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Factors controlling dissimilatory nitrate reduction processes in constructed stormwater urban wetlands

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Abstract Water treatment wetlands are increasingly being used to reduce pollutant loads including nitrogen (N) in urban runoff. Processes such as denitrification (DNF) and anaerobic ammonium oxidation (anammox), which remove N, and dissimilatory nitrate reduction to ammonium (DNRA), which recycles N, play an important role in controlling NOx ($NO_3^- + NO_2^-$) removal versus recycling in wetlands. The relative importance of DNF, anammox and DNRA was investigated in four constructed stormwater urban wetlands in Melbourne, Australia. Rates of DNF and DNRA were variable and did not differ significantly among wetlands. However, rates of DNF and DNRA were significantly different ($p < 0.05$) in different seasons. The relationship between NOx reduction processes and measured concentrations of water column NOx, chlorophyll a (chl-*a*), sediment organic carbon (OC), porewater ferrous iron (Fe^{2+}) and sulfide (S^{2-}) and water column temperature were examined using multiple regression analysis (MRA). Anammox was an insignificant pathway (< 0.05% of total nitrate reduction). During winter when average water column temperatures were 12 °C, DNRA was consistently higher than DNF averaging 67 ± 23% of total NOx reduction. The MRA revealed that DNF was positively associated with NOx concentration whereas DNRA was negatively associated with temperature, and porewater Fe^{2+}, and positively associated with chl-*a*. The ratio between DNF and DNF:(DNF + DNRA) showed a positive correlation with both temperature and NOx concentration in the MRA. At higher temperatures and higher NOx concentrations, DNF increased over DNRA. Overall, this study suggests that at low NOx concentrations, N is recycled internally in these urban stormwater wetlands, but the portion of N removed by DNF increases as NOx concentrations increase.

Keywords Denitrification · DNRA · Organic carbon · Temperature · Wetland

Introduction

Constructed wetlands are increasingly being used to remove pollutants including nitrogen (N) in urban stormwater runoff. As a consequence, N removal in wetlands has received considerable attention. It has been found that constructed wetlands can be highly efficient at N removal, and this depends primarily on the hydraulic loading rate (flow/surface area) to the wetland, highlighting the importance of sediment contact time as a variable controlling removal. Most

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studies of N removal in wetlands, however, focus simply on N removal efficiencies, with relatively few studies investigating the actual removal processes taking place in wetlands (Kadlec and Wallace 2009). A detailed understanding of the underlying processes controlling nitrogen cycling within wetlands is critical to designing wetlands that remove nitrogen effectively to avoid low nitrogen removal efficiencies over their lifetime under a range of environmental conditions. Processes of key importance controlling nitrogen removal in wetlands include assimilation by plants as well as NO_3^- reduction controlled by bacterial processes (Payne et al. 2014). Of these processes, bacterial recycling remains particularly enigmatic with numerous possible pathways and very few systematic studies on the factors controlling them in constructed wetlands (Gold et al. 2017; Koch et al. 2014; Rosenzweig et al. 2011). Nitrogen cycling in freshwater ecosystem have been found to be enhanced by the presence of bioturbating invertebrates (tubificid oligochaetes) (Nogaro and Burgin 2014). Rosenzweig et al. (2011) observed that seasonal variability might play an important role in nitrogen retention in an urban stormwater detention pond, with highest nitrogen retention observed during the summer sampling period. Gold et al. (2017) found that mixing of water column and excavation of pond sediment could improve the capacity of wet ponds to permanently remove nitrogen. However, none of these studies focused on the factors controlling the removal versus recycling of nitrogen in freshwater ecosystem such as constructed wetlands.

There are three competing pathways of dissimilatory NO_3^- reduction that can determine the degree of N recycling versus loss as N_2 from a system (Dalsgaard et al. 2012; Dong et al. 2009; Kartal et al. 2007; Sørensen 1978; Tiedje 1988). Denitrification (DNF) reduces nitrogen oxides (NO_3^- and NO_2^-) to N_2 and removes N permanently from a system (Bernard et al. 2015; Tiedje 1988). Similar to DNF, anaerobic ammonium oxidation (anammox) removes N permanently as N_2 by simultaneously oxidising NH_4^+ and reducing NO_3^- to N_2 (Dalsgaard et al. 2012; Dong et al. 2009; Kartal et al. 2007). In contrast, dissimilatory nitrate reduction to ammonium (DNRA) is another process which reduces nitrate to biologically available NH_4^+ (Bernard et al. 2015; Burgin and Hamilton 2007; Tiedje 1988). Despite the fact that these competing processes have been well studied in marine and coastal environments, few studies have examined the factors that control the partitioning between DNF and DNRA in constructed wetlands.

Nitrogen removal within constructed wetlands is based on the assumption that DNF is a major nitrogen removal pathway (Bachand and Horne 1999; Hunt et al. 2003; Poe et al. 2003), however conditions that promote DNRA over DNF may limit the N removal capacity of these systems. Whilst previous studies have observed both DNF and DNRA across a range of environments, there is a paucity in DNRA measurements in urban constructed wetlands and the putative factors that promote DNRA over DNF in other environments are currently unknown for urban constructed wetlands. Previous studies (Brunet and Garcia-Gil 1996; Jones et al. 2017; Kelly-Gerreyn et al. 2001; Robertson and Thamdrup 2017; Straub et al. 1996; van den Berg et al. 2016; Yoon et al. 2015) have typically identified the following factors as controls over the partitioning of DNF and DNRA: (1) the ratio of organic carbon:nitrate, (2) the concentration of free sulfide, (3) the availability of Fe^{2+}, and (4) temperature.

Heterotrophic DNF and DNRA both utilise carbon as an electron donor, however studies have shown DNRA, carried out by fermentative bacteria (e.g., *E. coli* sp.), is favoured over DNF when conditions are nitrate limited and rich in organic carbon (Bonin 1996; Nijburg et al. 1997; Tiedje 1988). A study on the effect of carbon sources on DNRA in coastal wetland sediments showed that rates of DNRA from the sediments without addition of exogenous carbon were low compared to those in which various sources of organic carbon was added (Liu et al. 2016). This result was attributed to the low C:NO_3^- ratio in the sediment without external sources of organic carbon (Liu et al. 2016). Previous studies in wetlands have shown DNF rates are related to organic carbon concentration with the highest rates reported under conditions of balanced supply of dissolved organic carbon and NO_3^-, that is, at DOC:NO_3^- (electron donor:electron acceptor) ratios that were near the microbial requirement for DNF (Hansen et al. 2016).

Alternatively, chemolithoautotrophic DNF and DNRA can utilise free sulfur (S^{2-} or S^0) or ferrous iron (Fe^{2+}) to reduce nitrate to N_2 or NH_4^+, respectively (Brunet and Garcia-Gil 1996; Otte et al. 1999; Straub et al. 1996; Weber et al. 2001, 2006a). In coastal environments, it has been shown that sulfur

oxidising bacteria (*Thioploca* sp. and *Beggiatoa* sp.) can carry out DNRA (Otte et al. 1999; Schulz and Jørgensen 2001). In a study of Laguna Madre/Baffin Bay in the USA, An and Gardner (2002) observed DNRA contributed up to 75% of total nitrate reduction in the presence of high sulfide concentrations, postulating high DNRA rates were due to several factors including sulfide stimulation of DNRA in combination with inhibition of nitrification and heterotrophic DNF. Moreover, it was observed from the nitrate-amended sediment slurries of anaerobic freshwater sediments that the initial concentration of free sulfide was the factor determining the type of nitrate reduction. When the concentration of free sulfide was extremely low, nitrate was reduced through DNF whereas DNRA and incomplete DNF to gaseous nitrogen oxides occurred at higher sulfide concentrations (Brunet and Garcia-Gil 1996).

Previous studies have also linked ferrous iron to nitrate reduction (Benz et al. 1998; Straub et al. 1996; Straub and Buchholz-Cleven 1998) with the major product being N_2 (DNF). However, Weber et al. (2006b) linked DNRA to the oxidation of Fe^{2+} in a study where bacteria were cultured from wetland sediments. More recently, in the Yarra River estuary and Lake Almind, a freshwater lake in Denmark, Fe-driven DNRA was shown to be a significant contributor to overall NO_3^- reduction with rates comparable to DNF (Robertson et al. 2016; Robertson and Thamdrup 2017).

Finally, temperature can play an important role in determining the partitioning between DNF and DNRA (Dong et al. 2011; Gardner and McCarthy 2009; Kelly-Gerreyn et al. 2001; Smyth et al. 2013). There is a strong link between increased temperatures and an increase in the sediment oxygen demand that favours organisms that carry out DNRA because conditions become more reducing (Dong et al. 2011; Gardner and McCarthy 2009; Kelly-Gerreyn et al. 2001; Smyth et al. 2013). Previous studies have shown that bacteria that undertake DNRA predominated over denitrifiers at high temperatures in summer whereas denitrifiers predominated in autumn and winter (Jørgensen 1989; King and Nedwell 1984). Ogilvie et al. (1997) showed from estuarine sediments that nitrate ammonifiers had the higher affinity for nitrate compared to denitrifiers at higher temperatures. Moreover, Kelly-Gerreyn et al. (2001) found that although both DNRA and DNF occur at all temperatures, DNRA is the favoured process at the extremes (< 14 and > 17 °C) of the observed temperatures whereas DNF is favoured only in a narrow range (14 to 17 °C) of temperatures.

In this study, we measured rates of DNF, anammox and DNRA in intact cores taken from four constructed stormwater urban wetlands in Melbourne Australia, across a range of seasonal conditions to include natural variations in water column and sediment condition. Based on previous studies, we hypothesised that heterotrophic DNRA would be the dominant nitrate reduction pathway because urban constructed wetlands are well vegetated to assist in nitrogen removal and therefore are often rich in organic carbon. We hypothesised that DNRA would be favoured over DNF in urban wetlands with low concentrations of water column NO_3^- and high sediment organic carbon content. Furthermore, we postulated that rates of DNRA would increase during the warmer months. To test these hypotheses, rates of DNF and DNRA were measured as response variables while concentrations of water column NOx, sediment organic carbon, chl-a in sediment, free sulfide and Fe^{2+} in the porewater and water column temperature were measured as predictor variables. Multiple regression analysis was used to identify the significance of each of these factors in controlling the rates of DNF and DNRA and the relative partitioning of these two processes.

