Dynamics of nitrate-nitrogen removal in experimental stormwater biofilters under intermittent wetting and drying

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Abstract

High concentrations of nitrate-nitrogen degrade the quality of aquatic environments. Primary mechanism that removes nitrate-nitrogen (denitrification) requires anoxic condition and electron donors. While removal of total nitrogen and ammonium-nitrogen are often high in stormwater biofilters, poor removal or even release of nitrate-nitrogen in the outflow was often observed. Five Perspex™ biofilter columns (94 mm internal diameter) were fabricated with a filter layer that contained 8% organic material. Columns were operated at 875 mm/h and fed with simulated stormwater with different antecedent dry days and concentration of nitrate-nitrogen. Samples were collected from the outflow at different time intervals between 2 – 150 minutes and were tested for nitrate-nitrogen. Removal of nitrate-nitrogen varied during an event from a high removal percentage (60-90%) in the initial outflow that gradually
decreased in the first 30 minutes and settled at 0-15% removal thereafter. This was consistent during all simulated events independent of number of antecedent dry days (ADD) or inflow concentrations. ADD and previous event feed concentration affected the outflow nitrate-nitrogen concentration in the first 30 minutes of the current event. Therefore, from this study, we conclude that denitrification within stormwater biofilters occurs mainly over the drying period rather than the wetting period.

Key Words: Antecedent Dry Days, nitrate-nitrogen, stormwater biofilters, simulated stormwater

**Non-standard abbreviations**

ADD – Antecedent Dry Days (number of day between two consecutive rainfall events)

EN – Event Number (corresponds to age of filter)

NO3IN – Concentration of nitrate-nitrogen in the feed of current event

NO3PRE – concentration of nitrate-nitrogen in the feed immediately preceding an event

min2, min7, min12, min20, min30, min60, min90, min120 and min150 – concentration of nitrate-nitrogen in the outflow at 2, 7, 12, 20, 30, 60, 90, 120 and 150 min from the start of outflow, respectively
Stormwater biofilters are designed to manage stormwater both quantitatively by reducing peak flow runoff and qualitatively by removing nutrients, solids and heavy metals (Blecken et al., 2008; Blecken et al., 2009b; Davis, 2007; Davis et al., 2007; Davis et al., 2006; Davis et al., 2003). Nutrients including nitrogen, phosphorous and carbon degrade water resources quality when present in high concentrations and stormwater runoff has often been shown to contain high concentrations of such nutrients (Ice, 2004; Liu, 2011). Nutrient removal in stormwater management systems including stormwater biofilters are therefore required to be enhanced. Several studies that have monitored the efficiency of stormwater biofilters in removing nutrients from stormwater runoff reported that removal of nitrogen depended specifically on the species of nitrogen concerned. For example, even though ammonium-nitrogen has commonly been observed to be removed in stormwater biofilters, concentration of nitrate-nitrogen has often been reported to be higher in the outflow compared with that in the inflow (Blecken et al., 2011; Bratieres et al., 2008b; Davis et al., 2006). This observation of higher concentrations of nitrate-nitrogen in the outflow indicated leaching of nitrate-nitrogen from the system.

Stormwater biofilters in general, are designed to transport stormwater rapidly through the system and hence filter beds may not operate exclusively under saturated conditions (Browne et al., 2008). Saturated filter beds are believed to have stratified zones based on dissolved oxygen content (aerobic/anoxic/anaerobic) with the depth of the filter, while unsaturated filters contain air pockets that inhibit formation of such stratified zones. In addition, during a rainfall event rapid transport of stormwater through the system can distribute dissolved oxygen throughout the filter layer more effectively compared to a system with higher retention time. The filter layer of stormwater biofilters therefore may operate under aerobic
conditions during rainfall, and depending on the efficiency of microorganisms within the
layer in consuming dissolved oxygen, the filter may develop zones/micro-zones or pockets of
anoxic and anaerobic conditions (Browne et al., 2008).

According to Davis et al (2010) high interstitial velocity of water moving through the pores
that results from rapid infiltration through the filter layer creates an environment not
conducive (in terms of time of contact or retention time) for effective removal of ammonium-
or nitrate-nitrogen by biologically mediated processes. Results of this study go on to suggest
that ammonium-nitrogen is readily adsorbed to charged sites in the filter material and is
subsequently nitrified to nitrate-nitrogen during the dry-phase in the biofilter. They
substantiated this view by their observation of higher nitrate-nitrogen concentration
(leaching) in outflow in the subsequent event, which supposedly resulted from the residue of
the nitrification of ammonium-nitrogen during the dry-phase that was eventually washed off
during the next rainfall event.

