



CRC for
Water Sensitive Cities

Effectiveness of nitrogen removal using urban wetlands – summary report

Monash University component of Project B2.2/2.3

Keryn Roberts, Md. Moklesur Rahman, Wei Wen Wong,
Perran Cook and Mike Grace



Australian Government
Department of Industry,
Innovation and Science

Business
Cooperative Research
Centres Programme

Effectiveness of nitrogen removal using urban wetlands – summary report

Monash University component of Project B2.2/2.3

Protection and restoration of urban freshwater ecosystems: informing management and planning (Project B2.23)
B2.23-1-2018

Authors

Dr Keryn Roberts¹, Mr Md. Moklesur Rahman¹, Dr Wei Wen Wong¹, Assoc. Prof. Perran Cook¹, Assoc. Prof. Mike Grace¹

¹ Water Studies Centre, School of Chemistry, Monash University

© 2018 Cooperative Research Centre for Water Sensitive Cities Ltd.

This work is copyright. Apart from any use permitted under the Copyright Act 1968, no part of it may be reproduced by any process without written permission from the publisher. Requests and inquiries concerning reproduction rights should be directed to the publisher.

Publisher

Cooperative Research Centre for Water Sensitive Cities
Level 1, 8 Scenic Blvd, Clayton Campus
Monash University
Clayton, VIC 3800

p. +61 3 9902 4985

e. admin@crcwsc.org.au

w. www.watersensitivecities.org.au

Date of publication: October 2018

An appropriate citation for this document is:

Roberts, K.L., Rahman, Md, M., Wong, W.W., Cook, P.L.M., & Grace, M.R. (2018). *Effectiveness of nitrogen removal using urban wetlands - summary report: Monash University component of Project B2.2/B2.3*. Melbourne, Australia: Cooperative Research Centre for Water Sensitive Cities.

Disclaimer

The CRC for Water Sensitive Cities has endeavoured to ensure that all information in this publication is correct. It makes no warranty with regard to the accuracy of the information provided and will not be liable if the information is inaccurate, incomplete or out of date nor be liable for any direct or indirect damages arising from its use. The contents of this publication should not be used as a substitute for seeking independent professional advice.

Table of contents

Executive summary4

Introduction5

Part 1 – Stable isotopes of NO₃⁻ at the natural abundance level.....6

Part 2 – Summary of findings13

Conclusion.....15

References16

Executive summary

This report summarises the Monash University research outcomes for Project B2.2/2.3—*Protection and restoration of urban freshwater ecosystems: informing management and planning*.

Nitrogen management is imperative

Synthetically derived nitrogen and an increase in impervious surfaces have rapidly increased the amount of nitrogen in rivers and coastal waters. These greater nitrogen loads can lead to economic and environmental losses, so their careful management is imperative.

As urban development increases, constructed wetlands are becoming more common as tools for reducing nitrogen and other pollutant loads, but their effectiveness is variable and limited. In particular, information about temporal changes and the important processes involved in removal is limited, which makes effectively managing nitrogen loads difficult.

Urban wetlands show promising potential as a management tool

We used two different approaches to investigate the effectiveness of constructed wetlands at removing nitrogen:

- We examined whether natural abundance stable isotopes of nitrogen ($\delta^{15}\text{N}$) and oxygen ($\delta^{18}\text{O}$) could be used as a functional indicator of nitrogen processing in constructed wetlands. We also examined the effectiveness of natural abundance stable isotopes as a potential monitoring tool (part 1).
- We examined the isotope pairing technique using two competing nitrogen pathways: denitrification and dissimilatory nitrate reduction to ammonium (DNRA) (part 2). Isotope pairing uses labelled NO_3^- to measure actual rates of nitrogen pathways (denitrification and DNRA) under in situ conditions, to determine the effectiveness of denitrification as a removal process across temperature and carbon conditions.

Our study has some important implications for nitrogen management:

- Nitrogen processing is dynamic on a temporal scale (seasonal and diurnal), which should be considered when developing monitoring programs.
- The nitrogen processing capacity of the Melbourne wetlands we studied was variable, and as such, more site-specific management strategies may be appropriate.
- Denitrification is an important nitrogen removal pathway. To better understand the importance of assimilation, ammonium uptake should be considered because primary producers prefer NH_4^+ over NO_3^- .
- This is one of the first studies to simultaneously measure denitrification and DNRA with a suite of co-predictors (temperature, organic carbon, porewater iron and sulfide, nitrate, and chlorophyll-a), and it emphasises that DNRA can be favoured under certain conditions that promote nitrogen recycling.
- Groundwater-fed constructed wetlands give rise to new complexities for management and monitoring because of significant variability on both a temporal and spatial scale (within the wetland).

More research is needed into wetland function over time

This study highlights the importance of gathering a 'whole picture' view of wetland function. From our results, it is unclear whether constructed wetlands effectively remove a significant amount of nitrogen from stormwater over time, without studying nitrogen removal efficiency over a wetland's lifetime. In addition, more research is needed into how wetlands behave during event conditions when a significant portion of the annual nitrogen load is put into the system.

Introduction

It is well known that synthetically derived nitrogen (for example, from the Haber-Bosch process) and an increase in impervious surfaces have led to a rapid increase in the amount of nitrogen transported into rivers and coastal waters (Brabec et al., 2002; Bernhardt et al., 2008). These increased nitrogen loads can lead to eutrophication and enhanced primary productivity (algal blooms) and result in both economic and environmental losses.

In the urban landscape, constructed wetlands are commonly used to mitigate nitrogen loads in stormwater runoff, because they increase the hydraulic residence time and allow for removal through natural processes (Lee et al., 2009). The main processes that remove nitrogen in a constructed wetland system are physical removal through settling, assimilation into plant material, and microbially mediated removal through denitrification (Vyzamal, 2007; Bernhardt et al., 2008) (Figure 1).

With increasing urban development, constructed wetlands are becoming more common as management tools for reducing nitrogen and other pollutant loads (Scholes et al., 1998; Taylor et al., 2005; Wetzel et al., 2001), but the effectiveness of these systems can change over time. Several studies show long term nitrogen removal efficiencies are frequently at less than 50% (Taylor et al., 2005; Lee et al., 2009). At present, monitoring of constructed wetlands after construction largely looks at nutrient concentrations in the initial establishment phase (two to five years after construction), and often ignores the change in removal efficiency throughout the lifetime of the wetland (Wetzel, 2001; Farrell & Scheckenberger, 2003). Monitoring using concentrations from the inlet to the outlet allows removal efficiencies to be calculated, but gives little or no information about temporal changes and the important processes involved in this removal and, therefore, little indication of where nitrogen management should focus.