Materials and methods

Site description

Nitrate reduction pathways were quantified in four constructed stormwater urban wetlands in Melbourne, Australia (Huntingdale Road (HR), Cascades on Clyde (CC), Koolamara Blvd (KB) and Namatjira Reserve (NR) wetlands) from December 2014 to February 2016. The HR wetland (37°53′38″S, 145°6′39″E) was constructed in early 2003 (Melbourne Water, pers. comm.) and is located on the floodplain of Scotchmans Creek. The area of the HR wetland is 18,500 m^2 (Melbourne Water, pers. comm.) and responds quickly to rain events leading to short periods of high water levels. The CC wetland is located in North Clyde (38°6′10″S, 145°19′37″E) and was constructed in May 2008 (Melbourne Water, pers. comm.). The area of this wetland is 1,05,258 m^2 (Melbourne Water, pers. comm.) and services runoff

from the adjacent urban and farming district. The KB wetland (37°53′50″S, 145°16′32″E) was constructed in 2005 and the area is 22,461 m^2 (Melbourne Water, pers. comm.). The main purpose of constructing this wetland was to improve water quality in the nearby Monbulk Creek, which flows through an urban and agricultural catchment. The NR wetland is within parkland (Namatjira Park) and is located at Clayton South (37°56′5″S, 145°6′40″E). The wetland was constructed in 2012, with an area of 45,000 m^2 (Melbourne Water, pers. comm.) and services a mixed urban and industrial catchment. The wetland naturally filters pollutants from stormwater, such as nitrogen and phosphorus that would otherwise flow into Port Phillip Bay (Melbourne Water, https://www.melbournewater.com.au). All of the studied wetlands range between 0.5 and 1 m in depth (under baseflow) and are predominantly surface flow wetlands (KB and NR wetlands have a small region of subsurface flow). The studied sites were dominated by *Juncus* sp. and to a lesser extent by *Typha* sp. DNF, anammox and DNRA were quantified from intact core incubations in April–May 2015 (except HR wetland), August–September 2015 and January–February 2016 which represent the annual temporal variability in terms of temperature as indicated by air temperature during the study period. Sediment cores for slurry incubations were collected from the inlet, mid-point and outlet of all four wetlands. Slurry incubations were performed in December–January 2014–2015, and in April–May 2015 (HR wetland was excluded from the measurements due to construction works on the wetland during the sampling period), and in January–February 2016 simultaneously with intact core incubations from all four wetlands.

Intact sediment core incubation for denitrification and DNRA

Rates of DNF and DNRA were measured in intact sediment cores from April 2015 to February 2016 using the isotope pairing technique (Dalsgaard et al. 2000; Nielsen 1992). Four cores from the inlet, mid-point and outlet of each wetland on each sampling date were collected using stoppered polyethylene cylinders (6.5 cm inner diameter; 27–29 cm height) for the intact core incubations. The depth of the sediment collected ranged from 10 to 18 cm. In addition, six sediment cores were collected at each location to measure parameters in sediment and sediment porewater. Sediment cores and water sampling was carried out between 10 am and 12 pm on each sampling trip to allow for comparisons between the in situ variables of water column on different dates and to remove the artefact of possible shifts over a diurnal cycle. Site water was collected in a 20 L carboy for use in the experiments from the mid-point of each wetland. Sediment cores were kept cool until they were transferred into a temperature controlled water bath maintained at in situ site water temperature within 1–2 h of collection. The cores were recirculated overnight at in situ oxygen concentration and mixed using a magnetic stirrer ($\sim$ 40 rpm) suspended $\sim$ 3 cm above the sediment surface to prevent disturbance of sediment.

Rates of DNF and DNRA were measured using the isotope pairing technique (IPT) when the dissolved oxygen (DO) was within $\pm$ 30 μmol-O_2 L^{-1} of the in situ oxygen concentration (Nielsen 1992; Risgaard-Petersen 2003; Dalsgaard et al. 2000). All four cores from a location in a wetland on a date were used to create one time series. At the start of the incubation, a 12 mL filtered (0.2 μm PES filter, Sartorius) NOx sample was collected before the addition of 250 μL of 0.1 mol L^{-1} ^{15}N-NO_3^- (^{15}N at. %, 99.6%, Novachem Pty Ltd, Australia) to the overlying water column. The water column was mixed to ensure the tracer was homogenously dispersed throughout the water column, also ensuring there was no disturbance to the sediment surface. Another 12 mL filtered (0.2 μm PES filter, Sartorius) NOx sample was also collected to determine the initial NOx concentration after the addition of ^{15}N-NO_3 in the overlying water column. Water removed was replaced with site water and the cores were sealed. Before sacrificing each intact core, the DO was measured, and then 1 mL of 50% (w/v) $ZnCl_2$ was added to the typically 1 L overlying water column to ensure microbial activity ceased. The core was gently slurried to minimize loss of $^{15}N_2$ and allowed to settle for a minute before a N_2 gas sample was collected in a 12 mL Exetainer (Labco) ensuring there were no bubbles in the sampling procedure or exetainer, which was then preserved with 250 μL of 50% (w/v) $ZnCl_2$ until analysis. For DNRA, 20 mL of homogenised slurry was transferred into a 50 mL centrifuge tube (Falcon) and frozen until extraction with KCl and subsequent conversion of $^{15}NH_4^+$ to $^{15}N_2$ via alkaline hypobromite (Risgaard-Petersen

et al. 1995; Roberts et al. 2014). A random core was slurried at approximate time intervals of 1, 3, 5, and 7 h during which time the DO reduced by no more than 20% of the initial DO.

A 4 mL helium headspace was introduced into the 12 mL Exetainer and the sample shaken vigorously to ensure the N_2 in the sample was transferred to the helium headspace before analysis on a Sercon 20-22 isotope mass spectrometer connected to a gas chromatograph. To increase the background concentration of ^{14}N in the sample and accurately allow the calculation of excess ^{15}N-N_2 from the N_2 ratios of 28/29 and 28/30, the headspaces for both DNF and DNRA were pre-treated with 50 µL of air (Roberts et al. 2014). Rates of DNF were determined from the linear production of $^{29}N_2$ and $^{30}N_2$ over time (Dalsgaard et al. 2000; Nielsen 1992) and were used to calculate the rate of DNF produced from the consumption of ^{14}N-NO_3^- (D_{14}). The method has an isotope ratio precision of $\sim$ 0.01%, which translates to a rate equivalent to < 0.5 µmol m^{-2} h^{-1}.

The accumulation of $^{15}NH_4^+$ over the incubation was determined after extraction of the sediment slurry with 1:1 slurry to 2 M KCl extraction (1 h, 120 rpm) to release adsorbed $^{15}NH_4^+$ into solution. 8 mL of extract was pipetted into an Exetainer, sealed with a gas tight rubber septum and then purged with helium to remove any residual $^{15}N_2$. 200 µL of alkaline hypobromite was added to convert $^{15}NH_4^+$ to $^{15}N_2$, using a vent needle to prevent overpressure within the Exetainer, and shaken for 24 h at 130 rpm to ensure complete conversion of $^{15}NH_4^+$ to $^{15}N_2$. A series of standards were prepared in the same matrix to test the recovery of $^{15}NH_4^+$. Recovery for all standards ranged from 102 ± 4.4 to 114 ± 2%. The $^{15}N_2$ produced was analysed on a Sercon 20-22 isotope ratio mass spectrometer connected to a gas chromatograph. Rates of DNRA were calculated from the linear production of ^{15}N-NH_4^+ over time. The rate of DNRA ($DNRA_{14}$) or production of ^{14}N-NH_4^+ produced in the intact cores was determined similar to DNF (D_{14}) using Eq. 1, based on the assumption that ^{14}N-NO_3^- and ^{15}N-NO_3^- were mixed uniformly and there was no difference in the reduction rates of the isotopes by the DNRA or denitrifying bacteria. Therefore, DNRA rates ($DNRA_{14}$) were determined from the production of ^{15}N-NH_4^+ and the frequency of NO_3^- reduction used in the DNF calculation (D_{14}) (Eq. 1) (Nielsen 1992; Risgaard-Petersen et al. 1995)

$$D_{14} = D_{15} \times f_{14}/f_{15} \quad (1)$$

where $f_{14}/f_{15} = (^{15}N^{14}N)/(2*(^{15}N^{15}N))$.

The instrument precision was $< 0.01\%$ for 29/28 and 30/28 ratios allowing us to detect an equivalent rate of < 0.5 µmol m^{-2} h^{-1} for DNF and DNRA.

Slurry assays for denitrification, DNRA and anammox

Slurry experiments were undertaken to quantify the significance of the anammox reaction. Anammox was only assessed in slurries because a definitive test of this process is not possible in intact cores (Risgaard-Petersen et al. 2004). Sediment cores and water were collected from the same sites using the same method as described above. Sediment slurries were prepared by homogenising the surface 1 cm sediment slice with site water at a ratio of 1:10 (v:v) for each treatment: DNF and DNRA or anammox (Meyer et al. 2005; Thamdrup and Dalsgaard 2002). 8 mL of homogenised slurry was transferred into a 12 mL gas tight Exetainer (Labco), then purged with helium for 5 min to remove residual oxygen. The slurries in Exetainers were mixed at 130 rpm overnight in the dark at room temperature to eliminate any remaining oxygen and NOx.

To determine the contribution of anammox to nitrate reduction, three parallel incubations were performed: incubation I, amended with $^{15}NH_4^+$ (^{15}N at. %, 98%, Cambridge) used as a control; incubation II, amended with $^{15}NH_4^+ + {}^{14}NO_2^-$ used to confirm the presence of anammox; incubation III, amended with $^{15}NO_3^-$ (^{15}N at. %, 99.6%, Novachem Pty Ltd, Australia) to determine combined DNF and anammox. In all incubations, the tracers were amended to a final concentration of 100 µM. Throughout the incubation the slurries were mixed at 130 rpm and the incubation terminated at $\sim$ 0, 1, 2 and 3 h by adding 250 µL $ZnCl_2$ (50% w:v). Potential rates of DNF, DNRA and anammox were determined according to Bernard et al. (2015), Meyer et al. (2005), Rysgaard et al. (2004), and Thamdrup and Dalsgaard (2002). DNF and DNRA rates were determined as described above.

