Biological Nitrogen Removal from Stormwater in Bioretention Cells: A Critical Review

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Abstract

Excess nitrogen in stormwater degrades surface water quality via eutrophication and related processes. Bioretention has been recognized as a highly effective low impact development (LID) technology for management of high runoff volumes and reduction of nitrogen (N) pollutants through various mechanisms. This paper provides a comprehensive and critical review of recent developments on the biological N removal processes occurring in bioretention systems. The key plant- and microbe-mediated N transformation processes include assimilation (N uptake by plants and microbes), nitrification, denitrification, and anammox (anaerobic ammonia oxidation), but denitrification is the major pathway of permanent N removal. Overall, both lab- and field-scale bioretention systems have demonstrated promising N removal performance (TN: > 70%). The phyla *Bacteroidetes* and *Proteobacteria* are the most abundant microbial communities found to be enriched in biofilter media. Furthermore, the denitrifying communities contain several functional genes (e.g., *nirK*/*nirS* and *nosZ*), and their concentrations increase near the surface of media depth. The N removal effectiveness of bioretention systems is largely impacted by the hydraulics and environmental factors. When a bioretention system operates at low hydraulic/N loading rate, containing a saturation zone, vegetated with native plants, having deeper and multilayer biofilter media with warm climate temperature and wet storm events periods, the N removal efficiency can be high. This review highlights shortcomings and current knowledge gaps in the area of total nitrogen removal using bioretention systems as well as identifies future research directions on this topic.

Keywords: Stormwater runoff; Bioretention cells; Nitrogen removal; Nitrification; Denitrification; Microbial community.

Introduction

Increased urbanization has led to creation of impervious surfaces (e.g., roads, highways, sidewalks, rooftops, parking lots and urban lawn) that cause flash floods in cities after intense and prolonged rainfall (1). Impervious surfaces also change the hydrological flow regime and the quality of urban runoff even at a low proportion of impervious cover (5 -15%) (2) as some reports have suggested a positive relationship between the proportion of impervious surface cover and their hydrologic/environmental impacts (3, 4). Notable hydrological changes include increased storm runoff volume with a high peak flow and flow velocity, while water quality changes of concern include increased concentrations and mass loads of diverse pollutants (5). Urban stormwater contains a wide variety of chemical pollutants (e.g., nutrients, heavy metals, organic compounds and particulate matter) (6–8) and microbial pathogens (e.g., *Escherichia coli* and *Enterococci*) (9, 10). Thus, discharge of stormwater into a stream could adversely impact the quality of aquatic ecosystems and cause health risk to aquatic organisms (11, 12).

Among the pollutants in stormwater, nitrogen (N) is recognized as an important pollutant that causes eutrophication of receiving waters when discharged in large amounts (13–15). Stormwater from residential areas usually contains a high amount of inorganic nitrogen pollutants (mainly nitrate) (16). Atmospheric deposition and inorganic/organic fertilizers are the major nitrogen sources in stormwater in urban areas (16). Nitrogen in stormwater is present in dissolved (mainly inorganic-N) and/or particulate (mostly organic-N) forms (13, 17). The chemical forms of dissolved inorganic nitrogen include nitrate (NO_3^-), nitrite (NO_2^-) and ammonium (NH_3 and NH_4^+) (13, 17, 18). Concentrations of various forms of N species detected in stormwater generated from different impervious sources are given in Table 1. Nitrogen in stormwater is usually present in dissolved forms (~80%) among which NO_3^- is the most (~47%) and NH_4^+/NH_3 is the least abundant (~11%) pollutant (17). In order to protect public

health and the environment, it is necessary to treat stormwater to decrease contaminant levels prior to discharge to receiving waters, or before using it as a resource to alleviate water stress.

Low-impact development (LID) has recently been adopted globally as an environmentally and economically viable technology to manage stormwater runoff and mitigate pollution in aquatic ecosystems (19, 20). Bioretention cells (BRCs) (also called as bioretention systems, rain gardens or biofilters) are an engineered soil- and plant-based LID technology. BRCs have shown high performance in the removal of various stormwater pollutants including nitrogen (mainly particulate N) (13, 15). The key advantages of BRCs are that they require small space compared to engineered wetlands, consume low energy and are cost effective (21). The key components of a BRC include vegetation, the top layer (mulch, soil media), and the bottom layer (gravel layer) (Fig. 1) (22, 23). Frequently a subsurface saturated zone is created as a special engineered layer to promote denitrification and N removal. In BRCs, stormwater is directed for infiltration through the engineered filter media. The infiltrated water is stored and transferred to an underdrain system, then released into nearby surface water bodies, or directly allowed to percolate to groundwater (24). Potential mechanisms for removal of nitrogen pollution from runoff through BRC using plants-media-microorganisms include physical (filtration), chemical (e.g., adsorption and ion exchange), and biological (e.g., transpiration, assimilation, denitrification, immobilization, decomposition) processes (25).

Many studies have reported poor NO_3-N removal efficiency (15, 26). As a consequence, high concentrations of NO_3-N were observed in the treated effluent since this anion is highly soluble and mobile. It is thus clear that physicochemical processes namely soil adsorption are not effective in capturing NO_3-N in runoff (27). Recently, researchers have examined microbial community composition enriched in bioretention media for pollutant removal (21, 28–31). Efforts have also been directed at enhancing plant-microbe driven biological nitrogen

removal by controlling operational conditions (e.g., hydraulic loading rate) and engineering BRC filter media conditions for enrichment of oxic (e.g., nitrifiers) and/or anoxic (e.g., denitrifiers) N-transforming microorganisms (32–34).

To date, a few reviews have been published on the removal of nitrogen from stormwater using BRCs (18, 35–38). Most of the past reviews have reported bioretention design considerations (18, 35, 36, 38), summarized regulatory measures (18), synthesized knowledge on nitrogen fate and removal mechanisms, and discussed the impact of environmental factors (35, 36, 38).

This review specifically covers recent developments to expand on information provided in past reviews: (1) shift of microbial community composition in BRC filter media (28–31, 39), (2) the occurrence of different biological N processes (nitrification, denitrification, anaerobic ammonia oxidation (anammox)), and (3) dissimilatory nitrate reduction to ammonium (DNRA) (15, 40–42). The abundance of key functional enzymes (e.g., *amoA*, *nirK/nirS* and *nosZ*) (15, 43) and their importance under lab- and field-scale studies also merits attention.

The Scopus database shows that an increasing number of research articles have been published in the last ten years (2011 – 2020) on N removal from stormwater in BRCs (supplementary material, Fig. S1). The bibliographic records (number of articles, conference papers, reviews, conference reviews and book chapters) on the review topic published during 2011 - 2020 were collected using the keywords, namely, 'nitrogen', 'stormwater', and 'bioretention' in the Scopus search engine. This review aims to update the research community by summarizing recent research findings and developments on biological N removal from stormwater in BRCs. The relative contributions of various biological processes on N removal in lab- and field-scale studies and the underlying molecular level mechanisms, and the responsible functional enzymes are discussed. Moreover, the composition of the microbial community enriched in the BRC media is highlighted. The impact of various environmental

factors on N fate and its removal, possible methods for augmentation of plant-microbe driven N removal process and the need for future investigations for improvement of bioretention performance are described. We believe that this review paper would contribute to better understanding of the fate and biological transformation of N contaminants, as well as the modification of existing designs, operational and media characteristics of a BRC to enhance its effectiveness for removal of nitrogen.

Plant and microbe-driven biological nitrogen removal in bioretention cells

Biological N cycling in plant-soil ecosystems

An overview of biological N cycling in soil and the associated enzymes is shown in Fig. 2. Nitrogen in soil can exist as organic, inorganic, dissolved and particulate forms with a wide range of oxidation states from -3 (NH_4^+/NH_3) to +5 (NO_3^-) (44, 45). The physicochemical and thermodynamic properties of various nitrogen compounds are given in supplementary material (supplementary material, Table S1).

In soil, the N transformations can be described by a series of oxidation–reduction reactions catalyzed by both plants and microorganisms (bacteria, archaea, and fungi) (46). Nitrogen is one of the essential elements which limits the growth of plants, and plant biomass typically contains 2–5% N by dry weight (47). Rhizosphere microbes play a vital role in the transformation of N to plant-usable forms (45). Among different N forms, only NH_4^+ and NO_3^- are used by organisms for new biomass generation (48). In stormwater, both organic and inorganic N species are present depending on the source of N generation, and their fate and transformation processes are different when runoff passes through the soil-based engineered bioretention media. It is important to understand the microbiology, physiology and biochemistry of microbe-driven N cycle processes in the soil/plant rhizosphere in order to enhance the removal efficiency of N contaminants (specifically dissolved N species) in a BRC.

The key N transformation processes, reactions, enzymes and physicochemical/thermodynamic properties including redox potential are summarized in Table 2.

In BRCs, the major biological N transformation processes include assimilation (e.g., vegetative N uptake), ammonification (mineralization), nitrification, denitrification, anammox, and DNRA (38, 49). In plant-mediated assimilation, inorganic N compounds (e.g., NH_4^+ and NO_3^-) are converted to amino acids. Generally, $NH_4^+$is more favorable than NO_3^- for assimilation by plants since NO_3^- (ΔG^0 : - 1492.8 KJ/N atom) reduction requires more energy than NH_4^+ (ΔG^0 : -1797.4 KJ/N atom) (supplementary material, Table S2) (50). In BRCs, ammonium removal up to 80% can be achieved via adsorption and biological process (e.g., nitrification) (23).

Ammonification (mineralization) is the process in which organic nitrogen compounds (e.g., urea, $CO(NH_2)_2$) are transformed in enzymically-catalyzed reactions into an inorganic bioavailable N form, ammonium (NH_4^+) (Table S2) (51). This species subsequently can be taken up by plants and microbes (22).

Nitrification is a dual-step process of sequential oxidation of NH_4^+ to NO_3^- through NO_2^- (Table S2) (52). The process is mediated by two groups of microorganisms: first ammonia-oxidizing bacteria/archaea that oxidize NH_4^+ to NO_2^-, then nitrite-oxidizing bacteria, which oxidize NO_2^- to NO_3^- (45, 48). The key enzymes in the nitrification reaction are ammonia monooxygenase (*amo*) and hydroxylamine oxidoreductase (*hao*) and nitrite oxidoreductase (*nxr*) (45).

Denitrification involves multistep reactions of reduction of NO_3^- to dinitrogen gas (N_2) (Table S2, with $C_3H_4O_3$ as an example organic electron donor) (53), which is released to the atmosphere, or returned to the soil through plant roots by N_2 fixation (reduction of N_2 to NH_3) (38). Each reaction step is catalyzed by a specific enzyme including nitrate reductase (Nar), nitrite reductase (Nir), nitric oxide reductase (Nor), and nitrous oxide reductase (Nos) (54). In

BRCs, the process is performed by mostly heterotrophic microbes (denitrifiers), which use nitrate instead of O_2 as a terminal electron acceptor during respiration. A few studies have also reported autotrophic denitrification in BRCs using inorganic electron donors such as reduced inorganic sulfur compounds (e.g. elemental sulfur (S^0) (55) and iron-based sulfide minerals (e.g. pyrite, FeS_2) (56); the nitrate reduction reactions are presented in elsewhere (Table S2) (57, 58). Complete denitrification results in the endpoint product of N_2 gas, which is not generally bioavailable and promotes permanent removal of N from stormwater in BRCs (22). However, incomplete denitrification is undesirable since it generates nitrous oxide (N_2O), a potent greenhouse gas (59).

DNRA is the reduction of nitrate to ammonium (Table S2) (52). This process is carried out by anaerobic and facultative anaerobic bacteria (45). The DNRA reaction is catalyzed by a cytochrome C nitrite reductase (Nrf) that converts NO_2^- to NH_4^+ (60, 61). Denitrification causes N loss, but DNRA activity conserves/recycles nitrogen in the ecosystem as the end-product, NH_4^+, a biologically reactive N that can be used by plants and microbes or recycled (by oxidation) back to NO_3^- (62).

The DNRA process is highly competitive with denitrification as both processes use the same inorganic N species (NO_3^-) as electron acceptors and environmental conditions (e.g., anoxic). The fate of NO_3^- in bioretention media due to DNRA has been generally overlooked and no published reports were found. The plants used in bioretention technology could release organic compounds through roots (root exudates), and these compounds may impact the selectivity between denitrification and DNRA activity in the rhizosphere (46). Future investigations should focus on these topics to unravel nitrate fate and potential DNRA activity in BRCs.

Anaerobic ammonium oxidation (anammox) is the production of N_2 from NO_2^- and NH_3 under anoxic conditions via intermediates such as nitric oxide (NO) and hydrazine (N_2H_2) (Table S2) (63, 64). The responsible organisms are slow growing microbes that belong to the order *Brocadiales*, and are associated with the phylum *Planctomycetes* (60). The key enzymes that catalyze the anammox reaction are hydrazine hydrolase (*hh*), producing N_2H_4 and hydrazine dehydrogenase (*hdh*)/hydrazine-oxidizing enzyme (*hzo*), converting N_2H_4 to N_2 (64, 65). A few recent studies have examined anammox bacteria for stormwater treatment using mathematical models in BRC and in constructed wetlands (66, 67); no reports are yet available on experimental works on anammox bacteria enrichment in BRCs for stormwater treatment. Further research on this topic is warranted.

In biological nitrogen transformation process (e.g., nitrification and denitrification), nitric oxide (NO, a free radical gas) is produced as a byproduct. NO is recognized as one of the important air pollutants which can create several environmental problems including acid rain, haze and photochemical smog (68). Moreover, NO acts as a signaling molecule that impacts plants growth and development and influences different pathways involved in plant-microbe interactions (69). For example, in plant-bacterial interactions, NO involves in abiotic (oxygen, heat and salt stress) and biotic (pathogen, NO acts as antimicrobial agent) stress response, root architecture, root hair formation, nodule development, lateral root formation, etc. (69). From the perspective of N removal from stormwater in plant and soil-based engineered systems (e.g., bioretention cells), enrichment of NO-consuming microorganisms may help to achieve better N removal performance which needs to be verified in future studies.

In addition to bioretention cells, other plant-based systems, specifically green roofs and constructed wetlands, are used for removal of excess nitrogen from stormwater (70, 71). Several studies have reported that plant traits and plant species diversity significantly impact pollutant removal efficiency of plant-based constructed ecosystems (47, 72). Plant traits

namely plant mass, growth rate, root length, root mass, root thickness, root architecture as well as plant tolerance to nutrients and salts are commonly used to study the relationahip between plants traits and pollutant removal performance of a specific plant species (47, 72, 73). In lab-scale phytoremediation experiments, Chen et al. (72) showed that plant root, leaf and total dry biomass had moderate to strong correlation with nitrate removal. Moreover, fast growing plants demonstrated high performance for nitrate removal, bur slow growing plants were mostly effective for phosphate removal (72). Among native and exotic plant species, native plants were efficient for removal of both nitrate and phosphate (72). Hunt et al. (74) screened 30 plant species for their capability for removal of nitrate and phosphate from stormwater in bioretention columns, and noticed that 24 out of 30 plants showed more than 50% uptake of nitrate from stormwater, and two plants namely Arundo donax var. versicolor and Bougainvillea 'Sakura Variegata' contributed highest nitrate removal (96%). Read et al. (73) investigated the performance of 20 diverse plant species on removal of N and P from stormwater in biofilter systems, and authors have found that among 20 plants, Carex appressa (a grasslike plant) was the strongest contributor for decontamination of stormwater, and C. appressa possessed traits such as high growth rate, high root mass and long root length. Plants with high tolerance to salt and nutrients are effective for nitrogen removal from water and wastewater (47, 75). Plant-based systems usually contain monoculture (i.e., single species) or mix diversity of plant communities (76). In general, several studies have suggested for plantation of diverse species which could enhance ecosystem services in addition to the primary role of pollutant removal (71, 77).

Perspectives: Urban stormwater is generally characterized by its low strength (mainly low in organic carbon) and high dissolved O_2 content, which makes it difficult for the application of microbially-driven processes for effective removal of N pollutants (66). To enhance N removal (e.g., denitrification), carbon amendment with addition of external carbon source is required.

Biological N removal offers several advantages over physicochemical processes, namely low-cost, no chemical additions, less negative environmental impacts, and most importantly, high removal efficiency of nitrogen by transforming it to inert N_2 gas (78, 79). Hence, increased attention has recently been given to understand the dynamics of microbial communities in bioretention media, then modify the design parameters and/or operational/environmental conditions to increase population of desired functional bacteria (e.g., nitrifiers and denitrifiers) to achieve higher N removal efficiency.

Dynamics of microbial communities in engineered bioretention media

Microorganisms present within the engineered biofiltration media during installation, microbial colonization from the environment, and/or development of microbial biofilms over the course of operation are responsible for driving the various N transformation reactions to permanently remove N through denitrification, or conversion to another form of N (29, 80). Ecological conditions in the bioretention media may be different at different depths (top, middle and bottom), which could impact the community composition and their functions (e.g., enzyme activity) and ultimately the nature of N cycling (30, 39, 81). Moreover, the microbial community composition at the upper layer of the media could be greatly impacted by the plant species and density of plant roots, while the presence/absence of anaerobic saturated zone and C source (or other electron donor) could shape the microbial community composition in the bottom layer (39). In heterotrophic N removal, the materials used as electron donor include woodchip, mulch, newspaper, sawdust, wheat-straw, and others (9, 15), whereas in autotrophic process, elemental sulfur (S^0), pyrite (FeS_2), natural zeolite and magnetite (Fe_3O_4) are used as electron donor (55, 56, 82). Understanding the composition and stability of microbial communities present within the biofiltration system could help to develop better stormwater management strategies and efficient N removal.

Molecular techniques including 16S rRNA gene-based sequencing (29–31, 39) and terminal restriction fragment length polymorphism (TRFLP) (28, 83) are commonly employed for characterization of microbial communities. Additionally quantitative polymerase chain reaction (qPCR) is another popular molecular method that has been used for quantification of functional genes encoding enzymes responsible for nitrate, nitrite and ammonia transformations (15, 84). A study on engineered infiltration systems (with stormwater) using the 16S rRNA sequencing showed that the phyla *Proteobacteria* (51%) was dominant, followed by *Bacteroidetes* (18%), *Firmicutes* (9%) and *Saccharibacteria* (< 4%) (29). However, *Firmicutes* (42%), *Proteobacteria* (34%) and *Bacteroidetes* (11%) were the key microbial candidates in the non-inoculated columns (without stormwater). A mesocolumn-based research revealed that the phyla *Bacteroidetes* and *Proteobacteria* were abundant in all the media samples and accounted for nearly 40% and 30% of the total assigned reads, respectively (39).

A few studies have looked into the variability of bacterial communities in a BRC at various depths and they observed that the most noticeable microbial activities occur in the top layer and the microbial population decreased noticeably with depth (81). The top two abundant phyla among the communities were *Bacteriotides* and *Proteobacteria*, and their proportion changed with depth. In another work, the columns filled with the homogenous media mix containing sand, soil and fly ash (ratio: 1:1:1), the proportion of phylum *Proteobacteria* decreased from 57.09% (20 cm) to 45.72% (40 cm), and then increased to 68.32% (60 cm) (30). Igielski et al. analyzed the microbial diversity in the biofilm developed on the surface of woodchips and the effluent pipe in a lab-scale BRC configured with internal water storage zone (85). They found that both denitrifying communities and anaerobic lignocellulose degrading bacteria were enriched in the system. In the woodchip biofilm, the major communities (class level) were *α-proteobacteria* (12.87%), *β-proteobacteria* (11.37%) and *Opituta* (8.96%),

whereas significant change of community abundance/composition was observed in the effluent tube biofilm, i.e., *α-proteobacteria* (47.21%), *β-proteobacteria* (24.58%) and *Acidobacteria* (9.0%) were predominantly enriched.

A recent study examined changes of microbial diversity in bioretention columns where each column was planted with three different aquatic plants (31). They noticed that the abundance of *Proteobacteria* and *Saccharibacteria* in the control sample (without vegetation) was elevated by up to 40 times during the operation, whereas the abundance of *Actinobcteria*, *Acidobacteria*, *Cyanobacteria*, and *Nitrospirae* decreased with operation time. Conversely, the selected three plants exhibited different effects on the microbial population, i.e., the plant, *Iris pseudacorus* L enhanced the proportion of *Actinobacteria*, *Canna indica* L encouraged growth of *Acidobacteria*, while *Lythrum salicaria* L. also favored enrichment of *Chloroflexi* and *Saccharibacteria*.

Although heterotrophic denitrifiers are the dominant communities in the bioretention media due to use of organic carbon rich materials as a source of electron donor, recently, a few researchers have investigated the diversity of autotrophic communities in BRCs supplied with S and Fe-based inorganic electron donors (56, 82). In simulated BRCs augmented with natural pyrite or zeolite as electron donor, abundances of sulfur/Fe-based denitrifiers including genera *Thauera*, *Sulfuritalea* and *Thiobacillus* were higher when the column was operated with pyrite (2.1%, 1.7% and 2.6%, respectively) compared to zeolite (< 0.1%, 0.3% and < 0.1%, respectively) as an electron donor (56). Deng et al. found enhancement of the anammox reaction in biofilter media with iron as an electron donor and higher DNRA rate with iron plus sulfur as electron donors (82).

In a TRFLP-based study, a total of 33 different terminal restriction fragments were detected in biofilter columns (28). Moreover, the bacterial community structure changed with the increase in biofilter operation time, and considerable correlations were observed between

bacterial communities and effluent water chemistry (e.g., concentration of NO_3-N). In another constructed stormwater wetland study, cluster analysis of nitrous oxide reductase (*nosZ*) gene TRFLP fingerprints revealed that the samples collected from the rhizospheric sediment (13 fragments) contained a higher number of denitrifying communities than unvegetated sediments (9 fragments) (83).