As a result of increased nitrogen inputs, and the potential of wetland sediments to process and remove nutrients and pollutants, nitrogen cycling in wetlands has received considerable attention (Bowden, 1986; Dierberg and Brezonik, 1983; Valiela & Teal, 1979). In this study, we used two different approaches to determine the effectiveness of constructed wetlands at removing nitrogen:

1. stable isotopes of NO_3^- at the natural abundance level
2. isotope pairing technique using two competing nitrogen pathways: denitrification and DNRA.

We conducted the study in two parts:

1. Part 1 looked at whether natural abundance stable isotopes of nitrogen ($\delta^{15}\text{N}$) and oxygen ($\delta^{18}\text{O}$) could be used as a functional indicator of nitrogen processing in constructed wetlands. We aimed to determine how nitrogen is transformed within a wetland system, and therefore how it could be better managed. We examined surface water constructed wetlands (Cascades on Clyde and Kelletts Road wetlands, Melbourne) and groundwater-fed constructed wetlands (Anvil Way Compensation Basin, Perth) during summer and winter. We also examined the effectiveness of natural abundance stable isotopes as a potential monitoring tool. The post-doctoral fellow employed on this project, Dr Keryn Roberts, conducted the research for Part 1.
2. Part 2 applied the isotope pairing technique, which uses labelled NO_3^- to measure actual rates of nitrogen pathways (denitrification and dissimilatory nitrate reduction to ammonium or DNRA) under in situ conditions, to determine the effectiveness of denitrification as a removal process across a wide range of temperature and carbon conditions. The PhD candidate supported by the project, Mr Md Moklesur Rahman, conducted the detailed mechanistic studies on several Melbourne wetlands used in Part 2.

Part 1 – Stable isotopes of NO_3^- at the natural abundance level

For this part of the study, we asked whether natural abundance stable isotopes of nitrogen ($\delta^{15}\text{N}$) and oxygen ($\delta^{18}\text{O}$) could be used as a functional indicator of nitrogen processing in constructed wetlands. We wanted to understand how nitrogen is transformed within a wetland system so its management can be better targeted.

We used natural abundance stable isotopes of nitrate (NO_3^-) and auxiliary water quality information to determine the effectiveness of surface water constructed wetlands and groundwater-fed constructed wetlands at removing nitrogen during the two contrasting seasons of summer and winter. The surface water constructed wetlands we studied were Cascades on Clyde and Kelletts Road wetlands in Melbourne, and the groundwater-fed constructed wetlands we studied were at Anvil Way Compensation Basin in Perth. We also examined the effectiveness of natural abundance stable isotopes as a potential monitoring tool.

In brief, natural abundance stable isotopes of nitrogen can be used in aquatic environments to determine two key pieces of information: source and transformation of nitrogen. We focused on using natural abundance stable isotopes to determine the transformation of nitrogen by measuring the $\delta^{15}\text{N}$ signature of nitrogen in the residual (or reactant) pool and the likely process determined from the level of fractionation¹ (Fry, 2006; Michener & Lajtha, 2007). Fractionation of $\delta^{15}\text{N}\text{-NO}_3^-$ during assimilation, for example, will lead to an increase in $\delta^{15}\text{N}\text{-NO}_3^-$ signature in the residual pool because the lighter isotope (^{14}N) is an energetically more favourable reactant (Figure 1A). Several studies have shown the level of fractionation is predictable and varies by pathway, such as denitrification, assimilation or nitrification (Michener and Lajtha 2007) (Figure 1 (A)).

¹ Fractionation is the process by which the relative abundance of the two stable isotopes of nitrogen, ^{14}N and ^{15}N , will change when they undergo a reaction. The more energetically favourable reactant is the lighter isotope (^{14}N), leading to a predictable preference towards the lighter isotope in the product and a concentration of the heavier isotope (^{15}N) in the reactant or residual pool (Fry 2006) (Figure 2). Fractionation is the amount the isotopic signature changes. Enrichment factor (ϵ) indicates the amount and direction of fractionation; for example, a positive ϵ indicates a depletion in the residual pool, while a negative ϵ indicates an enrichment in the residual pool.