Nitrate is a very stable compound, removal of which is conventionally attributed to plant
uptake and reduction process called denitrification. Because of the fact that nitrate-nitrogen
taken up by the plant returns to the system when the plant dies, nitrate-nitrogen is not
considered to have been removed from the system unless the plant itself is removed (Payne et
al., 2014). Due to the fact that stormwater biofilters are rarely maintained after installation,
nitrate-nitrogen removal due to phytoremediation in stormwater biofilters is still considered a
moot question (Payne et al., 2014). Denitrification in contrast, reduces nitrogen compounds
including NO$_3^-$ and NO$_2^-$ eventually to N$_2$ mediated by microorganisms such as
Pseudomonas, Achromobacter and Bacillus (Joshi et al., 2007). In addition, denitrification is
active in anoxic environments and in the presence of organic carbon as a substrate for
heterotrophic denitrifiers and as an electron donor for the reduction process of denitrification
(Cheremisinoff, 2002; Sperling and Chernicharo, 2005).
Nitrification of ammonium

\[
2\text{NH}_4^+ + 3\text{O}_2 \xrightarrow{\text{microorganisms}} 2\text{NO}_2^- + 4\text{H}^+ + 2\text{H}_2\text{O}
\]

Nitrification of nitrite

\[
2\text{NO}_2^- + \text{O}_2 \xrightarrow{\text{microorganisms}} \text{NO}_3^-
\]

The two crucial parameters in denitrification are the presence of electron donors and an anoxic environment. Past studies therefore, have attempted to enhance nitrate-nitrogen removal in stormwater biofilters by adding organic material (an organic carbon source) in association with a permanent saturated zone to provide electron donors and to induce anoxic environmental conditions, respectively (Blecken et al., 2009a, b, 2010b; Kim et al., 2003).

Most of these studies have analysed the performance of the systems from event mean concentrations and also after extended continuous feeding of the columns. In addition, these experiments analysed inflow and outflow of the same event, thereby focusing only on the wet-phase of an event. An important aspect of stormwater biofilters however, is that they are subjected to sporadic wetting and drying cycles. Stormwater biofilters, after rapid transportation of water through the system become dry and remain dry in between rainfall events indicating that an event in stormwater biofilters has a longer dry-phase than the length of the wet-phase (Subramaniam et al., 2014). Therefore, the dynamics of the system during the dry-phase that is much longer compared with the wet-phase is crucial in stormwater biofilter performance analysis.

**Conceptual Model of biofilms**

An event in stormwater biofilters can be considered as having two phases: 1) a wet-phase – the phase during the rainfall and 2) a dry-phase – the phase of the stormwater biofilters in between two rainfall events. During the wet-phase, stormwater is transported continuously
through the filter layer. The flow through the pores has a gradient across the flow channel, with the highest flow velocity in the middle of the channel and stagnant water present around the stationary solid surfaces (filter layer particles) (Ives, 1966) as shown in Figure 1. The thickness of this stagnant zone depends on the interstitial velocity of water. Slower interstitial velocity results in the formation of a thicker stagnant zone compared with the thickness of that during higher interstitial velocity flow.

Influent (fresh) stormwater is rich in dissolved oxygen and this dissolved oxygen is transported in the system as it infiltrates through the filter layer. Oxygen is further transported to zones where depletion of dissolved oxygen had occurred, through diffusion (Cheremisinoff, 1996; Newcombe and Dixon, 2006). Depletion of dissolved oxygen occurs primarily due to oxidation of chemical compounds and respiration by microorganisms. Growth of microorganisms occurs as two types (Cheremisinoff, 2002; Sperling and Chernicharo, 2005):

1. Attached growth: the communities of microorganisms that grow on the solid surfaces in the system;
2. Suspended growth: the communities grow in suspension in the system.

The filter media provides solid surfaces and hence facilitate attached growth of microorganisms. As such, microorganisms congregate in the stagnant water that is retained as a film around the solid surfaces, which is referred to as a biofilm (Sperling and Chernicharo, 2005). The presence of different species of microorganisms will cause development of zones based on the relative availability of dissolved oxygen, in the biofilm. Dissolved oxygen is used by heterotrophic microorganisms that also require nutrients, especially organic carbon and nitrogen (Williamson and McCarty, 1976). At the beginning of an event, the biofilm
receives a continuous supply of dissolved oxygen as fresh water with a high content of dissolved oxygen percolates through the system and when microbial communities are still in the growth phase. Following the availability of oxygen and organic carbon, the heterotrophic bacteria that prefer aerobic environments will become dominant in such environments and utilise the available dissolved oxygen. As the thickness of the biofilm grows, and when the depletion rate of dissolved oxygen is higher than the rate of diffusion of oxygen into the biofilm, the core of the biofilm adjacent to the solid surface will turn anoxic and eventually into an anaerobic environment (Barnes et al., 1981; Sperling and Chernicharo, 2005). After development of the different zones, a concert of different types of bacteria will remove different types of pollutants in different zones, as shown in Figure 2.