Predictor variables

Filtered surface water samples for NH_4^+, filterable reactive phosphorus (FRP) and NO_x were collected

from the inlet, mid-point and outlet by filtering through 0.2 μm PES filters (Sartorius). Nutrient concentrations were then measured via flow injection analysis (Lachat Quickchem 8000 Flow Injection Analyser with spectrophotometric detection) according to APHA (2005) methods. Matrix spikes and standard reference materials (SRM) were prepared and analysed according to APHA (2005) and were found within the limits of 100 ± 10%. Water temperature, pH, dissolved oxygen (DO), turbidity and conductivity were measured in situ at each site using a pre-calibrated Horiba (U-10 or U-50 model) water quality multiprobe.

Sediment cores for porewater extraction were collected concurrently with cores for intact core and slurry incubations. Immediately after returning to the laboratory, the top 1 cm of sediment was sliced, transferred into a 50 mL Falcon tube, capped and then centrifuged for 10 min at 2000 rpm. The supernatant was then filtered through a 0.2 μm PES filter (Sartorius) for Fe^{2+} and S^{2-}. Fe^{2+} and S^{2-} from sediment porewater were analysed using the ferrozine (Stookey 1970; Viollier et al. 2000) and zinc acetate (Fonselius et al. 2007) methods, respectively. Standards for both Fe^{2+} and S^{2-} were prepared within the range of 0–1 mg L^{-1} and the absorbance was measured at 562 nm and 670 nm for Fe^{2+} and S^{2-}, respectively using a UV–Vis Spectrophotometer (GBC UV Visible Spectrophotometer—918).

In addition, total Fe (T-Fe), total phosphorus (TP), total nitrogen (TN), total carbon (TC), particle size (% sand, silt and clay) and porosity (%) from the surface 1 cm of sediment were analysed in April–May 2015 to January–February 2016 to characterise the sediment. Dry sediments were digested with concentrated nitric acid (Suprapur, 65%, Merck) according to APHA (2005). T-Fe was analysed using flame AAS (XplorAA, GBC Scientific Equipment). To determine TP, dry sediments were digested with persulphate oxidizing solution according to APHA (2005) then analysed via flow injection analysis (Lachat Quickchem 8000 Flow Injection Analyser with spectrophotometric detection). TC, after acidifying with HCl, and TN from dry sediments, were analysed using a Sercon 20-22 continuous flow isotope ratio mass spectrometer (IRMS) fitted to an elemental analyser at 1050 °C. Chl-*a* from wet sediments was determined fluorometrically after extraction in 90% acetone (Bernard et al. 2015; Sun et al. 1991). Particle sizing was determined using the modified pipette method (Gavlak et al. 2003) while sediment porosities were determined from the difference between the wet and dry weight of sediments in a known volume after drying at 60 °C.

Statistical analysis

SigmaPlot (SigmaPlot 13.0) was used to perform the *t* test and one-way ANOVA to compare the DNF data for a given site and wetland with the DNRA data for the same site and wetland. One-way ANOVA was also used to compare OC content among wetlands. Rates of DNF and DNRA for each site within a wetland across all sampling dates were used to investigate the variability among wetlands, in different seasons and the interaction between wetlands and seasons using a two-way ANOVA in SPSS (IBM SPSS Statistics 25). Relationships between environmental parameters and nitrate reduction pathways were assessed using multiple linear regression models. The response variables were rates of DNF, DNRA and the DNF:(DNF + DNRA) ratio. Response variables were right skewed and therefore were ln(x + 1) transformed. Distributions were corrected to approximate normality by using this measure. The predictor variables were included as continuous predictors in the models. Predictors were screened for collinearity with pairwise correlations. The predictors included in the models were not correlated ($R < 0.7$). All predictors were standardized by subtracting means and dividing by standard deviations prior to analyses to limit the effects of different ranges of predictors. Multiple regression analysis was performed by using a set of additive models with all combinations of predictor variables (Fe^{2+}, S^{2-}, intact core NOx concentration, surface water temperature, OC, chl-*a*, silt particle content) to each of the response variables (DNF, DNRA, DNF:(DNF + DNRA)). We performed both AIC and BIC models and compared their outcomes. The outcomes from both models were similar. We choose to report the BIC outcomes because they are more tolerant than AIC to free parameters when the sample size is small, as was usually the case in this study. BIC values were converted to BIC weights (BICw) to provide normalized relative model likelihoods. BICw represent the probability that a model is the best model given the data and the suite of candidate models assessed. BICw ranges from 0 to 1. A BICw of zero indicates zero probability that a given model is

the best. BICw values approaching 1 indicate high probability that a given model is the best (Wagenmakers and Farrell 2004). Model performance was assessed using R^2 values and p values of predictors. Analyses were done in R (Version: R.3.2.2). Predictor variables were checked for collinearity in SPSS (IBM SPSS Statistics 22). Normality was tested using the normality test of residuals for all regression models. The normality test of residuals (Shapiro–Wilk) passed for both temperature ($p = 0.270$) and porewater reduced iron ($p = 0.277$) (Fig. 3). Significance of the statistical test result was determined at $\alpha = 0.05$ level.

Results

Physico-chemical parameters and air temperature

Physico-chemical parameters of the surface water were recorded from all sites during each sampling occasion (See Appendix Table 8). The mean surface water temperature ranged from 12 to 24 °C from December 2014 to February 2016. The annual mean minimum air temperature during the study period was 7 °C whereas the mean maximum temperature was 27 °C (BOM). The surface water temperature measured in situ and applied for the intact core incubations were within this annual air temperature range. DO saturation during the study period ranged from 26 to 235%. Water column concentrations ranged from 0.7 to 24 μM for NH_4^+, 1.4 to 57.3 μM for NOx and 0.4 to 3.2 μM for FRP.

Sediment characteristics

Characterisation of sediments from all sampling sites from April–May 2015 to January–February 2016 are presented in Table 1. T-Fe content in dry sediments ranged from 0.5 to 4.1 (%). TP content in sediments ranged from 0.01 to 0.1 (%) while TN in sediment samples ranged from 0.1 to 1.2% with most samples below 1%. A difference in %OC content among wetlands is apparent, which indicates that KB and HR wetlands group together as high in %OC while NR and CC wetlands group together as low in %OC. The content of %OC in the KB and HR wetlands are significantly higher ($p < 0.05$) compared to the NR and CC wetlands. The abundance of Chl-*a* in sediments ranged from 4 to 7.4 mg m^{-2}. Sediments in the CC and the HR wetlands are dominated by silt particles while sand is the dominant particle type in sediments of the KB and the NR wetlands. Parameters measured from sediment porewater are presented in Table 2. Fe^{2+} in the porewater ranged from 6 to 750 μM with typically higher concentrations during warmer periods. S^{2-} in the porewater ranged from below the method detection limit of 1.5 to a maximum of 12.3 μM. Dissolved NH_4^+ in porewater was highly variable ranging from below detection (0.143 μM) to a maximum of 1000 μM whereas NOx concentration ranged from 0.1 to 7.1 μM.

Denitrification and DNRA from intact cores

The rates of DNF observed from intact cores of four wetlands were highly variable and ranged from 8.5 ± 1.3 μmol m^{-2} h^{-1} in April 2015 to 200 ± 50 μmol m^{-2} h^{-1} in September 2015 (Fig. 1). The differences in rates of DNF among wetlands were not significant ($p > 0.05$) but rates of DNF in different seasons were significantly different ($p < 0.05$). A Tukey HSD post hoc analysis showed that DNF rates between autumn and spring, spring and winter, and spring and summer were significantly different ($p < 0.05$) (Table 3). However, the interaction of wetlands and seasons did not have any significant effect on the rates of DNF ($p > 0.05$) from four wetlands.

Rates of DNRA from intact cores ranged from 1.8 ± 0.4 μmol m^{-2} h^{-1} in January 2016 to 140 ± 40 μmol m^{-2} h^{-1} in April 2015 (Fig. 1). Similar to DNF, rates of DNRA were also not significantly different ($p > 0.05$) among wetlands but DNRA rates varied significantly ($p < 0.05$) among seasons. The post hoc analysis showed a significant difference ($p < 0.05$) in rates of DNRA between autumn and summer, and spring and summer (Table 3). There was no interaction between wetlands and seasons on rates of DNRA ($p > 0.05$). DNRA dominated in intact cores (Fig. 1) and in 60% cases, DNRA was significantly higher ($p < 0.05$) than DNF in intact cores. The ratio of DNF:(DNF + DNRA) ranged from 0.1 to 0.9 (Fig. 1). The DNF:(DNF + DNRA) ratios were not significantly ($p > 0.05$) different among wetlands but varied significantly ($p < 0.05$) among seasons (Table 3). The interaction effect of wetlands and seasons on the DNF:(DNF + DNRA) ratios were not significant ($p > 0.05$).