In addition to metagenomics and TRFLP methods, a few researchers have employed quantitative PCR (qPCR) to quantify the microbial biomass at different layers of the filter medium (15, 29, 86). Chen et al. demonstrated that the 16S rDNA concentration was higher at the middle zone (30-45 cm) (6.4×10^8 copies per gram soil (c/gs)), but decreased for the samples collected from the deepest regions (45-60 cm and > 60 cm) ($1.2 \times 10^8 – 1.3 \times 10^8$ c/gs) (15). Another study also reported a similar level (in the order of ~$10^8 – 10^{10}$ c/gs) of 16S rDNA concentrations in bioretention columns packed with different filter materials (single or double layers with woodchips and/or vermiculite). However, the biomass density increased/decreased along the column depths, depending on the packing material type and the packing pattern (86). Overall, 16S rDNA concentration is a surrogate for total biomass enriched in the different layers of the stormwater treatment biofilters. However, metagenomics characterization (e.g., 16s rRNA gene-based sequencing) is performed to determine enrichment of specific microbial communities (nitrifiers, denitrifiers, etc.,), and qPCR analysis is done for quantification of specific nitrogen processing genes (e.g., *amoA*, *nirK*, *nirS*, *norB*, *nosZ*, etc.,).

For better understanding about the fate and transport of microorganisms in bioretention systems, and the associated mechanisms for removal of nitrogen from runoff in bioretention systems, controlled studies using pure culture are required. A few studies have been carried out using *Escherichia coli* as a model bacterium to elucidate bacteria transport mechanisms through stormwater biofilters (87, 88). Although little information is available about nitrogen removal from stormwater using pure culture system, numerous reports are published on N

removal (specifically by denitrification) from groundwater and wastewater employing pure culture of denitrifying bacterium (various species of *Pseudomonas* and *Bacillus*). Among *Pseudomonas* Spp., *Pseudomonas denitrificans* and *Pseudomonas aeruginosa* were frequently used in past works and authors observed high N removal efficiency (>75%) (89, 90). A number of *Bacillus* Spp. namely *Bacillus cereus* and *Bacillus subtilis* show promising denitrifying capacity (> 68%) (91, 92). In future research, these denitrifying microorganisms can be considered to test their performance for N removal from stormwater in bioretention systems.

Stormwater characteristics, i.e., presence of inorganic pollutants (N species namely nitrate, nitrite and ammonium, phosphate, heavy metals) and organic pollutants in runoff could impact the abundance and composition of microbial communities in the bioretention systems (28, 93, 94). Stormwater rich in inorganic nitrogen species (nitrate, nitrite and ammonium) could promote enrichment N transforming bacteria namely nitrifiers, denitrifiers and ammonifiers (95). Wang et al. (95) analyzed microbial communities enriched in a conventional bioretention system supplied with N-containing synthetic stormwater and found that the genus *Pseudomonas* was the major bacteria which drive the N removal in the bioretention system. The stormwater containing organic contaminants could promote enrichment of organic degraders since some studies have reported the presence organic degrading bacteria (e.g., genus *Flavobacterium* and *Clostridium* spp.) in bioretention systems (22, 33, 95). A recent report indicated the presence of antibiotic resistant bacteria and antibiotic resistance genes in stormwater which could be linked to the presence of antibiotics in stormwater (96). Another study also noticed an increase in the concentration of antibiotics (sulfadiazine) and antibiotic resistant bacteria (cefazolin- and sulfamethazole- resistant bacteria) in the surface water and surface sediments of a urban lake after strong storm events (97). Together, these studies indicate that the type of pollutants in stormwater could affect the dynamics of microbial communities in bioretention cells.

Perspectives: Together, the findings of the above studies suggest that microbial community composition and abundance vary widely within bioretention media. Multiple studies have revealed that the phyla *Bacteroidetes* and *Proteobacteria* are the most abundant among the communities. *Bacteroidetes* are normally recognized as organic degraders (98). They may degrade high molecular weight and complex organic pollutants in stormwater, and make them bioavailable as a C source for other microbes (e.g., nitrifiers and denitrifiers). The higher abundance of *Bacteroidetes* in BRCs indicates possible high amounts of carbon resources in the upper layer. *Proteobacteria* represent diverse microorganisms including denitrifying bacteria (specifically sub-classes *α-* and *β-Proteobacteria*) (99). A few other members (mainly *β-* and *γ-Proteobacteria*) are also involved in the initial step of nitrification (100, 101). The synergistic growth and function of *Bacteroidetes* and *Proteobacteria* may predominantly contribute to biological N removal in BRCs.

Microbially-driven N removal in lab-scale and field-scale studies

The mutual effects of plants, soil, and microorganisms in BRCs create favorable conditions for nitrogen removal (43). The key microbially-driven processes involved in the removal of ammonium and nitrate in a BRC are nitrification and denitrification, respectively. In a few studies, phenotypic observations were further verified by genotypic analysis, i.e. quantification of nitrification (e.g., *amoA*) and denitrification genes (e.g., *nirK*, *nirS*, *norB*, and *nosZ*) using the qPCR method and identification of key nitrifiers and denitrifiers enriched in filter media by metagenomic techniques (15, 29, 39). Frasser et al. investigated the dynamics of microbial communities and changes of *nosZ* gene (encoding nitrous oxide reductase) in lab-scale sand columns, and found that the abundance of *nosZ* gene increased from $\sim 1.0 \times 10^3$ copies/g from day 1 to nearly 7.0×10^3 copies/g on day 24 (29). Moreover, a total of 10 potential denitrifying taxa detected in the communities, all belonging to *α-, β-,* and *γ-Proteobacteria*.

A mesocosm study, which used a ^{15}N isotope tracer technique, stated that assimilation (plant and microbial) was the major pathway of N transformation (77−98%) in columns having saturated zones (39). Moreover, a control test on only soil showed nearly 38% N assimilation rate, and plant assimilation rates were found between 39−60% (39). However, only 1−7% N transformation was due to denitrification reactions. The functional gene, *nirK* was mainly enriched in the phylum *Bacteroidetes* (abundance: nearly 70%), while the *nosZ* gene was distributed in phyla *Bacteroidetes* (abundance: ~40%) and *Proteobacteria* (abundance: ~30%). The authors have also assessed the effect of different plant species. The relative abundance of the genus *Nitrospira* (nitrite oxidizing bacteria) was high in the non-saturated zone (both upper and bottom layers) in systems containing three different types of plants including *Buffalo*, *Carex appressa* and *Dianella tasmanica*.

A report on the treatment of stormwater in a BRC using Fe-biochar and incorporation of saturated zones demonstrated that the microbial denitrification enzyme assay (DEA) rate at the bottom layer was higher (~ 1.12 times) compared to the top layer samples (102). Wan et al. explored N removal in bioretention columns in which woodchips and vermiculite were packed in different patterns (i.e., column 1: only vermiculite (control), column 2: only woodchips, column 3: vermiculite (upper) + woodchips (lower), and column 4: woodchips (upper) + vermiculite (lower)) (86). Here, more than 80% of nitrate removal occurred in all the column configurations. The abundance of denitrification genes namely *narG*, *nirS* and *nirK* at various column depths increased when woodchips were employed. These findings suggest that denitrification activity may be higher with addition of woodchips, which provide carbon source for denitrifier communities (86).

A field-scale study reported that the combined nitrification-denitrification process contributed 33% and 56% of nitrate and total nitrogen (TN) removal, respectively (15). The concentrations of denitrifying genes (*nirK, nirS*, *norB*, and *nosZ*) varied between 10^5 and 10^8

gene copies/gram soil. The nitrification gene (*amoA*) was observed at a significantly lower level, i.e., between 10^4 and 10^6 gene copies/gram soil. This observation suggests that denitrification may be the predominant N removal process. In most cases, the samples collected from the top layer of filter media contained high concentrations of functional genes, which declined at various degrees as a function of media depth. Another field-scale study reached the same conclusion about the reduction of denitrification functional genes (only *nirK* and *nosZ* were tested) with depth since the abundance of *nirK* and *nosZ* genes as well as denitrification potential rates in the top layer were on average 5.7, 3.6, and 23 times, respectively, greater than the bottom layer samples (84).

In a field-scale study by Willard et al., researchers assessed the long-term performance of a BRC seven years post-construction, and observed high removal efficiency for several pollutants including TN (median % reduction nearly 100, detection limit: 0.001 mg/L) (103). The *nirK* gene concentration varied between 3.7×10^7 and 1.7×10^9 copies/gram of soil, while the level of *nosZ* gene ranged between 2.4×10^5 and 3.6×10^6 copies/gram of soil. Although the BRC had an internal water storage (IWS) system in the bottom layer, the quantity of the two functional genes decreased with an increase in depths, possibly due to insufficient amounts of organic carbon (103).

Although in most of the studies, the primary focus is to study nitrification plus denitrification-driven N removal in BRCs, no information is available about anammox, which is often observed in wastewater deficient in organic carbon (104). Thus, it is expected that anammox technology may be useful for treatment of stormwater since it generally is limited in the quantity of organic compounds. A few studies have demonstrated the enrichment of anammox bacteria with other microbes (nitrifier, denitrifier or DNRA) in a similar plant-based engineered system (constructed wetland) built for stormwater treatment (67, 83) .

Rahman et al. evaluated the relative contribution of various biological processes on nitrate removal in constructed stormwater urban wetlands, and reported that the denitrification rate varied between 6 ± 1 and 27 ± 9 μmol L slurry^{-1} h^{-1}, and the DNRA ranged from 0.6 ± 0.2 to 11 ± 2 μmol L slurry^{-1} h^{-1} (67). However, the anammox rate was low (only 0 – 0.01 μmol L slurry^{-1} h^{-1}; less than 0.05% of total NO_3^- reduction). In contrast, results from another study revealed a high proportion of anammox-mediated N transformation in unvegetated sediments (29%) and rhizopheric sediments (26%) in a constructed wetland (83). Furthermore, in the plant rhizospheric material, the denitrification and anammox rates were 14.41 ± 7.95 and 2.03 ± 1.76 nmol N/g sed. wet wt./hr, respectively (83). Although molecular data for the anammox enzyme were not available, qPCR results of the *nosZ* gene indicated that the rhizospheric denitrifying communities contained up to 4 × 10^4 copies/ng of DNA. A mathematical modelling-based study revealed that up to 71.1% N removal through partial nitrification, followed by anammox, can be achieved in urban stormwater due to the presence of adequate NH_4^+ (66).

Denitrification kinetics: To evaluate denitrification kinetics in BRCs, researchers have analyzed nitrate removal data using primarily two reaction orders, namely first order (Eq. 17) and zero order (Eq. 18) (32, 105). In most studies, it has been observed that first order kinetics most appropriately describe the denitrification rate (32, 106) (supplementary material, Table S3). In a lab-scale column having media components consisting of woodchips and pea gravel, and an initial nitrate concentration of 3 mg-N/L, Peterson et al. found that the denitrification process can be more accurately fit to a pseudo-first-order model (rate constant, k=11.4 day^{-1}) (32). Using microcosm-based stormwater biofilters, Lynn et al. explored changes of denitrification kinetics with varying media components (e.g., wood, sand plus wood, and gravel plus wood) (105). They found that the denitrification reaction can be represented by both first-order and zero order models, and the first order denitrification constant for the three types of

media were: wood (k = 0.75 hr^{-1}) > gravel-wood (k = 0.58 hr^{-1}) > sand-wood (k = 0.27 hr^{-1}), i.e. the wood-based system showed the greatest nitrate removal performance. Among the two models, the first-order model described the denitrification data slightly better than zero order.

In woodchip bioreactors which were fed with 2 – 11 mg NO_3-N/L, Halaburka et al. reported that the denitrification rate at constant temperature can be appropriately described using zero order kinetics (rate constant: 0.13 (mg-N/mg-biomass-hr) (107). A batch experiment in which woodchip was used as organic substrate (solid-to-liquid ratio of 1:3 by volume) reported that nearly 100% nitrate reduction (decreased from 0.3 to < 0.02 mg-N/L) achieved within 2.6 days; the reaction followed first order kinetics with a rate constant equal to 0.0011 min^{-1} (106). The key factors that impact the denitrification rate constant include dissolved organic carbon level, dissolved oxygen level and influent nitrate concentration (105, 107).

The kinetic expressions for batch systems are:

$$dC/dt = k[C]^n \quad \text{(general equation for zero, first, or higher order rate)} \qquad (16)$$

$$C = C_0 exp^{-k_1 t} \qquad (17)$$

$$C = C_0 - k_0 t \qquad (18)$$

Where, C_0 and C = influent and effluent nitrate concentration, respectively, k_1 and k_0 = first order and zero order rate constant, respectively, and t = time.

Perspectives: Denitrification appears to be the major biological N removal process although some studies noted the importance of plant assimilation. The denitrification rate data were mostly fit by a first order model. More studies need to be carried out to obtain in-depth knowledge about the contribution of other processes including anammox and DNRA on total N removal. Significant amounts of organic N (dissolved organic N: 28% and particulate organic N: 24%) are present in stormwater (17). Hence, future research should be conducted to elucidate the fate and removal mechanisms of organic N in BRCs. Multiple studies have pointed out that the N removal efficiency in BRCs can be influenced by numerous factors.

These factors include hydraulics, climatic conditions, filter media characteristics, plants selection, and stormwater qualities (35, 36, 38), which are briefly discussed in the following section.

Factors affecting N removal in bioretention cells

Hydraulic factors

Hydraulic loading rates (HLR) for stormwater are generally variable, but can be controlled by integrating flow control regulators at the bioreactor outlet (33). For denitrifying bioreactors in the field, installation of a regulated outlet control device could enhance the HLR for denitrification (33). The major hydraulic factors that impact N removal in BRCs are runoff volume, flow rate, hydraulic conductivity and retention time (22, 38). N removal improves with higher retention time, or lower infiltration rates (108). Kim et al. evaluated the impact of various HLR (4 – 20 cm/hr) on N removal in lab-scale bioretention columns and reported that nearly 100% nitrate removal could be achieved at lower HLR (i.e., 4 cm/hr) (55). However, nitrate removal declined to nearly 20% at higher HLR (20 cm/hr) with woodchips as a solid-phase electron-donor and carbon source. The significant deterioration of biofilter performance at higher HLR could be due to the washout of functional microorganisms, enzymes, and/or organic substrates (55), or simply contact time. Based on the results obtained using other electron donors (e.g., newspaper and sulfur/limestone), the authors have suggested that with the optimum HLR of 12 cm/hr, nitrate could be removed efficiently.

Other field-scale/pilot-scale tests also showed similar findings on HLR effects on N removal. Results from a conventional field-scale BRC (planted) showed that with the variation of HLR from 4.1 to 13.9 cm/hr, the removal efficiency of total ammonium, NO_x (nitrate + nitrite) and TN decreased from 85 to 74%, 61 to 56% and 59 to 53%, respectively (34). Another field-scale experiment with woodchips as a C source observed nearly an average of 55% NO_x-N removal at lower HLR (0.93 – 1.38 cm/hr), but the efficiency decreased at higher HLR (109).

Osman et al. found the most appropriate hydraulic conductivity range for BRCs to be between 1.3 and 20 cm/hr; if the hydraulic conductivity exceeds the recommended range, then soil moisture would not be adequate for plant growth (38). However, at values below the stipulated range, clogging with ineffective capture of runoff would result (110). Overall, the findings of these studies suggest that lower HLR can increase hydraulic retention time (HRT) and enhance nitrogen removal rate.

Role of a saturated zone

In recent years, many studies have recommended installation of a saturated zone (SZ) into BRCs to increase nitrogen removal (specifically nitrate) by encouraging microbial denitrification and attenuating plant water stress (47, 111, 112). One of the easiest options to create a SZ in bioretention columns is by raising their outlet pipe, hence providing a constant water level in the bottom layer of biofilter (113). In field-scale tests, the SZ is termed as internal water storage zone (IWS) (36). In addition to an elevated pipe configuration, anoxic saturation conditions can be created by placing a layer of materials that act as sources of organic carbon and support the development of microbial biofilm (woodchips, newspaper, sawdust, wheat straw, sugar cane mulch, pine chips, etc.) below the primary filter media to facilitate heterotrophic denitrification (33, 112, 114) (Table 3).

A mesocolumn study by Morse et al. found higher proportions of NO_x removal in SZ columns (89%) than the columns without a SZ (72%) (39). Another lab-scale investigation also reported a similar trend in that the vegetated columns installed with a SZ (87%) demonstrated greater TN reduction than non-SZ columns (75%) (114). A recent field-scale study also reached the same conclusion that BRCs (planted) having an internal water storage (IWS) zone showed better performance with respect to ammonium (NH_4^+-N) (with IWS: 86% and without IWS: 81%) and NO_x-N removal (with IWS: 88% and without IWS: 54%) (34).

Although installation of a SZ enhances N removal as demonstrated in several lab-scale studies, a few field-scale tests reported minimum or no significant improvement of N removal with incorporation of the SZ. In a previous work where authors compared the pollutant removal efficiency of two field-scale BRCs, with one having a standard design and the other with creation of an anaerobic sump by adding a layer of newspaper and sand mix (mass ratio: 0.017:1.0) (115). The mean event concentration (EMC) reduction for nitrate (NO_3-N) in the anaerobic sump-containing cell and the standard design cell was 79 and 86%, respectively (115). Field-scale experiments also found an insignificant impact of IWS because the concentration of denitrifying functional genes (*nirK* and *nosZ*) decreased with an increase of depth (15, 103). Altogether, inconsistent results have been observed on the impact of SZ on N removal in BRCs. Part of this lack of improvement may be related to inadequate HRT in the field installations or lack of continued stored water. Thus, additional research is needed on this topic, including more accurate determination of N transformations using ^{15}N tracer techniques.

Plant species

Plants are considered as an essential component of BRCs. Roles of plants in the BRCs include: (1) planted cells are highly effective for contaminants removal compared to non-planted cells, (2) biofiltration efficiency differs with the type of plant species used, (3) native plants show better performance than exotic ones, (4) diverse plant systems are more effective compared to single-plant systems (77). Vegetation contributes treatment of pollutants in BRCs both directly and indirectly. Direct effects include degradation and/or uptake of pollutants. However, indirect impacts include an influence on rhizosphere microbial community composition through release of organic compounds (root exudates) (22). Vegetation also contributes to bioretention hydrologic functions of the filter media through various routes including plant transpiration, plant interception of rainwater, regulation of surface flow, and modification of water infiltration (47). Most lab- and field-scale studies have concluded that the efficiency of

removal of pollutants is higher in planted BRCs compared to non-planted systems (Table 4) (34, 39). Additional information on the efficiency of different plant species (single or multiple plantings) for removal of various nitrogen species (mainly nitrate and total nitrogen) from stormwater is given elsewhere (Table S4). Among the reported findings, two plant species namely *Arundo donax* var. versicolor and *Bougainvillea* 'Sakura Variegata' were most effective for removal of nitrate (96% removal by both species) from stormwater (74).

A field-scale trial showed that the average NO_x (NO_3^-+NO_2^-) removal efficiency was higher for the planted than non-planted systems (34). For a conventional BRC, the NO_x removal efficiency increased from 15 to 54% (each system was planted with five local plants) (34). Bioretention mesocosms-based study noted that TN retention was 81% in the shrubs/grasses vegetated systems compared to 41% in the non-vegetation systems (116). Another pilot scale trial on street tree BRCs found that the TN load removal from the planted (*Lophostemon confortes*) systems was more (95%) than the unplanted systems (only 36%) (117).

Plant diversity also influences the treatment performance since Morse et al. found that five out of six selected plants (*Juncus krassii, Buffalo, Carex appressa, Allocasurina littoralis, and Leptospermum continentale*) showed lower denitrification (mean: 1−3%) than the other plant species evaluated (*Dianella tasmanica* - mean: 7%) (39). Another study also reported that the columns vegetated with *Medicago sativa* (L.). demonstrated low nitrogen removal rate (TN: − 29.8% to − 123.0%), whereas in columns vegetated with *Radermachera hainanensis* (Merr.), *Juncus effusus* (L.), *Ophiopogon japonicus* (Linn. f.) and *Vetiveria zizanioides* (L.), the removal efficiency was significantly enhanced (TN: 52.8% to 84.2%) (118).

A lab-scale column test involving ^{15}N isotope analysis observed a large variations of nitrification efficiencies with the application of three types of bioretention grasses. namely *Ophiopogon japonicus* (27–53%), *Iris tectorum Maxim* (16–37%) and *Hosta plantaginea* (12–

39%) (43). However, the denitrification efficiencies were lower than nitrification, i.e., 9–2%, 5–11%, and 8–11%, respectively. Interestingly, this study also revealed that the rhizosphere oxygen level regulates N transformation reactions since both nitrification and denitrification were higher (2 - 3 fold) at the top layer of the BRC. Another study with three types of vegetation (grassed, landscaped and overgrown) found that the denitrification efficiency among the three types of vegetation was in the order of grassed < landscaped < overgrown.

Together, research has found that vegetated BRCs show better N removal performance than non-vegetated cells. Although impacts of plant diversity on N removal efficiency has been investigated in many studies, several issues are still unclear. For example, how N removal efficacy may change by the plant growth/age is not fully understood yet which needs further investigation.

N pollutant loads and characteristics

Stormwater events can vary in terms of their frequency, intensity, and duration (22), which may impact the quality of runoff. Prevailing climatic conditions may also influence the runoff quality. For example, during warmer and dry weather conditions, more pollutants may accumulate on impervious surfaces. These pollutants tend to be washed out with the first flush of rainfall, which causes an increase in the concentration of pollutants at the initial period of storm events (22). The nature of nitrogen pollutants and their concentrations in stormwater should influence the fate of biological N removal process in BRCs (38).