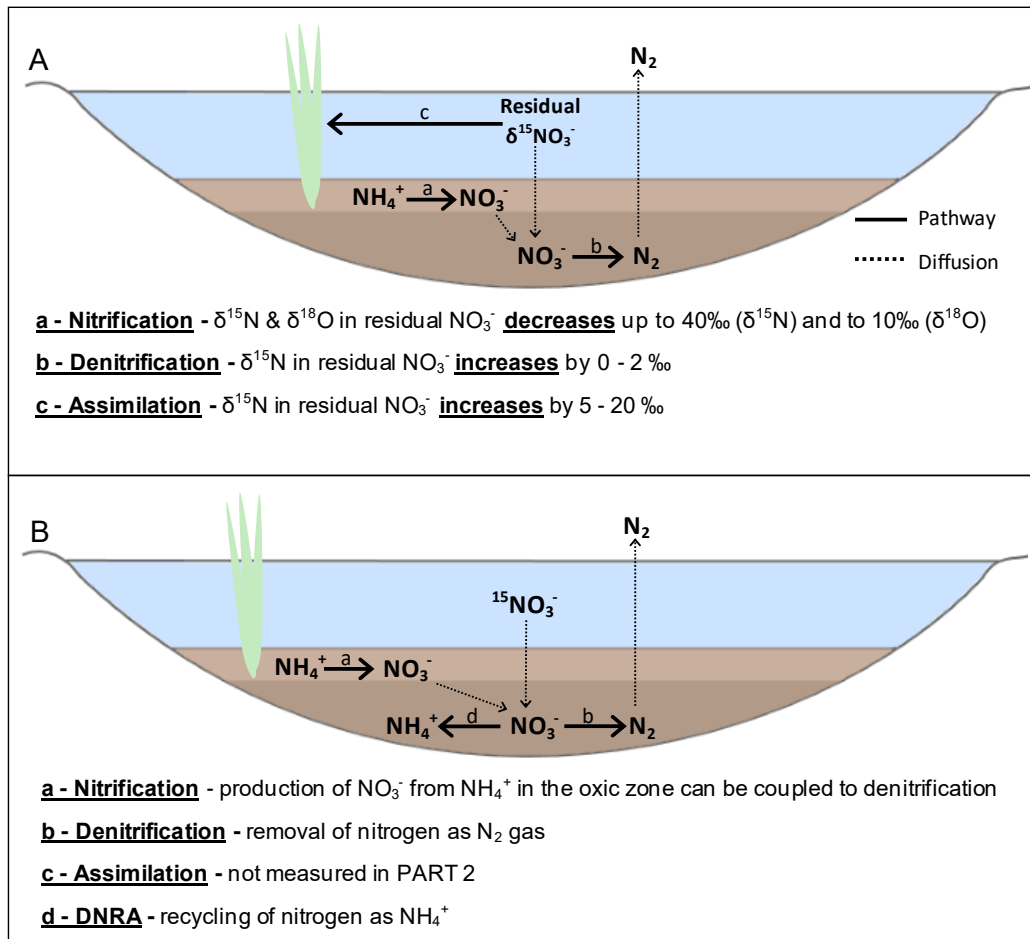


Figure 1: (A) Conceptual model of important nitrogen pathways in a constructed wetland system and the expected levels of fractionation in the residual NO_3^- pool. (B) Nitrogen pathways measured in constructed wetlands using the isotope pairing technique

In wetlands, permanent nitrate removal through denitrification is preferable over temporary sequestration into plant material, and understanding the balance between these two processes could optimise wetland performance (Lund et al. 2000). Based on the principles of fractionation during transformation pathways, we tested the efficacy of natural abundance stable isotopes of nitrogen as qualitative functional indicators of nitrogen processing in constructed wetlands. By using natural abundance stable isotopes in addition to the nutrient concentrations already measured, this method could provide more information about the processes that are important in nitrogen removal.

Several studies have examined denitrification in constructed wetlands using the dual isotopic composition of NO_3^- (Sovik & Mørkved, 2008; Lund et al., 2000), but few have examined assimilation and nitrification in addition to denitrification using natural stable isotopes in wetlands (Itoh et al., 2011; Erler et al., 2010). In those studies, the wetlands were heavily influenced by sewage waste. In this study, we examined the effectiveness of this method in constructed urban wetlands that receive and treat stormwater (Melbourne) and groundwater (Perth).

Part 2 – Isotope pairing technique using two competing nitrogen pathways: denitrification and DNRA

For this part, we applied the isotope pairing technique, which uses labelled NO_3^- to measure actual rates of nitrogen pathways (denitrification and dissimilatory nitrate reduction to ammonium or DNRA) under in situ conditions, to determine the effectiveness of denitrification as a removal process across a wide range of temperature and carbon conditions.

There are two competing pathways of dissimilatory nitrate reduction that can determine the degree of nitrogen retention within a system (Sørensen 1978):

1. **denitrification**, a microbially mediated reduction pathway, reduces nitrogen oxides (NO_3^- and NO_2^-) to nitrogen gas (N_2) and permanently removes nitrogen from a system (Bernard et al. 2015a). As such, denitrification counteracts nitrogen inputs into constructed wetland systems and acts as a 'sink' of nitrogen
2. **dissimilatory nitrate reduction to ammonium (or DNRA)** competes with denitrification for nitrate, converting nitrate into bioavailable ammonium and retaining nitrogen in the system, which potentially exacerbates eutrophication (An and Gardner 2002).

Factors controlling nitrate reduction pathways vary both temporally and spatially across different ecosystems. For example, the availability of NO_3^- , organic carbon (OC), and concentration of dissolved oxygen (DO) have been identified as controlling factors on nitrate reduction processes in aquatic sediments (Burgin and Hamilton 2007; Inwood et al. 2007; Sgouridis et al. 2011). Further, the ratio of dissolved organic carbon (DOC) to NO_3^- (Dalsgaard and Thamdrup 2002), temperature (Dong et al. 2011), porewater concentrations of NO_3^- , sulfide and iron (Brunet and Garcia-Gil 1996; Fossing et al. 1995; Reyes-Avila et al. 2004; Straub et al. 1996), and salinity (Giblin et al. 2010) have been reported as important factors controlling the partitioning of nitrate reduction into denitrification and DNRA in different ecosystems. That said, very few studies have examined the role of DNRA in constructed wetlands and determined the potential impact of this process on retaining bioavailable nitrogen within the system and limiting permanent nitrogen removal via denitrification.

Our study determined rates of nitrate reduction pathways (denitrification and DNRA) using the isotope pairing technique in four constructed wetlands of varying degrees of sediment organic carbon content (a known predictor of the partitioning between denitrification and DNRA) and temperatures. As well as measuring denitrification and DNRA rates under in situ conditions, we collected sediment fluxes of nutrients and oxygen, and auxiliary water quality and sediment data.

Part 1 – Summary of findings

We looked at nitrogen cycling across a seasonal cycle² within groundwater and surface water-fed constructed wetland systems in Perth and Melbourne. We used natural abundance stable isotopes of nitrogen ($\delta^{15}\text{N}$) as a qualitative measure to assess important nitrogen cycling pathways, such as denitrification, assimilation and nitrification.

The results indicate that the shift in different nitrogen pathways on a seasonal cycle in both system types is important, and system management should consider seasonal shifts in efficiency and nitrogen transformation pathways.

For both of the Melbourne wetlands we studied, denitrification was an important nitrogen removal pathway during summer, supported by a decrease in NO_3^- concentrations throughout the wetland and minimal fractionation of $\delta^{15}\text{N}-\text{NO}_3^-$. In summer, water column nitrification was also an important pathway contributing to the depletion of water column $\delta^{15}\text{N}-\text{NO}_3^-$ moving towards the outlet (Figure 2). In contrast, during winter, we observed minimal fractionation within the wetland, which was consistent with limited NO_3^- removal (Figure 2). This study showed that the removal efficiency in winter significantly decreased compared with summer months, and processes such as denitrification and assimilation were not significantly contributing to nitrogen removal. Many factors contributed to the limited nitrogen removal we observed during winter: high nutrient inputs, decreased residence time of the water within the wetland, high wind events leading to mixing of the water column and resuspension of particulate material, and lower temperatures decreasing rates of microbial activity.

