Monitoring nitrogen processing in constructed wetlands: two stable isotope approaches.

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Abstract
The increased pressure of anthropogenic nitrogen inputs on our waterways has increased the importance of nitrogen management strategies such as constructed wetlands. Several studies have assessed the effectiveness of wetland operation by examining wetland components in the design process, however, long term management of operational wetlands is often reliant on monitoring only nutrient concentrations. Stable isotopes of nitrogen can be a useful tool in investigating nitrogen processing and are often applied to detailed mechanistic studies but rarely utilised in ongoing monitoring strategies. Here we present a conceptual model for two stable isotope approaches, one quantitative and one qualitative, to assess their feasibility as management tools to improve our current understanding of nitrogen removal over the lifetime of a constructed wetland system. The first approach is the direct measurement of NO_3^- reduction pathways (denitrification) to assess nitrogen removal whilst the second approach uses the dual isotopic composition ($\delta^{15}N$ and $\delta^{18}O$) of NO_3^- at the natural abundance level in the surface water to qualitatively assess denitrification and assimilation. We propose the natural abundance stable isotope approach, with further research, would complement current monitoring programs providing further information on the behaviour of nitrogen in wetlands.

Key Words
Denitrification, assimilation, stable isotope, constructed wetlands.

Introduction
Synthetically derived nitrogen e.g. from the Haber-Bosch process, and an increase in impervious surfaces has led to a rapid increase in the amount of nitrogen transported into rivers and coastal waters (Brabec et al 2002; Bernhardt et al 2008). Constructed wetlands are commonly used in the urban landscape to mitigate nitrogen loads in storm water runoff by increasing the hydraulic residence time allowing for removal through natural processes (Lee et al 2009). The main processes that aid in the removal of 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), however long term removal efficiencies are often still less than 50 % (Taylor et al 2005; Lee et al 2009).

Constructed wetlands are highly engineered systems with several studies examining vegetation types, hydraulic residence time, temperature, water depth, oxygen concentration and sediment conditions in the design process (Brix 1993; Persson et al 1999; Bachand et al 2000). However, monitoring of these systems after construction is based largely on nutrient concentrations in the initial establishment phase (2-5 years after construction) and the change in removal efficiency throughout the lifetime of the wetland is often ignored (Wetzel 2001; Farrell and Scheckenberger, 2003). Monitoring using concentrations from the inlet to the outlet allows for removal efficiencies to be calculated but there is little or no information on the important processes involved in this removal and in turn where management should be focused.

Stable isotopes are a useful tool in determining how nutrients are transformed within a system. For example, the isotope pairing technique can determine rates of denitrification and therefore nitrogen removal (Nielsen 1992) whilst natural abundance stable isotopes can be used qualitatively to determine what processes are important, for example denitrification and/or assimilation, and therefore provide a more targeted management approach. Here we provide a conceptual model for two stable isotope methods that could provide further information on the removal capacity of a wetland to assist in long term management of these systems.

Methods

Approach One – Isotope pairing technique

Rates of nitrate reduction pathways such as denitrification and dissimilatory nitrate reduction to ammonium (DNRA) can be determined through the ^{15}N-stable isotope pairing technique (Nielsen 1992). Combining the nitrate reduction rates with sediment-water fluxes of nutrients enables more robust estimates of nutrient budgets for a system. Sediment cores were collected from the inlet, midpoint and outlet of the wetland and maintained at *in situ* conditions until nutrient fluxes and rates of nitrate reduction pathways were measured (for specific method details see Roberts et al 2012). On the first day after collection, sediment-water fluxes of NO_3^-, NH_4^+, O_2 and filterable reactive phosphorus (FRP) are determined over an incubation period of ~8 hours. After the incubation, the overlying water in the sediment cores is re-circulated with new site water at *in situ* conditions. On day two, ^{15}N-NO_3^- is added to the overlying water and the sediment core sealed throughout the incubation period; denitrification and DNRA are determined from the accumulation of ^{15}N-N_2 and ^{15}N-NH_4^+ over time, respectively. The stable isotope samples are analysed on an isotope ratio mass spectrometer couple to a gas chromatograph. The isotope pairing technique is based on a number of assumptions outlined in Risgaard-Petersen et al (2003).

