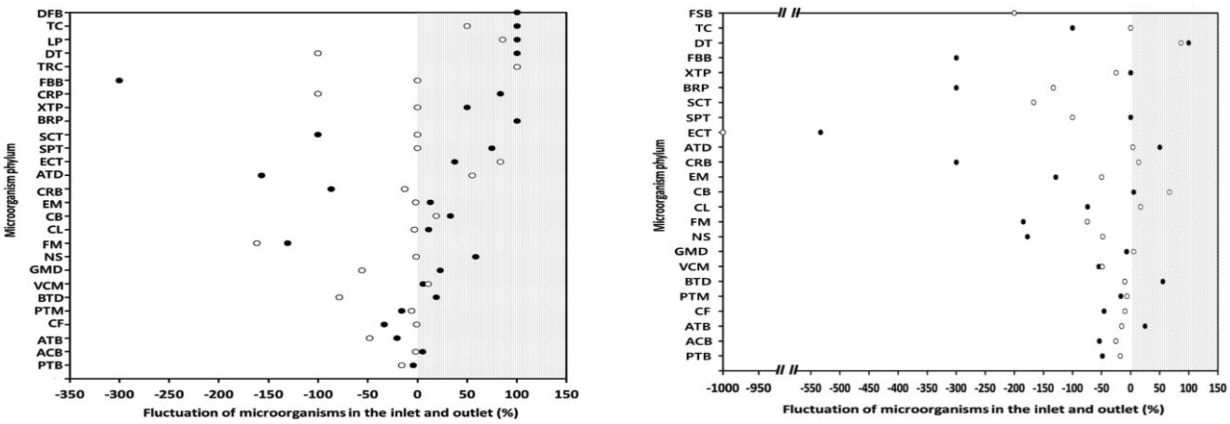
3.3. Considerations of LID design factor for nutrients and metals

Fig. 4 shows the fluctuation of microorganism count between the inlet and outlet media part, determined by dividing the difference in the microorganism count by the inlet count. The stormwater runoff passed through the media where the concentration and form of the pollutant changes due to the physicochemical and biological pollutant removal mechanisms, affecting the type and count of the microorganism phylum. Positive values implied that the microorganism count at the outlet decreased compared to that at the inlet after passing through the media part while negative values signified an increase in microorganism count. For the top and bottom media layers of the HSSF facility, it was found that approximately 70% of the microorganism phylum increased count in the outlet media part compared to the inlet. However, in the top media layer of the IT, approximately 66% of microorganism phylum was observed to have decreased count, while 66% of the phylum in the bottom media layer was observed to have increased. These findings were associated with the sufficient water content, stable wetland temperature, and nutrient contribution of wetland plant roots to microorganisms present in HSSF. For IT which has infiltration mechanism, the decreased microbial count was related to the difficulty in growth, except for the microorganisms that thrive in harsh environments, since the top layer is frequently exposed to the atmosphere and has no long term water retention capabilities. However, for the bottom media layer of IT, there was an increase in the number of microorganisms species with increased count due to the nutrients provided by the filler woodchips in the top media layer and appropriate water content and temperatures.

Among the microorganism phylum, Gemmatimonadetes (GMD) was found to increase in the count by approximately 55% in IT bottom media layer outlet, while ACB was found to increase in the count by approximately 55% in the top media layer and 25% in the bottom media layer of the HSSF facility. GMD and ACB were classified as microorganisms with excellent phosphorus absorption characteristics which contributed partly to the TP removal efficiencies of both IT and HSSF [3,19]. In general, it was reported that GMD grows actively in aerobic conditions and temperature ranging from 25°C to 35°C, and ACB grows well in soil with high water content and low pH [3,20]. Nitrospirae (NS) is a microorganism that plays an important role in the nitrogen cycle and the microorganism count increased by 1.3%, 177%, and 45% in the IT facility bottom layer, HSSF facility top layer, and HSSF facility bottom layer, respectively. The increase in the count of microorganisms was related to the nitrogen cycle wherein higher nitrogen removal efficiency was observed in HSSF compared to IT. ATB and Firmicutes (FM) are microorganisms that affect the biological removal of heavy metals, and their counts were observed to increase at the outlet of both HSSF and IT. In particular, their counts increased by approximately 50% to 160% in the IT facility. Among the two microorganism species, FM is a microorganism with Fe absorption, so the microorganism count increase was higher in IT, where there was higher inflow Fe concentration. When such microorganism characteristics are reflected in the LID facility design, cost-effective LID facility design becomes possible by implementing additional biological removal efficiency to the physicochemical pollutant removal efficiency of the systems. Thus, phosphorus removal efficiency is expected to increase if the environmental conditions considering the growth of GMD and ACB are reflected in the design of the LID receiving high phosphorus concentration stormwater runoff. For the processing of high nitrogen content stormwater runoff, environmental conditions considering the growth of NS can be reflected in the design, and for high heavy metal content stormwater runoff, environmental conditions considering the growth of ATB and FM can be reflected in the design to enhance the pollutant removal efficiency.