In Perth, we could only use natural abundance stable isotopes of NO_3^- in winter 2015 because of low ($< 5\mu\text{mol L}^{-1}$) NO_3^- in the oxygen-depleted inlet water during summer 2016. During winter, the isotopic signature was driven by the inlet signatures of $\delta^{18}\text{O}$ and $\delta^{15}\text{N}-\text{NO}_3^-$, with limited variation in both $\delta^{18}\text{O}$ and $\delta^{15}\text{N}-\text{NO}_3^-$ observed throughout the wetland. The values of $\delta^{18}\text{O}$ and $\delta^{15}\text{N}-\text{NO}_3^-$ changed during and after a rain event: after receding back to baseflow, the isotope values returned to pre-event conditions driven by a change in the inlet source of NO_3^- . Within the wetland, denitrification was the most important NO_3^- removal pathway in winter, supported by minimal fractionation of $\delta^{15}\text{N}-\text{NO}_3^-$ and measured rates of denitrification using the $^{15}\text{N}-\text{NO}_3^-$ isotope pairing technique (Figure 3). In winter, there was limited to no removal of NH_4^+ , DON and TN.

We also reported limited removal of DON and TN for summer, with increases in both analytes observed throughout the wetland most likely explained by leaching of DON from plant material (Figure 3). Gross primary production increased throughout the wetland, supported by an increase in O_2 concentration and uptake of NH_4^+ (Figure 3). NO_3^- was low during the summer months, but if present uptake via denitrification occurs as confirmed by the $^{15}\text{N}-\text{NO}_3^-$ isotope, additional experiments to determine denitrification rates should be undertaken (Figure 3).

² The classification of seasons 'summer' and 'winter' for both Melbourne and Perth are based on typical rainfall and temperature conditions for the region.

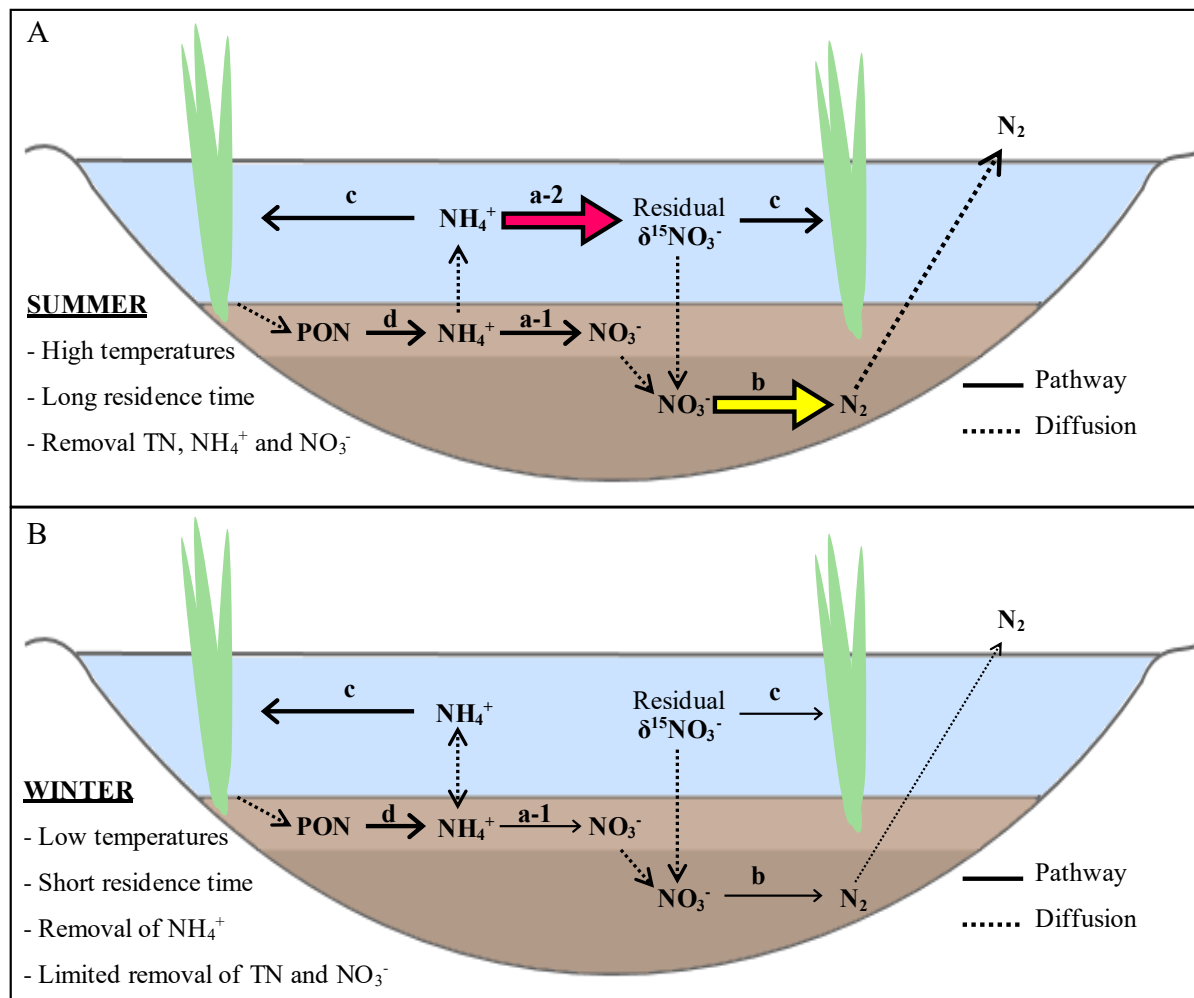


Figure 2: Conceptual model of the main nitrogen cycling processing pathways during summer (A) and winter (B) in Melbourne constructed wetlands, including benthic nitrification (a-1), water column nitrification (a-2), denitrification (b), assimilation (c), and mineralisation (d). The water column is represented in blue; light brown sediment is oxic and darker brown sediment is anoxic. The size of the arrow is indicative of the importance of the process within the system. For example, water column nitrification and denitrification were the main pathways that influenced the residual $\delta^{15}\text{N-NO}_3^-$ during summer, while very little processing was observed during the winter months, with little effect observed on the residual $\delta^{15}\text{N-NO}_3^-$.