In a column reactor, Kim et al. assessed the effects of different influent nitrate loading rates (NLR) (6.5 – 24.9 mg/day as N) on the denitrification rate using three types of solid-phase substrates (electron donors: newspaper, woodchips, and sulfur/limestone) (55). The nitrate removal efficiency was nearly 100% when tested at the lower loading rate (6.5 mg/day), but the removal efficiency decreased constantly with the rise of loading rates, i.e., the efficiency decreased to ~90% at 11.8 mg/day and varied between ~40 – 60% at 24.9 mg/day NLR.

Using a stepped BRCs, Wang et al. observed variations of the N removal efficiency with change of the influent nitrate/ammonium concentrations (118). By increasing the nitrate EMC from 3.04 ± 2.64 to 3.17 ± 2.01 mg/L, the mean removal rate slightly increased by 7.4% (i.e. from 45.4 to 52.8%). However, the removal of ammonium was not impacted significantly because with the increasing load from 1.73 ± 2.01 to 2.22 ± 2.41 mg/L (EMC), the removal rate of ammonium decreased only slightly (95.3% to 94.7%) (118). This may be because the ammonium removal was primarily controlled by the media. In a review article by Davis et al., the authors reported that the TN removal efficiency in both field- and laboratory-scale studies largely varied within a wide range (32 – 99%) when the influent concentrations fluctuated between 1.2 – 6.0 mg/L (119).

Variable influent nutrient loads (e.g., nitrate and ammonium levels) could change the rhizosphere dissolved oxygen (DO) and pH levels, which are believed to be influential factors that affect microbial N transformations (43). In column-based BRCs, Chen et al. observed that the root DO level was constantly enhanced with increased nutrient loads (43). However, the increase in loading rates did not have significant effects on pH, which could be due to the natural buffering capability of soil. Furthermore, the authors detected that the rate of nitrification, denitrification and DNRA was greater at higher nutrients loads, but among them, nitrification was the dominant and DNRA was the least important N removal pathway (43).

Altogether, research has shown inconsistent results about the impact of N loading rate on bioretention performance, which may be due to variations of the BRC configuration, study modes (lab-scale, pilot-scale or field-scale), vegetation diversities, filter media composition, carbon substrates, the availability of saturation zone and/or the nature of N pollutants. It is important to evaluate removals based on consistent criteria, such as rates, not just relative metrics such as percent removals. The key outcome of these investigations is that to achieve

higher removal performance, inlet N (e.g., nitrate) loads could be considered as one of the bioretention design factors.

Characteristics and depth of the engineered media

The structure of the engineered media and its depth generally regulate the stormwater pollutant removal efficiency in BRCs (38, 120). The bioretention media are broadly divided into three layers (top/upper, middle, and bottom), and each layer is designed to meet specific objectives (22). The upper layer is mainly designed to support the growth of plants as well as to enhance microbially-driven treatment mechanisms, while the middle filter layer improves several mechanical processes including screening and sorption performance, and the bottom gravel layer provides drainage (22).

Multiple studies have been performed on nitrogen removal in BRCs using different media compositions (121–123). Glaister et al. compared NO_x (nitrate + nitrite) and ammonium (NH_4^+) removal efficiency of two types of biofilter media, loamy sand (Fe: 1000 mg/kg and Al: 900 mg/kg) and skye sand (Fe: 21000 mg/kg and Al: 1000 mg/kg) (123). They found that the N removal was higher in skye sand (NO_x: 93% and NH_3: 96%) than loamy sand (NO_x: 81% and NH_3: 88%) under drying periods. In laboratory column experiments using synthetic/actual stormwater and three types of filter media such as concrete sand (sand: 88%, silt: 10% and clay 2%), compost free media (termed as COA - sand: 73%, silt: 18% and clay 9%) and masonry sand (sand: 94%, silt: 2% and clay 4%), Barrett et al. observed different removal trends for stormwater pollutants (122). For example, greater N removal was achieved in the columns filled with COA (NO_x: 62% and NH_3: 79%) compared to masonry sand (NO_x: 56% and NH_3: 72%); both columns were planted with a native Texas plant Big Muhly (*Muhlenbergia lindheimeri*) and had a saturation zone (122).

A recent study on bilayer media bioretention columns found more N (89%) removal in the column which contained 5% fly ash (other media: 90% sand +5% crushed straw) than the

column that contained 5% clay (85.9%) (121). The major reason for the higher performance in the fly ash-based system was due to the smaller permeability of fly ash compared to clay, which caused an increase of the hydraulic retention time and possibly more denitrification. Using two sets of loamy-sand-filled BRCs having 0.6 and 0.9 media depths, Brown and Hunt, noted that for both configurations, the effluent ammonia concentration was considerably lower than the influent, but a significant increase of NO_x-N concentration was noticed in the effluent (124). This trend is due to potential nitrification of organic N and/or lower denitrification is possibly due to the absence of internal water storage zones. In this field-scale study, the lower media depth was effective with estimated annual total nitrogen load reductions of 21% for the cell with 0.6-m depth and 19% for the 0.9-m depth. Chen et al. also noticed that the top layer (nitrification: 7 - 28%, denitrification: 2 - 5%) of their biofilter media produced higher N removal than the bottom layer (nitrification: 2 - 12%, denitrification: 1 - 3%) (43). A lab-scale column trial reported around 20% increase of ammonium (NH_4^+-N) removal due to addition of iron-rich soil to the biofilter containing initially sandy loam (21). In a recent study where three columns were filled with different filter materials such as woodchips, woodchips plus biochar (33% by wt.) or woodchips plus straw, it was observed that the three types of woodchip bioreactors showed high performance for nitrate removal from stormwater. The concentration of nitrate in the effluent decreased by above 99% to concentrations below the detection limit (less than 0.05 mg-N/L) (125).

Overall, many studies have recommended the use of a layered media bioretention system to deliver the highest outcomes for stormwater treatment with the appropriate media depth (86, 121, 126) (Table 5). In most cases, higher degree of denitrification occurred at the bottom layer of the biofilter. During engineering and construction, it is important to select soil plus sand-based media compared to only sand-based media in order to decrease the infrastructure and maintenance cost of the BRC while meeting the treatment objectives.

Effects of storm events frequency (wet vs dry periods)

Stormwater events vary in their frequency, size, and duration. Thus, BRCs will experience a high degree of alternating wet and dry periods (22). Dry conditions can alter the media properties (e.g., increase of porosity due to formation of aggregates) and biological activities (e.g., decrease of plant and microbially-driven pollutants degradation/transformation rates) (22). Many lab- and field-scale studies have been conducted to understand the fate of N pollutants during wet and dry conditions (Table 6).

A column experiment (containing loamy sand media, vegetation, and a saturated zone) showed that the NO_x removal during the dry period was 81%, but varied between 80 – 86% in two wet cycles (first: August -November, second: April – July) (123). However, the ammonia removal was lower during the dry (88%) than the wet periods (89 – 99%). Using single-plant biofilter columns with a saturation zone, Payne et al. found that the TN removal was greater during the wet cycle (79 – 93%) compared to the dry cycle (12 – 78%); the large variations in both conditions were mainly due to plant diversity (114). Subramaniam et al. evaluated the dynamics of nitrate removal in lab-scale biofilter columns and it is observed that the NO_3-N removal fluctuated during an event from a high removal proportion (60–90%) in the first outflow that slowly decreased in the initial operation period (0.5 hr), then the removal rate stabilized at 0–15% (127). Additionally, this study concluded that the denitrification process was more active during the dry period of an event compared to the wet period.

Results from a field-scale woodchip BRC showed that the cell exhibited denitrification during both the wet and dry phases. Nevertheless, a major fraction of nitrate removal was observed during the wet phase (TN: > 26.3%) compared to the dry phase (TN: < 9.9%) (109). Another study from the same research group using a layered BRC containing woodchips as a C source demonstrated more than 80% nitrate removal (86) and the nitrate removal mainly occurred during the wet period.

In total, wet conditions mainly support denitrification, whereas nitrification and ammonification are predominant in dry conditions (86, 109, 121). Long dry periods have displayed negative impacts on the capacity of BRCs to remove pollutants because of increases of metal and nitrogen leaching observed in several studies (22). To keep BRCs operating with high performance in hot and dry climates, it is necessary to select appropriate drought-tolerant plant species, which may assist with plant growth, as well as assist in the survival of microorganisms in the rhizosphere.

Temperature effects (cold vs warm)

Temperature will affect most nitrogen removal mechanisms in BRCs. Nitrogen uptake by plants is generally higher at warm temperature (128). Microbial activities leading to N transformation processes tend to increase to an optimum temperature (around 20–35°C, depending on locations and soil types) (129). Successful operation of BRCs in cold climates can be a great challenge because of several reasons, namely, cold temperatures, ice cover, cold water, de-icing salts, repeating freeze-thaw cycles, etc. (130). These characteristics may impact the biological processes, soil infiltration rates, and vegetation health.

To date, limited information is available about temperature effects on BRCs (Table 7). In a recent study by Halaburka et al. (131), authors have investigated the impacts of a wide ranges of temperatures (4 – 30 °C) on nitrate removal rate in woodchips bioreactors. They found that temperature considerably influences the nitrate reduction (e.g. denitrification). The nitrate removal rate (mg-N/L/h) was −0.00340 at 4 °C, while it was −0.360 at 30 °C (131). A biofilter mesocosms-based study investigated the influence of three temperatures (2, 7 and 20°C) on NO_x-N and NH_4-N removal, and observed that the ammonium removal was positively correlated with the temperature (i.e., 18, 51 and 74% at 2, 7 and 20 °C, respectively) (132). However, the removal of other nitrogen species (nitrate-N: NO_x-N) was not effective, i.e., significant leaching was observed at higher temperature (20 °C). At lower temperature (2 °C),

a slight change in the concentration of N species was observed, i.e., 2-fold rise in nitrate and nearly 18% reduction of ammonium concentration, which suggests that at lower temperature, nitrification may occur. Chang et al. evaluated the impacts of three temperatures (10, 23, and 28 °C) on nitrate removal from stormwater under lab-scale column experiments (133). Nitrate removal efficiency increased with increase of temperature, 63.2, 77.9 and 93.6 % at 10, 23 and 28 °C, respectively. Another recent study from the same research group evaluated the impacts of four different temperatures (4, 12, 23 and 35°C) on the removal of nutrients (nitrate and total phosphorus) from stormwater in lab-scale (134). Overall, no significant changes in the nitrate removal was observed with the variations of temperature because the removal efficiency varied between 85 – 90% at all temperatures (4 – 35 °C).

The kinetics of N removal are impacted by variations in environmental temperature. Chang et al., (2011) evaluated the reaction kinetics for nitrate removal in a column packed with multi-media components including fine sand (50%), sawdust (25%), tire crumb (15%), limestone (10%), and operated under three different temperature levels (10, 23 and 28 °C) (133). They found that the nitrate transformation was zero order with the rate constant increasing with increases of temperature, i.e., k (M/s) = 0.047, 0.076 and 0.07 at 10, 23 and 28 °C, respectively. Interestingly, the reaction changed to first order with change of the filter media components to fine sand (50%), tire crumb (30%) and sawdust (20%) with k values (s^{-1}) were 0.012, 0.017 and 0.05 at 10, 23 and 28 °C, respectively, and the change of order may be related to the bioavailability of carbon. In another study using a column packed with fine sand (96.2%) and iron filings (3.8%) and tested under 4, 12, 23 and 35 °C, the reaction was zero order, but the rate constants did not significantly change with temperature.

Taken together, researchers have shown that environmental temperature considerably influences N transformations. Additionally, availability of dissolved organic carbon impacts

the denitrification rate. A few reports have shown that temperature has a positive effect on stormwater denitrification (36, 129). To improve our understanding about climate effects on microbially-mediated N transformation in BRCs, more lab-scale and field-scale studies are required.

Future research directions

- Little research has been performed on the role of anammox in the BRCs. Comprehensive studies employing ^{15}N isotope techniques are needed to understand the fate of N in the BRCs as well as the relative contribution of various bioprocesses to the total N removal.
- The filter media redox conditions may control the fate of N biotransformation reactions since oxic conditions mainly favor nitrification and anoxic environments encourage denitrification (135). Therefore, in-depth research investigations should be done to evaluate changes of redox and oxygen gradient patterns as a function of media depths.
- Although a few reports are available on the dynamics of bacterial communities in biofilter media (30, 81), archaeal communities may synergistically work with bacteria and contribute to N removal. Thus, in future studies, researchers should also consider assessing the dynamics of archaeal communities in BRCs.
- The rhizosphere could facilitate interactions between microbes and N species. Plants influence the composition and function of rhizosphere communities by releasing organic compounds through roots, which need to be verified to select an appropriate plant species or species mix. Moreover, additional studies are needed to understand N removal by other rhizospheric phenomena such as the role of fungal communities, plant root-formed preferential flow paths and their impact on nutrient transport, the role of legumes in nitrogen fixation in bioretention systems, and finally, the electron shuttling of wood-derived biochar amended filter media to facilitate denitrification (136, 137).

- To date, most of the studies on BRCs have been carried out under controlled lab-scale environments and field-scale trials at normal climate, but limited information is presently available on the impacts of challenging climates, namely, cold or tropical weather conditions, on stormwater treatment efficiency of BRCs; research on this topic merits further consideration.
- For bioaugmentation of denitrification rate in BRCs, one of the important criteria is to increase C/N ratio of stormwater (138), thus future works should consider augmentation of filter media using carbon-rich materials such as biochar, softwood chips, etc. Other potential parameters that can accelerate the nitrogen removal efficiency in BRCs include low hydraulic loading rates (HRT), incorporation of a saturation zone (SZ)/internal water storage (IWS) with deeper depth, mixed vegetation, mixed layer filter media, wet periods, and warmer climates (22, 38). Additional information on bioaugmentation of N removal in BRCs is given in supplementary materials.
- Besides controlled experimental work in the laboratory, a few studies have explored modeling of denitrifying stormwater biofilters under different simulated storm conditions (139, 140). More robust numerical models should be developed to assess the overall TN reduction efficiency of BRCs. Such simulation studies may provide useful data for designers to select suitable parameters according to the treatment objectives set for BRCs.

Conclusions

This paper presents a state-of-the-art review of the recent developments that have been made on the biological nitrogen removal from stormwater in BRCs. Plant- and microbially-driven N transformation processes that occur in BRCs include the uptake of nitrogen (assimilation) by both plants and microorganisms, nitrification, denitrification, and anammox. However, denitrification is the major process for N removal (especially nitrate) from runoff. Biofilters

are generally enriched with diverse microbial communities, but the phyla *Bacteroidetes* and *Proteobacteria* are the most abundant.

High N removal efficiency (TN: > 70%) has been achieved in both lab- and field-scale studies. However, large variations have been observed among the studies. The lack of consistency can be attributed to the fluctuations of hydraulics (hydraulic loading rate or N loading rate) and environmental factors. The key factors to consider are the presence/absence of saturation zones, the composition and height of the filter media, the type of plant species, the frequency of storm events (wet and dry periods) and the prevailing ambient temperature (warm and cold climate) (Fig. 3). In general, BRCs show better N removal performance when they are operated at low hydraulic/N loading rates, installed with a saturation zone, vegetated with native plants, having deeper and multilayer biofilter media with warm climate temperature and wet periods.

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Disclosure statement

The authors report no conflict of interest.

Supplementary online material

Supplementary data to this study are submitted.

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List of Tables and Figures

Table 1. Concentration of different nitrogen species detected in stormwater runoff

Stormwater source	Different chemical forms of nitrogen (mg/L)[e]					
	Nitrate (NO_3-N)	Nitrate + Nitrite (NO_x-N)	Ammonia (NH_3-N)	Organic-N	Total nitrogen (TN)	Reference
Road	1.0	NA	0.29	NA[d]	2.0	(15)
Roadway	NA	0.66	NA	NA	1.3	(141)
Parking lot	NA	0.19±0.11	0.29 ±0.48	0.45±0.39	0.94±0.87	(124)
Carpark	NA	0.4 ± 0.2	0.04 ± 0.06	0.6 ± 0.3	1.1 ± 0.5	(142)
Asphalt	0.6±0.9	NA	0.18±0.36	NA	NA	(6)
Paver	0.3±1.2	NA	0.05±0.14	NA	NA	(6)
Crushed stone	0.3±0.4	NA	0.11±0.24	NA	NA	(6)
Asphalt	NA	0.3	0.31	0.75	1.33	(143)
Highway	1.1	NA	1.1	NA	NA	(144)
Interstate highway	NA	0.20±0.17	0.12±0.23	1.50±2.04	1.64±2.1	(145)
Mixed[a]	NA	0.12±0.16	0.10±0.13	0.89±0.79	1.01±0.81	(145)
Mixed[b]	0.39±0.58	NA	NA	0.66±1.24	1.61±1.97	(146)
Mixed[c]	NA	0.74±0.56	0.29±0.39	1.1±0.99	2.13±1.68	(17)

[a] Mixed: Parking lot, maintenance building, picnic area

[b] Mixed: Rooftops, driveways and sidewalks, roads and patios.

[c] Mixed: Residential, commercial, and/or parkland

[d] NA: Not available

[e] Standard deviation associated with some data is missing since it is not available, or the data is extracted from the figure in the cited reference.

Table 2. Nitrogen transformation process, reaction, enzymes and their properties

Process/Reaction	Condition	Enzyme	Redox potential (E_0' in mV)	Location	Reference
Dissimilatory nitrate reduction (DNRA)					
$NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O$	Anoxic	Nitrate reductase (NR: eukNR, Nar, Nap and Nas)	+433	Membrane associated, periplasm or cytoplasm	(60, 61)
$NO_2^- + 8H^+ + 6e^- \rightarrow NH_4^+ + 2H_2O$	Anoxic	Nitrite reductase (Nrf)	+340	Cytoplasmic membrane	(60, 61)
Denitrification					
$NO_2^- + 2H^+ + e^- \rightarrow NO + H_2O$	Anoxic	Nitrite reductase (NiR)	+350	Periplasm	(60, 61)
$2NO + 2H^+ + 2e^- \rightarrow N_2O + H_2O$	Anoxic	Nitric oxide reductase (NoR)	+1175	Transmembrane	(60, 61)
$N_2O + 2H^+ + 2e^- \rightarrow N_2 + H_2O$	Anoxic	Nitrous oxide reductase (NoS)	+1335	Periplasm	(60, 61)
Anammox					
$NO + NH_3 + 3H^+ + 3e^- \rightarrow N_2H_4 + H_2O$	Anoxic	Hydrazine hydrolase (HH)	+340	Anammoxosome	(60, 61)
$N_2H_4 \rightarrow N_2 + 4H^+ + 4e^-$	Anoxic	Hydrazine dehydrogenase (HDH)	-230	Anammoxosome	(60, 61)
Nitrification					
$NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^-$	Oxic	Nitrite oxidase (NO)	+420	Membrane associated	(60, 61)
$NH_2OH + H_2O \rightarrow NO_2^- + 5H^+ + 4e^-$	Oxic	Hydroxylamine oxidoreductase (HAO)	+60	Periplasm	(60, 61)
$NH_3 + O_2 + 2H^+ + 2e^- \rightarrow NH_2OH + H_2O$	Oxic	Ammonia oxidase (AMO)	+730	Transmembrane	(60, 61)
Nitrogen fixation					
$N_2 + 6H^+ + 6e^- \rightarrow 2NH_3$	Oxic/Anoxic	Nitrogenase (Nif)	-92	Cytoplasm	(60, 61, 147)

Table 3. Nitrogen removal efficiency in bioretention cells with and without saturated zones.

Study site	N removal (%)[b] NOx[b]	N removal (%)[b] TN[c]	Saturation zone (SZ)	HLR[a]	Carbon source	Planted	Reference
Mesocolumn	89	NA[d]	Yes	10 - 30 cm/hr	Sugar cane mulch and pine chips	Single-plant	(39)
	72	NA	No				
Lab-scale column	NA	87	Yes	10 - 30 cm/hr	Sugar cane mulch and pine chips	Single-plant	(114)
	NA	72	No				
Field-scale	NA	90	Yes	NA	Newspaper	Single-plant	(115)
	NA	95	No				
Field-scale	81	83	Yes	4.1 - 13.9 cm/hr	Eucalyptus Woodchips	Mixed-plant	(34)
	29	74	No				
Lab-scale column[e]	81	82	Yes	20–40 cm/hr	Pine woodchips and pine flour	Single plant	(123)
	9	33	No				
Lab-scale column[f]	93	89	Yes	20–40 cm/hr	Pine woodchips and pine flour	Single plant	(123)
	27	44	No				
Lab-scale column	-23[h]	73	Yes	~2 cm/hr	Newspaper	Single plant[g]	(148)
	62[h]	35	No				
Lab-scale column	66.1[h]	81.2	Yes	NA	Woodchips	Single plant	(149)
	30.5[h]	59.4	No				

[a] HLR: Hydraulic loading rates

[b] NOx: Nitrate + Nitrite

[c] TN: Total nitrogen

[d] NA: Data not available

[e] Columns were operated under wet period.

[f] Columns were operated under dry period.

[g] 10 - 40 plants (*Phragmites australis*) per column.

[h] It refers to only nitrate (NO_3-N).

Table 4. Nitrogen removal efficiency in planted and nonplanted bioretention cells/constructed wetlands.

Study type	N removal efficiency (%)	Plantation	Main filter media	Reference
Mesocolumn[a]	NO_x: 39 - 60 (AS)[b], 1 - 7 (DN)[c]	Yes	Loamy sand (30 cm), sand (20 cm) and gravel (10 cm)	(39)
	NO_x: 38 (AS), 15 (DN)	No		
Field-scale	NO_x: 54	Yes	Sand (30 cm), River rock (5 cm) and #57 stone (30 cm)	(34)
	NO_x: 15	No		
	NO_x: 88	Yes	Sand (30 cm), River rock and wood chip (30 cm), River rock (5 cm) and #57 stone (30 cm)	
	NO_x: 78	No		
Mesocosms	TN: 81	Yes	Sandy loam (80 cm)	(108)
	TN: 41	No		
Pilot-scale	TN: 95	Yes	Sandy soils[f]	(117)
	TN: 32	No		
Lab-scale column	NO_x: 93, NH_3: 96	Yes	Skye sand (30 cm), coarse sand (20 cm), pea gravel (70 mm), and gravel (30 mm).	(123)
	NO_x: 41, NH_3: 84	No		
Constructed wetlands[a]	NO_x: 78 (DN)	RS[d]	Not applicable	(83)
	NO_x: 71 (DN)	BS[e]		

[a] These studies have used ^{15}N tracer technique to find out the different N transformation processes.