Table 1 Sediment properties determined from four wetlands from April–May 2015 to January–February 2016

	Sampling dates	Sites	T-Fe (%)	TP (%)	TN (%)	OC (%)	Chl-a (mg m^{-2})	Porosity (%)	Sand (%)	Silt (%)	Clay (%)
Koolamara Blvd (KB)	April 2015	In	2.0	0.1	0.2	3.7	2.2	81.4	82.6	10.9	6.5
		M-P	1.7	0.1	0.7	8.7	4.8	72.1	52.2	14.1	33.8
		Out	2.1	0.1	0.7	10.6	14.5	81.1	67.1	18.2	14.7
	August 2015	In	2.3	0.0	0.6	9.9	5.0	83.3	46.1	52.5	1.4
		M-P	1.7	0.0	0.6	7.9	13.1	84.8	28.1	61.7	10.2
		Out	1.9	0.0	0.7	9.8	2.9	87.9	51.4	44.6	4.0
	February 2016	In	2.2	0.0	0.7	10.6	2.8	90.9	64.6	28.4	7.0
		M-P	1.4	0.0	0.7	7.4	12.0	83.4	23.0	64.5	12.5
		Out	1.9	0.0	1.2	15.5	7.2	90.8	51.4	46.6	2.0
Namatjira Reserve (NR)	May 2015	In	2.9	0.1	0.5	8.7	22.9	77.6	18.3	15.5	66.2
		M-P	0.8	0.0	0.1	2.4	2.8	78.2	89.4	5.6	5.0
		Out	1.4	0.0	0.1	1.1	13.4	66.0	94.8	2.4	2.8
	September 2015	In	2.2	0.0	0.5	7.2	8.3	77.9	42.5	51.0	6.5
		M-P	1.0	0.0	0.1	1.6	5.6	62.9	92.9	1.5	5.6
		Out	0.5	0.0	0.2	1.3	6.0	70.3	96.1	2.4	1.5
	January 2016	In	1.8	0.0	0.5	9.7	5.1	93.4	54.6	40.5	4.9
		M-P	0.8	0.0	0.1	0.7	1.8	68.2	94.1	5.0	0.9
		Out	0.8	0.0	0.1	1.9	0.6	78.9	88.5	9.7	1.8
Cascades on Clyde (CC)	April 2015	In	4.0	0.1	0.2	2.3	3.4	68.3	25.9	11.7	62.5
		M-P	3.9	0.1	0.3	2.5	3.5	74.2	23.6	24.6	51.8
		Out	3.2	0.1	0.3	3.4	3.9	85.2	20.3	32.2	47.6
	September 2015	In	3.8	0.0	0.3	4.4	5.1	87.5	19.5	74.0	6.5
		M-P	3.8	0.0	0.4	3.7	5.6	83.1	27.0	71.6	1.4
		Out	3.4	0.0	0.8	11.4	7.2	83.9	55.5	43.1	1.4
	February 2016	In	3.7	0.0	0.2	3.7	3.1	82.2	19.0	67.8	13.2
		M-P	4.1	0.0	0.4	3.9	20.8	81.2	44.4	38.9	16.7
		Out	2.6	0.0	0.4	5.1	3.3	91.2	41.6	18.9	39.5
Huntingdale Road (HR)	August 2015	In	1.4	0.0	0.7	14.1	7.4	79.3	41.3	55.2	3.5
		M-P	1.3	0.0	0.7	13.5	2.6	83.4	37.7	55.1	7.2
		Out	0.6	0.0	0.3	4.2	5.0	81.7	51.9	39.6	8.5
	January 2016	In	1.6	0.0	0.7	13.4	4.4	87.7	42.9	44.7	12.4
		M-P	1.3	0.0	0.9	15.7	2.8	92.8	54.9	26.7	18.4
		Out	0.6	0.0	0.3	4.4	2.0	81.1	39.5	60.2	0.3

In for inlet; M-P for mid-point and Out for outlet

Multiple regression analysis

The multiple regression model results revealed that the water column NOx in concert with OC and chl-*a* in the sediments, were positively associated with DNF rates from intact cores (Table 4). The best fitting model showed that these three predictor variables together explain 55% of the variation in DNF from intact cores from all 4 wetlands combined. DNRA was best described by a model containing temperature, porewater Fe^{2+}, chl-*a* and silt content (Table 5). These predictors jointly explained 49% of the variation in DNRA from intact cores. DNRA rates were negatively associated with temperature and porewater Fe^{2+} and positively associated with chl-*a* and silt content. The DNF:(DNF + DNRA) ratio from intact cores was

Table 2 Dissolved iron, sulfide and nutrient concentrations in porewater and surface water of four wetlands from December–January 2014 to January–February 2016

Sampling location	Dates	Sites	Porewater					Surface water		
			Fe(II) (μM)	S^{2-} (μM)	NH_4^+ (μM)	NOx (μM)	FRP (μM)	NH_4^+ (μM)	NOx (μM)	FRP (μM)
Koolamara Blvd (KB)	Dec 2014	In	62.4	12.3	88.6	0.6	0.9	14.8	14.2	1.0
		Mid	325.0	8.3	194.3	0.6	0.3	4.6	3.8	0.8
		Out	269.9	2.2	138.6	0.8	0.4	2.1	1.4	0.7
	April 2015	In	216.6	0.5	996.4	0.1	1.0	9.6	15.4	0.9
		Mid	20.6	0.8	60.6	0.4	0.9	24.2	6.0	0.7
		Out	218.3	1.1	2.4	0.4	0.9	1.1	2.1	0.7
	August 2015	In	749.6	0.0	407.1	0.1	0.3	3.0	26.5	0.3
		Mid	130.2	0.0	50.9	0.2	0.1	6.4	16.8	0.1
		Out	301.2	1.9	169.3	0.1	0.1	1.9	4.1	0.1
	February 2016	In	130.5	0.1	0.0	2.5	0.8	3.5	50.4	0.4
		Mid	216.8	1.3	233.4	0.6	0.4	2.5	13.6	0.7
		Out	137.5	0.1	491.3	2.2	0.5	0.6	1.9	0.8
Namatjira Reserve (NR)	Jan 2015	In	–	–	–	–	–	5.4	41.6	3.2
		Mid	75.9	5.6	156.4	0.9	0.5	22.0	15.9	0.6
		Out	39.8	5.0	31.2	1.0	0.7	0.7	2.2	0.7
	May 2015	In	17.7	0.1	74.1	0.5	0.9	6.0	53.4	1.2
		Mid	30.9	0.1	74.6	0.9	0.9	9.1	16.1	0.5
		Out	125.8	0.2	61.3	0.1	0.9	7.9	9.3	1.8
	September 2015	In	212.3	0.3	125.4	0.7	0.4	10.1	37.9	1.1
		Mid	50.8	0.4	38.3	0.1	0.1	6.8	35.6	0.3
		Out	61.8	0.6	30.0	0.1	0.1	3.2	29.0	0.2
	January 2016	In	422.5	5.6	57.8	1.7	0.6	31.2	0.3	0.7
		Mid	324.8	10.3	133.4	7.1	1.9	4.1	0.6	0.7
		Out	574.6	0.1	61.0	3.3	0.5	45.7	0.2	0.2
Cascades on Clyde (CC)	Jan 2015	In	104.5	5.8	104.3	1.0	0.6	9.2	15.2	0.4
		Mid	51.6	2.3	16.1	1.2	0.4	1.4	1.0	0.5
		Out	347.3	0.6	43.9	1.4	0.4	3.2	3.7	0.4
	April 2015	In	50.0	1.5	89.9	0.2	0.7	6.3	57.3	0.6
		Mid	25.2	0.1	1.5	0.2	0.7	4.6	17.0	0.4
		Out	104.4	0.2	2.7	0.1	0.7	1.8	1.5	0.5
	September 2015	In	191.7	0.2	102.1	0.4	0.2	7.5	147.1	1.3
		Mid	124.2	0.3	70.3	0.3	0.1	9.7	105.0	1.3
		Out	5.8	0.3	35.3	0.1	0.1	7.3	75.0	0.6
	February	In	328.4	1.3	100.1	3.4	0.4	0.4	29.2	0.2
	2016	Mid	601.4	0.3	93.1	4.5	0.5	0.3	1.7	0.2
		Out	739.4	0.2	17.0	0.4	0.3	0.6	0.2	0.1

Table 2 continued

Sampling location	Dates	Sites	Porewater					Surface water		
			Fe(II) (μM)	S^{2-} (μM)	NH_4^+ (μM)	NOx (μM)	FRP (μM)	NH_4^+ (μM)	NOx (μM)	FRP (μM)
Huntingdale Road (HR)	August 2015	In	105.7	0.7	148.2	0.1	0.5	3.7	30.6	0.2
		Mid	15.4	1.2	57.8	0.1	0.2	7.9	30.2	0.2
		Out	43.6	0.1	30.5	1.9	0.4	6.5	32.3	0.2
	January 2016	In	324.7	0.4	520.6	2.2	0.3	3.9	13.1	0.2
		Mid	243.8	1.3	261.8	0.2	0.4	3.2	4.1	0.2
		Out	715.5	1.0	226.7	1.6	0.2	0.2	0.2	0.1

In for inlet; M-P for mid-point and Out for outlet

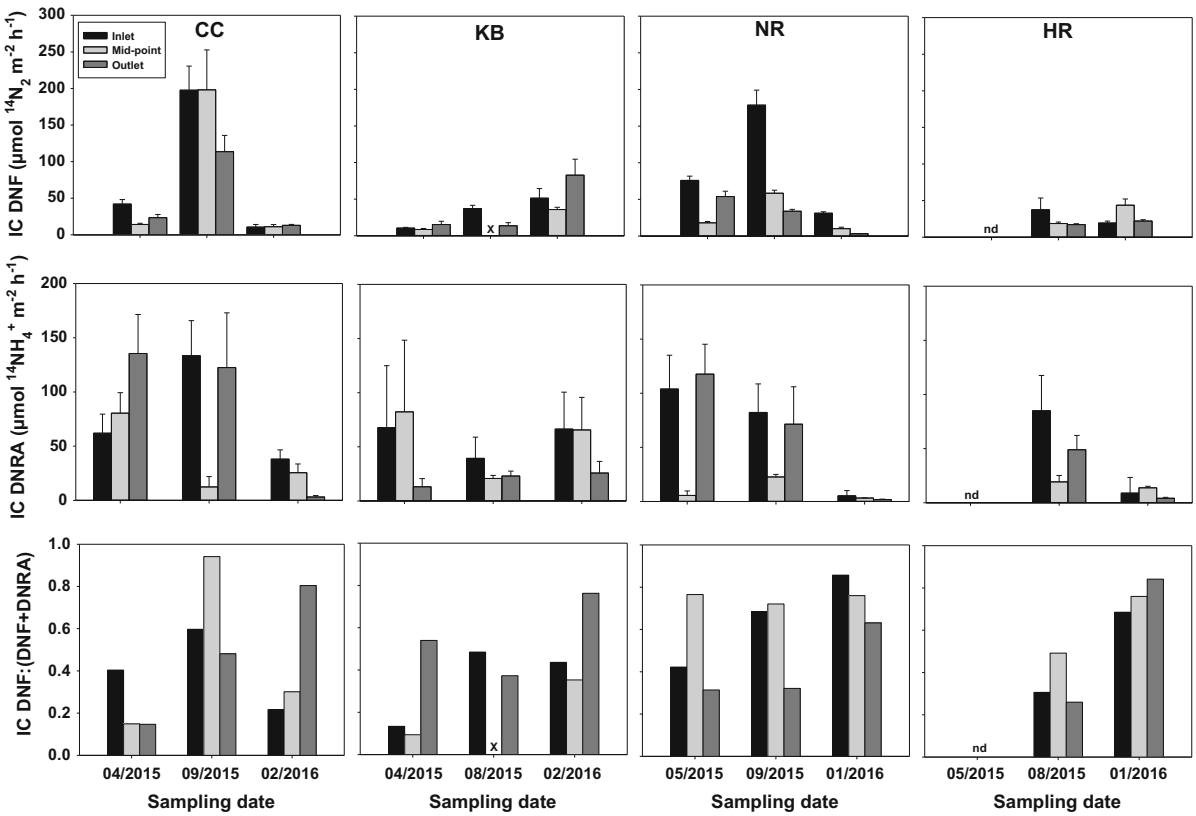

Fig. 1 Rates of denitrification, DNRA and the DNF:(DNF + DNRA) ratio from intact core (IC) incubations from April 2015 to February 2016. Error bars represent ± 1SE and the SE is based on the slope of the time series. nd represents no data for that month

best described by a model including water column NOx, temperature and porewater Fe^{2+} (Table 6). These three predictor variables together explained 23% of the variation in the DNF:(DNF + DNRA) ratio from intact core measurements. The DNF:(DNF + DNRA) was positively correlated to all these three predictor variables.