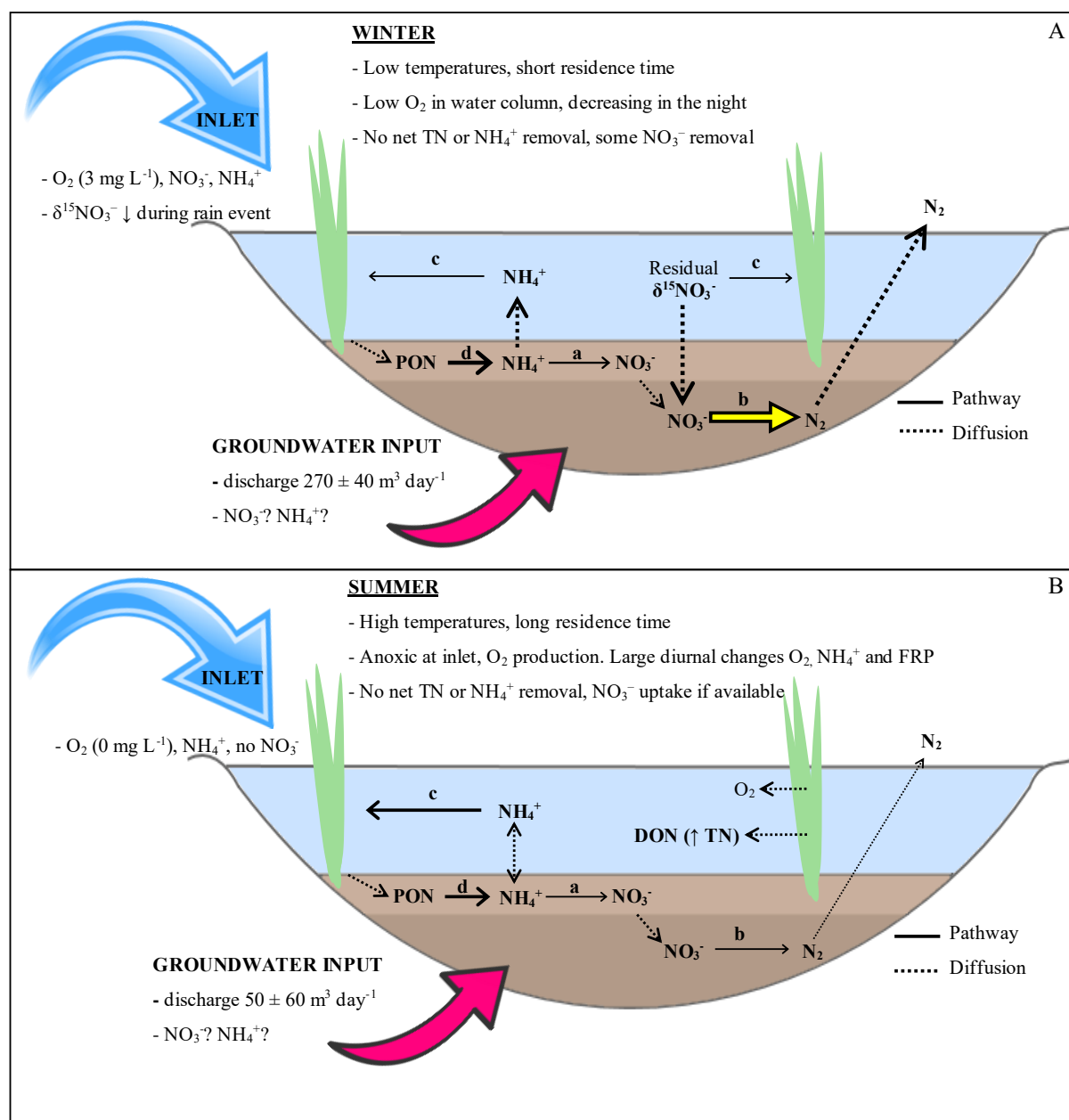


Figure 3: Conceptual model of the main nitrogen cycling processing pathways during summer (A) and winter (B) in Anvil Way Compensation Basin, including benthic nitrification (a), denitrification (b), assimilation (c), and mineralisation (d). The water column is represented in blue; light brown sediment is oxic and darker brown sediment is anoxic. The size of the arrow is indicative of the importance of the process within the system. For example, during winter, denitrification was the main NO_3^- removal pathway.

Groundwater discharge contributed up to 20–30 per cent of the total discharge to the wetland during the sampling period (Figure 3), but constraints within the study meant we could not confirm the specific contribution to nutrient concentrations and the effect of groundwater on nutrient dynamics. Most of the groundwater bores within Anvil Way Compensation Basin were anoxic, with low concentrations of NO_3^- , but DON, TN and NH_4^+ were reported within a similar concentration range to the wetland surface waters, which suggests shallow groundwater discharge within the wetland may be an important source of nitrogen to the wetland. This requires further investigation.

In Perth's Anvil Way Compensation Basin, we carried out a tracer release experiment over several days in both summer and winter, and identified large changes in O_2 , NH_4^+ and filterable reactive phosphorus (not reported) concentrations on a diurnal cycle, which indicates a shift in nutrient transformation pathways on this diurnal cycle. For example, summer NH_4^+ concentrations increased during the night when oxygen concentrations were low resulting from the cessation of nitrification leading to less uptake of NH_4^+ from the water column and at some sites an efflux of mineralised NH_4^+ from the sediment (Figure 3). During daylight, NH_4^+ concentrations in the water column decreased because of an increase in dissolved oxygen (from enhanced primary production) and uptake via assimilation and nitrification (Figure 3).

These large diurnal changes in nutrient concentrations need to be considered when monitoring constructed wetlands. For example, the wetland may shift from a net 'source' to a net 'sink' of nitrogen on a diurnal cycle; therefore, the time of day at which the monitoring sample is taken is important because of both diurnal and seasonal variations. Misinterpretation of nutrient removal efficiencies caused by a sampling artefact may have implications for wetland management and should be considered when monitoring wetlands with large changes in diurnal O_2 concentrations.

These data are a preliminary assessment of a technique that could be used in monitoring constructed wetlands in conjunction with the nutrient concentrations already measured, but this technique is only effective in wetlands with well constrained inputs and outputs, high nitrate inputs ($\sim 1\text{mg/L}$ inlet concentration for the method to be effective), and a mixed water column (to account for grab sampling). In wetlands that meet these criteria, the method proved to be effective, but we have some recommendations for future assessment:

1. Using stable isotopes of NO_3^- ($\delta^{15}N$ and $\delta^{18}O$) is more effective if the sources of $\delta^{15}N$ are known; for example, NH_4^+ (nitrification), plant matter-N, groundwater-N, and porewater $\delta^{15}N$ constituents.
2. Within the wetlands, several nitrogen cycling pathways occur simultaneously, which makes interpreting $\delta^{15}N$ - NO_3^- without supporting information complex; as such, auxiliary information, in addition to nutrient concentrations, is required.
3. To determine whether the method is effective across a broad range of wetlands, and to gather more information on the fractionation effects of different pathways in wetland systems, further testing is required, including on wetlands with different input sources in both groundwater-fed and surface water-fed systems.
4. Seasonal shifts in nutrient removal were present for both groundwater-fed and surface water-fed systems, and this should be considered in monitoring wetlands and calculating annual nitrogen removal budgets. Further, in Perth, nutrient and oxygen concentrations changed drastically on a diurnal cycle and, as such, sampling time during monitoring may be important in interpreting wetland function. These diurnal shifts in $\delta^{15}N$ need to be investigated before this method can be effective in monitoring programs.