As mentioned earlier, one of the important aspects of a stormwater biofilter is that it is subjected to intermittent wetting and drying cycles. Most of the studies on the performance of stormwater biofilters have focussed on removal of pollutants solely during the wet-phase of a rainfall event attributing any removal of nitrate-nitrogen to pollutant removal processes in the wet-phase (Blecken et al., 2010a; Blecken et al., 2009a; Bratieres et al., 2008a; Bratieres et al., 2008b; Davis et al., 2001; Davis et al., 2006; Lucas and Greenway, 2008, 2011). The dynamics of zones development (based on availability of dissolved oxygen) in the biofilms however, is likely to be affected significantly by the alternating wetting and drying cycles, and the development of the various zones are more dynamic during these occasions than during a continuous wet-phase event. The current study focuses on identifying the dynamics of pollutant removal in stormwater biofilters, that have been subjected to intermittent wetting and drying similar to that which occurs in field-scale installations.
Methodology

Laboratory-scale bioretention basins

Five Perspex™ columns each of 94 mm internal diameter and of length 1.5 m were used as experimental stormwater biofilters. Each column was packed according to guidelines (Gold Coast City Council 2003, South East Queensland Healthy Waterways 2010) and all three materials (filter, transition and drain layers) were obtained from an industry standard material supplier in Brisbane and the Gold Coast, Australia. Figure 3 shows the dimensions of the packing and a photograph of a packed column with three different materials as described below.

Filter zone - Engineered filter media: Engineered filter media consisted of primarily loamy sand. The particle size distribution was engineered to include particles with diameter less than 1 mm ($D_{60} = 300$ microns). The engineered mix was intended to have a hydraulic conductivity of 50 – 500 mm/h (180 – 200 mm/h optimum) according to the guidelines, and the observed saturated hydraulic conductivity varied between 300 – 450 mm/h as monitored during the experiment. Engineered filter media also included approximately 8% of a mixture of natural organic matter (by weight) added to enhance nitrate-nitrogen removal. Organic matter added however, had negligible levels of total nitrogen and total phosphorus.

Drain zone: drain zone had two layers (transition layer and gravel layer)

a. Transition layer: A transition zone is included if the ratio between particle size of gravel media and filter media are more than an order of ten. A transition zone was therefore included in this laboratory-scale stormwater biofilters using transition media supplied by the industrial supplier. Transition media provided by the supplier was engineered to have particles of diameter between 1 – 2 mm ($D_{60} = 1.18$ mm).
b. Gravel layer: Primary purpose of drain zone is to rapidly transport infiltrated (treated) stormwater to drain channel that followed or to temporarily store infiltrated stormwater prior to infiltrating in the native soil in systems that were designed to recharge groundwater. In this experiment, drain zone operates to rapidly transport infiltrated stormwater into the drain channel that was also a water sampling port in this study. Gravel media provided by the supplier was engineered to comprise of particles of sizes between 2 – 5 mm in diameter ($D_{50} = 4$ mm).

**Ponding zone:** Ponding zone is included in design specifications to provide temporary storage of stormwater runoff, to control over flow quantities and to provide head to initiate and facilitate infiltration process through the filter.

**Vegetation:** Based on the argument that phytoremediation is not a nitrate-nitrogen removal process in stormwater biofilters and the fact that there are several field-scale installations designed without any vegetation other than surface turf-grass, nitrate-nitrogen removal in this study is based in the filter zone only. Impact of vegetation on nitrate-nitrogen removal is therefore beyond the scope of this study.

**Simulation event**

A simulated rainfall event was designed according to the 3 month ARI (Annual Recurrence Interval) for South-East Queensland, Australia. From the data it was computed that a 3 month ARI was a rainfall event with 34 mm/h intensity that lasted for approximately 30 minutes (Parker, 2010). The other assumption considered was that the area of bioretention basins (stormwater biofilters) covered approximately 3% of the catchment area with a catchment runoff coefficient of 0.8. The biofilter column feed rate for an event was computed as 105 mL/min which was approximated to 100 mL/min (875 mm/h). While the feed rate for a simulated event in this study was computed based on 3 month ARI as
discussed above, the length (duration of wet-phase) of the events was prolonged to three hours. This was done in order to better understand the dynamics of nitrate-nitrogen removal with time in the wet-phase of the event.

Preliminary stabilisation of stormwater biofilters

After installation and packing of stormwater biofilter columns, they were preliminarily stabilised using tapwater for two weeks. During preliminary stabilisation two events were simulated during a week-long period, with a total of four events in two weeks. Preliminary stabilisation was intended to remove any loose particles in the filter zone after packing, and to settle the filter as no other compaction was done during the packing of the columns. Biofilter columns were fed with tapwater alone, with the feeding rate and duration as mentioned under simulation event. The packing was designed so that the zones settled to a height as shown in the diagram (Figure 3) within two weeks. Observations from monitoring the process of draining of biofilter columns following an event showed that it took approximately 16 – 20 hours for draining to stop. This was similar to observations from a field-scale operation of a bioretention basin (Parker, 2010). Accordingly, simulated events with a day lapse (24 hours) were defined as zero antecedent dry days.