Table 3 The results of the Tukey HSD post hoc from the two-way ANOVA analysis showing the significant differences in rates of DNF, DNRA and DNF:(DNF + DNRA) in different seasons

Seasons	Denitrification	DNRA	DNF:(DNF + DNRA)
Autumn versus spring	< 0.05	> 0.05	< 0.05
Autumn versus summer	> 0.05	< 0.05	< 0.05
Autumn versus winter	> 0.05	> 0.05	> 0.05
Spring versus summer	< 0.05	< 0.05	> 0.05
Spring versus winter	< 0.05	> 0.05	> 0.05
Summer versus winter	> 0.05	> 0.05	> 0.05

Table 4 Best model results of multiple regression analysis of influence of predicted variables on intact core denitrification rates from four wetlands

Adjusted R^2 = 0.55; Standard error of estimation: 0.63; F = 13.83; p < 0.001, BICw = 0.14			
	Coefficient, B	Standard error of B	Significance level, p
Intercept	2.36	0.25	< 0.001*
NOx concentration (NOx)	0.02	0.00	< 0.001*
Organic carbon (OC)	0.04	0.02	0.086
Chl-a	0.04	0.02	0.062

*Values are statistically significant

Table 5 Best model results of multiple regression analysis of influence of predicted variables on intact core DNRA rates from four wetlands

Adjusted R^2 = 0.49; Standard error of estimation: 0.84; F = 8.44; p < 0.001, BICw = 0.08			
	Coefficient, B	Standard error of B	Significance level, p
Intercept	4.13	0.53	< 0.001*
Temperature (T)	− 0.06	0.03	0.038*
Fe^{2+} (Fe)	− 0.00	0.00	0.019*
Chl-a	0.07	0.03	0.025*
Silt	0.01	0.01	0.097
Model equation: DNRA = − 0.06 T − 0.00 Fe + 0.07 Chl-a + 0.01 Silt + 4.13 ± 0.84			

*Values are statistically significant

Table 6 Best model results of multiple regression analysis of influence of predicted variables on intact core DNF:(DNF + DNRA) ratio from four wetlands

Adjusted R^2 = 0.23; Standard error of estimation: 0.14; F = 5.65; p < 0.01, BICw = 0.08			
	Coefficient, B	Standard error of B	Significance level, p
Intercept	0.09	0.09	< 0.010*
Temperature (T)	0.01	0.00	0.003*
NOx concentration (NOx)	0.00	0.00	0.019*
Model equation: DNF:(DNF + DNRA) = 0.01 T + 0.00 NOx + 0.09 ± 0.14			

*Values are statistically significant

Denitrification, DNRA and anammox from slurry samples

Potential rates of DNF from slurry samples ranged from 6 ± 1 μmol L slurry^{-1} h^{-1} in January 2016 at the outlet of the HR wetland to 27 ± 9 μmol L slurry^{-1} h^{-1} in January 2015 from the inlet of the NR wetland (Fig. 2). Mean rates of DNF among wetlands were not significantly different ($r > 0.05$). Potential rates of DNRA from slurry samples were always lower compared to DNF and ranged from 0.6 ± 0.2 μmol L slurry^{-1} h^{-1} to 11 ± 2 μmol L slurry^{-1} h^{-1}. Although incubations were done at ambient laboratory temperature (23 °C), potential rates of DNRA were higher in all samples collected in warmer periods (24 °C) than those collected in cooler periods (14 °C). Therefore, the DNF:(DNF + DNRA) ratio was, in general, lower in the warmer period with the exception of the NR wetland in summer 2016 due to lower DNRA rates. Similar to the potential rates of DNF, there was no significant difference ($r > 0.05$) in potential rates of DNRA among wetlands. The percentage contribution of potential DNRA to total nitrate reduction from slurry samples was 3–47% with most measurements below 35%. Rates of anammox from slurries, which ranged from 0 to 0.01 μmol L slurry^{-1} h^{-1}, were negligible compared to DNF rates, which indicates anammox is not an important process in the studied wetlands. We conducted intact cores and slurry experiments simultaneously in April–May 2015 (autumn) and January–February 2016 (summer). During this period the DNF:(DNF + DNRA) ratio from intact cores ranged from 0.1 to 0.9 (Fig. 1) whereas the ratio from slurry samples ranged from 0.6 to 1 (Fig. 2). DNRA dominated in intact cores, however, the partitioning shifted from DNRA to DNF in slurry samples.

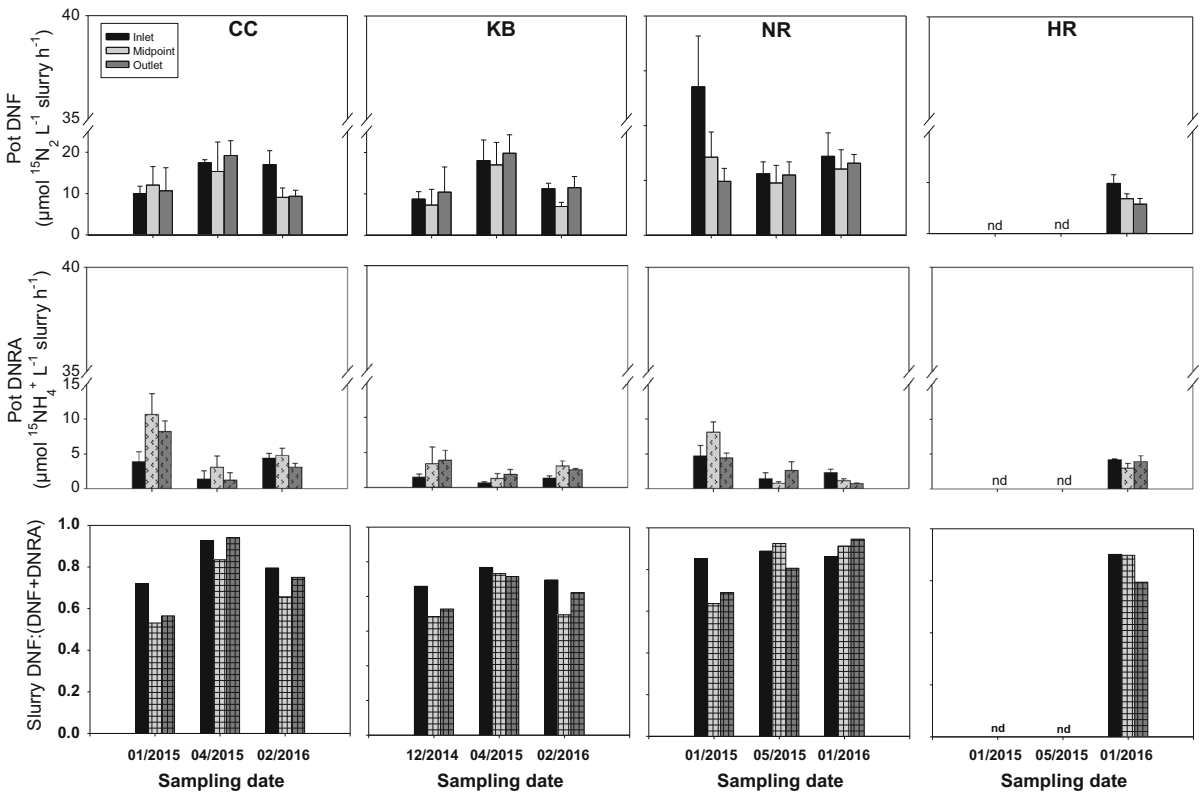

Fig. 2 Potential rates of denitrification, DNRA and DNF:(DNF + DNRA) ratio from slurry samples amended with ^{15}N-NO_3^- from December 2014 to February 2016. Error bars represent ± 1SE and the SE is based on the slope of the time series. nd represents no data for that month

Discussion

Factors controlling denitrification and DNRA

Although there are many studies that have reported both DNF and DNRA from marine environments (An and Gardner 2002; Behrendt et al. 2013; Christensen et al. 2000; Giblin et al. 2013), there are few studies of DNF and DNRA in intact cores from freshwater wetlands (Nogaro and Burgin 2014; Scott et al. 2008). However, the few studies that do exist for freshwater wetlands did not undertake a detailed analysis of the factors governing DNF and DNRA. To unravel the factors controlling DNF, DNRA and the relative importance of DNF and DNRA in the intact core measurements, we used a multiple regression analysis (MRA) approach to investigate which combination of environmental variables control these processes. The advantage of this approach is that it allows identification of important factors influencing DNF and DNRA under environmentally relevant conditions without performing numerous manipulation experiments that are both highly time consuming and are impractical without perturbing the sediment–water interface. The disadvantage is that this approach only uses correlation, not necessarily causation, so any observed relationships should be considered with reference to the literature and ideally followed up with manipulative experiments. We now discuss the best models describing the controlling factors of DNF and DNRA in intact cores.