Approach Two – Dual isotopic composition of nitrate

The dual isotopic composition of nitrate at the natural abundance level can be used to determine which key processes are transforming nitrate within a system. During assimilation or denitrification, for example, the lighter isotope is energetically more favourable and therefore used preferentially, leading to a predictable pattern in isotope enrichment of the residual (unreacted) pool of nitrate. Common literature examples of enrichment factors (ε) are given for processes such as assimilation (ε -30 to 0‰ $\delta^{15}N$) and benthic denitrification (ε -4 to -1‰ $\delta^{15}N$; Michener and Lajtha 2007), however for this method to be feasible, specific enrichment factors for wetland components need to be determined (preliminary estimates for Melbourne wetlands are periphyton -25‰, algae -16‰ and bare sediment -2 ‰, Roberts et al unpubl.).

To assess the isotopic composition of NO_3^-, a 125mL filtered (0.2µm) water sample was collected from the inlet, midpoint and outlet. The samples were pre-treated with sulfamic acid to remove residual NO_2^- (Granger and Sigman 2009). The NO_3^- was then converted to NO_2^- through a cadmium reduction step and then to N_2O in a final 1:1 azide:acetic acid step (McIlvin and Altabet 2005). Alternatively, samples of NO_3^- can be converted to N_2O using the denitrifier method described in Sigman et al (2001). The samples for $\delta^{15}N$ and $\delta^{18}O$ of N_2O are then analysed on an isotope ratio mass spectrometer coupled to a gas chromatograph fitted to a cyroprep system cooled using liquid N_2. International isotope standards of nitrate IAEA, USGS 35, USGS 34 are used to adjust the N_2O isotope values to the actual $\delta^{15}N$ and $\delta^{18}O$ of NO_3^-.

Results and Discussion

To explain how the two stable isotope approaches could be utilised in a wetland monitoring program, we have developed a conceptual model (Figure 1) which will be referred to throughout the discussion. A number of studies have examined denitrification and assimilation in wetlands using the presented approaches (Vymazal 2007, Scott et al 2008, Matheson et al 2010). However few studies have looked at applying these principles to a wetland monitoring program. The addition of labelled isotopes (approach one) to sediment cores or mesocosm experiments directly traces the fate of the nitrate through a system (Figure 1) providing a detailed examination of the nitrogen cycle. Rates determined from these measurements clarify how NO_3^- consumption is partitioned within the wetland, for example, removal via denitrification or temporary storage via assimilation. This approach, however, is labour intensive and would result in a significant expense in the initial set up phase and ongoing cost of experimentation and analysis (Table 1). The isotope pairing technique in addition to sediment water fluxes would be particularly useful when the functionality of a wetland is in dispute, but unlikely to be financially feasible in a monitoring program with many wetlands.

The second approach using natural abundance stables isotopes of nitrate in the surface water is a low effort alternative to the isotope pairing technique (Table 1). A nutrient sample collected from the inlet and outlet in a normal monitoring program could be used to determine the dual isotopic composition ($\delta^{15}N$ and $\delta^{18}O$) of NO_3^-. A number of studies have used the dual isotopic composition of nitrate to examine source inputs into aquatic systems, however, few have used this approach to specifically examine nitrogen processing in constructed wetlands (Søvik and Mørkved, 2008; Lund et al 1999, Itoh et al 2010). Søvik and Mørkved (2008) showed the use of $\delta^{15}N$-NO_3^- was a valuable tool in verifying the presence of denitrification, however this study also highlighted the importance of understanding the enrichment effect of other processes such as assimilation and nitrification. To apply this method to a monitoring program, a known range of enrichment factors for denitrification and assimilation in wetlands would need to be determined and the efficacy of this method tested across a broad range of systems before implementation. However, in the future this approach could provide a valuable qualitative method for further understanding wetland function.