Part 2 – Summary of findings

We investigated two competing nitrate reduction pathways (denitrification and DNRA) across four urban wetlands in Melbourne. We used the isotope pairing technique to quantitatively measure rates of denitrification: a nitrogen removal process, and DNRA, a nitrogen recycling process. Since these two pathways compete for available nitrate—a significant constituent in Melbourne’s stormwater (Taylor et al. 2005)—it is particularly important to determine the partitioning between these processes and to identify under what conditions denitrification is favoured.

The results indicate that DNRA, the recycling pathway, is an important process throughout the year, contributing up to 60 per cent of total nitrate reduction, but this result is concerning because a significant portion of the nitrate that is reduced is recycled within the system rather than removed.

We measured denitrification and DNRA in wetland sediments using two techniques that employ the labelled $^{15}\text{N-NO}_3^-$ method³:

1. We used the isotope pairing technique in intact cores to measure in situ rates of denitrification and DNRA, as well as in situ predictor variables, such as temperature, carbon, and iron and sulfide concentration in the porewater. We also used multiple regression analysis to determine co-predictors of nitrate reduction rates in wetland sediments.
2. We measured rates of denitrification and DNRA in sediment slurries to examine the partitioning between the processes under optimal conditions.

Under in situ conditions, DNRA was the dominant nitrate reduction pathway in the wetlands we studied, irrespective of season or site. The multiple regression analysis identified porewater iron and sulfide and chlorophyll-a as important controls on the DNRA rate. DNRA bacteria can use porewater iron and sulfide as an alternative energy source to carbon, thereby promoting DNRA over denitrification. Interestingly, chlorophyll-a was considered an important predictor of DNRA rates, which could be attributed to assimilatory nitrate reduction to ammonium (ANRA) that can be carried out by plants and benthic bacteria (Silver et al. 2001). It is difficult to exclude ANRA, and define the contribution to the overall DNRA rate, but the implication for management is the same: a large proportion of the nitrate in wetlands is recycled at the expense of denitrification.

The concentrations of nitrate, organic carbon and chlorophyll-a were important predictors of denitrification rates, which conforms to the traditional paradigm. Generally, under conditions of high nitrate, denitrification is the favoured pathway of nitrate reduction. When the nitrate concentration increases, the ratio of organic carbon to nitrate decreases. Under these conditions, denitrifying bacteria are a more efficient user of nitrate (Holmes et al. 1996; King & Nedwell 1985; Martin et al. 2001; Pattinson et al. 1998) and, as a result, denitrification increases.

The ratio between denitrification and DNRA was most strongly associated with temperature and nitrate concentration, which suggests the DNRA is favoured under conditions of low nitrate and temperatures less than 15°C (Figure 4). While the immediate concern is the high rates of DNRA observed in constructed wetlands, DNRA was only favoured under conditions of low NO_3^- (mean $\sim 20 \mu\text{mol L}^{-1}$). These results suggest that if NO_3^-

³ PhD student Md Moklesur Rahman carried out a mechanistic study on the controls of denitrification and DNRA as part of the PhD project due for submission in December 2017. Please refer to the thesis for a more detailed analysis of denitrification and DNRA in wetlands.

concentrations were to increase (for example, during a storm event), denitrification would become the dominant pathway and lead to more permanent nitrate removal (Figure 4).

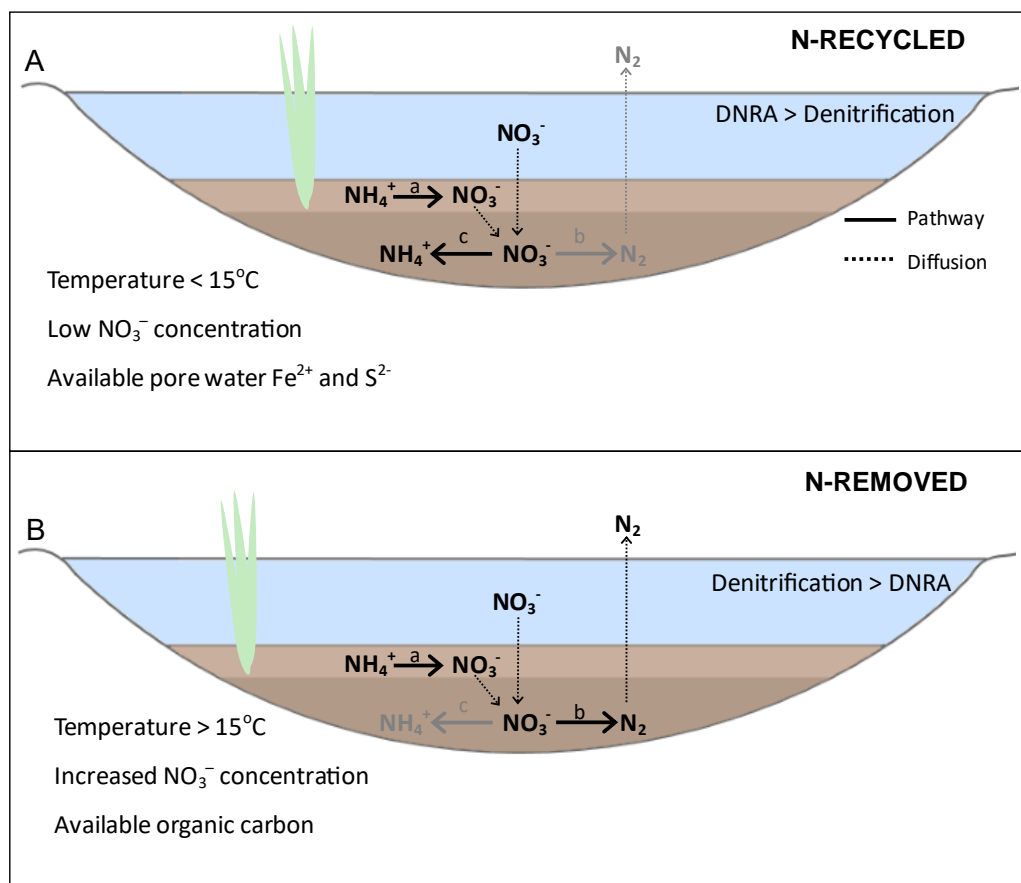


Figure 4: Conceptual model of the main nitrogen cycling processing pathways examined in this study (A): a system dominated by DNRA, and (B) a system dominated by denitrification.