Simulated Stormwater

The nature of this study requires a controlled environment since dynamics of nitrate-nitrogen concentrations need to be monitored for varied EN, ADD and inflow concentrations (NO3IN and NO3PRE). In addition, several storm events had to be simulated within a short period of time that required large amounts of feed. The quality of stormwater feed to the experimental biofilter columns therefore, had to be consistently regulated across the experimental schedule. In such occasions, it has been a common practice to use simulated stormwater for laboratory studies (Blecken et al., 2009a; Bradford et al., 2003; Davis et al., 2006; Davis et al., 2003;
Simulated stormwater for this study was prepared by mixing the following materials in tapwater:

1. Ammonium nitrate (NH$_4$NO$_3$): to represent ammonium-nitrogen and nitrate-nitrogen in stormwater
2. Glycine (C$_2$H$_5$NO$_2$): to represent organic-nitrogen in stormwater
3. Montmorillonite and kaolinite (1:1 by weight): to represent solids in suspension in stormwater.

Insignificant level of chlorine was observed in tapwater from tests using DPD tablets and therefore, dechlorination was not considered. Since stormwater quality in various studies in South East Queensland varied extensively based on several factors including catchment characteristics and land use, standard simulated stormwater in this study was designed for 5.0 ppm of total nitrogen (TN, with NO$_3$-N: 2.0 mg/L, NH$_4$-N: 1.5 mg/L and organic-N: 2.5 mg/L) and 100 mg/L suspended solids (kaolinite : montmorillonite – 1:1 by weight) (Liu, 2011; Miguntanna, 2009; Parker, 2010).

**Experimental runs**

Events were simulated as explained earlier, with simulated stormwater on the first four biofilter columns and with tapwater alone on the fifth biofilter column. Level of water in the ponding zone was maintained at or below 350 mm as shown in Figure 3 by maintaining the feed rate at a reduced level (equal to outflow rate), once the ponding level had reached 350 mm. This corresponds to a situation of overflow (reduced flow) in field-scale stormwater biofilters. Since columns were not re-packed between events, the sequence of events was numbered (EN– event number) to represent the age of the filter in field-scale operations. For Experiment 1, the first four biofilter columns (C1-C4) were fed with standard simulated...
stormwater with a nitrate-nitrogen concentration of 2.00 ± 0.22 mg/L while varying antecedent dry days (ADD) from 0 – 56 days (Table 1). In Experiment 1, different ADD’s were scheduled for each biofilter column ensuring that it did not follow a pattern. For example, C1 had events with 4, 0, 2, 21, 56, 12, 7 and 13 days while C2 had events with 0, 2, 7, 12, 21, 0, 4 and 31 days. Events were simulated this way to avoid any impact of certain pattern affecting performance of a column in a unique way. This is to contrive field-scale condition to laboratory-scale study where events are subjected to spontaneous ADD and inflow quality. During Experiment 2, the first four columns were fed with varying concentration of pollutants and ADD, and variations in inflow nitrate-nitrogen concentration was spontaneously varied in each column similar to variation of ADD in Experiment 1. The range of ADD’s and inflow nitrate-nitrogen concentration are given in Table 1. During Experiment 1 and 2, the fifth column was continued to be fed with tapwater alone that had approximately 0.6 mg/L of nitrate-nitrogen with different ADD and increasing EN.

**Water quality monitoring**

Samples (250 mL) were collected from the inflow and tap water during each experimental trial. Additionally, samples (250 mL) were collected from the outflow stream at 2, 7, 12, 20, 30, 60, 90, 120, 150 min from the beginning of outflow. Samples were tested for nitrate-nitrogen – (4500-NO₃-E) based on Standard Methods for Examination of Water and Wastewater (APHA, 2005).

**Data analysis**

Experiment 01 was conducted by maintaining inflow concentrations at a constant level, and varying ADD and EN. Initially graphical representation techniques were used to interpret general trend in data obtained from Experiment 01. Trends in stabilisation, occurrence of peak concentrations, and variability in removal of pollutants with time were some of the
common observations made from graphical techniques. In contrast, all four variables were varied in Experiment 02, where interpretation of graphical representation of data was highly limited. However, general trends on the impact of PRE (the previous event: EN-1) and IN (the current event: EN) were identified and observations were made on variation of their impacts on outflow quality depending on ADD and EN. For a comprehensive analysis of data to confirm the variation in the impacts of each variable on the outflow quality, multivariate and statistical modelling tools were required to be employed.

For statistical analysis, nitrate-nitrogen concentration in the outflow at different times (2, 7, 12, 20, 30, 60, 90, 120 and 150 min) were considered as individual dependent variables (min2, min7, min12, min20, min30, min60, min90, min120 and min150, respectively). These dependent variables were analysed statistically with independent variables, ADD, EN, NO3IN (nitrate-nitrogen concentration in the inflow of the current event) and NO3PRE (nitrate-nitrogen concentration in the inflow of the previous event).