[b] AS: Assimilation

[c] DN: Denitrification

[d] RS: Rhizosphere sediment (called as vegetated system)

[e] BS: Bare sediment (called as nonvegetated system)

[f] Detail media composition is not available.

Table 5. Changes of N removal efficiency with various filter media composition and depth.

Study type	Key filter media composition	Overall N removal efficiency (%)	Change of N removal efficiency (%) with depth	Reference
Bilayer media columns	90% sand+5% fly ash+5% crushed straw	NO_3-N: 91.5–97.4	NO_3-N: 25 (0-75cm), 85.1 (75-95cm)	(121)
	90% sand+5% clay+5% crushed straw	NO_3-N: 87.5–96.9	NO_3-N: 13.8 (0-75cm), 80.8 (75-95cm)	
	Quartz sand	NO_3-N: 34.5–46.2	NA[a]	
	Quartz sand+5% crushed straw	NO_3-N: 42.5–51.9	NA	
Laboratory column	Sand (73%)+silt (18)+clay (9%)	NO_x: 62, NH_3: 79	NA	(122)
	Sand (94%)+silt (2)+clay (4%)	NOx: 56, NH_3: 72	NA	
Biofilter columns	Skye sand (Fe: 21000 mg/kg and Al: 1000 mg/kg)	NO_x: 93, NH_3: 96	NA	(123)
	Loamy sand (Fe: 1000 mg/kg and Al: 900 mg/kg)	NO_x: 81, NH_3: 88	NA	
lab-scale columns	Sandy loam (100 kg)	NH_4^+-N: 76.5	NA	(21)
	Sandy loam (100 kg)+iron-rich soil (15 kg)	NH_4^+-N: > 95	NA	
Lab-scale columns	Loamy sand (30cm)+gravel (15 cm)+pebble (30cm)	NA	^{15}N-NO_3^-: 2- 5 (top 10 cm), 1- 3 (bottom 10 cm)	(43)
		NA	^{14}N-NO_3^-: 7- 28 (top 10 cm), 2- 12 (bottom 10 cm)[d]	
Pilot-scale columns	Mixed structure[b]	NA	TN: 64.8 (20cm), 75 (40cm), 86.8 (60cm)	(30)
	Layered structure[c]	NA	TN: 63.3 (20cm), 72.1 (40cm), 83.9 (60cm)	
Field-scale	Sand (87.5%)+silt and clay (10%)+compost (2.5%)	NA	TN: 21 (60cm), 19 (90cm)	(124)

[a] NA: Data not available.

[b] Mixed structure: Soil: sand: fly ash (1:1:1) (60 cm)

[c] Layered structure: [Soil (10cm) + sand (10cm) + fly ash (10 cm)] (two layers)

[d] This refers to ^{14}N–NO_3^- produced by nitrification

Table 6. Impacts of stormwater events variability (wet and dry periods) on nitrogen removal efficiency.

Study type	Condition	N removal efficiency (%)	Other conditions	Reference
Biofilter columns	Wet1[a]	NOx: 80, NH_3: 89, TN: 70	Loamy sand media, vegetated, and saturated zone	(123)
	Wet2[b]	NOx: 86, NH_3: 99, TN: 85		
	Dry	NOx: 81, NH_3: 88, TN: 69		
lab-scale columns	Wet	TN: 79 - 93	Loamy sand filter, single-plant, and saturated zone	(114)
	Dry	TN: 12 - 78		
Field-scale	Wet	TN: > 26.3	Sand, soil, and wood chips, single-plant, no saturation zone	(109)
	Dry	TN: < 9.9		
Bioretention columns	Wet	NO_3^-: ~ -20	Wood chips, sandy loam, river sand, vegetation, saturation zone	(148)
	Dry	NO_3^-: ~ 100		

Table 7. Impacts of various temperatures on nitrogen removal efficiency.

Study type	Temperature/Season	N removal efficiency/rate (nmol N/g sed. wet wt./hr)	Initial N concentrations (mg/L)	Reference
Biofilter mesocosms	2 °C	NH_4-N: 18 ± 26%, NOx-N: -208 ± 101%	NOx-N: 0.40 ± 0.16, NH_4-N: 0.22 ± 0.05	(132)
	7 °C	NH_4-N: 51 ± 15%, NOx-N: -320 ± 127%		
	20 °C	NH_4-N: 74 ± 18%, NOx-N: -944 ± 359%		
Laboratory column	22.9 °C	NO_3^-: > 98%	NO_3^--N: 5.65	(150)
	10 to +10 °C	NO_3^-: > 96%		
Laboratory column	10 °C	NO_3^-: 63.2%	NA	(133)
	23 °C	NO_3^-: 77.9%		
	28 °C	NO_3^-: 93.6%		
Constructed stormwater wetlands			NO_3^-: ~ 0.004 - 0.22	(83)
Unvegetated sediments	Summer	DN: 0.67, AN: 0.04		
	Fall	DN: 3.77, AN: 0.20		
	Winter	DN: 4.57, AN: 0.65		
Plant rhizospheric	Summer	DN: 16.3, AN: 2.2		
	Fall	DN: 8.88, AN: 1.67		
	Winter	NA[a]		

[a] NA: Data not available

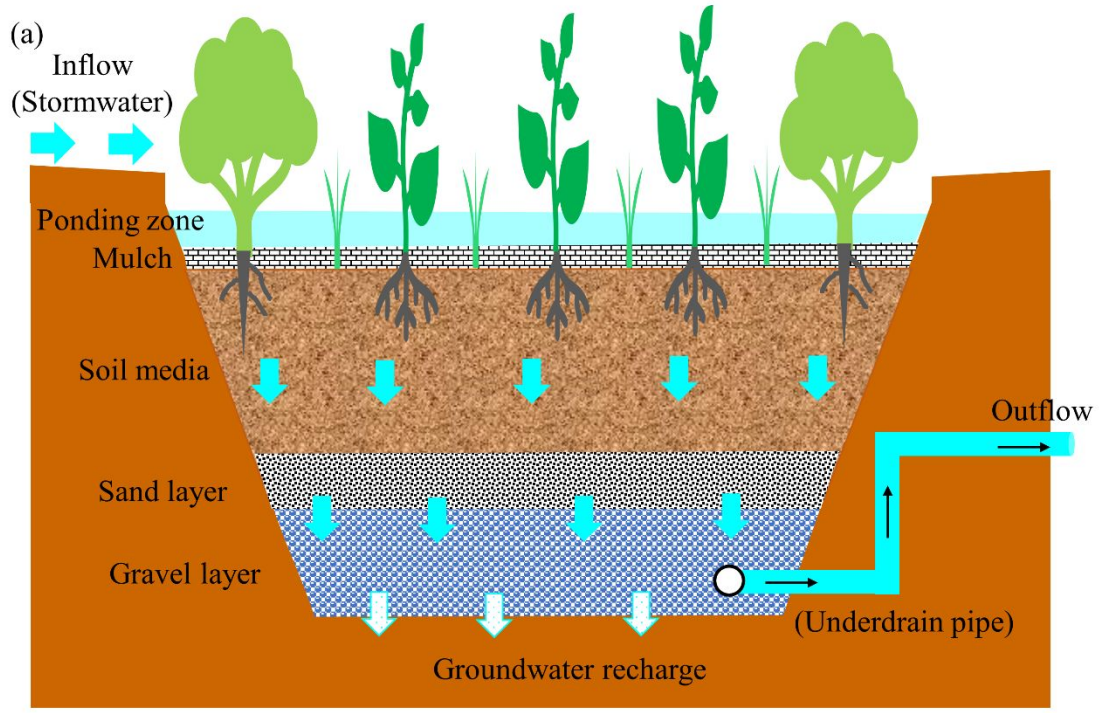

Fig.1. Schematic showing different components of a typical field-scale stormwater bioretention cell (a), and image of a bioretention facility installed at National University of Singapore.

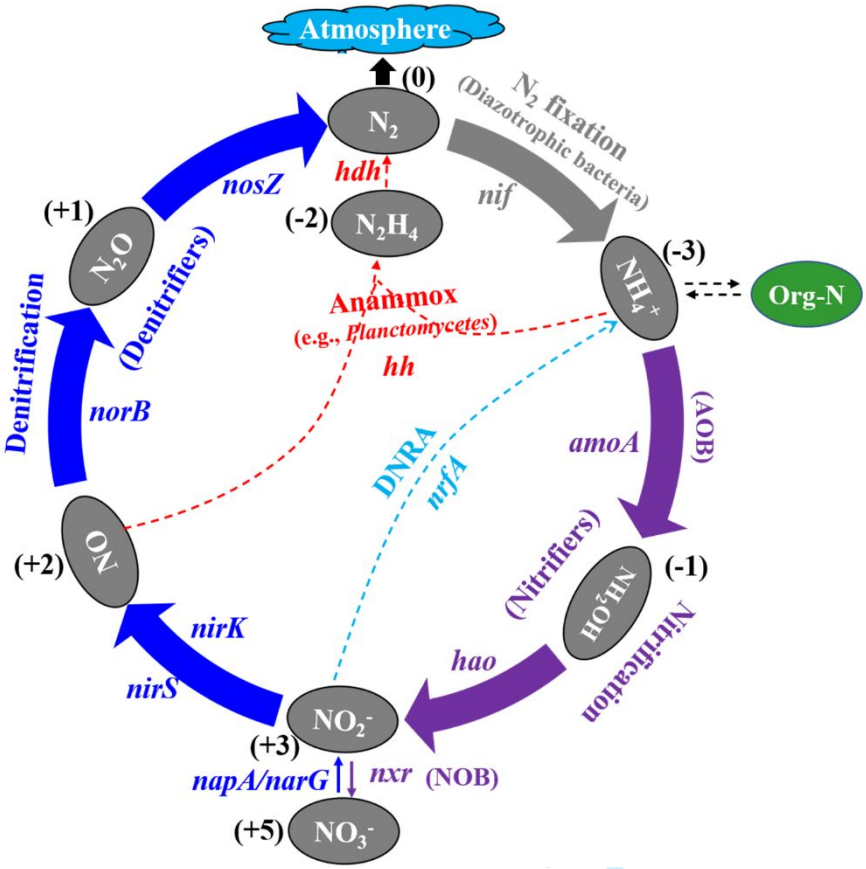

Fig. 2. An overview of the major biological nitrogen cycle in soil (42, 61, 65). Specific enzymes responsible for various nitrogen transformations are: denitrification - *narG/napA*: nitrate reductase; *nirS/nirK*: nitrite reductase; *norB*: nitric oxide reductase; *nosZ*: nitrous oxide reductase; N_2-fixation - *nif*: nitrogen fixation; nitrification - *amoA*: ammonia monooxygenase; *hao*: hydrazine oxidoreductase; DNRA - *nrfA*: respiratory nitrite ammonification; anammox - *nxr*: nitrite oxidoreductase; *hh*: hydrazine hydrolase; *hdh*: hydrazine dehydrogenase. AOB : ammonia-oxidizing bacteria; NOB : nitrite-oxidizing bacteria. Numerical values shown in the bracket indicate the oxidation state of N in the compounds.

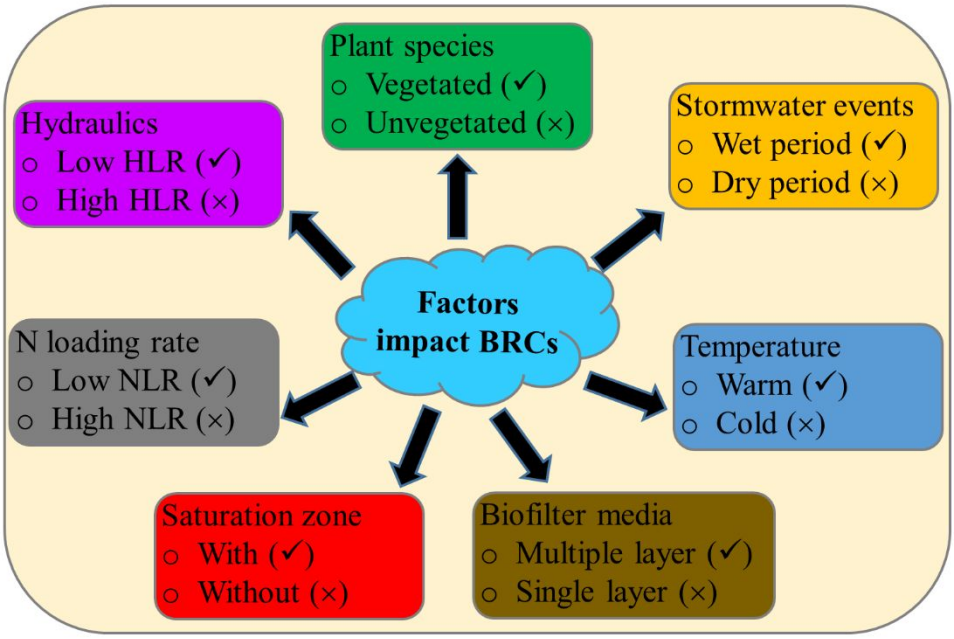

Fig. 3. Key environmental factors that impact the nitrogen removal performance in bioretention cells (BRCs). The symbol tick (✓) means an increase and cross (×) means a decrease of N removal efficiency which are observed in most studies.

Biological Nitrogen Removal from Stormwater in Bioretention Cells: A Critical Review

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Abstract

Excess nitrogen in stormwater degrades surface water quality via eutrophication and related processes. Bioretention has been recognized as a highly effective low impact development (LID) technology for management of high runoff volumes and reduction of nitrogen (N) pollutants through various mechanisms. This paper provides a comprehensive and critical review of recent developments on the biological N removal processes occurring in bioretention systems. The key plant- and microbe-mediated N transformation processes include assimilation (N uptake by plants and microbes), nitrification, denitrification, and anammox (anaerobic ammonia oxidation), but denitrification is the major pathway of permanent N removal. Overall, both lab- and field-scale bioretention systems have demonstrated promising N removal performance (TN: > 70%). The phyla *Bacteroidetes* and *Proteobacteria* are the most abundant microbial communities found to be enriched in biofilter media. Furthermore, the denitrifying communities contain several functional genes (e.g., *nirK*/*nirS* and *nosZ*), and their concentrations increase near the surface of media depth. The N removal effectiveness of bioretention systems is largely impacted by the hydraulics and environmental factors. When a bioretention system operates at low hydraulic/N loading rate, containing a saturation zone, vegetated with native plants, having deeper and multilayer biofilter media with warm climate temperature and wet storm events periods, the N removal efficiency can be high. This review highlights shortcomings and current knowledge gaps in the area of total nitrogen removal using bioretention systems as well as identifies future research directions on this topic.

Keywords: Stormwater runoff; Bioretention cells; Nitrogen removal; Nitrification; Denitrification; Microbial community.

Introduction

Increased urbanization has led to creation of impervious surfaces (e.g., roads, highways, sidewalks, rooftops, parking lots and urban lawn) that cause flash floods in cities after intense and prolonged rainfall (1). Impervious surfaces also change the hydrological flow regime and the quality of urban runoff even at a low proportion of impervious cover (5 -15%) (2) as some reports have suggested a positive relationship between the proportion of impervious surface cover and their hydrologic/environmental impacts (3, 4). Notable hydrological changes include increased storm runoff volume with a high peak flow and flow velocity, while water quality changes of concern include increased concentrations and mass loads of diverse pollutants (5). Urban stormwater contains a wide variety of chemical pollutants (e.g., nutrients, heavy metals, organic compounds and particulate matter) (6–8) and microbial pathogens (e.g., *Escherichia coli* and *Enterococci*) (9, 10). Thus, discharge of stormwater into a stream could adversely impact the quality of aquatic ecosystems and cause health risk to aquatic organisms (11, 12).

Among the pollutants in stormwater, nitrogen (N) is recognized as an important pollutant that causes eutrophication of receiving waters when discharged in large amounts (13–15). Stormwater from residential areas usually contains a high amount of inorganic nitrogen pollutants (mainly nitrate) (16). Atmospheric deposition and inorganic/organic fertilizers are the major nitrogen sources in stormwater in urban areas (16). Nitrogen in stormwater is present in dissolved (mainly inorganic-N) and/or particulate (mostly organic-N) forms (13, 17). The chemical forms of dissolved inorganic nitrogen include nitrate (NO_3^-), nitrite (NO_2^-) and ammonium (NH_3 and NH_4^+) (13, 17, 18). Concentrations of various forms of N species detected in stormwater generated from different impervious sources are given in Table 1. Nitrogen in stormwater is usually present in dissolved forms (~80%) among which NO_3^- is the most (~47%) and NH_4^+/NH_3 is the least abundant (~11%) pollutant (17). In order to protect public

health and the environment, it is necessary to treat stormwater to decrease contaminant levels prior to discharge to receiving waters, or before using it as a resource to alleviate water stress.

Low-impact development (LID) has recently been adopted globally as an environmentally and economically viable technology to manage stormwater runoff and mitigate pollution in aquatic ecosystems (19, 20). Bioretention cells (BRCs) (also called as bioretention systems, rain gardens or biofilters) are an engineered soil- and plant-based LID technology. BRCs have shown high performance in the removal of various stormwater pollutants including nitrogen (mainly particulate N) (13, 15). The key advantages of BRCs are that they require small space compared to engineered wetlands, consume low energy and are cost effective (21). The key components of a BRC include vegetation, the top layer (mulch, soil media), and the bottom layer (gravel layer) (Fig. 1) (22, 23). Frequently a subsurface saturated zone is created as a special engineered layer to promote denitrification and N removal. In BRCs, stormwater is directed for infiltration through the engineered filter media. The infiltrated water is stored and transferred to an underdrain system, then released into nearby surface water bodies, or directly allowed to percolate to groundwater (24). Potential mechanisms for removal of nitrogen pollution from runoff through BRC using plants-media-microorganisms include physical (filtration), chemical (e.g., adsorption and ion exchange), and biological (e.g., transpiration, assimilation, denitrification, immobilization, decomposition) processes (25).

Many studies have reported poor NO_3-N removal efficiency (15, 26). As a consequence, high concentrations of NO_3-N were observed in the treated effluent since this anion is highly soluble and mobile. It is thus clear that physicochemical processes namely soil adsorption are not effective in capturing NO_3-N in runoff (27). Recently, researchers have examined microbial community composition enriched in bioretention media for pollutant removal (21, 28–31). Efforts have also been directed at enhancing plant-microbe driven biological nitrogen

removal by controlling operational conditions (e.g., hydraulic loading rate) and engineering BRC filter media conditions for enrichment of oxic (e.g., nitrifiers) and/or anoxic (e.g., denitrifiers) N-transforming microorganisms (32–34).

To date, a few reviews have been published on the removal of nitrogen from stormwater using BRCs (18, 35–38). Most of the past reviews have reported bioretention design considerations (18, 35, 36, 38), summarized regulatory measures (18), synthesized knowledge on nitrogen fate and removal mechanisms, and discussed the impact of environmental factors (35, 36, 38).

This review specifically covers recent developments to expand on information provided in past reviews: (1) shift of microbial community composition in BRC filter media (28–31, 39), (2) the occurrence of different biological N processes (nitrification, denitrification, anaerobic ammonia oxidation (anammox)), and (3) dissimilatory nitrate reduction to ammonium (DNRA) (15, 40–42). The abundance of key functional enzymes (e.g., *amoA*, *nirK/nirS* and *nosZ*) (15, 43) and their importance under lab- and field-scale studies also merits attention.

The Scopus database shows that an increasing number of research articles have been published in the last ten years (2011 – 2020) on N removal from stormwater in BRCs (supplementary material, Fig. S1). The bibliographic records (number of articles, conference papers, reviews, conference reviews and book chapters) on the review topic published during 2011 - 2020 were collected using the keywords, namely, 'nitrogen', 'stormwater', and 'bioretention' in the Scopus search engine. This review aims to update the research community by summarizing recent research findings and developments on biological N removal from stormwater in BRCs. The relative contributions of various biological processes on N removal in lab- and field-scale studies and the underlying molecular level mechanisms, and the responsible functional enzymes are discussed. Moreover, the composition of the microbial community enriched in the BRC media is highlighted. The impact of various environmental

factors on N fate and its removal, possible methods for augmentation of plant-microbe driven N removal process and the need for future investigations for improvement of bioretention performance are described. We believe that this review paper would contribute to better understanding of the fate and biological transformation of N contaminants, as well as the modification of existing designs, operational and media characteristics of a BRC to enhance its effectiveness for removal of nitrogen.

Plant and microbe-driven biological nitrogen removal in bioretention cells

Biological N cycling in plant-soil ecosystems

An overview of biological N cycling in soil and the associated enzymes is shown in Fig. 2. Nitrogen in soil can exist as organic, inorganic, dissolved and particulate forms with a wide range of oxidation states from -3 (NH_4^+/NH_3) to +5 (NO_3^-) (44, 45). The physicochemical and thermodynamic properties of various nitrogen compounds are given in supplementary material (supplementary material, Table S1).

In soil, the N transformations can be described by a series of oxidation–reduction reactions catalyzed by both plants and microorganisms (bacteria, archaea, and fungi) (46). Nitrogen is one of the essential elements which limits the growth of plants, and plant biomass typically contains 2–5% N by dry weight (47). Rhizosphere microbes play a vital role in the transformation of N to plant-usable forms (45). Among different N forms, only NH_4^+ and NO_3^- are used by organisms for new biomass generation (48). In stormwater, both organic and inorganic N species are present depending on the source of N generation, and their fate and transformation processes are different when runoff passes through the soil-based engineered bioretention media. It is important to understand the microbiology, physiology and biochemistry of microbe-driven N cycle processes in the soil/plant rhizosphere in order to enhance the removal efficiency of N contaminants (specifically dissolved N species) in a BRC.