In contrast to the in situ measurements of denitrification and DNRA, rates measured in slurries indicated denitrification was the dominant pathway at all sites. These results support the hypothesis that under optimal conditions (high nitrate and organic carbon), denitrification will be the main removal pathway. The increased rate of denitrification relative to DNRA observed in the slurries compared to in situ measurements, shows a dynamic shift between nitrate reduction pathways under changing nitrate conditions.

This study has important implications for management. The results show wetlands will respond rapidly to change, including increases in nitrate loading. Further, DNRA was favoured at lower temperatures, which implies that increasing temperatures from global warming and the urban heat island effect will not negatively impact nitrogen processing in the urban stormwater wetlands studied here.

Conclusion

In summary, nitrogen processing in urban constructed wetlands is dynamic at both the temporal (seasonal and diurnal) and spatial scale. Greater focus on the nitrogen removal efficiency of a constructed wetland over the lifetime of the wetland is necessary to completely assess whether these systems effectively remove a significant amount of nitrogen from stormwater over the wetlands lifecycle.

The results from this study emphasise the need for further investigation into how wetlands behave during event conditions, when a significant portion of the annual nitrogen load is put into the system. The results from Part 1 of this study suggest the wetlands studied were ineffective during event conditions, but Part 2 showed denitrification would increase under these conditions. Although denitrification may increase under conditions of high nitrate, such as an event, other compounding factors may influence nitrogen removal efficiency, including residence time, wind resuspension of sediment, and temperature. The combined results of Parts 1 and 2 highlight the importance of gathering a 'whole picture' view of wetland function, to make informed management decisions.

Our assessment of natural abundance stable isotopes of nitrogen as a potential management tool provided promising results, but these should be used with care. To obtain significant meaning from natural abundance stable isotopes a substantial amount of supporting data, such as water quality and other nitrogen sources was required. This method could be useful in constructed wetland systems with well constrained source inputs, however further research is required before it can be implemented in management.

Our key findings are:

- Nitrogen processing is dynamic on a temporal scale (seasonal and diurnal), which should be considered when developing monitoring programs.
- The nitrogen processing capacity of the wetlands we studied in the Melbourne region was variable, and as such, more site-specific management strategies may be appropriate.
- In both Parts 1 and 2, it was determined that denitrification is an important nitrogen removal pathway. To improve understanding of the importance of assimilation, ammonium uptake should also be considered because it has been shown that primary producers preferentially uptake NH_4^+ over NO_3^- (Cohen et al., 2004; Nichols and Keeney, 1976; Dudley et al., 2001).
- This is one of the first studies to simultaneously measure denitrification and DNRA with a suite of co-predictors (temperature, organic carbon, porewater iron and sulfide, nitrate, and chlorophyll-a) and it emphasises that DNRA can be favoured under certain conditions that promote nitrogen recycling.
- Groundwater-fed constructed wetlands give rise to new complexities for management and monitoring with significant variability on both a temporal and spatial scale (within the wetland).

References

- An, S. & Gardner, W.S. (2002). Dissimilatory nitrate reduction to ammonium (DNRA) as a nitrogen link, versus denitrification as a sink in a shallow estuary (Laguna Madre/Baffin Bay, Texas). *Marine Ecology Progress Series*, 237, pp. 41–50.
- Bernard, R., Mortazavi, B. & Kleinhuizen, A. (2015). Dissimilatory nitrate reduction to ammonium (DNRA) seasonally dominates NO₃ – reduction pathways in an anthropogenically impacted sub-tropical coastal lagoon. *Biogeochemistry*, 125(1), pp. 47–64.
- Bernhardt, E.S., Band, L.E., Walsh, C.J. & Berke, P.E. (2008). Understanding, managing, and minimizing urban impacts on surface water nitrogen loading. *Annals of the New York Academy of Sciences*, 1134, pp. 61–96.
- Bowden, W.B. (1986). Nitrification, nitrate reduction, and nitrogen immobilization in a tidal freshwater marsh sediment. *Ecology*, 67(1), pp. 88–99.
- Brabec, E., Schulte, S. & Richards, P.L. (2002). Impervious surfaces and water quality: a review of current literature and its implications for watershed planning. *Journal of Planning Literature*, 16(4), pp. 499–514.
- Brunet, R.C. & Garcia-Gil, L.J. (1996). Sulfide-induced dissimilatory nitrate reduction to ammonia in anaerobic freshwater sediments. *FEMS Microbiology Ecology*, 21(2), pp. 131–138.
- Burgin, A.J. & Hamilton, S.K. (2007). Have we overemphasized the role of denitrification in aquatic ecosystems? A review of nitrate removal pathways. *Frontiers in Ecology and the Environment*, 5(2), pp. 89–96.
- Cohen, R.A. & Fong, P. (2004). Nitrogen uptake and assimilation in *Enteromorpha intestinalis* (L.) Link (Chlorophyta): using ¹⁵N to determine preference during simultaneous pulses of nitrate and ammonium. *Journal of Experimental Marine Biology and Ecology*, 309, pp. 67–77.
- Dalsgaard, T. & Thamdrup, B. (2002). Factors controlling anaerobic ammonium oxidation with nitrite in marine sediments. *Applied and Environmental Microbiology*, 68(8), pp. 3802–3808.
- Dierberg, F.E. & Brezonik, P.L. (1983). 'Nitrogen and phosphorus mass balances in natural and sewage-enriched cypress domes. *Journal of Applied Ecology*, 20(1), pp. 323–337.
- Dong, L.F., Sobey, M.N., Smith, C.J., Rusmana, I., Phillips, W., Stott, A., Osborn, A.M. & Nedwell, D.B. (2011). Dissimilatory reduction of nitrate to ammonium, not denitrification or anammox, dominates benthic nitrate reduction in tropical estuaries. *Limnology and Oceanography*, 56(1), pp. 279–291.
- Dudley, B.J., Gahnström, A.M.E & Walker, D.I. (2001). 'The role of benthic vegetation as a sink for elevated inputs of ammonium and nitrate in a mesotrophic estuary. *Marine Ecology Progress Series*, 219, pp. 99–107.
- Erler, D.V. & Eyre, B.D. (2010). Quantifying nitrogen process rates in a constructed wetland using natural abundance stable isotope signatures and stable isotope amendment experiments'. *Journal of Environmental Quality*, 39, pp. 2191–2199.
- Farrell, A.C. & Scheckenberger, R.B. (2003). An assessment of long-term monitoring data for constructed wetlands for urban highway runoff control. *Water Quality Research Journal of Canada*, 38(2), pp. 283–315.
- Fossing, H., Gallardo, V.A., Jorgensen, B.B., Huttel, M., Nielsen, L.P., Schulz, H., Canfield, D.E., Forster, S., Glud, R.N., Gundersen, J.K., Kuver, J., Ramsing, N.B., Teske, A., Thamdrup, B. & Ulloa, O. (1995). Concentration and transport of nitrate by the mat-forming sulphur bacterium *Thioploca*. *Nature*, 374(6524), pp. 713–715.
- Fry, B. (2006). *Stable isotope ecology*. New York, USA: Springer Science + Business Media LLC.
- Giblin, A., Weston, N., Banta, G., Tucker, J. & Hopkinson, C. (2010). The effects of salinity on nitrogen losses from an oligohaline estuarine sediment. *Estuaries and Coasts*, 33(5), pp. 1054–1068.
- Holmes, R.M., Jones, Jr J.B., Fisher, S.G. & Grimm, N.B. (1996). Denitrification in a nitrogen-limited stream ecosystem. *Biogeochemistry*, 33(2), pp. 125–146.
- Inwood, S.E., Tank, J.L. & Bernot, M.J. (2007). Factors controlling sediment denitrification in midwestern streams of varying land use. *Microbial Ecology* 53(2), pp. 247–258.
- Itoh, M., Takemon, Y., Makabe, A., Yoshimizu, C., Kohzu, A., Ohte, N., Tumurskh, D., Tayasu, I., Yoshida, N. & Nagata, T. (2011). 'Evaluation of wastewater nitrogen transformation in a natural wetland (Ulaanbaatar, Mongolia) using dual-isotope analysis of nitrate'. *Science of the Total Environment*, 409, pp. 1530–1538.