Correlation Analysis (Pearson’s correlation)

Correlation analysis is a statistical tool often employed in research studies to identify any linear relationships between the variables, and is often carried out in conjunction with PCA analysis. Relationships observed in a PCA analysis could be further validated if correlations between variables are significant in a subsequent correlation analysis.

Pearson correlation analysis is a specific type of correlation analysis that is used in this study to verify PCA observations. Results of Pearson correlation analysis reveal two entities:

1. Pearson’s correlation coefficient
2. Significance of correlation
The Pearson’s correlation coefficient measures the strength and direction of the linear correlation between two continuous variables. A positive correlation indicates that the variable is directly proportional to the other, while negative correlations coefficient indicates that the variable is inversely proportional to each other.

Pearson’s correlation coefficients are accompanied by significance as stated earlier. Lower the significance, generally p = 0.05 or less (for 95% confidence), indicates higher significance of correlation between the two variables. Therefore, a high Pearson’s correlation coefficient with p<0.05 indicates a very strong correlation between the two variables that is statistically highly significant (95% confidence). However, a high Pearson’s correlation coefficient with p>0.05 indicates a strong correlation between the two variables yet, not statistically significant.
Results

Nitrate-nitrogen

Figure 4 shows nitrate-nitrogen concentration (a) and removal efficiency (b) for events of Experiment 1. Unlike reports from earlier experiments where leaching of nitrate-nitrogen was observed, nitrate-nitrogen was removed in this study for all simulated rainfall events irrespective of antecedent dry days (ADD) or event number (EN) (Figure 4 a and b). The results shown in the graphs (Figure 4) are the concentration/removal of nitrate-nitrogen in the outflow of the experiments that were fed with synthetic stormwater of similar strength (2.00 ± 0.22 ppm of nitrate-nitrogen). Removal of nitrate-nitrogen however, decreased with time, i.e. the concentration of nitrate-nitrogen in the outflow steadily increased, for the first 30 min in all events. After 30 min of outflow, the concentration of nitrate-nitrogen in the outflow settled to concentrations equal to that of the concentration of nitrate-nitrogen in the inflow (NO3IN – current event inflow concentrations), indicating a settled concentration but only very limited removal.

Figure 5 shows concentration (a) and removal efficiency (b) of nitrate-nitrogen in the outflow with volume of outflow in porevolumes. The first 30 minutes of fluctuation in nitrate-nitrogen concentration observed in the previous graphs (Figure 4) corresponds to approximately 0.75 porevolumes of outflow.

In the subsequent experiments, the concentration of nitrate-nitrogen in the feed of the experimental columns was varied between 1 ppm and 6 ppm. In addition, the column C5 (5th column) that was fed with tapwater alone had a concentration of approximately 0.6 ppm of nitrate-nitrogen. The concentration of nitrate-nitrogen was varied between 0.6 ppm and 6 ppm nitrate-nitrogen in the feed of the experimental columns, considering the control column as an experimental column for this phase of the analysis. The concentration of nitrate-
nitrogen in the outflow and removal percentages are shown in the figure below (Figure 6a and b, respectively) where NO3IN represents the concentration of nitrate-nitrogen in the feed of the current event (EN) while NO3PRE represents the concentration of nitrate-nitrogen in the immediately preceding event (EN-1).

In contrast to the observation of nitrate-nitrogen in the previous set of results shown in Figure 4 where the trend of removal of nitrate-nitrogen was similar across all experiments (with constant initial concentrations), the removal of nitrate nitrogen during the first 30 min varied significantly across experiments with varying initial concentrations as shown in Figure 6. More importantly, very poor removal and negative removal (leaching) of nitrate-nitrogen was observed in the first 30 min in some of the experiments in this phase of the study, which was not observed until the initial concentrations were varied.

Analysis by graphical interpretation of outflow nitrate-nitrogen concentrations with volume of outflow in porevolumes is given in Figure 7a with removal efficiency in Figure 7b. The trend observed here was very similar to that was observed in Experiment 1 (Figure 5), with stabilisation evidently occurring after 0.75 porevolumes of outflow. Neither time nor outflow volume taken for stabilisation was affected by variation of either of independent variables in this study (ADD, EN or inflow concentrations).