The key N transformation processes, reactions, enzymes and physicochemical/thermodynamic properties including redox potential are summarized in Table 2.

In BRCs, the major biological N transformation processes include assimilation (e.g., vegetative N uptake), ammonification (mineralization), nitrification, denitrification, anammox, and DNRA (38, 49). In plant-mediated assimilation, inorganic N compounds (e.g., NH_4^+ and NO_3^-) are converted to amino acids. Generally, $NH_4^+$is more favorable than NO_3^- for assimilation by plants since NO_3^- (ΔG^0 : - 1492.8 KJ/N atom) reduction requires more energy than NH_4^+ (ΔG^0 : -1797.4 KJ/N atom) (supplementary material, Table S2) (50). In BRCs, ammonium removal up to 80% can be achieved via adsorption and biological process (e.g., nitrification) (23).

Ammonification (mineralization) is the process in which organic nitrogen compounds (e.g., urea, $CO(NH_2)_2$) are transformed in enzymically-catalyzed reactions into an inorganic bioavailable N form, ammonium (NH_4^+) (Table S2) (51). This species subsequently can be taken up by plants and microbes (22).

Nitrification is a dual-step process of sequential oxidation of NH_4^+ to NO_3^- through NO_2^- (Table S2) (52). The process is mediated by two groups of microorganisms: first ammonia-oxidizing bacteria/archaea that oxidize NH_4^+ to NO_2^-, then nitrite-oxidizing bacteria, which oxidize NO_2^- to NO_3^- (45, 48). The key enzymes in the nitrification reaction are ammonia monooxygenase (*amo*) and hydroxylamine oxidoreductase (*hao*) and nitrite oxidoreductase (*nxr*) (45).

Denitrification involves multistep reactions of reduction of NO_3^- to dinitrogen gas (N_2) (Table S2, with $C_3H_4O_3$ as an example organic electron donor) (53), which is released to the atmosphere, or returned to the soil through plant roots by N_2 fixation (reduction of N_2 to NH_3) (38). Each reaction step is catalyzed by a specific enzyme including nitrate reductase (Nar), nitrite reductase (Nir), nitric oxide reductase (Nor), and nitrous oxide reductase (Nos) (54). In

BRCs, the process is performed by mostly heterotrophic microbes (denitrifiers), which use nitrate instead of O_2 as a terminal electron acceptor during respiration. A few studies have also reported autotrophic denitrification in BRCs using inorganic electron donors such as reduced inorganic sulfur compounds (e.g. elemental sulfur (S^0) (55) and iron-based sulfide minerals (e.g. pyrite, FeS_2) (56); the nitrate reduction reactions are presented in elsewhere (Table S2) (57, 58). Complete denitrification results in the endpoint product of N_2 gas, which is not generally bioavailable and promotes permanent removal of N from stormwater in BRCs (22). However, incomplete denitrification is undesirable since it generates nitrous oxide (N_2O), a potent greenhouse gas (59).

DNRA is the reduction of nitrate to ammonium (Table S2) (52). This process is carried out by anaerobic and facultative anaerobic bacteria (45). The DNRA reaction is catalyzed by a cytochrome C nitrite reductase (Nrf) that converts NO_2^- to NH_4^+ (60, 61). Denitrification causes N loss, but DNRA activity conserves/recycles nitrogen in the ecosystem as the end-product, NH_4^+, a biologically reactive N that can be used by plants and microbes or recycled (by oxidation) back to NO_3^- (62).

The DNRA process is highly competitive with denitrification as both processes use the same inorganic N species (NO_3^-) as electron acceptors and environmental conditions (e.g., anoxic). The fate of NO_3^- in bioretention media due to DNRA has been generally overlooked and no published reports were found. The plants used in bioretention technology could release organic compounds through roots (root exudates), and these compounds may impact the selectivity between denitrification and DNRA activity in the rhizosphere (46). Future investigations should focus on these topics to unravel nitrate fate and potential DNRA activity in BRCs.

Anaerobic ammonium oxidation (anammox) is the production of N_2 from NO_2^- and NH_3 under anoxic conditions via intermediates such as nitric oxide (NO) and hydrazine (N_2H_2) (Table S2) (63, 64). The responsible organisms are slow growing microbes that belong to the order *Brocadiales*, and are associated with the phylum *Planctomycetes* (60). The key enzymes that catalyze the anammox reaction are hydrazine hydrolase (*hh*), producing N_2H_4 and hydrazine dehydrogenase (*hdh*)/hydrazine-oxidizing enzyme (*hzo*), converting N_2H_4 to N_2 (64, 65). A few recent studies have examined anammox bacteria for stormwater treatment using mathematical models in BRC and in constructed wetlands (66, 67); no reports are yet available on experimental works on anammox bacteria enrichment in BRCs for stormwater treatment. Further research on this topic is warranted.

In biological nitrogen transformation process (e.g., nitrification and denitrification), nitric oxide (NO, a free radical gas) is produced as a byproduct. NO is recognized as one of the important air pollutants which can create several environmental problems including acid rain, haze and photochemical smog (68). Moreover, NO acts as a signaling molecule that impacts plants growth and development and influences different pathways involved in plant-microbe interactions (69). For example, in plant-bacterial interactions, NO involves in abiotic (oxygen, heat and salt stress) and biotic (pathogen, NO acts as antimicrobial agent) stress response, root architecture, root hair formation, nodule development, lateral root formation, etc. (69). From the perspective of N removal from stormwater in plant and soil-based engineered systems (e.g., bioretention cells), enrichment of NO-consuming microorganisms may help to achieve better N removal performance which needs to be verified in future studies.

In addition to bioretention cells, other plant-based systems, specifically green roofs and constructed wetlands, are used for removal of excess nitrogen from stormwater (70, 71). Several studies have reported that plant traits and plant species diversity significantly impact pollutant removal efficiency of plant-based constructed ecosystems (47, 72). Plant traits

namely plant mass, growth rate, root length, root mass, root thickness, root architecture as well as plant tolerance to nutrients and salts are commonly used to study the relationahip between plants traits and pollutant removal performance of a specific plant species (47, 72, 73). In lab-scale phytoremediation experiments, Chen et al. (72) showed that plant root, leaf and total dry biomass had moderate to strong correlation with nitrate removal. Moreover, fast growing plants demonstrated high performance for nitrate removal, bur slow growing plants were mostly effective for phosphate removal (72). Among native and exotic plant species, native plants were efficient for removal of both nitrate and phosphate (72). Hunt et al. (74) screened 30 plant species for their capability for removal of nitrate and phosphate from stormwater in bioretention columns, and noticed that 24 out of 30 plants showed more than 50% uptake of nitrate from stormwater, and two plants namely Arundo donax var. versicolor and Bougainvillea 'Sakura Variegata' contributed highest nitrate removal (96%). Read et al. (73) investigated the performance of 20 diverse plant species on removal of N and P from stormwater in biofilter systems, and authors have found that among 20 plants, Carex appressa (a grasslike plant) was the strongest contributor for decontamination of stormwater, and C. appressa possessed traits such as high growth rate, high root mass and long root length. Plants with high tolerance to salt and nutrients are effective for nitrogen removal from water and wastewater (47, 75). Plant-based systems usually contain monoculture (i.e., single species) or mix diversity of plant communities (76). In general, several studies have suggested for plantation of diverse species which could enhance ecosystem services in addition to the primary role of pollutant removal (71, 77).

Perspectives: Urban stormwater is generally characterized by its low strength (mainly low in organic carbon) and high dissolved O_2 content, which makes it difficult for the application of microbially-driven processes for effective removal of N pollutants (66). To enhance N removal (e.g., denitrification), carbon amendment with addition of external carbon source is required.

Biological N removal offers several advantages over physicochemical processes, namely low-cost, no chemical additions, less negative environmental impacts, and most importantly, high removal efficiency of nitrogen by transforming it to inert N_2 gas (78, 79). Hence, increased attention has recently been given to understand the dynamics of microbial communities in bioretention media, then modify the design parameters and/or operational/environmental conditions to increase population of desired functional bacteria (e.g., nitrifiers and denitrifiers) to achieve higher N removal efficiency.

Dynamics of microbial communities in engineered bioretention media

Microorganisms present within the engineered biofiltration media during installation, microbial colonization from the environment, and/or development of microbial biofilms over the course of operation are responsible for driving the various N transformation reactions to permanently remove N through denitrification, or conversion to another form of N (29, 80). Ecological conditions in the bioretention media may be different at different depths (top, middle and bottom), which could impact the community composition and their functions (e.g., enzyme activity) and ultimately the nature of N cycling (30, 39, 81). Moreover, the microbial community composition at the upper layer of the media could be greatly impacted by the plant species and density of plant roots, while the presence/absence of anaerobic saturated zone and C source (or other electron donor) could shape the microbial community composition in the bottom layer (39). In heterotrophic N removal, the materials used as electron donor include woodchip, mulch, newspaper, sawdust, wheat-straw, and others (9, 15), whereas in autotrophic process, elemental sulfur (S^0), pyrite (FeS_2), natural zeolite and magnetite (Fe_3O_4) are used as electron donor (55, 56, 82). Understanding the composition and stability of microbial communities present within the biofiltration system could help to develop better stormwater management strategies and efficient N removal.

Molecular techniques including 16S rRNA gene-based sequencing (29–31, 39) and terminal restriction fragment length polymorphism (TRFLP) (28, 83) are commonly employed for characterization of microbial communities. Additionally quantitative polymerase chain reaction (qPCR) is another popular molecular method that has been used for quantification of functional genes encoding enzymes responsible for nitrate, nitrite and ammonia transformations (15, 84). A study on engineered infiltration systems (with stormwater) using the 16S rRNA sequencing showed that the phyla *Proteobacteria* (51%) was dominant, followed by *Bacteroidetes* (18%), *Firmicutes* (9%) and *Saccharibacteria* (< 4%) (29). However, *Firmicutes* (42%), *Proteobacteria* (34%) and *Bacteroidetes* (11%) were the key microbial candidates in the non-inoculated columns (without stormwater). A mesocolumn-based research revealed that the phyla *Bacteroidetes* and *Proteobacteria* were abundant in all the media samples and accounted for nearly 40% and 30% of the total assigned reads, respectively (39).

A few studies have looked into the variability of bacterial communities in a BRC at various depths and they observed that the most noticeable microbial activities occur in the top layer and the microbial population decreased noticeably with depth (81). The top two abundant phyla among the communities were *Bacteriotides* and *Proteobacteria*, and their proportion changed with depth. In another work, the columns filled with the homogenous media mix containing sand, soil and fly ash (ratio: 1:1:1), the proportion of phylum *Proteobacteria* decreased from 57.09% (20 cm) to 45.72% (40 cm), and then increased to 68.32% (60 cm) (30). Igielski et al. analyzed the microbial diversity in the biofilm developed on the surface of woodchips and the effluent pipe in a lab-scale BRC configured with internal water storage zone (85). They found that both denitrifying communities and anaerobic lignocellulose degrading bacteria were enriched in the system. In the woodchip biofilm, the major communities (class level) were *α-proteobacteria* (12.87%), *β-proteobacteria* (11.37%) and *Opituta* (8.96%),

whereas significant change of community abundance/composition was observed in the effluent tube biofilm, i.e., *α-proteobacteria* (47.21%), *β-proteobacteria* (24.58%) and *Acidobacteria* (9.0%) were predominantly enriched.

A recent study examined changes of microbial diversity in bioretention columns where each column was planted with three different aquatic plants (31). They noticed that the abundance of *Proteobacteria* and *Saccharibacteria* in the control sample (without vegetation) was elevated by up to 40 times during the operation, whereas the abundance of *Actinobcteria*, *Acidobacteria*, *Cyanobacteria*, and *Nitrospirae* decreased with operation time. Conversely, the selected three plants exhibited different effects on the microbial population, i.e., the plant, *Iris pseudacorus* L enhanced the proportion of *Actinobacteria*, *Canna indica* L encouraged growth of *Acidobacteria*, while *Lythrum salicaria* L. also favored enrichment of *Chloroflexi* and *Saccharibacteria*.

Although heterotrophic denitrifiers are the dominant communities in the bioretention media due to use of organic carbon rich materials as a source of electron donor, recently, a few researchers have investigated the diversity of autotrophic communities in BRCs supplied with S and Fe-based inorganic electron donors (56, 82). In simulated BRCs augmented with natural pyrite or zeolite as electron donor, abundances of sulfur/Fe-based denitrifiers including genera *Thauera*, *Sulfuritalea* and *Thiobacillus* were higher when the column was operated with pyrite (2.1%, 1.7% and 2.6%, respectively) compared to zeolite (< 0.1%, 0.3% and < 0.1%, respectively) as an electron donor (56). Deng et al. found enhancement of the anammox reaction in biofilter media with iron as an electron donor and higher DNRA rate with iron plus sulfur as electron donors (82).

In a TRFLP-based study, a total of 33 different terminal restriction fragments were detected in biofilter columns (28). Moreover, the bacterial community structure changed with the increase in biofilter operation time, and considerable correlations were observed between

bacterial communities and effluent water chemistry (e.g., concentration of NO_3-N). In another constructed stormwater wetland study, cluster analysis of nitrous oxide reductase (*nosZ*) gene TRFLP fingerprints revealed that the samples collected from the rhizospheric sediment (13 fragments) contained a higher number of denitrifying communities than unvegetated sediments (9 fragments) (83).

In addition to metagenomics and TRFLP methods, a few researchers have employed quantitative PCR (qPCR) to quantify the microbial biomass at different layers of the filter medium (15, 29, 86). Chen et al. demonstrated that the 16S rDNA concentration was higher at the middle zone (30-45 cm) (6.4×10^8 copies per gram soil (c/gs)), but decreased for the samples collected from the deepest regions (45-60 cm and > 60 cm) ($1.2 \times 10^8 – 1.3 \times 10^8$ c/gs) (15). Another study also reported a similar level (in the order of ~$10^8 – 10^{10}$ c/gs) of 16S rDNA concentrations in bioretention columns packed with different filter materials (single or double layers with woodchips and/or vermiculite). However, the biomass density increased/decreased along the column depths, depending on the packing material type and the packing pattern (86). Overall, 16S rDNA concentration is a surrogate for total biomass enriched in the different layers of the stormwater treatment biofilters. However, metagenomics characterization (e.g., 16s rRNA gene-based sequencing) is performed to determine enrichment of specific microbial communities (nitrifiers, denitrifiers, etc.,), and qPCR analysis is done for quantification of specific nitrogen processing genes (e.g., *amoA*, *nirK*, *nirS*, *norB*, *nosZ*, etc.,).

For better understanding about the fate and transport of microorganisms in bioretention systems, and the associated mechanisms for removal of nitrogen from runoff in bioretention systems, controlled studies using pure culture are required. A few studies have been carried out using *Escherichia coli* as a model bacterium to elucidate bacteria transport mechanisms through stormwater biofilters (87, 88). Although little information is available about nitrogen removal from stormwater using pure culture system, numerous reports are published on N

removal (specifically by denitrification) from groundwater and wastewater employing pure culture of denitrifying bacterium (various species of *Pseudomonas* and *Bacillus*). Among *Pseudomonas* Spp., *Pseudomonas denitrificans* and *Pseudomonas aeruginosa* were frequently used in past works and authors observed high N removal efficiency (>75%) (89, 90). A number of *Bacillus* Spp. namely *Bacillus cereus* and *Bacillus subtilis* show promising denitrifying capacity (> 68%) (91, 92). In future research, these denitrifying microorganisms can be considered to test their performance for N removal from stormwater in bioretention systems.

Stormwater characteristics, i.e., presence of inorganic pollutants (N species namely nitrate, nitrite and ammonium, phosphate, heavy metals) and organic pollutants in runoff could impact the abundance and composition of microbial communities in the bioretention systems (28, 93, 94). Stormwater rich in inorganic nitrogen species (nitrate, nitrite and ammonium) could promote enrichment N transforming bacteria namely nitrifiers, denitrifiers and ammonifiers (95). Wang et al. (95) analyzed microbial communities enriched in a conventional bioretention system supplied with N-containing synthetic stormwater and found that the genus *Pseudomonas* was the major bacteria which drive the N removal in the bioretention system. The stormwater containing organic contaminants could promote enrichment of organic degraders since some studies have reported the presence organic degrading bacteria (e.g., genus *Flavobacterium* and *Clostridium* spp.) in bioretention systems (22, 33, 95). A recent report indicated the presence of antibiotic resistant bacteria and antibiotic resistance genes in stormwater which could be linked to the presence of antibiotics in stormwater (96). Another study also noticed an increase in the concentration of antibiotics (sulfadiazine) and antibiotic resistant bacteria (cefazolin- and sulfamethazole- resistant bacteria) in the surface water and surface sediments of a urban lake after strong storm events (97). Together, these studies indicate that the type of pollutants in stormwater could affect the dynamics of microbial communities in bioretention cells.

Perspectives: Together, the findings of the above studies suggest that microbial community composition and abundance vary widely within bioretention media. Multiple studies have revealed that the phyla *Bacteroidetes* and *Proteobacteria* are the most abundant among the communities. *Bacteroidetes* are normally recognized as organic degraders (98). They may degrade high molecular weight and complex organic pollutants in stormwater, and make them bioavailable as a C source for other microbes (e.g., nitrifiers and denitrifiers). The higher abundance of *Bacteroidetes* in BRCs indicates possible high amounts of carbon resources in the upper layer. *Proteobacteria* represent diverse microorganisms including denitrifying bacteria (specifically sub-classes *α*- and *β-Proteobacteria*) (99). A few other members (mainly *β*- and *γ-Proteobacteria*) are also involved in the initial step of nitrification (100, 101). The synergistic growth and function of *Bacteroidetes* and *Proteobacteria* may predominantly contribute to biological N removal in BRCs.

Microbially-driven N removal in lab-scale and field-scale studies

The mutual effects of plants, soil, and microorganisms in BRCs create favorable conditions for nitrogen removal (43). The key microbially-driven processes involved in the removal of ammonium and nitrate in a BRC are nitrification and denitrification, respectively. In a few studies, phenotypic observations were further verified by genotypic analysis, i.e. quantification of nitrification (e.g., *amoA*) and denitrification genes (e.g., *nirK*, *nirS*, *norB*, and *nosZ*) using the qPCR method and identification of key nitrifiers and denitrifiers enriched in filter media by metagenomic techniques (15, 29, 39). Frasser et al. investigated the dynamics of microbial communities and changes of *nosZ* gene (encoding nitrous oxide reductase) in lab-scale sand columns, and found that the abundance of *nosZ* gene increased from $\sim 1.0 \times 10^3$ copies/g from

day 1 to nearly 7.0×10^3 copies/g on day 24 (29). Moreover, a total of 10 potential denitrifying taxa detected in the communities, all belonging to *α-, β-,* and *γ-Proteobacteria*.

A mesocosm study, which used a ^{15}N isotope tracer technique, stated that assimilation (plant and microbial) was the major pathway of N transformation (77−98%) in columns having saturated zones (39). Moreover, a control test on only soil showed nearly 38% N assimilation rate, and plant assimilation rates were found between 39−60% (39). However, only 1−7% N transformation was due to denitrification reactions. The functional gene, *nirK* was mainly enriched in the phylum *Bacteroidetes* (abundance: nearly 70%), while the *nosZ* gene was distributed in phyla *Bacteroidetes* (abundance: ~40%) and *Proteobacteria* (abundance: ~30%). The authors have also assessed the effect of different plant species. The relative abundance of the genus *Nitrospira* (nitrite oxidizing bacteria) was high in the non-saturated zone (both upper and bottom layers) in systems containing three different types of plants including *Buffalo*, *Carex appressa* and *Dianella tasmanica*.

A report on the treatment of stormwater in a BRC using Fe-biochar and incorporation of saturated zones demonstrated that the microbial denitrification enzyme assay (DEA) rate at the bottom layer was higher (~ 1.12 times) compared to the top layer samples (102). Wan et al. explored N removal in bioretention columns in which woodchips and vermiculite were packed in different patterns (i.e., column 1: only vermiculite (control), column 2: only woodchips, column 3: vermiculite (upper) + woodchips (lower), and column 4: woodchips (upper) + vermiculite (lower)) (86). Here, more than 80% of nitrate removal occurred in all the column configurations. The abundance of denitrification genes namely *narG*, *nirS* and *nirK* at various column depths increased when woodchips were employed. These findings suggest that denitrification activity may be higher with addition of woodchips, which provide carbon source for denitrifier communities (86).

A field-scale study reported that the combined nitrification-denitrification process contributed 33% and 56% of nitrate and total nitrogen (TN) removal, respectively (15). The concentrations of denitrifying genes (*nirK, nirS*, *norB*, and *nosZ*) varied between 10^5 and 10^8 gene copies/gram soil. The nitrification gene (*amoA*) was observed at a significantly lower level, i.e., between 10^4 and 10^6 gene copies/gram soil. This observation suggests that denitrification may be the predominant N removal process. In most cases, the samples collected from the top layer of filter media contained high concentrations of functional genes, which declined at various degrees as a function of media depth. Another field-scale study reached the same conclusion about the reduction of denitrification functional genes (only *nirK* and *nosZ* were tested) with depth since the abundance of *nirK* and *nosZ* genes as well as denitrification potential rates in the top layer were on average 5.7, 3.6, and 23 times, respectively, greater than the bottom layer samples (84).

In a field-scale study by Willard et al., researchers assessed the long-term performance of a BRC seven years post-construction, and observed high removal efficiency for several pollutants including TN (median % reduction nearly 100, detection limit: 0.001 mg/L) (103). The *nirK* gene concentration varied between 3.7×10^7 and 1.7×10^9 copies/gram of soil, while the level of *nosZ* gene ranged between 2.4×10^5 and 3.6×10^6 copies/gram of soil. Although the BRC had an internal water storage (IWS) system in the bottom layer, the quantity of the two functional genes decreased with an increase in depths, possibly due to insufficient amounts of organic carbon (103).