- King, D. & Nedwell, D.B. (1985). The influence of nitrate concentration upon the end-products of nitrate dissimilation by bacteria in anaerobic salt marsh sediment. *FEMS Microbiology Ecology*, 1(1), pp. 23–28.
- Lee, C., Fletcher, T.D. & Sun, G. (2009). Nitrogen removal in constructed wetland systems. *Engineering in Life Sciences*, 9(1), pp. 11–22.
- Lund, L.J., Horne, A.J. & Williams, A.E. (2000). Estimating denitrification in a large constructed wetland using stable nitrogen isotope ratios. *Ecological Engineering*, 14, pp. 67–76.
- Martin, L.A., Mulholland, P.J., Webster, J.R. & Valett, H.M. (2001). Denitrification potential in sediments of headwater streams in the southern Appalachian Mountains, USA. *Journal of the North American Benthological Society*, 20(4), pp. 505–519.
- Michener, R. & Lajtha, K. (2007). *Stable isotopes in ecology and environmental science*. 2nd Ed Oxford, UK: Blackwell Publishing.
- Nichols, D.S. & Keeney, D.R. (1976). Nitrogen nutrition of *Myriophyllum spicatum*: uptake and translocation of ¹⁵N by shoots and roots. *Freshwater Biology*, 6, pp. 145–154.
- Pattinson, S.N., García-Ruiz, R., & Whitton, B.A. (1998). Spatial and seasonal variation in denitrification in the Swale–Ouse system, a river continuum. *Science of the Total Environment*, 210, pp. 289–305.
- Reyes-Avila, J., Razo-Flores, E. & Gomez, J. (2004). Simultaneous biological removal of nitrogen, carbon and sulfur by denitrification. *Water Research*. 38(14–15), pp. 3313–3321.
- Scholes, L., Shutes, R.B.E., Revitt, D.M., Forshaw, M. & Purchase, D. (1998). The treatment of metals in urban runoff by constructed wetlands. *The Science of the Total Environment*, 214, pp. 211–219.
- Sgouridis, F., Heppell, C.M., Wharton, G., Lansdown, K. & Trimmer, M. (2011). Denitrification and dissimilatory nitrate reduction to ammonium (DNRA) in a temperate re-connected floodplain. *Water Research*, 45(16), pp. 4909–4922.
- Silver, W.L., Herman, D.J. & Firestone, M.K. (2001). Dissimilatory nitrate reduction to ammonium in upland tropical forest soils. *Ecology*, 82(9), pp. 2410–2416.
- Sørensen, J. (1978). Denitrification rates in a marine sediment as measured by the acetylene inhibition technique. *Applied and Environmental Microbiology*, 36(1), pp. 139–143.
- Søvik, A.K. & Mørkved, P.T. (2008). Use of stable nitrogen isotope fractionation to estimate denitrification in small constructed wetlands treating agricultural runoff. *Science of the Total Environment*, 392, pp. 157–165.
- Straub, K.L., Benz, M., Schink, B. & Widdel, F. (1996). Anaerobic, nitrate-dependent microbial oxidation of ferrous iron. *Applied and Environmental Microbiology*, 62(4), pp. 1458–1460.
- Taylor, G.D., Fletcher, T.D., Wong, T.H.F., Breen, P.F. & Duncan, H.P. (2005). Nitrogen composition in urban runoff – implications for stormwater management. *Water Research*, 29, pp. 1982–1989.
- Valiela, I. & Teal, J.M. (1979). The nitrogen budget of a salt marsh ecosystem. *Nature*, 280(5724), pp. 652–656.
- Vymazal, J. (2007). Removal of nutrients in various types of constructed wetlands. *Science of the Total Environment*, 380, pp. 48–65.
- Wetzel, R.G. (2001). Fundamental processes within natural and constructed wetland ecosystems: short-term versus long-term objectives. *Water Science and Technology*, 44(11), pp. 1–8.



Cooperative Research Centre for Water Sensitive Cities



Level 1, 8 Scenic Boulevard
Monash University
Clayton VIC 3800



info@crwsc.org.au



www.watersensitivecities.org.au