Even though the duration of stabilisation was constant across all events, the peaks varied extensively. In order to understand the impact of ADD, EN and inflow concentrations (NO3IN and NO3PRE) on outflow nitrate-nitrogen concentrations, statistical tools were used. The results of statistical analysis on independent (ADD, EN, NO3PRE and NO3IN) and dependent variables (min2, min7, min12, min20, min30, min60, min90, min120 and min150) are discussed below.
Statistical analysis (correlation analysis) clearly shows some significant correlation between some dependent and independent variables. The most significant and pronounced correlation exists between nitrate-nitrogen inflow concentrations (both NO3PRE and NO3IN) and outflow nitrate-nitrogen concentration at different times. The relationship between them however, is no static as it would have been represented in analyses based on event-mean concentrations. For example, NO3PRE is very significantly and strongly correlated with concentration of nitrate-nitrogen in the beginning of outflow, while the strength of correlation gradually decreased and became weak after 20 minutes. Contrastingly, NO3IN was weakly and insignificantly correlated to min2 and then gradually increased in strength and significance, and became prominently correlated from min20. Strong and significant correlation between variables imply the impact of independent variables on dependent variables. For examples, the impact of NO3PRE is strong in the outflow concentration of nitrate-nitrogen in the beginning of the outflow, and gradually faded and failed to have any impact after 20 minutes. Similarly, the impact of NO3IN on the outflow concentration of nitrate-nitrogen was weak in the beginning and then gradually increased and became dominant after 20 minutes. Another important feature of this analysis reveals that the correlations are positive, which indicates that an increase in NO3PRE or NO3IN results in the increase in the outflow concentration in the respective impact ranges.

Discussion

The trend observed here essentially illustrates the change in concentration of nitrate-nitrogen in the outflow in detail, which was not evident in analyses based on event mean concentrations. Even though observations in studies mentioned earlier (based on event mean concentrations) showed leaching of nitrate-nitrogen, results in the current study still did not
observe leaching of nitrate-nitrogen occurring at any time in the outflow after stabilisation (beyond 30 minutes of outflow). In addition, the factors that impact the concentration of nitrate-nitrogen in the beginning of the outflow are related to either the wet-phase of the previous event (NO3PRE) or the dry-phase of the previous event (ADD and EN). Furthermore, very limited removal (0 – 10%) occurred in the outflow beyond the stabilisation phase, that indicated that removal of nitrate-nitrogen was not significant in the wet-phase of the event. The removal observed in the beginning of the outflow was therefore, may not be related to the wet-phase of the current event.

Two factors discussed earlier, that were crucial for denitrification of nitrate-nitrogen were

1. Soil moisture with anoxic zones;
2. Organic carbon in sufficient concentrations.

Stormwater biofilters retain significant amounts of water by the end of an event, where several studies have quoted this observation to justify removal of cumulative mass of pollutants despite of leaching of the same observed in analysis based on event mean concentration (Davis, 2007; Davis, 2008; Davis et al., 2006; Davis et al., 2003). In addition, another study from these experiments showed very high concentrations of organic carbon being present in the retained water, over the dry-phase of an event (Subramaniam et al., 2014).

Retention of water in the filter layer at the end of draining can be of two types:

- Water retained as a film around the solid surfaces;
- Water retained as a result of capillary forces in the filter.

With continuous evaporation, water retained by both processes may disappear and reach a stable soil moisture level that is resistant to further evaporation or further draining. Figure 8
shows degree of saturation (volume of water / volume of voids *100) of biofilter columns at different depths and on 0 and 40 days ADD. It was evident that the bottom layers of biofilter columns retained very significant amounts of water (40% degree of saturation) even after 40 days of drying. In contrast, the top layer dropped from approximately 40% to 10% of degree of saturation by the end of 40 days of drying. This can be depicted conceptually as shown in Figure 9. The top layer which dries rapidly would have a thin biofilm layer, and intrusion of air into void pores is higher, producing a fresh supply of oxygen around the biofilm. Diffusion of oxygen into the thin biofilm therefore, turns the whole film into an aerobic environment. The bottom filter layer on the other hand, holds more moisture and will have a thicker biofilm around any solid surfaces. Reduction of fresh air into lesser void pores further restricts diffusion of oxygen into the core of the biofilm, resulting in retention of stratified zones for a longer period. This will facilitate denitrification for longer period of time extending into the drying-phase of the event.

Furthermore, the complete length of the filter layer in the experiment was exposed to the environment separated only by the wall of the columns. Therefore, the filter layer was exposed to more heating during the drying period due to heating of the columns from the external ambient temperature. Filter zone of the field-scale installations would however, surrounded by native soil, which might be at a temperature considerably lower than ambient temperature of this experiment. Therefore, the soil moisture content in the filter layer in field-scale installations could be expected to be even higher (in absence of vegetation) than that observed in the current laboratory-scale experiment. However, field-scale systems with vegetation may be subjected to more drying due to evapotranspiration, which will also vary depending on the type of vegetation. In addition, the conceptual model proposed above assumes that drying is uniform across the complete cross-section of the column and that it is equally divided with area. In addition, it assumes that the whole filter bed is saturated during
the wetting cycle of the event. Consideration of short wetting during an event however, would not ensure saturation of the complete column. Drying of an unsaturated column may not be uniform as it is depicted in the conceptual model.

