Plant carbohydrate limitation on nitrate reduction in wetland microcosms

Noah P. Hume*, Maia S. Fleming, Alexander J. Horne

Ecological Engineering Group, Department of Civil & Environmental Engineering, University of California at Berkeley, Berkeley, CA 94720-1710, USA

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Abstract

Although nitrate limitation in most natural wetlands results in pseudo-first-order reductions, large site-to-site variations in apparent denitrification rates cannot be easily explained by water quality (e.g., pH, Temp, DOC) or plant productivity. Our microcosm results show increasing nitrate removal efficiencies at higher ratios of total applied plant carbon to nitrate reduced, suggesting that denitrification rates may be limited by the rates of supply of both electron donor or acceptor, described by an applied carbon to nitrate (C_{App}:N_{Red}) ratio. However, the observed first-order rate constants varied more strongly ($r^2 = 0.77, p < 0.0001$) with the acid-soluble carbohydrates to nitrate (CH$_2$O_{App}:N_{Red}) ratio than the total C_{App}:N_{Red} ratio. Although observed rate constants for bulrush (Scirpus sp.) were significantly lower ($0.01 < p < 0.06$) than for other plant sources (Hydrocotyle sp., Lemna sp., Typha sp.), there were no significant differences in the plant-specific rate constants when compared at the same CH$_2$O_{App}:N_{Red} ratio. In full-scale wetlands, this suggests that either high plant productivity or low NO$_3^{-}$/C$_0$ loading (high CH$_2$O_{App}:N_{Red}) may contribute to a high effective denitrification rate. In contrast, low productivity or a high NO$_3^{-}$ loading (low CH$_2$O_{App}:N_{Red}) would promote a lower denitrification rate. This co-limitation between plant carbon and nitrate may confound first-order rate comparisons in full scale denitrification wetlands since highly N-loaded systems may become carbon limited, requiring higher order reaction kinetics to better describe performance variations. In addition to hydraulic residence time, temperature and nitrate removal data, extending these results to compare large wetlands will require estimation of the CH$_2$O_{App}:N_{Red} ratio from an inventory of plant species, productivity estimates and carbon quality. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Carbon quality; Denitrification; Kinetics; Plants; Wetland

1. Introduction

Respiratory denitrification, the bacterially mediated electron transfer between highly reduced natural organic matter (NOM) and electron poor nitrogen oxides (NO$_3^{-}$, NO$_2^{-}$), yields only slightly less free energy than the transfer between the carbon/oxygen couple [1]. Early investigations of denitrification in agricultural soils centered upon preventing the unintended loss of valuable fertilizer [2]. More recent applied research has focused upon ameliorating the historical nitrate contamination of surface waters that enhances eutrophication and often disqualifies its use as a drinking water supply [3]. Constructed wetlands have become increasingly favored as a simple and economical means for reduction of high concentrations of nitrate in both agricultural runoff and treated municipal wastewater (e.g., [4–7]).

In wetlands dominated by emergent reeds, the loose aggregations of intact stems and semi-decayed litter provide the microaerophilic zones required for denitrification to occur [8,9]. In addition to such controlling factors as dissolved oxygen, temperature and pH, high
rates of denitrification depend upon a readily available source of electron rich carbon [7,30]. Although aquatic plants provide the largest supply of readily oxidized carbon in wetlands, the wide site-to-site variation in apparent denitrification rates (i.e., 200 to over 5000 mg N m⁻² d⁻¹) in constructed wetlands [3,10] cannot be explained easily by plant species or the factors above.

Although common wetland plants differ only slightly in carbon content, the differences in lignin and cellulose content strongly influence the degradation rates of wetland litter [11]. This suggests denitrification activity in wetlands is affected by the carbon quality (e.g., C: N, lignin, carbohydrates) of the different plants that make up the litter. On a dry weight basis, aquatic plants with the highest acid-soluble carbohydrate content reduce greater amounts of NO₃⁻N per gram of added material [12]. The reduction efficiency or “denitrification potential” also varied with changes in carbon and NO₃⁻N loading, with greater efficiency at either lower carbon or higher NO₃⁻N additions. In this study, we examined the kinetic effects of co-limitation between the two primary resources required for denitrification, both carbohydrate carbon and nitrate.