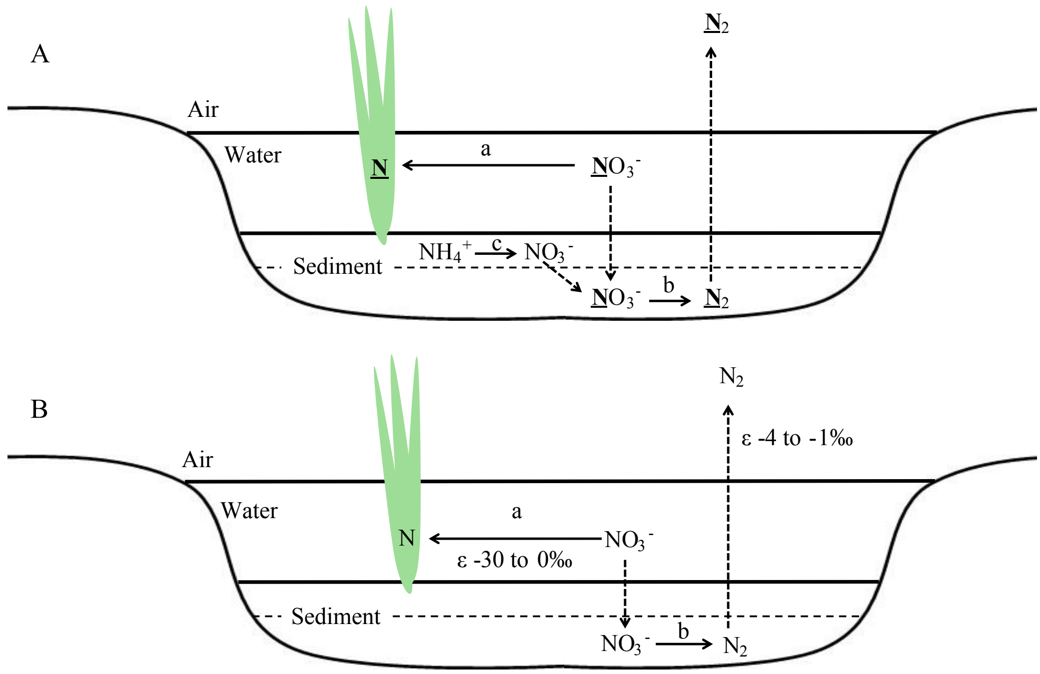

Figure 1 A) Approach One – ^{15}N-labelled NO_3^- used to measure the rate of plant uptake (a), denitrification (b) and the portion of denitrification driven by nitrification (c). The N represents the ^{15}N labelled nitrogen moving through the system; and B) Approach Two – dual isotopic composition (δ^{15}N and δ^{18}O) of NO_3^- with the expected fractionation factors (ε) in the residual NO_3^- pool of -4 to -1‰ for benthic denitrification and -30 to 0‰ for assimilation. Dashed arrows represent diffusion whilst solid arrows represent a process. The sediment is separated by a dotted line representing the oxygen penetration depth.

Table 1: Outline of the requirements for the two stable isotope approaches

	Approach 1 Isotope Pairing Technique	Approach 2 Dual Isotopic Composition of NO3-
Initial Set Up	Sediment core incubation set up	Research to develop initial database of expected wetland fractionation factors for denitrification and assimilation
Sampling Effort	Intensive – intact sediment and water	Minimal – filtered water sample
Experimental Effort	Intensive – two full lab days	NA
Sample preparation for analysis	Medium effort – headspace gas sample, extract and convert NH_4^+ samples	Medium effort – conversion of NO_3^- to N_2O (2 days) – outsourced at cost.
Analysis Costs	~$20 per sample and $100 per rate measurement	~$40 per sample only ~$70 – 100 per sample including analysis/ conversion step
Specific Site Requirements	NA	Defined inlet and outlet
Implementation into monitoring	Difficult – training, equipment requirements	Easy – additional filtered water sample, no training required.

Conclusion

Both approaches provide valuable information on nitrogen processing in wetlands, however, at present neither approach is suitable for ongoing monitoring. We do not recommend approach one for ongoing monitoring due to cost and time considerations (Table 1), however, it could be used in a scenario where more detailed information on nitrogen behavior is required, for example, when a wetland is not functioning as expected. Whilst approach two requires some further research to develop a working range of expected enrichment factors applicable to the wetlands in question, the ease of implementation into an existing monitoring program and the relatively low cost of sample analysis (Table 1) makes the dual isotopic composition of NO_3^- at the natural abundance level a feasible option in future. Stable isotopes could be a useful tool in wetland monitoring complementing existing programs by providing some insight into nitrogen processing allowing for more targeted management of constructed wetlands over the lifetime of the system.

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