The MRA showed that DNF for intact cores was positively related to the concentration of NO_3^- in the water column and that this had the highest significance of all the parameters considered (Table 4). This agrees well with the literature. As NO_3^- concentrations increase, more nitrate will be transported into the anoxic zone of the sediment where DNF takes place (Seitzinger 1988). In addition, organic carbon and chl-*a* also contributed to the best fitting models. Given that heterotrophic DNF requires organic carbon to take place, the positive influence of this is to be expected. This finding has importance for constructed wetlands, particularly when they are relatively young and have not had time for organic carbon to accumulate. The weak positive relationship between chl-*a* from benthic algae and DNF contrasts with previous studies that have shown benthic algae has a negative impact on nitrification and DNF (Risgaard-Petersen 2003). Under N replete conditions, however, it has been shown that benthic algae may stimulate nitrification and hence DNF (Risgaard-Petersen et al. 2004).

The MRA showed DNRA from intact cores is negatively related to temperature, with the highest rates being observed at low temperatures (Table 5 and Fig. 3a). This contrasts with many previous studies that have shown higher temperatures favour DNRA over DNF, most likely because this leads to more reducing conditions (Brunet and Garcia-Gil 1996; Burgin and Hamilton 2007; Dong et al. 2011; Gardner and McCarthy 2009; Jørgensen 1989; Ogilvie et al. 1997; Smyth et al. 2013). Under highly reducing conditions, both reduced iron and sulfur enhance DNRA more than DNF (Brunet and Garcia-Gil 1996; Burgin and Hamilton 2007). Kelly-Gerreyn et al. (2001) observed that over a temperature range of 5–22 °C, DNF was the favoured nitrate reduction pathway only in a narrow window of 14 to 17 °C. In contrast, DNRA was the dominant nitrate reduction pathway at temperatures less than 14 °C and greater than 17 °C although both processes occurred at all temperatures due to the adaptive response of different nitrate reducing bacteria to temperature (Kelly-Gerreyn et al. 2001). The temperature for the intact core incubations in this work replicated field conditions of 11 to 28 °C; most of the higher DNRA rates from intact cores lay within the range of 10 to 15 °C. This suggests that the bacteria that carry out DNRA in this study have a relatively low temperature optimum compared to most previous studies.

Interestingly, DNRA was significantly and negatively related to porewater Fe^{2+} concentrations. It has recently been shown that DNRA can be driven by Fe^{2+} oxidation in estuarine sediments and that the addition of Fe^{2+} can stimulate DNRA (Roberts et al. 2014; Robertson et al. 2016). Similarly, accumulation of ammonium was observed through the rapid oxidation of Fe^{2+} after adding nitrate to reduced, acetate depleted sediment suspensions (Coby et al. 2011). The negative relationship observed here suggests that rather than Fe^{2+} controlling the rate of DNRA, the rate of DNRA controls the Fe^{2+} pool size (Fig. 3b). In support of this result, we have observed a strong link between DNRA and Fe^{2+} in slurry samples incubated with Fe^{2+} from the same wetlands (Rahman et al. in review). As for DNF, there was a significant positive relationship between DNRA and chl-*a*. As previously discussed, one explanation for this could be that

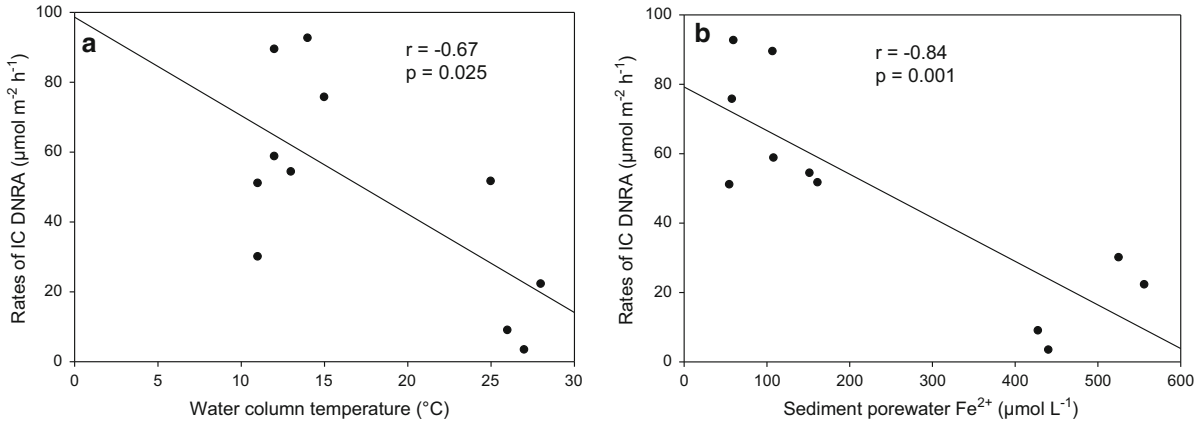

Fig. 3 Relationship between the average (inlet, mid-point and outlet) rates of intact core (IC) DNRA and water column incubation temperature (**a**) and sediment porewater Fe^{2+} concentration (**b**)

benthic algae stimulate nitrification by supplying oxygen and thus, increasing the amount of nitrate to reach the zone of DNRA within the sediment. Alternatively, it is possible that benthic algae assimilate NO_3^- and release it as NH_4^+ after assimilatory nitrate reduction to ammonium (Guerrero et al. 1981; Hewitt 1975), which cannot be excluded from our experiments.

Of fundamental importance to this study are the factors that control the relative importance of DNF and DNRA. We note that the adjusted R^2 of the best model was only 0.23 (Table 6), suggesting that factors we did not measure are important contributors to the variance in the partitioning between the two nitrate reduction pathways. The MRA showed that temperature and NOx concentration were the most important factors describing the variation in the DNF:(DNF + DNRA) ratio, meaning that DNRA increased relative to DNF at low temperatures and low NO_3^- concentrations. The increased rates of DNRA relative to DNF at low nitrate concentrations is consistent with previous studies (Bu et al. 2017; Dong et al. 2011; Silver et al. 2001). Studies in the past demonstrated that nitrate ammonifying bacteria are the most efficient user of nitrate than the denitrifying bacteria under nitrate-limited conditions (Bonin 1996; Nijburg et al. 1997; Tiedje 1988). This is because nitrate ammonifying bacteria transfer eight electrons during DNRA while denitrifying bacteria transfer only five electrons during DNF (Holmes et al. 1996; King and Nedwell 1985; Martin et al. 2001; Pattinson et al. 1998).

In Table 7, we have provided a comparison between DNF and DNRA rates with NOx concentrations measured in this study and from other freshwater wetlands, lakes and estuaries. Rates of DNF and DNRA and their relative proportion in this study are within the ranges observed from similar ecosystems (Table 7). However, the DNF:(DNF + DNRA) ratio in this study is comparatively lower than others in Table 7 owing to higher DNRA rates measured in this study. The greater partitioning toward DNRA over DNF, as discussed previously, is likely due to low NO_3^- concentrations, with similar observations made in Little Lagoon. Rates of DNF in the Little Lagoon were seasonally lower compared to DNRA mainly due to sulfide inhibiting nitrification and thus, limiting the supply of NO_3^- for DNF (Bernard et al. 2015). In contrast, due to less sulfidic conditions and stimulation by newly fixed N in freshwater sediments, DNF was the dominant dissimilatory nitrate reduction pathways in the Lake Waco freshwater wetland (Scott et al. 2008). Within 3 tropical estuaries, benthic nitrate reduction was nitrate limited, however, the increase in DNRA was more than DNF with increasing nitrate availability (Dong et al. 2011). Of all studies compared in Table 7, the constructed freshwater lake in south-eastern Poland showed the highest rate of DNF which were higher by an order of magnitude compared to DNRA rates in the same study. In that study, temperature and sediment organic matter content controlled the partitioning between DNF and DNRA. The DNF:DNRA ratio decreased suggesting that rates of DNRA increased with increasing temperature and

Table 7 Rates of denitrification and DNRA with NOx concentrations reported globally from wetlands, freshwater lakes, and estuarine intact core sediments

Study sites	Concentrations of NOx (μM)	Denitrification (μmol m^{-2} h^{-1})	DNRA (μmol m^{-2} h^{-1})	Denitrification:DNRA	References
Huntingdale Road (HR) Wetland	0.2–32	17–44	4–85	0.4–5	This study
Namatjira Reserve (NR) Wetland	0.2–53	3–76	2–118	0.5–6	This study
Cascades on Clyde (CC) Wetland	0.2–147	11–198	3–136	0.2–16	This study
Koolamara Blvd (KB) Wetland	1–50	9–83	13–82	0.1–3	This study
Lake Waco Wetland*	0.3–37	54–278	1.3–33	2–68	Scott et al. (2008)
Freshwater lakes	1–259**	31–171	2.9–5	11–42	Nizzoli et al. (2010)
Constructed freshwater lake	51–171	26–610	0.2–41	8–101	Gruca-Rokosz et al. (2009)
Colne Estuary	–	3–394	3–319	1–2	(Dong et al. 2009)
Tropical estuaries	0.3–312	0–103	0–1137	0–10	Dong et al. (2011)
Little Lagoon*	0.5–1.2	0–55	0–237	0–18	Bernard et al. (2015)

**NO_3^- concentrations

*Rates of $^{15}NO_3^-$ denitrification (D_{15}) and DNRA ($DNRA_{15}$)

organic matter content (Gruca-Rokosz et al. 2009). In a recent study, it was observed that the contribution of DNF to total nitrate reduction in groundwater flowing underneath an integrated constructed wetland was only 14–16% whereas DNRA contributed 40–63%. In that study, the higher contribution of DNRA over DNF was attributed to high carbon content and high TC:NO_3^--N ratio (Jahangir et al. 2017).

Implications for management

This study showed that DNF is likely to be favoured over DNRA at higher nitrate concentrations as a larger amount of NO_3^- is removed by DNF relative to DNRA at higher NO_3^- concentrations. At low nitrate concentrations (mean: 23 μmol L^{-1}), DNRA often exceeded DNF in these four urban stormwater wetlands. As such, the high DNRA rates observed here do not imply a wetland malfunction but highlights a dynamic shift in nitrogen processing pathways in response to nitrogen loading. The timescale of this shift, however, remains unknown and further research is required to investigate this given the highly dynamic nature of nitrate concentrations in wetlands. In addition to increased nitrate availability, high organic carbon loading may enhance DNF and hence higher removal of nitrate from these urban wetlands. Also of key relevance to management, was the finding that DNRA increased at lower temperatures relative to DNF. This implies that increasing temperatures from global warming and the urban heat island affect will not negatively affect the nitrate removal capability of the urban stormwater wetlands studied here.