For effective nitrogen removal, that encompasses both nitrification and denitrification which require aerobic and anoxic environments respectively, should happen in close proximity due to limitations in transportation of nitrogen species, more specifically during the dry-phase where percolation of water does not occur (nitrate-nitrogen resulting from nitrification in aerobic zone needs to be transported to anoxic zones for denitrification) (Baldwin and Mitchell, 2000; Brune et al., 2000; Payne et al., 2014; Tiedje et al., 1982). Although some researchers speculated that micro-zones contribute to enhanced removal of nitrate-nitrogen through denitrification, Payne et al., (2014) and Baldwin and Mitchel (2000) argue that the dynamics of these micro-zones during the dry-phase may in turn negatively impact nitrate-nitrogen removal by hindering microbial activities due to isolation (Hunt and Jarret, 2004; Hunt et al., 2003). Such isolation between water retained in the system around filter particles would in fact enhance the opportunity of having both aerobic and anoxic zones in close proximity, that would in turn enhance both nitrification and denitrification processes in succession. Figure 10 shows the conceptual depiction of saturated (a) and unsaturated (b) filter and the zones where nitrification (zone I – aerobic zone) and denitrification (zone II – anoxic zone) could possibly occur. Nitrate-nitrogen resulting from nitrification process in zone I will have to be transported to zone II for denitrification to reduce it to nitrogen. Transportation of nitrate-nitrogen over this distance (Figure 10a) is unlikely to occur, especially during dry-phase when water is stagnant (Payne et al., 2014). In such occasions, denitrification would be active, only near the boundary of zone I and II while denitrifiers in the rest of the anoxic zone will not receive nitrate-nitrogen. In contrast, in isolated zones produced in unsaturated zone brings both zone I and zone II to such close proximity where
process of diffusion can effectively transport nitrate-nitrogen. In this situation, greater area of
anoxic zone would actively support denitrification process, more efficiently removing it from
the system.

Presence of soil moisture and organic carbon, and the hypothesis that there are micro-
environments with anoxic and anaerobic zones illustrate a conducive environment for
denitrification during the dry-phase of an event, provided that there was nitrate-nitrogen
present in retained water. Another important conclusion from the observation of limited
removal of nitrate-nitrogen in the outflow after 30 min is that there was very limited or no
significant removal of nitrate-nitrogen in the wet-phase of the event. The water that is
retained in the biofilter at the end of an event, will consist of nitrate-nitrogen in
concentrations comparable to inflow concentrations of the preceding event. This retained
water that would have high nitrate-nitrogen may or may not undergo denitrification during
the dry-phase that follows the event. However, it will need to be drained during the wetting in
the subsequent event that could occur either as a plug flow pattern or as diffusion and mixing
of old and new water that eventually constitutes the outflow. Similar observation on two
different parameters (total suspended solids and total organic carbon) were made in another
study (Subramaniam et al., 2015). It has been discussed there, about the process of mixing
that must occur in stormwater biofilters in the beginning of outflow in each event. If a plug
flow was to occur, the outflow for the first few minutes in the current event would have been
solely from the water retained from the previous event. Should a plug flow occur while the
retained water failed to undergo denitrification, the initial outflow of the current event should
bear concentrations comparable to inflow concentration of the previous event (NO3PRE). On
the other hand, should a plug flow occur while the retained water underwent denitrification,
the initial outflow of the current event should have reduced or zero nitrate-nitrogen for a
period, followed by a sudden increase to NO3IN concentrations. Furthermore, should
diffusion and mixing occur while no denitrification occurred during dry-phase, the initial outflow should have an average concentration of NO3PRE and NO3IN. However, the results in this study reflected that the impact of NO3PRE gradually decreased while impact of NO3IN gradually increased in the outflow, simultaneously in first 20 – 30 minutes of outflow. The analysis on the porevolumes shows that it took approximately 0.75 porevolumes of outflow for the concentration of nitrate-nitrogen to settle. Therefore, diffusion and mixing of old (retained) and new (current inflow) was essentially driving stabilisation phase in the first 30 minutes.

According to the conceptual model, the water that is retained in the system that undergoes denitrification reducing nitrate-nitrogen concentrations in retained water. Thus, the old water (retained water from preceding event) with a reduced concentration of nitrate-nitrogen mixes with fresh infiltrating water (inflow of current event) with higher concentrations of nitrate-nitrogen and constitute the outflow. The proportion of mixing that varies with time can explain the gradual increase in concentration of nitrate-nitrogen observed in this study and the gradual decrease and increase of the impact of NO3PRE and NO3IN respectively, on the concentration of nitrate-nitrogen in the outflow. The fact that the concentration of nitrate-nitrogen in the outflow is affected by the number of ADD for the first 30 min (first flush) adds support to this idea.