Although in most of the studies, the primary focus is to study nitrification plus denitrification-driven N removal in BRCs, no information is available about anammox, which is often observed in wastewater deficient in organic carbon (104). Thus, it is expected that anammox technology may be useful for treatment of stormwater since it generally is limited in the quantity of organic compounds. A few studies have demonstrated the enrichment of

anammox bacteria with other microbes (nitrifier, denitrifier or DNRA) in a similar plant-based engineered system (constructed wetland) built for stormwater treatment (67, 83) .

Rahman et al. evaluated the relative contribution of various biological processes on nitrate removal in constructed stormwater urban wetlands, and reported that the denitrification rate varied between 6 ± 1 and 27 ± 9 μmol L slurry^{-1} h^{-1}, and the DNRA ranged from 0.6 ± 0.2 to 11 ± 2 μmol L slurry^{-1} h^{-1} (67). However, the anammox rate was low (only 0 – 0.01 μmol L slurry^{-1} h^{-1}; less than 0.05% of total NO_3^- reduction). In contrast, results from another study revealed a high proportion of anammox-mediated N transformation in unvegetated sediments (29%) and rhizopheric sediments (26%) in a constructed wetland (83). Furthermore, in the plant rhizospheric material, the denitrification and anammox rates were 14.41 ± 7.95 and 2.03 ± 1.76 nmol N/g sed. wet wt./hr, respectively (83). Although molecular data for the anammox enzyme were not available, qPCR results of the *nosZ* gene indicated that the rhizospheric denitrifying communities contained up to 4 × 10^4 copies/ng of DNA. A mathematical modelling-based study revealed that up to 71.1% N removal through partial nitrification, followed by anammox, can be achieved in urban stormwater due to the presence of adequate NH_4^+ (66).

Denitrification kinetics: To evaluate denitrification kinetics in BRCs, researchers have analyzed nitrate removal data using primarily two reaction orders, namely first order (Eq. 17) and zero order (Eq. 18) (32, 105). In most studies, it has been observed that first order kinetics most appropriately describe the denitrification rate (32, 106) (supplementary material, Table S3). In a lab-scale column having media components consisting of woodchips and pea gravel, and an initial nitrate concentration of 3 mg-N/L, Peterson et al. found that the denitrification process can be more accurately fit to a pseudo-first-order model (rate constant, k=11.4 day^{-1}) (32). Using microcosm-based stormwater biofilters, Lynn et al. explored changes of denitrification kinetics with varying media components (e.g., wood, sand plus wood, and gravel

plus wood) (105). They found that the denitrification reaction can be represented by both first-order and zero order models, and the first order denitrification constant for the three types of media were: wood ($k = 0.75\ hr^{-1}$) > gravel-wood ($k = 0.58\ hr^{-1}$) > sand-wood ($k = 0.27\ hr^{-1}$), i.e. the wood-based system showed the greatest nitrate removal performance. Among the two models, the first-order model described the denitrification data slightly better than zero order.

In woodchip bioreactors which were fed with 2 – 11 mg NO_3-N/L, Halaburka et al. reported that the denitrification rate at constant temperature can be appropriately described using zero order kinetics (rate constant: 0.13 (mg-N/mg-biomass-hr) (107). A batch experiment in which woodchip was used as organic substrate (solid-to-liquid ratio of 1:3 by volume) reported that nearly 100% nitrate reduction (decreased from 0.3 to < 0.02 mg-N/L) achieved within 2.6 days; the reaction followed first order kinetics with a rate constant equal to 0.0011 min^{-1} (106). The key factors that impact the denitrification rate constant include dissolved organic carbon level, dissolved oxygen level and influent nitrate concentration (105, 107).

The kinetic expressions for batch systems are:

$$dC/dt = k[C]^n \quad \text{(general equation for zero, first, or higher order rate)} \qquad (16)$$

$$C = C_0 exp^{-k_1 t} \qquad (17)$$

$$C = C_0 - k_0 t \qquad (18)$$

Where, C_0 and C = influent and effluent nitrate concentration, respectively, k_1 and k_0 = first order and zero order rate constant, respectively, and t = time.

Perspectives: Denitrification appears to be the major biological N removal process although some studies noted the importance of plant assimilation. The denitrification rate data were mostly fit by a first order model. More studies need to be carried out to obtain in-depth knowledge about the contribution of other processes including anammox and DNRA on total N removal. Significant amounts of organic N (dissolved organic N: 28% and particulate organic N: 24%) are present in stormwater (17). Hence, future research should be conducted

to elucidate the fate and removal mechanisms of organic N in BRCs. Multiple studies have pointed out that the N removal efficiency in BRCs can be influenced by numerous factors. These factors include hydraulics, climatic conditions, filter media characteristics, plants selection, and stormwater qualities (35, 36, 38), which are briefly discussed in the following section.

Factors affecting N removal in bioretention cells

Hydraulic factors

Hydraulic loading rates (HLR) for stormwater are generally variable, but can be controlled by integrating flow control regulators at the bioreactor outlet (33). For denitrifying bioreactors in the field, installation of a regulated outlet control device could enhance the HLR for denitrification (33). The major hydraulic factors that impact N removal in BRCs are runoff volume, flow rate, hydraulic conductivity and retention time (22, 38). N removal improves with higher retention time, or lower infiltration rates (108). Kim et al. evaluated the impact of various HLR (4 – 20 cm/hr) on N removal in lab-scale bioretention columns and reported that nearly 100% nitrate removal could be achieved at lower HLR (i.e., 4 cm/hr) (55). However, nitrate removal declined to nearly 20% at higher HLR (20 cm/hr) with woodchips as a solid-phase electron-donor and carbon source. The significant deterioration of biofilter performance at higher HLR could be due to the washout of functional microorganisms, enzymes, and/or organic substrates (55), or simply contact time. Based on the results obtained using other electron donors (e.g., newspaper and sulfur/limestone), the authors have suggested that with the optimum HLR of 12 cm/hr, nitrate could be removed efficiently.

Other field-scale/pilot-scale tests also showed similar findings on HLR effects on N removal. Results from a conventional field-scale BRC (planted) showed that with the variation of HLR from 4.1 to 13.9 cm/hr, the removal efficiency of total ammonium, NO_x (nitrate + nitrite) and TN decreased from 85 to 74%, 61 to 56% and 59 to 53%, respectively (34). Another

field-scale experiment with woodchips as a C source observed nearly an average of 55% NO_x-N removal at lower HLR (0.93 – 1.38 cm/hr), but the efficiency decreased at higher HLR (109). Osman et al. found the most appropriate hydraulic conductivity range for BRCs to be between 1.3 and 20 cm/hr; if the hydraulic conductivity exceeds the recommended range, then soil moisture would not be adequate for plant growth (38). However, at values below the stipulated range, clogging with ineffective capture of runoff would result (110). Overall, the findings of these studies suggest that lower HLR can increase hydraulic retention time (HRT) and enhance nitrogen removal rate.

Role of a saturated zone

In recent years, many studies have recommended installation of a saturated zone (SZ) into BRCs to increase nitrogen removal (specifically nitrate) by encouraging microbial denitrification and attenuating plant water stress (47, 111, 112). One of the easiest options to create a SZ in bioretention columns is by raising their outlet pipe, hence providing a constant water level in the bottom layer of biofilter (113). In field-scale tests, the SZ is termed as internal water storage zone (IWS) (36). In addition to an elevated pipe configuration, anoxic saturation conditions can be created by placing a layer of materials that act as sources of organic carbon and support the development of microbial biofilm (woodchips, newspaper, sawdust, wheat straw, sugar cane mulch, pine chips, etc.) below the primary filter media to facilitate heterotrophic denitrification (33, 112, 114) (Table 3).

A mesocolumn study by Morse et al. found higher proportions of NO_x removal in SZ columns (89%) than the columns without a SZ (72%) (39). Another lab-scale investigation also reported a similar trend in that the vegetated columns installed with a SZ (87%) demonstrated greater TN reduction than non-SZ columns (75%) (114). A recent field-scale study also reached the same conclusion that BRCs (planted) having an internal water storage (IWS) zone showed

better performance with respect to ammonium (NH_4^+-N) (with IWS: 86% and without IWS: 81%) and NO_x-N removal (with IWS: 88% and without IWS: 54%) (34).

Although installation of a SZ enhances N removal as demonstrated in several lab-scale studies, a few field-scale tests reported minimum or no significant improvement of N removal with incorporation of the SZ. In a previous work where authors compared the pollutant removal efficiency of two field-scale BRCs, with one having a standard design and the other with creation of an anaerobic sump by adding a layer of newspaper and sand mix (mass ratio: 0.017:1.0) (115). The mean event concentration (EMC) reduction for nitrate (NO_3-N) in the anaerobic sump-containing cell and the standard design cell was 79 and 86%, respectively (115). Field-scale experiments also found an insignificant impact of IWS because the concentration of denitrifying functional genes (*nirK* and *nosZ*) decreased with an increase of depth (15, 103). Altogether, inconsistent results have been observed on the impact of SZ on N removal in BRCs. Part of this lack of improvement may be related to inadequate HRT in the field installations or lack of continued stored water. Thus, additional research is needed on this topic, including more accurate determination of N transformations using ^{15}N tracer techniques.

Plant species

Plants are considered as an essential component of BRCs. Roles of plants in the BRCs include: (1) planted cells are highly effective for contaminants removal compared to non-planted cells, (2) biofiltration efficiency differs with the type of plant species used, (3) native plants show better performance than exotic ones, (4) diverse plant systems are more effective compared to single-plant systems (77). Vegetation contributes treatment of pollutants in BRCs both directly and indirectly. Direct effects include degradation and/or uptake of pollutants. However, indirect impacts include an influence on rhizosphere microbial community composition through release of organic compounds (root exudates) (22). Vegetation also contributes to bioretention hydrologic functions of the filter media through various routes including plant

transpiration, plant interception of rainwater, regulation of surface flow, and modification of water infiltration (47). Most lab- and field-scale studies have concluded that the efficiency of removal of pollutants is higher in planted BRCs compared to non-planted systems (Table 4) (34, 39). Additional information on the efficiency of different plant species (single or multiple plantings) for removal of various nitrogen species (mainly nitrate and total nitrogen) from stormwater is given elsewhere (Table S4). Among the reported findings, two plant species namely *Arundo donax* var. versicolor and *Bougainvillea* 'Sakura Variegata' were most effective for removal of nitrate (96% removal by both species) from stormwater (74).

A field-scale trial showed that the average NO_x (NO_3^-+NO_2^-) removal efficiency was higher for the planted than non-planted systems (34). For a conventional BRC, the NO_x removal efficiency increased from 15 to 54% (each system was planted with five local plants) (34). Bioretention mesocosms-based study noted that TN retention was 81% in the shrubs/grasses vegetated systems compared to 41% in the non-vegetation systems (116). Another pilot scale trial on street tree BRCs found that the TN load removal from the planted (*Lophostemon confortes*) systems was more (95%) than the unplanted systems (only 36%) (117).

Plant diversity also influences the treatment performance since Morse et al. found that five out of six selected plants (*Juncus krassii, Buffalo, Carex appressa, Allocasurina littoralis, and Leptospermum continentale*) showed lower denitrification (mean: 1−3%) than the other plant species evaluated (*Dianella tasmanica* - mean: 7%) (39). Another study also reported that the columns vegetated with *Medicago sativa* (L.). demonstrated low nitrogen removal rate (TN: − 29.8% to − 123.0%), whereas in columns vegetated with *Radermachera hainanensis* (Merr.), *Juncus effusus* (L.), *Ophiopogon japonicus* (Linn. f.) and *Vetiveria zizanioides* (L.), the removal efficiency was significantly enhanced (TN: 52.8% to 84.2%) (118).

A lab-scale column test involving ^{15}N isotope analysis observed a large variations of nitrification efficiencies with the application of three types of bioretention grasses. namely *Ophiopogon japonicus* (27–53%), *Iris tectorum Maxim* (16–37%) and *Hosta plantaginea* (12–39%) (43). However, the denitrification efficiencies were lower than nitrification, i.e., 9–2%, 5–11%, and 8–11%, respectively. Interestingly, this study also revealed that the rhizosphere oxygen level regulates N transformation reactions since both nitrification and denitrification were higher (2 - 3 fold) at the top layer of the BRC. Another study with three types of vegetation (grassed, landscaped and overgrown) found that the denitrification efficiency among the three types of vegetation was in the order of grassed < landscaped < overgrown.

Together, research has found that vegetated BRCs show better N removal performance than non-vegetated cells. Although impacts of plant diversity on N removal efficiency has been investigated in many studies, several issues are still unclear. For example, how N removal efficacy may change by the plant growth/age is not fully understood yet which needs further investigation.

N pollutant loads and characteristics

Stormwater events can vary in terms of their frequency, intensity, and duration (22), which may impact the quality of runoff. Prevailing climatic conditions may also influence the runoff quality. For example, during warmer and dry weather conditions, more pollutants may accumulate on impervious surfaces. These pollutants tend to be washed out with the first flush of rainfall, which causes an increase in the concentration of pollutants at the initial period of storm events (22). The nature of nitrogen pollutants and their concentrations in stormwater should influence the fate of biological N removal process in BRCs (38).

In a column reactor, Kim et al. assessed the effects of different influent nitrate loading rates (NLR) (6.5 – 24.9 mg/day as N) on the denitrification rate using three types of solid-phase substrates (electron donors: newspaper, woodchips, and sulfur/limestone) (55). The nitrate

removal efficiency was nearly 100% when tested at the lower loading rate (6.5 mg/day), but the removal efficiency decreased constantly with the rise of loading rates, i.e., the efficiency decreased to ~90% at 11.8 mg/day and varied between ~40 – 60% at 24.9 mg/day NLR.

Using a stepped BRCs, Wang et al. observed variations of the N removal efficiency with change of the influent nitrate/ammonium concentrations (118). By increasing the nitrate EMC from 3.04 ± 2.64 to 3.17 ± 2.01 mg/L, the mean removal rate slightly increased by 7.4% (i.e. from 45.4 to 52.8%). However, the removal of ammonium was not impacted significantly because with the increasing load from 1.73 ± 2.01 to 2.22 ± 2.41 mg/L (EMC), the removal rate of ammonium decreased only slightly (95.3% to 94.7%) (118). This may be because the ammonium removal was primarily controlled by the media. In a review article by Davis et al., the authors reported that the TN removal efficiency in both field- and laboratory-scale studies largely varied within a wide range (32 – 99%) when the influent concentrations fluctuated between 1.2 – 6.0 mg/L (119).

Variable influent nutrient loads (e.g., nitrate and ammonium levels) could change the rhizosphere dissolved oxygen (DO) and pH levels, which are believed to be influential factors that affect microbial N transformations (43). In column-based BRCs, Chen et al. observed that the root DO level was constantly enhanced with increased nutrient loads (43). However, the increase in loading rates did not have significant effects on pH, which could be due to the natural buffering capability of soil. Furthermore, the authors detected that the rate of nitrification, denitrification and DNRA was greater at higher nutrients loads, but among them, nitrification was the dominant and DNRA was the least important N removal pathway (43).

Altogether, research has shown inconsistent results about the impact of N loading rate on bioretention performance, which may be due to variations of the BRC configuration, study modes (lab-scale, pilot-scale or field-scale), vegetation diversities, filter media composition, carbon substrates, the availability of saturation zone and/or the nature of N pollutants. It is

important to evaluate removals based on consistent criteria, such as rates, not just relative metrics such as percent removals. The key outcome of these investigations is that to achieve higher removal performance, inlet N (e.g., nitrate) loads could be considered as one of the bioretention design factors.

Characteristics and depth of the engineered media

The structure of the engineered media and its depth generally regulate the stormwater pollutant removal efficiency in BRCs (38, 120). The bioretention media are broadly divided into three layers (top/upper, middle, and bottom), and each layer is designed to meet specific objectives (22). The upper layer is mainly designed to support the growth of plants as well as to enhance microbially-driven treatment mechanisms, while the middle filter layer improves several mechanical processes including screening and sorption performance, and the bottom gravel layer provides drainage (22).

Multiple studies have been performed on nitrogen removal in BRCs using different media compositions (121–123). Glaister et al. compared NO_x (nitrate + nitrite) and ammonium (NH_4^+) removal efficiency of two types of biofilter media, loamy sand (Fe: 1000 mg/kg and Al: 900 mg/kg) and skye sand (Fe: 21000 mg/kg and Al: 1000 mg/kg) (123). They found that the N removal was higher in skye sand (NO_x: 93% and NH_3: 96%) than loamy sand (NO_x: 81% and NH_3: 88%) under drying periods. In laboratory column experiments using synthetic/actual stormwater and three types of filter media such as concrete sand (sand: 88%, silt: 10% and clay 2%), compost free media (termed as COA - sand: 73%, silt: 18% and clay 9%) and masonry sand (sand: 94%, silt: 2% and clay 4%), Barrett et al. observed different removal trends for stormwater pollutants (122). For example, greater N removal was achieved in the columns filled with COA (NO_x: 62% and NH_3: 79%) compared to masonry sand (NO_x: 56% and NH_3: 72%); both columns were planted with a native Texas plant Big Muhly (*Muhlenbergia lindheimeri*) and had a saturation zone (122).

A recent study on bilayer media bioretention columns found more N (89%) removal in the column which contained 5% fly ash (other media: 90% sand +5% crushed straw) than the column that contained 5% clay (85.9%) (121). The major reason for the higher performance in the fly ash-based system was due to the smaller permeability of fly ash compared to clay, which caused an increase of the hydraulic retention time and possibly more denitrification. Using two sets of loamy-sand-filled BRCs having 0.6 and 0.9 media depths, Brown and Hunt, noted that for both configurations, the effluent ammonia concentration was considerably lower than the influent, but a significant increase of NO_x-N concentration was noticed in the effluent (124). This trend is due to potential nitrification of organic N and/or lower denitrification is possibly due to the absence of internal water storage zones. In this field-scale study, the lower media depth was effective with estimated annual total nitrogen load reductions of 21% for the cell with 0.6-m depth and 19% for the 0.9-m depth. Chen et al. also noticed that the top layer (nitrification: 7 - 28%, denitrification: 2 - 5%) of their biofilter media produced higher N removal than the bottom layer (nitrification: 2 - 12%, denitrification: 1 - 3%) (43). A lab-scale column trial reported around 20% increase of ammonium (NH_4^+-N) removal due to addition of iron-rich soil to the biofilter containing initially sandy loam (21). In a recent study where three columns were filled with different filter materials such as woodchips, woodchips plus biochar (33% by wt.) or woodchips plus straw, it was observed that the three types of woodchip bioreactors showed high performance for nitrate removal from stormwater. The concentration of nitrate in the effluent decreased by above 99% to concentrations below the detection limit (less than 0.05 mg-N/L) (125).

Overall, many studies have recommended the use of a layered media bioretention system to deliver the highest outcomes for stormwater treatment with the appropriate media depth (86, 121, 126) (Table 5). In most cases, higher degree of denitrification occurred at the bottom layer of the biofilter. During engineering and construction, it is important to select soil

plus sand-based media compared to only sand-based media in order to decrease the infrastructure and maintenance cost of the BRC while meeting the treatment objectives.

Effects of storm events frequency (wet vs dry periods)

Stormwater events vary in their frequency, size, and duration. Thus, BRCs will experience a high degree of alternating wet and dry periods (22). Dry conditions can alter the media properties (e.g., increase of porosity due to formation of aggregates) and biological activities (e.g., decrease of plant and microbially-driven pollutants degradation/transformation rates) (22). Many lab- and field-scale studies have been conducted to understand the fate of N pollutants during wet and dry conditions (Table 6).

A column experiment (containing loamy sand media, vegetation, and a saturated zone) showed that the NO_x removal during the dry period was 81%, but varied between 80 – 86% in two wet cycles (first: August -November, second: April – July) (123). However, the ammonia removal was lower during the dry (88%) than the wet periods (89 – 99%). Using single-plant biofilter columns with a saturation zone, Payne et al. found that the TN removal was greater during the wet cycle (79 – 93%) compared to the dry cycle (12 – 78%); the large variations in both conditions were mainly due to plant diversity (114). Subramaniam et al. evaluated the dynamics of nitrate removal in lab-scale biofilter columns and it is observed that the NO_3-N removal fluctuated during an event from a high removal proportion (60–90%) in the first outflow that slowly decreased in the initial operation period (0.5 hr), then the removal rate stabilized at 0–15% (127). Additionally, this study concluded that the denitrification process was more active during the dry period of an event compared to the wet period.

Results from a field-scale woodchip BRC showed that the cell exhibited denitrification during both the wet and dry phases. Nevertheless, a major fraction of nitrate removal was observed during the wet phase (TN: > 26.3%) compared to the dry phase (TN: < 9.9%) (109). Another study from the same research group using a layered BRC containing woodchips as a

C source demonstrated more than 80% nitrate removal (86) and the nitrate removal mainly occurred during the wet period.

In total, wet conditions mainly support denitrification, whereas nitrification and ammonification are predominant in dry conditions (86, 109, 121). Long dry periods have displayed negative impacts on the capacity of BRCs to remove pollutants because of increases of metal and nitrogen leaching observed in several studies (22). To keep BRCs operating with high performance in hot and dry climates, it is necessary to select appropriate drought-tolerant plant species, which may assist with plant growth, as well as assist in the survival of microorganisms in the rhizosphere.

Temperature effects (cold vs warm)

Temperature will affect most nitrogen removal mechanisms in BRCs. Nitrogen uptake by plants is generally higher at warm temperature (128). Microbial activities leading to N transformation processes tend to increase to an optimum temperature (around 20–35°C, depending on locations and soil types) (129). Successful operation of BRCs in cold climates can be a great challenge because of several reasons, namely, cold temperatures, ice cover, cold water, de-icing salts, repeating freeze-thaw cycles, etc. (130). These characteristics may impact the biological processes, soil infiltration rates, and vegetation health.