Conclusions

DNRA was a significant nitrate reduction pathway in four constructed stormwater urban wetlands in this study. DNRA rates measured under environmental conditions (intact cores) were consistently high throughout the sampling period and particularly high under low temperature conditions (Table 5). In contrast to some previous studies, the majority of the higher DNRA rates were observed within the range of 10 to 15 °C in intact cores, suggesting that nitrate

ammonifying bacteria prefer this temperature range. MRA showed a negative relationship between iron and DNRA in intact cores, indicating that DNRA, by consuming the porewater Fe^{2+}, may control the Fe^{2+} pool size. As shown in the MRA, DNF was the dominant nitrate reduction pathway in intact cores when the nitrate concentrations were highest, indicating when there are periods of high NO_3^-, such as a rain event, DNF will be a significant removal pathway. This is consistent with the DNF rates measured in slurries, where DNF outcompeted DNRA when it was not limited by the supply of NO_3^-. Moreover, the MRA showed that the ratio of DNF to DNRA was most strongly associated with temperature and nitrate concentrations suggesting DNRA was favoured over DNF at low temperatures and low nitrate concentrations. As such, there is increasing permanent nitrogen removal as nitrate concentrations increase.

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Appendix

See Table 8.

Table 8 Physico-chemical parameters in the four study wetlands observed from December–January 2014–2015 to January–February 2016

Sampling location	Sampling dates	Sites	Temp (°C)	pH	DO (mg L^{-1})	%DO saturation	Conductivity (mS cm^{-1})	Turbidity (NTU)
Koolamara Blvd (KB)	Dec 2014	In	18.8	6.8	3.0	32.2	0.3	7.0
		M-P	19.2	6.9	5.3	57.3	0.2	17.0
		Out	17.8	6.9	3.6	37.9	0.2	1.0
	April 2015	In	12.4	6.5	9.2	86.1	0.2	5.3
		M-P	13.5	6.9	9.0	86.3	0.2	2.3
		Out	12.4	7.4	11.1	103.9	0.2	1.3
	Aug 2015	In	10.9	6.8	8.1	73.3	0.8	8.4
		M-P	11.7	7.1	7.7	70.9	0.6	4.5
		Out	10.6	7.0	4.9	44.0	0.6	44.0
	Feb 2016	In	22.7	6.5	6.4	74.2	0.4	16.7
		M-P	26.0	6.9	12.1	149.2	0.2	2.9
		Out	26.1	7.0	6.7	82.8	0.2	15.0
Namatjira Reserve (NR)	Jan 2015	In	22.1	6.8	2.3	26.4	0.2	8.0
		M-P	22.9	6.8	4.6	53.5	0.1	32.0
		Out	22.7	6.9	7.8	90.4	0.1	29.0
	May 2015	In	16.1	6.6	5.8	58.9	0.2	3.1
		M-P	15.0	6.9	4.7	46.6	0.1	15.1
		Out	15.2	7.0	6.5	64.7	0.1	47.0
	Sep 2015	In	12.4	7.0	9.0	84.2	0.1	18.9
		M-P	12.6	7.1	7.0	65.8	0.2	29.4
		Out	12.7	7.2	9.1	85.8	0.2	19.0
	Jan 2016	In	27.8	8.3	10.3	131.2	0.3	21.0
		M-P	26.5	7.4	14.9	185.4	0.3	27.0
		Out	28.8	75	14.1	182.8	0.2	19.5

Table 8 continued

Sampling location	Sampling dates	Sites	Temp (°C)	pH	DO (mg L^{-1})	%DO saturation	Conductivity (mS cm^{-1})	Turbidity (NTU)
Cascades on Clyde (CC)	Jan 2015	In	20.8	7.4	3.8	42.5	0.6	79.0
		M-P	22.6	7.8	5.6	64.8	0.5	51.0
		Out	20.6	7.1	4.0	44.5	0.6	34.0
	April 2015	In	13.6	7.3	9.6	92.3	0.5	55.9
		M-P	15.0	7.6	8.5	84.3	0.4	6.8
		Out	15.4	7.6	9.4	94.0	0.4	6.0
	Sep 2015	In	12.1	7.1	7.2	66.9	0.7	88.0
		M-P	12.3	7.4	5.8	54.2	0.3	162.0
		Out	12.9	7.3	7.3	69.1	0.3	139.0
	Feb 2016	In	26.0	8.4	12.8	157.8	0.6	35.2
		M-P	28.4	8.7	8.2	105.5	0.3	19.2
		Out	29.8	9.1	17.8	234.8	0.3	14.2
Huntingdale Road (HR)	Aug 2015	In	12.9	7.5	11.6	109.8	0.3	31.7
		M-P	11.0	7.2	7.2	65.3	0.3	51.8
		Out	10.7	6.7	7.1	63.9	0.3	23.4
	Jan 2016	In	27.7	7.3	8.8	111.9	05	80.4
		M-P	26.1	7.1	6.3	77.8	0.5	41.5
		Out	25.5	6.7	5.8	70.9	0.5	26.0

In for inlet; M-P for mid-point and Out for outlet

References

American Public Health Association (2005) Standard methods for the examination of water and wastewater. American Public Health Association (APHA), Washington, DC

An S, Gardner WS (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). Mar Ecol Prog Ser 237:41–50

Bachand PAM, Horne AJ (1999) Denitrification in constructed free-water surface wetlands: II. Effects of vegetation and temperature. Ecol Eng 14:17–32

Behrendt A, de Beer D, Stief P (2013) Vertical activity distribution of dissimilatory nitrate reduction in coastal marine sediments. Biogeosciences 10:7509–7523

Benz M, Brune A, Schink B (1998) Anaerobic and aerobic oxidation of ferrous iron at neutral pH by chemoheterotrophic nitrate-reducing bacteria. Arch Microbiol 169:159–165

Bernard RJ, Mortazavi B, Kleinhuizen AA (2015) Dissimilatory nitrate reduction to ammonium (DNRA) seasonally dominates NO_3^- reduction pathways in an anthropogenically impacted sub-tropical coastal lagoon. Biogeochemistry 125:47–64

Bonin P (1996) Anaerobic nitrate reduction to ammonium in two strains isolated from coastal marine sediment: a dissimilatory pathway. FEMS Microbiol Ecol 19:27–38

Brunet RC, Garcia-Gil LJ (1996) Sulfide-induced dissimilatory nitrate reduction to ammonia in anaerobic freshwater sediments. FEMS Microbiol Ecol 21:131–138

Bu C, Wang Y, Ge C, Ahmad HA, Gao B, Ni S-Q (2017) Dissimilatory nitrate reduction to ammonium in the Yellow River Estuary: rates, abundance, and community diversity. Sci Rep 7:6830

Burgin AJ, Hamilton SK (2007) Have we overemphasized the role of denitrification in aquatic ecosystems? A review of nitrate removal pathways. Front Ecol Environ 5:89–96

Christensen PB, Rysgaard S, Sloth NP, Dalsgaard T, Schwærter S (2000) Sediment mineralization, nutrient fluxes, denitrification and dissimilatory nitrate reduction to ammonium in an estuarine fjord with sea cage trout farms. Aquat Microb Ecol 21:73–84

Coby AJ, Picardal F, Shelobolina E, Xu H, Roden EE (2011) Repeated anaerobic microbial redox cycling of iron. Appl Environ Microbiol 77:6036–6042

Dalsgaard T, Nielsen LP, Brotas V, Viaroli P, Underwood G, Nedwell D, Sundbäck K, Rysgaard S, Miles A, Bartoli M (2000) Protocol handbook for NICE-Nitrogen Cycling in Estuaries: a project under the EU research programme: Marine Science and Technology (MAST III). National Environmental Research Institute, Silkeborg

Dalsgaard T, Thamdrup B, Farías L, Revsbech NP (2012) Anammox and denitrification in the oxygen minimum zone of the eastern South Pacific. Limnol Oceanogr 57:1331–1346

Dong LF, Smith CJ, Papaspyrou S, Stott A, Osborn AM, Nedwell DB (2009) Changes in benthic denitrification, nitrate ammonification, and anammox process rates and nitrate and nitrite reductase gene abundances along an estuarine nutrient gradient (the Colne Estuary, United Kingdom). Appl Environ Microbiol 75:3171–3179

Dong LF, Sobey MN, Smith CJ, Rusmana I, Phillips W, Stott A, Osborn AM, Nedwell DB (2011) Dissimilatory reduction of nitrate to ammonium, not denitrification or anammox, dominates benthic nitrate reduction in tropical estuaries. Limnol Oceanogr 56:279–291

Fonselius S, Dyrssen D, Yhlen B (2007) Determination of hydrogen sulphide. Methods of seawater analysis. Wiley, Weinheim, pp 91–100

Gardner W, McCarthy M (2009) Nitrogen dynamics at the sediment–water interface in shallow, sub-tropical Florida Bay: why denitrification efficiency may decrease with increased eutrophication. Biogeochemistry 95:185–198

Gavlak R, Horneck D, Miller RO, Kotuby-Amacher J (2003) Soil, plant and water reference methods for the western region. WCC-103 Publication, Fort Collins

Giblin AE, Tobias CR, Song B, Weston N, Banta GT, Rivera-Monroy VH (2013) The importance of dissimilatory nitrate reduction to ammonium (DNRA) in the nitrogen cycle of coastal ecosystems. Oceanography 26:124–131

Gold A, Thompson S, Piehler M (2017) Water quality before and after watershed-scale implementation of stormwater wet ponds in the coastal plain. Ecol Eng 105:240–251

Gruca-Rokosz R, Tomaszek JA, Koszelnik P (2009) Competitiveness of dissimilatory nitrate reduction processes in bottom sediment of Rzeszów reservoir. Environ Prot Eng 35(2):5–13

Guerrero MG, Vega JM, Losada M (1981) The assimilatory nitrate-reducing system and its regulation. Ann Rev Plant Physiol 32:169–204

Hansen AT, Dolph CL, Finlay JC (2016) Do wetlands enhance downstream denitrification in agricultural landscapes? Ecosphere 7:e01516