2. Experimental design

We conducted a series of microcosm experiments to test whether the ratio of applied plant carbon to NO₃⁻N explains differences in denitrification rates. The microcosms were established using equal carbon additions of four common wetland plants (marsh pennywort, duckweed, bulrush and cattail). Kinetic co-limitation of plant carbohydrates and NO₃⁻N during microbial denitrification is expressed by the ratio of applied carbon to NO₃⁻N. Here, this ratio is used to explain variations in first-order rate constants due to plant species, simulated wetland productivity and NO₃⁻N loading.

The experimental design consisted of eight applied carbon to NO₃⁻N ratios between 2 and 20 mg C/mg N, varying dissolved NO₃⁻N in the feed water (2–56 mg/l as N) with carbon additions simulating a wetland productivity of 500 and 2000 g C/m² yr. After estimation of denitrification rates, differences in the rate constants were tested by plant type, the ratios of total applied carbon and acid-soluble carbohydrates to NO₃⁻N.

3. Materials

3.1. Microcosms

To provide near steady-state conditions for the measurement of nitrate removal kinetics, we used flow-through microcosms (Fig. 1) to represent the low dissolved oxygen, organic slurry that defines the sediment-water interface of wetlands. Sixteen microcosms were constructed of 4-l plastic (PET) storage containers filled with 2.5 l of water and nutrients, providing a 1.5 l headspace. To ensure the added NO₃⁻N was the primary electron acceptor available for plant carbon oxidation in the microcosms, we excluded O₂ by sparging with N₂ gas and limited Fe³⁺, Mn⁴⁺ and SO₄²⁻ additions in the nutrient makeup water. Atmospheric O₂ contamination was limited to below 1 mg/l day by water seals and also sparged with N₂ whenever the microcosms were opened for feeding or sampling.

During startup, the microcosms were established in a random block design with seven grams (as C) each of starting litter with weekly amendments to provide 500 and 2000 g C/m² yr. Nutrients fed from an 8 μm filtered common recirculating header supplied by a N₂ sparged feed tank.

Fig. 1. Flow through microcosm assembly. Sixteen, water sealed microcosms, split into four treatments of wetland plant litter (pennywort, duckweed, bulrush and cattail) in a random block design. Seven grams (as C) each of starting litter with weekly amendments to provide 500 and 2000 g C/m² yr. Nutrients fed from an 8 μm filtered common recirculating header supplied by a N₂ sparged feed tank.

3.2. Wetland plant materials

Aboveground samples of hard stem bulrush (Scirpus acutus) and cattail (Typha latifolia) were collected along
with floating duckweed (Lemna minor) from drainage ditches on Sherman Island, CA, in the Sacramento/San Joaquin river delta. Marsh pennywort (Hydrocotyle umbellata) was collected from the margins of the treatment marsh at the City of Arcata, CA. After collection, the plants were oven dried at 40°C to a constant mass, milled and sieved (<2 mm) to near the same specific surface area (0.3 ± 0.1 m²/gDW).

3.3. Nutrients

Between 400 and 600 ml/day of fresh nutrients were supplied from a 120l, nitrogen sparged feed tank using 16 drip feeders (250–500 µl/min) mounted to a constant-pressure, 0.2 µm-filtered, recirculating header pipe. To ensure that NO₃-N and plant carbon were the only limiting resources for denitrifiers, we prepared the nutrient feed tank using NaNO₃ added to de-ionized water (DOC < 0.2 mg/l) along with trace metals (Fe²⁺, SeO₄²⁻, etc.), mineral salts (Ca²⁺, K⁺, Mg²⁺, etc.) and vitamins (Kit V-1, Sigma Chemical Co.). Micronutrient additions were based upon a low ionic strength (0.03 M) modification of standard methanogenic culture media [13]. A pH 6.5 phosphate buffer (9:5 KH₂PO₄ to K₂HPO₄) was substituted for more commonly used high MW organic acid buffers (e.g., BIS-TRIS, MOPS) to eliminate carbon analysis interference.