To date, limited information is available about temperature effects on BRCs (Table 7). In a recent study by Halaburka et al. (131), authors have investigated the impacts of a wide ranges of temperatures (4 – 30 °C) on nitrate removal rate in woodchips bioreactors. They found that temperature considerably influences the nitrate reduction (e.g. denitrification). The nitrate removal rate (mg-N/L/h) was −0.00340 at 4 °C, while it was −0.360 at 30 °C (131). A biofilter mesocosms-based study investigated the influence of three temperatures (2, 7 and 20°C) on NO_x-N and NH_4-N removal, and observed that the ammonium removal was positively correlated with the temperature (i.e., 18, 51 and 74% at 2, 7 and 20 °C, respectively) (132).

However, the removal of other nitrogen species (nitrate-N: NO_x-N) was not effective, i.e., significant leaching was observed at higher temperature (20 °C). At lower temperature (2 °C), a slight change in the concentration of N species was observed, i.e., 2-fold rise in nitrate and nearly 18% reduction of ammonium concentration, which suggests that at lower temperature, nitrification may occur. Chang et al. evaluated the impacts of three temperatures (10, 23, and 28 °C) on nitrate removal from stormwater under lab-scale column experiments (133). Nitrate removal efficiency increased with increase of temperature, 63.2, 77.9 and 93.6 % at 10, 23 and 28 °C, respectively. Another recent study from the same research group evaluated the impacts of four different temperatures (4, 12, 23 and 35°C) on the removal of nutrients (nitrate and total phosphorus) from stormwater in lab-scale (134). Overall, no significant changes in the nitrate removal was observed with the variations of temperature because the removal efficiency varied between 85 – 90% at all temperatures (4 – 35 °C).

The kinetics of N removal are impacted by variations in environmental temperature. Chang et al., (2011) evaluated the reaction kinetics for nitrate removal in a column packed with multi-media components including fine sand (50%), sawdust (25%), tire crumb (15%), limestone (10%), and operated under three different temperature levels (10, 23 and 28 °C) (133). They found that the nitrate transformation was zero order with the rate constant increasing with increases of temperature, i.e., k (M/s) = 0.047, 0.076 and 0.07 at 10, 23 and 28 °C, respectively. Interestingly, the reaction changed to first order with change of the filter media components to fine sand (50%), tire crumb (30%) and sawdust (20%) with k values (s^{-1}) were 0.012, 0.017 and 0.05 at 10, 23 and 28 °C, respectively, and the change of order may be related to the bioavailability of carbon. In another study using a column packed with fine sand (96.2%) and iron filings (3.8%) and tested under 4, 12, 23 and 35 °C, the reaction was zero order, but the rate constants did not significantly change with temperature.

Taken together, researchers have shown that environmental temperature considerably influences N transformations. Additionally, availability of dissolved organic carbon impacts the denitrification rate. A few reports have shown that temperature has a positive effect on stormwater denitrification (36, 129). To improve our understanding about climate effects on microbially-mediated N transformation in BRCs, more lab-scale and field-scale studies are required.

Future research directions

- Little research has been performed on the role of anammox in the BRCs. Comprehensive studies employing ^{15}N isotope techniques are needed to understand the fate of N in the BRCs as well as the relative contribution of various bioprocesses to the total N removal.
- The filter media redox conditions may control the fate of N biotransformation reactions since oxic conditions mainly favor nitrification and anoxic environments encourage denitrification (135). Therefore, in-depth research investigations should be done to evaluate changes of redox and oxygen gradient patterns as a function of media depths.
- Although a few reports are available on the dynamics of bacterial communities in biofilter media (30, 81), archaeal communities may synergistically work with bacteria and contribute to N removal. Thus, in future studies, researchers should also consider assessing the dynamics of archaeal communities in BRCs.
- The rhizosphere could facilitate interactions between microbes and N species. Plants influence the composition and function of rhizosphere communities by releasing organic compounds through roots, which need to be verified to select an appropriate plant species or species mix. Moreover, additional studies are needed to understand N removal by other rhizospheric phenomena such as the role of fungal communities, plant root-formed preferential flow paths and their impact on nutrient transport, the role of legumes in

nitrogen fixation in bioretention systems, and finally, the electron shuttling of wood-derived biochar amended filter media to facilitate denitrification (136, 137).

- To date, most of the studies on BRCs have been carried out under controlled lab-scale environments and field-scale trials at normal climate, but limited information is presently available on the impacts of challenging climates, namely, cold or tropical weather conditions, on stormwater treatment efficiency of BRCs; research on this topic merits further consideration.
- For bioaugmentation of denitrification rate in BRCs, one of the important criteria is to increase C/N ratio of stormwater (138), thus future works should consider augmentation of filter media using carbon-rich materials such as biochar, softwood chips, etc. Other potential parameters that can accelerate the nitrogen removal efficiency in BRCs include low hydraulic loading rates (HRT), incorporation of a saturation zone (SZ)/internal water storage (IWS) with deeper depth, mixed vegetation, mixed layer filter media, wet periods, and warmer climates (22, 38). Additional information on bioaugmentation of N removal in BRCs is given in supplementary materials.
- Besides controlled experimental work in the laboratory, a few studies have explored modeling of denitrifying stormwater biofilters under different simulated storm conditions (139, 140). More robust numerical models should be developed to assess the overall TN reduction efficiency of BRCs. Such simulation studies may provide useful data for designers to select suitable parameters according to the treatment objectives set for BRCs.

Conclusions

This paper presents a state-of-the-art review of the recent developments that have been made on the biological nitrogen removal from stormwater in BRCs. Plant- and microbially-driven N transformation processes that occur in BRCs include the uptake of nitrogen (assimilation) by both plants and microorganisms, nitrification, denitrification, and anammox. However,

denitrification is the major process for N removal (especially nitrate) from runoff. Biofilters are generally enriched with diverse microbial communities, but the phyla *Bacteroidetes* and *Proteobacteria* are the most abundant.

High N removal efficiency (TN: > 70%) has been achieved in both lab- and field-scale studies. However, large variations have been observed among the studies. The lack of consistency can be attributed to the fluctuations of hydraulics (hydraulic loading rate or N loading rate) and environmental factors. The key factors to consider are the presence/absence of saturation zones, the composition and height of the filter media, the type of plant species, the frequency of storm events (wet and dry periods) and the prevailing ambient temperature (warm and cold climate) (Fig. 3). In general, BRCs show better N removal performance when they are operated at low hydraulic/N loading rates, installed with a saturation zone, vegetated with native plants, having deeper and multilayer biofilter media with warm climate temperature and wet periods.

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Disclosure statement

The authors report no conflict of interest.

Supplementary online material

Supplementary data to this study are submitted.

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List of Tables and Figures

Table 1. Concentration of different nitrogen species detected in stormwater runoff

	Different chemical forms of nitrogen (mg/L)[e]					
Stormwater source	Nitrate (NO_3-N)	Nitrate + Nitrite (NO_x-N)	Ammonia (NH_3-N)	Organic-N	Total nitrogen (TN)	Reference
Road	1.0	NA	0.29	NA[d]	2.0	(15)
Roadway	NA	0.66	NA	NA	1.3	(141)
Parking lot	NA	0.19±0.11	0.29 ±0.48	0.45±0.39	0.94±0.87	(124)
Carpark	NA	0.4 ± 0.2	0.04 ± 0.06	0.6 ± 0.3	1.1 ± 0.5	(142)
Asphalt	0.6±0.9	NA	0.18±0.36	NA	NA	(6)
Paver	0.3±1.2	NA	0.05±0.14	NA	NA	(6)
Crushed stone	0.3±0.4	NA	0.11±0.24	NA	NA	(6)
Asphalt	NA	0.3	0.31	0.75	1.33	(143)
Highway	1.1	NA	1.1	NA	NA	(144)
Interstate highway	NA	0.20±0.17	0.12±0.23	1.50±2.04	1.64±2.1	(145)
Mixed[a]	NA	0.12±0.16	0.10±0.13	0.89±0.79	1.01±0.81	(145)
Mixed[b]	0.39±0.58	NA	NA	0.66±1.24	1.61±1.97	(146)
Mixed[c]	NA	0.74±0.56	0.29±0.39	1.1±0.99	2.13±1.68	(17)

[a] Mixed: Parking lot, maintenance building, picnic area

[b] Mixed: Rooftops, driveways and sidewalks, roads and patios.

[c] Mixed: Residential, commercial, and/or parkland

[d] NA: Not available

[e] Standard deviation associated with some data is missing since it is not available, or the data is extracted from the figure in the cited reference.

Table 2. Nitrogen transformation process, reaction, enzymes and their properties

Process/Reaction	Condition	Enzyme	Redox potential (E_0' in mV)	Location	Reference
Dissimilatory nitrate reduction (DNRA)					
$NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O$	Anoxic	Nitrate reductase (NR: eukNR, Nar, Nap and Nas)	+433	Membrane associated, periplasm or cytoplasm	(60, 61)
$NO_2^- + 8H^+ + 6e^- \rightarrow NH_4^+ + 2H_2O$	Anoxic	Nitrite reductase (Nrf)	+340	Cytoplasmic membrane	(60, 61)
Denitrification					
$NO_2^- + 2H^+ + e^- \rightarrow NO + H_2O$	Anoxic	Nitrite reductase (NiR)	+350	Periplasm	(60, 61)
$2NO + 2H^+ + 2e^- \rightarrow N_2O + H_2O$	Anoxic	Nitric oxide reductase (NoR)	+1175	Transmembrane	(60, 61)
$N_2O + 2H^+ + 2e^- \rightarrow N_2 + H_2O$	Anoxic	Nitrous oxide reductase (NoS)	+1335	Periplasm	(60, 61)
Anammox					
$NO + NH_3 + 3H^+ + 3e^- \rightarrow N_2H_4 + H_2O$	Anoxic	Hydrazine hydrolase (HH)	+340	Anammoxosome	(60, 61)
$N_2H_4 \rightarrow N_2 + 4H^+ + 4e^-$	Anoxic	Hydrazine dehydrogenase (HDH)	-230	Anammoxosome	(60, 61)
Nitrification					
$NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^-$	Oxic	Nitrite oxidase (NO)	+420	Membrane associated	(60, 61)
$NH_2OH + H_2O \rightarrow NO_2^- + 5H^+ + 4e^-$	Oxic	Hydroxylamine oxidoreductase (HAO)	+60	Periplasm	(60, 61)
$NH_3 + O_2 + 2H^+ + 2e^- \rightarrow NH_2OH + H_2O$	Oxic	Ammonia oxidase (AMO)	+730	Transmembrane	(60, 61)
Nitrogen fixation					
$N_2 + 6H^+ + 6e^- \rightarrow 2NH_3$	Oxic/Anoxic	Nitrogenase (Nif)	-92	Cytoplasm	(60, 61, 147)

Table 3. Nitrogen removal efficiency in bioretention cells with and without saturated zones.

Study site	N removal (%)[b] NOx[b]	N removal (%)[b] TN[c]	Saturation zone (SZ)	HLR[a]	Carbon source	Planted	Reference
Mesocolumn	89	NA[d]	Yes	10 - 30 cm/hr	Sugar cane mulch and pine chips	Single-plant	(39)
	72	NA	No				
Lab-scale column	NA	87	Yes	10 - 30 cm/hr	Sugar cane mulch and pine chips	Single-plant	(114)
	NA	72	No				
Field-scale	NA	90	Yes	NA	Newspaper	Single-plant	(115)
	NA	95	No				
Field-scale	81	83	Yes	4.1 - 13.9 cm/hr	Eucalyptus Woodchips	Mixed-plant	(34)
	29	74	No				
Lab-scale column[e]	81	82	Yes	20–40 cm/hr	Pine woodchips and pine flour	Single plant	(123)
	9	33	No				
Lab-scale column[f]	93	89	Yes	20–40 cm/hr	Pine woodchips and pine flour	Single plant	(123)
	27	44	No				
Lab-scale column	-23[h]	73	Yes	~2 cm/hr	Newspaper	Single plant[g]	(148)
	62[h]	35	No				
Lab-scale column	66.1[h]	81.2	Yes	NA	Woodchips	Single plant	(149)
	30.5[h]	59.4	No				

[a] HLR: Hydraulic loading rates

[b] NOx: Nitrate + Nitrite

[c] TN: Total nitrogen

[d] NA: Data not available

[e] Columns were operated under wet period.

[f] Columns were operated under dry period.

[g] 10 - 40 plants (*Phragmites australis*) per column.

[h] It refers to only nitrate (NO_3-N).

Table 4. Nitrogen removal efficiency in planted and nonplanted bioretention cells/constructed wetlands.

Study type	N removal efficiency (%)	Plantation	Main filter media	Reference
Mesocolumn[a]	NO_x: 39 - 60 (AS)[b], 1 - 7 (DN)[c]	Yes	Loamy sand (30 cm), sand (20 cm) and gravel (10 cm)	(39)
	NO_x: 38 (AS), 15 (DN)	No		
Field-scale	NO_x: 54	Yes	Sand (30 cm), River rock (5 cm) and #57 stone (30 cm)	(34)
	NO_x: 15	No		
	NO_x: 88	Yes	Sand (30 cm), River rock and wood chip (30 cm), River rock (5 cm) and #57 stone (30 cm)	
	NO_x: 78	No		
Mesocosms	TN: 81	Yes	Sandy loam (80 cm)	(108)
	TN: 41	No		
Pilot-scale	TN: 95	Yes	Sandy soils[f]	(117)
	TN: 32	No		
Lab-scale column	NO_x: 93, NH_3: 96	Yes	Skye sand (30 cm), coarse sand (20 cm), pea gravel (70 mm), and gravel (30 mm).	(123)
	NO_x: 41, NH_3: 84	No		
Constructed wetlands[a]	NO_x: 78 (DN)	RS[d]	Not applicable	(83)
	NO_x: 71 (DN)	BS[e]		

[a] These studies have used ^{15}N tracer technique to find out the different N transformation processes.

[b] AS: Assimilation

[c] DN: Denitrification

[d] RS: Rhizosphere sediment (called as vegetated system)

[e] BS: Bare sediment (called as nonvegetated system)

[f] Detail media composition is not available.

Table 5. Changes of N removal efficiency with various filter media composition and depth.

Study type	Key filter media composition	Overall N removal efficiency (%)	Change of N removal efficiency (%) with depth	Reference
Bilayer media columns	90% sand+5% fly ash+5% crushed straw	NO_3-N: 91.5–97.4	NO_3-N: 25 (0-75cm), 85.1 (75-95cm)	(121)
	90% sand+5% clay+5% crushed straw	NO_3-N: 87.5–96.9	NO_3-N: 13.8 (0-75cm), 80.8 (75-95cm)	
	Quartz sand	NO_3-N: 34.5–46.2	NA[a]	
	Quartz sand+5% crushed straw	NO_3-N: 42.5–51.9	NA	
Laboratory column	Sand (73%)+silt (18)+clay (9%)	NO_x: 62, NH_3: 79	NA	(122)
	Sand (94%)+silt (2)+clay (4%)	NOx: 56, NH_3: 72	NA	
Biofilter columns	Skye sand (Fe: 21000 mg/kg and Al: 1000 mg/kg)	NO_x: 93, NH_3: 96	NA	(123)
	Loamy sand (Fe: 1000 mg/kg and Al: 900 mg/kg)	NO_x: 81, NH_3: 88	NA	
lab-scale columns	Sandy loam (100 kg)	NH_4^+-N: 76.5	NA	(21)
	Sandy loam (100 kg)+iron-rich soil (15 kg)	NH_4^+-N: > 95	NA	
Lab-scale columns	Loamy sand (30cm)+gravel (15 cm)+pebble (30cm)	NA	^{15}N-NO_3^-: 2- 5 (top 10 cm), 1- 3 (bottom 10 cm)	(43)
		NA	^{14}N-NO_3^-: 7- 28 (top 10 cm), 2- 12 (bottom 10 cm)[d]	
Pilot-scale columns	Mixed structure[b]	NA	TN: 64.8 (20cm), 75 (40cm), 86.8 (60cm)	(30)
	Layered structure[c]	NA	TN: 63.3 (20cm), 72.1 (40cm), 83.9 (60cm)	
Field-scale	Sand (87.5%)+silt and clay (10%)+compost (2.5%)	NA	TN: 21 (60cm), 19 (90cm)	(124)

[a] NA: Data not available.

[b] Mixed structure: Soil: sand: fly ash (1:1:1) (60 cm)

[c] Layered structure: [Soil (10cm) + sand (10cm) + fly ash (10 cm)] (two layers)

[d] This refers to ^{14}N–NO_3^- produced by nitrification

Table 6. Impacts of stormwater events variability (wet and dry periods) on nitrogen removal efficiency.

Study type	Condition	N removal efficiency (%)	Other conditions	Reference
Biofilter columns	Wet1[a]	NOx: 80, NH_3: 89, TN: 70	Loamy sand media, vegetated, and saturated zone	(123)
	Wet2[b]	NOx: 86, NH_3: 99, TN: 85		
	Dry	NOx: 81, NH_3: 88, TN: 69		
lab-scale columns	Wet	TN: 79 - 93	Loamy sand filter, single-plant, and saturated zone	(114)
	Dry	TN: 12 - 78		
Field-scale	Wet	TN: > 26.3	Sand, soil, and wood chips, single-plant, no saturation zone	(109)
	Dry	TN: < 9.9		
Bioretention columns	Wet	NO_3^-: ~ -20	Wood chips, sandy loam, river sand, vegetation, saturation zone	(148)
	Dry	NO_3^-: ~ 100		

Table 7. Impacts of various temperatures on nitrogen removal efficiency.

Study type	Temperature/Season	N removal efficiency/rate (nmol N/g sed. wet wt./hr)	Initial N concentrations (mg/L)	Reference
Biofilter mesocosms	2 °C	NH_4-N: 18 ± 26%, NOx-N: -208 ± 101%	NOx-N: 0.40 ± 0.16, NH_4-N: 0.22 ± 0.05	(132)
	7 °C	NH_4-N: 51 ± 15%, NOx-N: -320 ± 127%		
	20 °C	NH_4-N: 74 ± 18%, NOx-N: -944 ± 359%		
Laboratory column	22.9 °C	NO_3^-: > 98%	NO_3^--N: 5.65	(150)
	10 to +10 °C	NO_3^-: > 96%		
Laboratory column	10 °C	NO_3^-: 63.2%	NA	(133)
	23 °C	NO_3^-: 77.9%		
	28 °C	NO_3^-: 93.6%		
Constructed stormwater wetlands			NO_3^-: ~ 0.004 - 0.22	(83)
Unvegetated sediments	Summer	DN: 0.67, AN: 0.04		
	Fall	DN: 3.77, AN: 0.20		
	Winter	DN: 4.57, AN: 0.65		
Plant rhizospheric	Summer	DN: 16.3, AN: 2.2		
	Fall	DN: 8.88, AN: 1.67		
	Winter	NA[a]		

[a] NA: Data not available

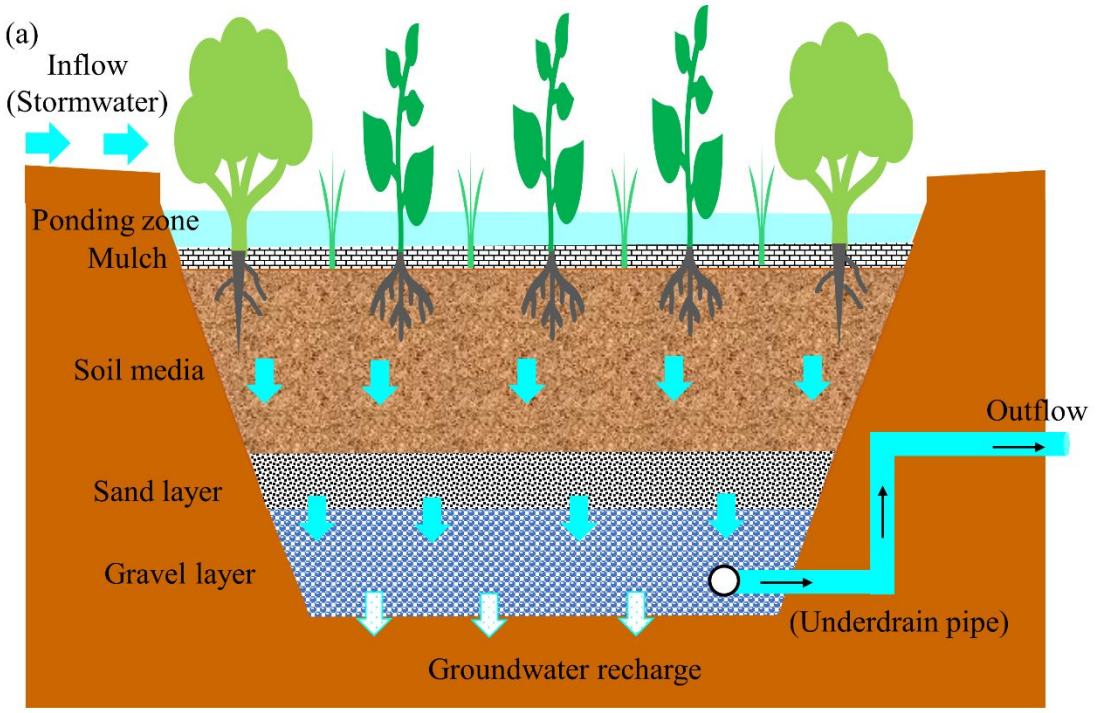

Fig.1. Schematic showing different components of a typical field-scale stormwater bioretention cell (a), and image of a bioretention facility installed at National University of Singapore.