Mixing of retained and percolating water however, is highly dependent on initial soil moisture profile and the preferential flow paths of the new wetting front. Owing to the spontaneity of drying and wetting of the columns in subsequent rainfall events, the mixed outflow will vary in proportion of mixing. This might have caused the variation in the data that was unexplained by the factors considered in this study.
Another important observation in this study was that there was lower removal and occasional leaching of nitrate-nitrogen occurring during stabilisation, that corresponds to events with high NO3PRE and low NO3IN. In order to investigate this observation, events were divided into two groups, based on NO3PRE (2.0 mg/L was chosen as this was the mean concentration used in this study):

(a) Events with NO3PRE less than 2.0 mg/L
(b) Events with NO3PRE greater than 2.0 mg/L

The outflow nitrate-nitrogen concentrations at different times and independent variables were used in a correlation analysis. Table 3 and 4 show correlation coefficients and significance for events with NO3PRE less than and greater than 2.0 mg/L, respectively. For events with NO3PRE less than 2.0 mg/L, NO3OUT was significantly correlated to NO3IN at all times while correlation with NO3PRE for this case is insignificant. This indicated insignificant impact from NO3PRE on NO3OUT at all times during these events. This contradicts the observation discussed earlier (using the complete data set).

In contrast, for events with NO3PRE greater than 2.0 mg/L, initial NO3OUT is significantly correlated to NO3PRE, while much lesser significant correlation displayed with NO3IN for the same period of outflow. In the first analysis, the impact of NO3PRE was insignificant due to the fact, that all nitrate-nitrogen retained from the previous event had been removed during the dry-phase, irrespective of the concentration (NO3PRE). On the other hand, the significant impact of NO3PRE on the second group indicates, that depending on NO3PRE, only a fraction was removed during the dry-phase with the remaining nitrate-nitrogen ending up in the initial outflow of the current event. It is therefore evident that there is a point of saturation, beyond which process of denitrification ceases to effectively remove nitrate-nitrogen from retained water, during the dry-phase.
Figure 12 shows presence of algae in a biofilter column. Although the biofilter columns were operated over a period of two years, presence of algae was not observed until the last couple of months. The filter material was clear during experimental runs with either standard synthetic stormwater or tapwater as the inflow feed. Algae appeared and multiplied in just 2 months when the columns were fed with higher strength synthetic stormwater, after approximately 1.5 years from the beginning of the study. The column that was fed with tapwater alone however, did not support any algal growth that could be observed even beyond 2 years. This suggested the presence of nitrate-nitrogen in biofilter over long periods of time when biofilter columns were fed with higher strength simulated stormwater. This indicated that substantial amounts of nitrate-nitrogen was present over the dry-phase of the events to support algal growth in events with higher strength synthetic stormwater, and that nitrate-nitrogen was not present in the biofilter during events with lower strength simulated stormwater or tapwater. This further confirms the findings discussed above, that the capability of denitrification processes in biofilters reach a point of saturation beyond which nitrate-nitrogen remains in the filter without being denitrified.

Transient pulses of nitrate-nitrogen were observed in the past, that lasted for few days due to rapid microbial immobilisation (Cui and Caldwell, 1997; Gomez et al., 2012; Kruse et al., 2004; Scholz et al., 2002). However, it was not clear how long it takes for the pulse to be evident from re-wetting. The current study had the wet-phase only for 3 hours, where no such pulses were observed. Increased removal during the first three days of ADD suggests that a pulse of activity could have been active during the initial dry-phase (after 3 hour long wet-phase). This study however, was not designed to quantify the concentration of nitrate-nitrogen that could be denitrified completely over the dry-phase of the event.
Conclusions

Efficient nitrate-nitrogen removal however, occurred in initial outflow, that gradually decreased and settled at limited or no removal after 30 minutes of outflow (corresponds to 0.75 porevolumes of outflow) in all events. Nitrate-nitrogen concentration in the first 30 minutes varied primarily depending on the inflow concentration of nitrate-nitrogen in the previous event, rather than the current event.

Significant amount of water is retained in the system at the end of a rainfall event, and the bottom layers of biofilter held significant amount of water even after 40 days of drying. Denitrification process is active during the dry-phase of the event, and removes nitrate-nitrogen from retained water during the dry-phase of the event. Initial outflow of the subsequent event is essentially a mixture of this old water (retained water) and fresh water (inflow of current event). The proportion of mixing gradually varies with time (retained water fraction gradually decrease) indicated by decreasing removal efficiency over the first 30 minutes. Old water ceases to contribute to outflow after 30 minutes of outflow (approximately 0.75 porevolumes of outflow) beyond which very limited or no removal of nitrate-nitrogen occurs.

Denitrification process during the dry-phase of an event reaches a point of saturation in removing denitrifying nitrate-nitrogen. Higher inflow concentrations from previous event (more than the saturation point concentration) leave a residue of nitrate-nitrogen in retained water that eventually causes increased nitrate-nitrogen concentration in the initial outflow of the event that follows. However, dry-phase denitrification process removes nitrate-nitrogen completely, when concentration of the same was below it saturation level. The process of denitrification is therefore more active during the drying phase of an event compared with the
wetting-phase and hence the drying-phase contributes most to nitrate removal in bioretention basins.

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