Hewitt EJ (1975) Assimilatory nitrate–nitrite reduction. Ann Rev Plant Physiol 26:73–100

Holmes RM, Jones JB Jr, Fisher SG, Grimm NB (1996) Denitrification in a nitrogen-limited stream ecosystem. Biogeochemistry 33:125–146

Hunt PG, Matheny TA, Szogi AA (2003) Denitrification in constructed wetlands used for treatment of swine wastewater. J Environ Qual 32:727–735

Jahangir MMR, Fenton O, Müller C, Harrington R, Johnston P, Richards KG (2017) In situ denitrification and DNRA rates in groundwater beneath an integrated constructed wetland. Water Res 111:254–264

Jones ZL, Jasper JT, Sedlak DL, Sharp JO (2017) Sulfide-induced dissimilatory nitrate reduction to ammonium supports anammox in an open-water unit process wetland. Appl Environ Microbiol 83:e00782-17

Jørgensen KS (1989) Annual pattern of denitrification and nitrate ammonification in estuarine sediment. Appl Environ Microbiol 55:1841–1847

Kadlec R, Wallace S (2009) Treatment wetlands. CRC Press, Boca Raton

Kartal B, Kuypers MM, Lavik G, Schalk J, Op den Camp HJ, Jetten MS, Strous M (2007) Anammox bacteria disguised as denitrifiers: nitrate reduction to dinitrogen gas via nitrite and ammonium. Environ Microbiol 9:635–642

Kelly-Gerreyn BA, Trimmer M, Hydes DJ (2001) A diagenetic model discriminating denitrification and dissimilatory nitrate reduction to ammonium in a temperate estuarine sediment. Mar Ecol Prog Ser 220:33–46

King D, Nedwell DB (1984) Changes in the nitrate-reducing community of an anaerobic saltmarsh sediment in response to seasonal selection by temperature. Microbiology 130:2935–2941

King D, Nedwell DB (1985) The influence of nitrate concentration upon the end-products of nitrate dissimilation by bacteria in anaerobic salt marsh sediment. FEMS Microbiol Ecol 1:23–28

Koch BJ, Febria CM, Gevrey M, Wainger LA, Palmer MA (2014) Nitrogen removal by stormwater management structures: a data synthesis. JAWRA 50:1594–1607

Liu X, Han J-G, Ma Z-W, Wang Q, Li L-H (2016) Effect of carbon source on dissimilatory nitrate reduction to ammonium in Costal Wetland sediments. J Soil Sci Plant Nutr 16:337–349

Martin LA, Mulholland PJ, Webster JR, Valett HM (2001) Denitrification potential in sediments of headwater streams in the southern Appalachian Mountains, USA. J N Am Benthol Soc 20:505–519

Meyer RL, Risgaard-Petersen N, Allen DE (2005) Correlation between anammox activity and microscale distribution of nitrite in a subtropical mangrove sediment. Appl Environ Microbiol 71:6142–6149

Nielsen LP (1992) Denitrification in sediment determined from nitrogen isotope pairing. FEMS Microbiol Lett 86:357–362

Nijburg JW, Coolen M, Gerards S, Gunnewiek P, Laanbroek HJ (1997) Effects of nitrate availability and the presence of glyceria maxima on the composition and activity of the dissimilatory nitrate-reducing bacterial community. Appl Environ Microbiol 63:931–937

Nizzoli D, Carraro E, Nigro V, Viaroli P (2010) Effect of organic enrichment and thermal regime on denitrification and dissimilatory nitrate reduction to ammonium (DNRA) in hypolimnetic sediments of two lowland lakes. Water Res 44:2715–2724

Nogaro G, Burgin AJ (2014) Influence of bioturbation on denitrification and dissimilatory nitrate reduction to ammonium (DNRA) in freshwater sediments. Biogeochemistry 120:279–294

Ogilvie BG, Rutter M, Nedwell DB (1997) Selection by temperature of nitrate-reducing bacteria from estuarine sediments: species composition and competition for nitrate. FEMS Microbiol Ecol 23:11–22

Otte S, Kuenen JG, Nielsen LP, Paerl HW, Zopfi J, Schulz HN, Teske A, Strotmann B, Gallardo VA, Jørgensen BB (1999) Nitrogen, carbon, and sulfur metabolism in natural thioploca samples. Appl Environ Microbiol 65:3148–3157

Pattinson SN, Garcia-Ruiz R, Whitton BA (1998) Spatial and seasonal variation in denitrification in the Swale-Ouse system, a river continuum. Sci Total Environ 210:289–305

Payne EGI, Pham T, Cook PLM, Fletcher TD, Hatt BE, Deletic A (2014) Biofilter design for effective nitrogen removal from stormwater: influence of plant species, inflow

hydrology and use of a saturated zone. Water Sci Technol 69:1312–1319
Poe AC, Piehler MF, Thompson SP, Paerl HW (2003) Denitrification in a constructed wetland receiving agricultural runoff. Wetlands 23:817–826
Risgaard-Petersen N (2003) Coupled nitrification-denitrification in autotrophic and heterotrophic estuarine sediments: on the influence of benthic microalgae. Limnol Oceanogr 48:93–105
Risgaard-Petersen N, Revsbech NP, Rysgaard S (1995) Combined microdiffusion-hypobromite oxidation method for determining nitrogen-15 isotope in ammonium. Soil Sci Soc Am J 59:1077–1080
Risgaard-Petersen N, Meyer RL, Schmid M, Jetten MS, Enrich-Prast A, Rysgaard S, Revsbech NP (2004) Anaerobic ammonium oxidation in an estuarine sediment. Aquat Microbial Ecol 36:293–304
Roberts KL, Kessler AJ, Grace MR, Cook PLM (2014) Increased rates of dissimilatory nitrate reduction to ammonium (DNRA) under oxic conditions in a periodically hypoxic estuary. Geochim Cosmochim Acta 133:313–324
Robertson EK, Thamdrup B (2017) The fate of nitrogen is linked to iron(II) availability in a freshwater lake sediment. Geochim Cosmochim Acta 205:84–99
Robertson EK, Roberts KL, Burdorf LD, Cook P, Thamdrup B (2016) Dissimilatory nitrate reduction to ammonium coupled to Fe(II) oxidation in sediments of a periodically hypoxic estuary. Limnol Oceanogr 61:365–381
Rosenzweig BR, Smith JA, Baeck ML, Jaffé PR (2011) Monitoring nitrogen loading and retention in an urban stormwater detention pond. J Environ Qual 40:598–609
Rysgaard S, Glud RN, Risgaard-Petersen N, Dalsgaard T (2004) Denitrification and anammox activity in arctic marine sediments. Limnol Oceanogr 49:1493–1502
Schulz HN, Jørgensen BB (2001) Big bacteria. Ann Rev Microbiol 55:105–137
Scott JT, McCarthy MJ, Gardner WS, Doyle RD (2008) Denitrification, dissimilatory nitrate reduction to ammonium, and nitrogen fixation along a nitrate concentration gradient in a created freshwater wetland. Biogeochemistry 87:99–111
Seitzinger SP (1988) Denitrification in freshwater and coastal marine ecosystems: ecological and geochemical significance. Limnol Oceanogr 33:702–724
Silver WL, Herman DJ, Firestone MK (2001) Dissimilatory nitrate reduction to ammonium in upland tropical forest soils. Ecology 82:2410–2416
Smyth AR, Thompson SP, Siporin KN, Gardner WS, McCarthy MJ, Piehler MF (2013) Assessing nitrogen dynamics throughout the estuarine landscape. Estuaries Coasts 36:44–55
Sørensen J (1978) Denitrification rates in a marine sediment as measured by the acetylene inhibition technique. Appl Environ Microbiol 36:139–143
Stookey LL (1970) Ferrozine—a new spectrophotometric reagent for iron. Anal Chem 42:779–781
Straub KL, Buchholz-Cleven BEE (1998) Enumeration and detection of anaerobic ferrous iron-oxidizing, nitrate-reducing bacteria from diverse european sediments. Appl Environ Microbiol 64:4846–4856
Straub KL, Benz M, Schink B, Widdel F (1996) Anaerobic, nitrate-dependent microbial oxidation of ferrous iron. Appl Environ Microbiol 62:1458–1460
Sun M, Aller RC, Lee C (1991) Early diagenesis of chlorophyll-a in Long Island Sound sediments: a measure of carbon flux and particle reworking. J Mar Res 49:379–401
Thamdrup B, Dalsgaard T (2002) Production of N2 through anaerobic ammonium oxidation coupled to nitrate reduction in marine sediments. Appl Environ Microbiol 68:1312–1318
Tiedje JM (1988) Ecology of denitrification and dissimilatory nitrate reduction to ammonium. Biol Anaerob Microorg 717:179–244
van den Berg EM, Boleij M, Kuenen JG, Kleerebezem R, van Loosdrecht MCM (2016) DNRA and denitrification coexist over a broad range of acetate/N-NO(3)(−) ratios, in a chemostat enrichment culture. Front Microbiol 7:1842
Viollier E, Inglett PW, Hunter K, Roychoudhury AN, Van Cappellen P (2000) The ferrozine method revisited: Fe(II)/Fe(III) determination in natural waters. Appl Geochem 15:785–790
Wagenmakers E-J, Farrell S (2004) AIC model selection using Akaike weights. Psychon Bull Rev 11:192–196
Weber KA, Picardal FW, Roden EE (2001) Microbially catalyzed nitrate-dependent oxidation of biogenic solid-phase Fe(II) compounds. Environ Sci Technol 35:1644–1650
Weber KA, Pollock J, Cole KA, O'Connor SM, Achenbach LA, Coates JD (2006a) Anaerobic nitrate-dependent iron(II) bio-oxidation by a novel lithoautotrophic betaproteobacterium, strain 2002. Appl Environ Microbiol 72:686–694
Weber KA, Urrutia MM, Churchill PF, Kukkadapu RK, Roden EE (2006b) Anaerobic redox cycling of iron by freshwater sediment microorganisms. Environ Microbiol 8:100–113
Yoon S, Cruz-Garcia C, Sanford R, Ritalahti KM, Loffler FE (2015) Denitrification versus respiratory ammonification: environmental controls of two competing dissimilatory NO_3^-/NO_2^- reduction pathways in *Shewanella loihica* strain PV-4. ISME J 9:1093–1104