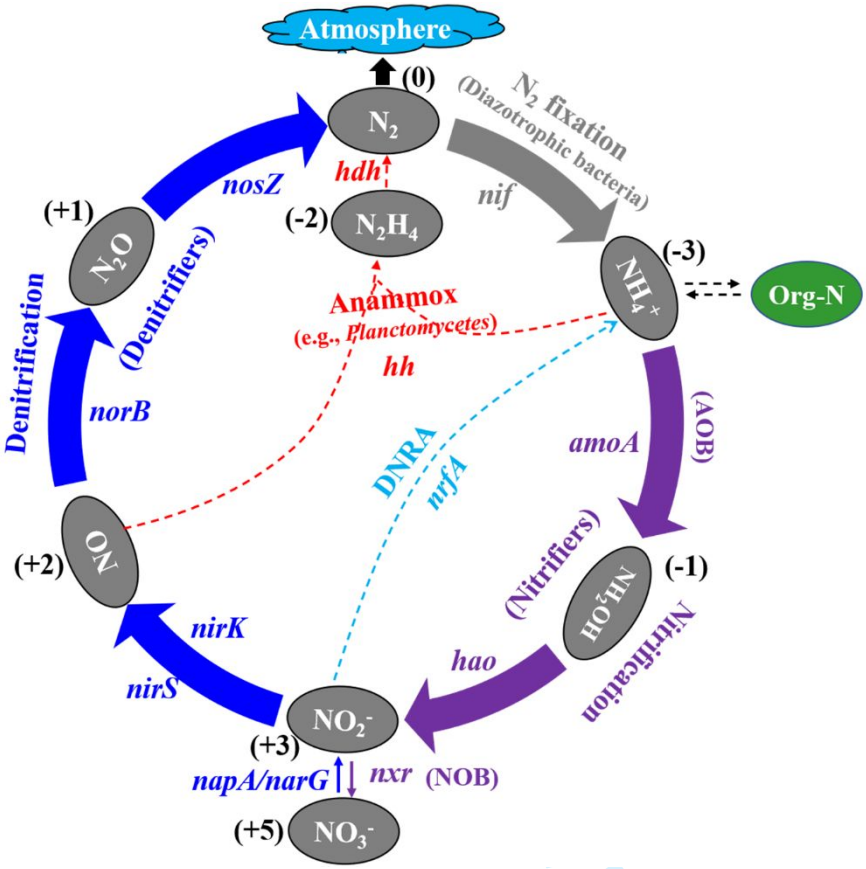

Fig. 2. An overview of the major biological nitrogen cycle in soil (42, 61, 65). Specific enzymes responsible for various nitrogen transformations are: denitrification - *narG/napA*: nitrate reductase; *nirS/nirK*: nitrite reductase; *norB*: nitric oxide reductase; *nosZ*: nitrous oxide reductase; N_2-fixation - *nif*: nitrogen fixation; nitrification - *amoA*: ammonia monooxygenase; *hao*: hydrazine oxidoreductase; DNRA - *nrfA*: respiratory nitrite ammonification; anammox - *nxr*: nitrite oxidoreductase; *hh*: hydrazine hydrolase; *hdh*: hydrazine dehydrogenase. AOB : ammonia-oxidizing bacteria; NOB : nitrite-oxidizing bacteria. Numerical values shown in the bracket indicate the oxidation state of N in the compounds.

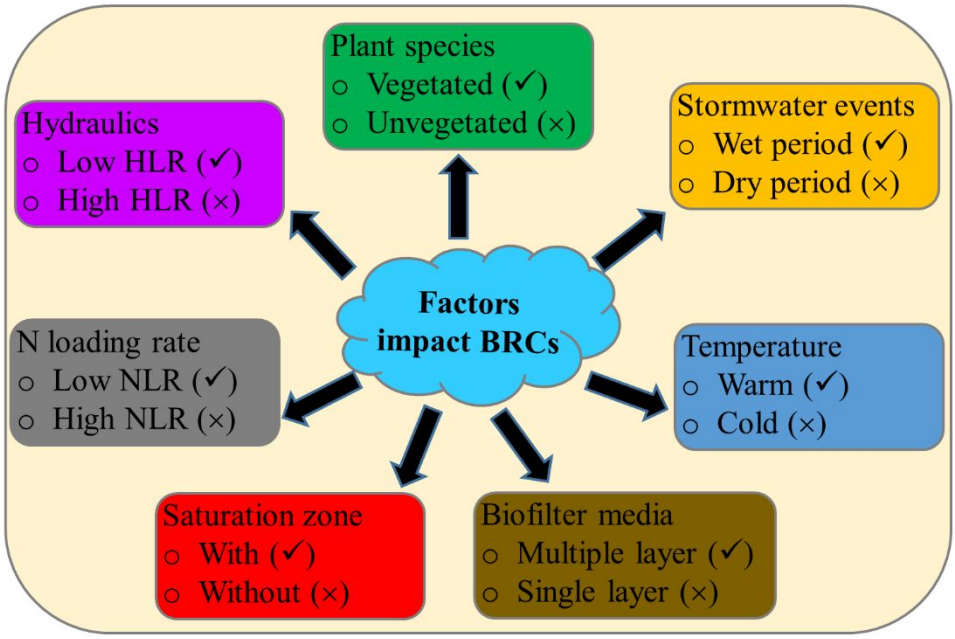

Fig. 3. Key environmental factors that impact the nitrogen removal performance in bioretention cells (BRCs). The symbol tick (✓) means an increase and cross (×) means a decrease of N removal efficiency which are observed in most studies.

Supplementary Material

Biological Nitrogen Removal from Stormwater in Bioretention Cells: A Critical Review

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Supplementary Tables and Figures

Table S1. Physicochemical and thermodynamic characteristics of various inorganic nitrogen species (adopted from previous studies (1, 2)).

N species (Formula)	$\Delta G_f°$ (kJ/mol)	$\Delta H_f°$ (kJ/mol)	S° (J/mol/K)	pK
Nitrate (NO_3^-)	−111.7	−208.2	−324	−1.5
Nitrite (NO_2^-)	−37.4	−105.0	−227	3.3
Nitric oxide (NO) (g)	86.9	90.6	12	NA[a]
Nitrous oxide (N_2O) (g))	104.6	82.4	−74	NA
Dinitrogen (N_2) (g)	0	0	0	NA
Hydroxylamine (NH_2OH) (aq)	−22.9	−98.7	−254	6
Hydrazine (N_2H_4) (aq)	128.5	34.4	−316	6.1
Ammonium (NH_4^+)	−79.4	133.1	713	9.2

[a] NA: Not applicable

Table S2: Biological nitrogen transformation processes.

Nitrogen transformation reactions	Free energy (ΔG^0)	Reference
Assimilation		
$NH_4^+ + HCO_3^- + 1\frac{1}{3}C_6H_{12}O_6 + 3\frac{1}{2}O_2 \rightarrow C_5H_9O_4N + 4CO_2 + 6H_2O$	-1797.4 KJ/N atom	(3)
$NO_3^- + H^+ + 1\frac{1}{3}C_6H_{12}O_6 + 1\frac{1}{2}O_2 \rightarrow C_5H_9O_4N + 3CO_2 + 4H_2O$	-1492.8 KJ/N atom	(3)
Ammonification		
$CO(NH_2)_2 + 2H_2O \rightarrow 2NH_3 + CO_2 + H_2O$	16.3 – 102.4 KJ/mol	(4)
Nitrification		
$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + H_2O + 2H^+$	-267.5 KJ/mol	(5)
$NO_2^- + 0.5O_2 \rightarrow NO_3^-$	-86.96 KJ/mol	(5)
Heterotrophic denitrification		
$C_3H_4O_3 + 5NO_3^- \rightarrow 5NO_2^- + 3CO_2 + 2H_2O$	-86.5 KJ/mol	(6)
$C_3H_4O_3 + 10NO_2^- + 10H^+ \rightarrow 10NO + 3CO_2 + 7H_2O$	-120.9 KJ/mol	(6)
$C_3H_4O_3 + 10NO \rightarrow 5N_2O + 3CO_2 + 2H_2O$	-159.1 KJ/mol	(6)
$C_3H_4O_3 + 5N_2O \rightarrow 5N_2 + 3CO_2 + 2H_2O$	-176.0 KJ/mol	(6)
Autotrophic denitrification		
$S^0 + \frac{6}{5}NO_3^- + \frac{2}{5}H_2O \rightarrow SO_4^{2-} + \frac{3}{5}N_2 + \frac{4}{5}H^+$	-547.6 KJ/mol	(7)
$6NO_3^- + 2FeS_2 + 4H_2O \rightarrow 3N_2 + 4SO_4^{2-} + 2Fe(OH)_3 + 2H^+$	-	(8)
Dissimilatory nitrate reduction to ammonium (DNRA)		
$NO_3^- \rightarrow NO_2^- \rightarrow NH_4^+$	-75.4 KJ/mol	(5)
Anammox		
$NH_4^+ + NO_2^- \rightarrow N_2H_4 \rightarrow N_2$	-357 KJ/mol	(9, 10)

Table S3. Nitrogen removal kinetics in biofilter systems for stormwater treatment.

Study type	NO_3-N (mg/L)	Media component	Nitrogen removal (denitrification/sorption) rate constant (k)				Reference
			First-order	R^2	Zero-order	R^2	
Lab-scale column	3.0	Woodchips-pea gravel	11.4 day^{-1}	NA[a]	6.6 mg/L-day	0.92	(21)
Microcosms	2.0	Wood	0.75 hr^{-1}	0.98	13.46 mg/L-day	0.86	(22)
Microcosms	2.0	Sand-wood	0.27 hr^{-1}	0.99	7.02 mg/L-day	0.98	(22)
Microcosms	2.0	Gravel-wood	0.58 hr^{-1}	0.95	9.24 mg/L-day	0.85	(22)
Microcosms	2.0	Sand	0.00 hr^{-1}	0.27	0.16 mg/L-day	0.28	(22)
Batch test	3.0	Woodchip	0.0011 min^{-1}	NA	NA	NA	(23)
			First-order	R^2	Second-order	R^2	
Lab-scale column[c]	5.0	Mix[b]	0.75 hr^{-1}	0.65	0.07 L/mg-hr	0.99	(24)
Lab-scale column[c]	2.5	Mix	0.33 hr^{-1}	0.99	0.30 L/mg-hr	0.92	(24)
Lab-scale column[c]	0.5	Mix	0.25 hr^{-1}	0.26	9.52 L/mg-hr	0.88	(24)
Lab-scale column	5.0	Natural soil	0.75 hr^{-1}	0.65	0.07 L/mg-hr	0.99	(24)
Lab-scale column	2.5	Natural soil	0.07 hr^{-1}	0.22	1.64 L/mg-hr	0.21	(24)
Lab-scale column	0.5	Natural soil	0.58 hr^{-1}	0.39	0.44 L/mg h	0.71	(24)

[a] NA: Not available

[b] Mix: Sand (50%), limestone (20%), sawdust (15%) and tire crumb (15%).

[c] It refers to mainly sorption-based experiments.

Table S4. Nitrogen removal efficiency of various plant species in bioretention systems.

Study mode	Plant species	N species	N removal efficiency (%)	Reference
Bioretention column	*Arundo donax* var. versicolor	Nitrate	96	(11)
Bioretention column	*Bougainvillea* 'Sakura Variegata'	Nitrate	96	(11)
Bioretention column	*Complaya trilobata*	Nitrate	95	(11)
Bioretention column	*Cymbopogon citratus* (DC.) Stapf	Nitrate	95	(11)
Bioretention column	*Ipomoea pes-caprae*	Nitrate	95	(11)
Bioretention column	*Chrysopogon zizanioides* (L.) Roberty	Nitrate	93	(11)
Bioretention column	*Pennisetum alopecuroides* (L.) Spreng.	Nitrate	93	(11)
Bioretention column	*Nerium oleander* 'Pink'	Nitrate	88	(11)
Bioretention column	*Scaevola taccada* (Gaertn.) Roxb.	Nitrate	88	(11)
Bioretention column	*Leucophyllum frutescens* (Berland.) I. M. Johnst.	Nitrate	87	(11)
Bioretention column	*Sanchezia oblonga* Ruiz & Pav.	Nitrate	87	(11)
Bioretention column	*Acalypha wilkesiana* cultivar	Nitrate	79	(11)
Bioretention column	*Ophiopogon jaburan*	Nitrate	77	(11)
Bioretention column	*Loropetalum chinense* (R. Br.) Oliv	Nitrate	71	(11)
Bioretention column	*Serissa japonica* (Thunb.) Thunb.	Nitrate	71	(11)
Bioretention column	*Pennisetum x advena* 'Rubrum'	Nitrate	70	(11)
Bioretention column	*Bulbine frutescens* (L.) Willd. 'Hallmark'	Nitrate	68	(11)
Bioretention column	*Ficus microcarpa* 'Golden'	Nitrate	68	(11)
Bioretention column	*Melastoma malabathricum* L.	Nitrate	68	(11)
Bioretention column	*Codiaeum variegatum* (L.) Rumph. ex A.Juss.	Nitrate	67	(11)
Bioretention column	*Osmoxylon lineare* (Merr.) Philipson	Nitrate	66	(11)
Bioretention column	*Galphimia glauca* Cav.	Nitrate	65	(11)
Bioretention column	*Dracaenaceae reflexa* 'Song of India'	Nitrate	64	(11)
Bioretention column	*Phyllanthus myrtifolius* Müll. Arg.	Nitrate	55	(11)
Bioretention column	*Vetiveria zizanioides* (L.) (2015)	Nitrate	45.4	(12)
Bioretention column	*Juncus effusus* (L.)	Nitrate	83.9	(12)
Bioretention column	*Radermachera hainanensis* (Merr.)	Nitrate	56.2	(12)
Mesocolumn	*Dianella tasmanica*	Nitrate/Nitrite	7[a]	(13)
Mesocolumn	Mixed plant speceies[b]	Nitrate/Nitrite	39 - 60[c]	(13)
Field-scale	Mixed plant speceies[d]	Nitrate + Nitrite	78 - 96	(14)
Mesocosms	Mixed plant speceies[e]	Total nitrogen	81	(15)
Pilot-scale	Mixed plant speceies[f]	Total nitrogen	82 - 95	(16)

Biofilter column	*Carex appressa*	Total nitrogen	89	(17)
Field-scale	Mixed plant speceies[g]	Total nitrogen	45	(18)
Bioretention column	*Vetiveria zizanioides* (L.) (2015)	Total nitrogen	66.2	(12)
Bioretention column	*Juncus effusus* (L.)	Total nitrogen	65.6	(12)
Bioretention column	*Radermachera hainanensis* (Merr.)	Total nitrogen	68.1	(12)
Bioretention column	*Pennisetum alopecuroides*	Total nitrogen	95.4	(19)
Field-scale	Mixed plant speceies[h]	Total nitrogen	56	(20)

[a] N removal by denitrification.

[b] *Allocasurina littoralis, Buffalo sp., Carex appressa, Dianella tasmanica, Gahnia siberiana, Hypocalmma angustifolium, Leptospermum continentale* and *Juncus krassii.*

[c] N removal by plant assimilation.

[d] Four dwarf pentas (*Pentas lanceolate*) and one blue daze (*Evolvulus glomeratus*).

[e] Swamp Foxtail Grass (*Pennisetum alopecurioides*) and Flax Lily (*Dianella brevipedunculata*), and two woody shrubs, Banksia (*Banksia integrefolia*) and Bottlebrush (*Callistemon pachyphyllus*).

[f] Three tree species (*Eucalyptus polyanthemos*,*Lophostemon confertus* and *Platanus orientalis*).

[g] Daylilies'Stella d'Oro' (*Hemerocallis spp.*) and Switchgrass 'Shenandoah' (*Panicum virgatum*).

[h] Prairie cord grass (*Spartina pectinata*) and sumpweed (*Iva annua*).

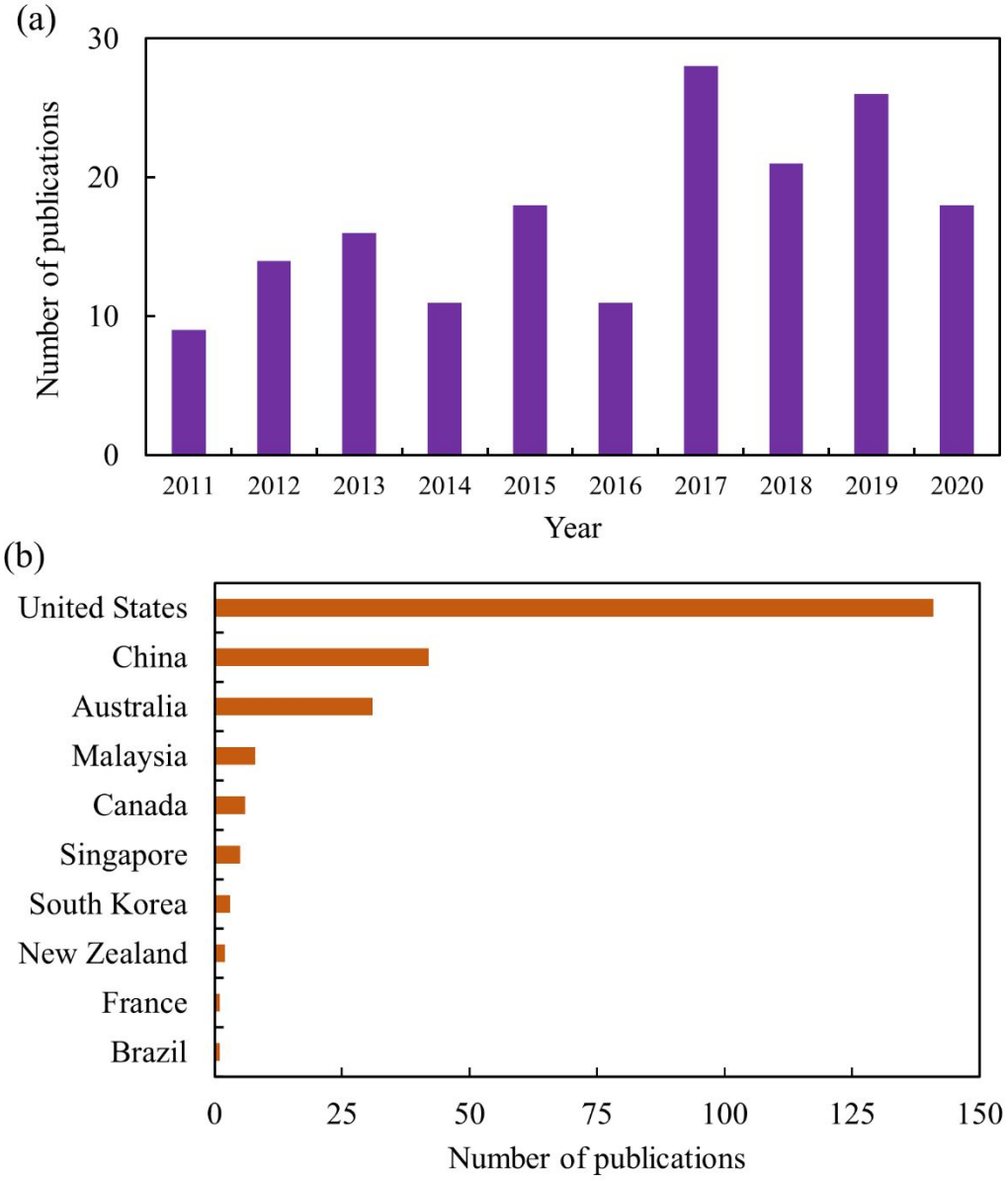

Fig. S1. Number of scientific publications (a) and their distribution among the top ten countries (b)) illustrating the trends in the recent research developments on nitrogen removal from stormwater runoff using bioretention cells in the last ten years (2011–2020). The raw data for this chart were collected from the Scopus database using the keywords such as 'nitrogen', 'stormwater', and 'bioretention'.

Supplementary text

Bioaugmentation of N removal in bioretention systems

Removal of nitrogen from stormwater using microbially-mediated processes continues to be a great challenge for bioretention systems. Researchers have shown that the denitrification process is more prone to be affected by the design of bioretention systems rather than the local environmental factors (13). The N removal efficiency can potentially be increased by simple design alterations including building bioretention systems with media composition that delivers suitable microorganisms with non-leachable nutrients, creating synergic relationships between vegetation and denitrifying microbes, and providing an external carbon source at the bottom layer of the biofilter to encourage and enhance denitrification activities (13). In biofilters, instead of using common soil media (e.g., sandy loam soil), researchers have recommended the use of iron-rich soil because it has the great ability to enhance the adsorption capacity of ammonium and phosphate, and stimulate nitrifier and denitrifier populations by providing microbially available phosphorus (25). Since denitrification rates are highly dependent on the nature of C substrates, selection of an appropriate C source is important. Generally, solid substrates (also called brown organic materials) that are rich in lignocellulose and can be easily hydrolyzed into dissolved organic carbon (DOC) by bacterial extracellular enzymes can be selected (26). Among them, woodchips have been widely used because they can support biofilm formation in addition to their primary purpose of acting as a C source (21, 26). In terms of assessing the impact of woodchip types and sizes on N removal, researchers have observed that large woodchips and softwood showed better TN removal performance than smaller sizes and hard woodchips (26, 27).

Perspectives: Overall, findings by various researchers imply that large size softwood chips can be beneficial to enhance denitrification in BRCs. Other potential approaches to augment denitrification activities include manipulation of the stormwater C/N ratio, adjustment of the

height of the outflow pipe, and addition of herbaceous plant detritus material to the filter media (28). Moreover, amendment of filter media with conventional/engineered biochar (modified using nanomaterials, chemicals, or microbial agents) can increase denitrification rates (29, 30). Other design factors that should be considered to enhance the N removal performance in BRCs include low hydraulic loading rates, incorporation of a saturation zone/internal water storage with deeper depth, mixed vegetation, mixed layer filter media, wet periods, and warmer climates (31, 32).

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