3.4. Comparison of microorganisms in media layers with the surrounding soil

IT and HSSF were developed to treat stormwater runoff containing nonpoint pollutant sources from the road and parking lot; in order to improve the pollutant removal efficiency, the soil was removed and sand and woodchips were used as filtration media. Such an environment and influent characteristic differences affected the number and count of microorganism species in the media part in the LID environment. However, the number and count of microorganism phylum in the bottom media layer of IT were lower than those in the soil which was due to the increased nutrient concentration from the grassroots in soil (Fig. 3). The number and count of microorganism species in HSSF were found to be higher than IT due to the water retained in the HSSF media part. Apparently, the number and count of microorganism



(a) IT (b) HSSF

Fig. 4. The fluctuation of microorganisms in the inlet and outlet ((inlet – outlet)/inlet × 100).

phylum in the HSSF media part in the outlet were higher compared to soil. From these results, it was concluded that the deep roots of the wetland plants provided a more favorable environment for microorganism growth compared to the grassroots of the soil. Therefore, the application of deep root plants in LID facilities can be utilized as a method of improving the biological pollutant removal capability considering microorganisms.

Among the microorganisms found in the soil and LID facilities, the microorganism PTB was found to have the highest count. This finding was due to its property of growing symbiotically within root nodule cells of legumes as nitrogen-fixing bacteroids, and as such has the highest count in areas with high nutrient concentration from plant roots and woodchips. The microorganisms with counts higher in the soil than in the media of LID facilities were ACB, CF, GMD, Streptophyta (SPT), and Xanthophyceae (XTP). ACB thrived in low pH, and CF, SPT, and XTP grew symbiotically with terrestrial plants and helped in the process of photosynthesis. GMD grows well in arid soils and its high count in the soil was due to the effect of soil-plant roots that have grown for a long time. Microorganisms that had higher counts in the LID facility than in the soil were PTM, Bacteroidetes (BTD), and Elusimicrobia (EM), which thrive in soil with high water content. PTM grows well in low-temperature ecosystems, while BTD and EM thrive in environments with high concentrations of organic materials. From these results, it can be observed that the microorganism growth in the LID facility was influenced the influent pollutant composition and water content; thus, a method to maintain suitable water content is necessary for facility designs considering biological mechanism.

4. Conclusions

Although various LID facility types are being applied for nonpoint pollution source removal, most studies only reported physicochemical mechanisms; studies about biological mechanisms are lacking. As a result, the adaptation of these facilities to the ecosystem was unstable causing problems in the pollutant removal efficiency and maintenance. Therefore, in this study, the influent composition, microorganisms, and pollutant removal efficiency were analyzed for LID facilities of different types to derive the biological factors necessary for LID facility design. The following conclusions were obtained in this study.

- Due to high vehicular activity, roads, in comparison to parking lots, discharge higher concentrations of pollutants including organic materials, nutrients, and heavy metals during rainfall. Long-term monitoring showed that the pollutant removal of IT is mainly due to the physicochemical mechanisms in the media and sedimentation tank with infiltration function. For the HSSF facility, physical filtration by the media and biological removal capability of the wetland plants were found to be major pollutant removal mechanisms.
- Influent water quality characteristics, presence of plant roots, and media characteristics influenced the number and count of the microorganism phyla found in each system. Also, the water content in LID facilities affected the microorganism growth; thus, the design of a facility considering its biological function needs to incorporate water retention in the system.
- In order to improve the biological processing capability of stormwater runoff with low organic material content, woodchips were used as a media, or plants with deep roots providing appropriate nourishment and a habitable environment for microorganisms.
- As the pollutant containing stormwater runoff passes through the media part, the pollutant form and type change due to the physicochemical and biological mechanisms. As a result, the type and count of microorganisms also varies. When such varying pollutant characteristics and microorganism species are reflected in the LID facility design, focused management of the target pollutant is expected to be possible.
- For the design of a LID facility for high phosphorus concentration stormwater runoff processing, the habitat conditions of GMD and ACB can be considered. Considering high nitrogen content, environmental conditions suitable for NS should be considered while environmental conditions for ATB and FM should be considered for high heavy metal concentrations.

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