4. Methods

4.1. Nitrogen species

The microcosms were sampled four times each week with samples collected six days after carbon feeding used to represent the near steady-state conditions of the carbon and the three major nitrogen species (i.e., NO₃⁻, NO₂⁻, NH₃). Samples were serially filtered to 0.45 µm and measured for NO₃⁻N by ion-specific electrode method NO₃⁻D [14]. To assure denitrification was the dominant NO₃⁻N reduction pathway, we measured ammonia and nitrite in the samples by standard methods NH₃-F and NO₂⁻-B [14]. Overall, effluent ammonia concentrations were within 5 ± 2% of the inlet NO₃⁻N, whereas nitrite concentrations was within 2 ± 1% (±1 SE).

4.2. Carbon quality

As added plant litter accumulated, the microcosms were periodically sub-sampled by decanting the contents, followed by centrifugation and homogenization of the solids. All plant samples were prepared for analysis of carbon and nitrogen content [15], and lignin by oven drying at 45°C and milling to pass a 40-mesh screen. Total lignin was estimated by the sum of the TAPPI Method 222 (Klason lignin) and Method 202, acid-soluble lignin fractions [16]. Acid-soluble carbohydrates were calculated by difference, subtracting the non-protein, acid-insoluble residue from the ash free dry weight (AFDW) of the prepared lignin samples.

4.3. Kinetic analysis

Denitrification kinetics was determined using a steady-state approach [1], operating the system for five weeks after startup to reach stable effluent DOC. The system was then operated for two weeks at each inlet NO₃⁻N condition, allowing six days after feeding prior to sampling for kinetic parameter estimates. First-order denitrification rates were estimated by log-linear regression of NO₃⁻-N removal fractions (C/Co) by hydraulic residence time [1].

During the experimental setup, we determined hydraulic residence time distributions for the microcosms by step-dose tracer tests using deionized water and a 30 mg N/L nitrate feed [17]. Fig. 2 shows clean water tracer tests behaved nearly equally as one or two mixed tank reactors in series (r² = 0.78 and 0.97). However, bubble ebullition of CO₂ and reduced nitrogen gases from the microbial community during the experiments provided sufficient mixing to use a single mixed reactor model for our kinetic parameter estimates.

![Fig. 2. Step-dose tracer test fits. Outlet nitrate measured for 2–3 weeks following step increase of inlet nitrate from zero to 30 mg-N/L to all sixteen 2.5 L microcosms. Although variance in outlet nitrate between replicate microcosms increased with time, they hydraulically behaved nearly equally as either one (r² = 0.57) or two (r² = 0.77) mixed tanks on a time scale of the 4–6 day hydraulic residence time.](image-url)
5. Experimental results

5.1. Nitrate removal efficiency

The steady-state NO$_3^-$-N removal efficiencies ($1 - C_{\text{out}}/C_{\text{in}}$) are plotted against the ratios of total applied carbon to NO$_3^-$-N reduced ($C_{\text{App}}:N_{\text{Red}}$) on Fig. 3. The removal efficiency makes an asymptotic approach toward complete removal with increasing $C_{\text{App}}:N_{\text{Red}}$ for data from both the low (500 gC/m$^2$yr) and high (2000 gC/m$^2$yr) simulated productivity experiments. This suggests the denitrification rates of the microbial community may be limited by rates of supply and availability of electron donor or acceptor, described by this ratio of applied carbon to NO$_3^-$-N.

5.2. Estimation of first-order rate constants

Nearly half of the thirty-two steady-state effluent NO$_3^-$ concentrations were below the 1 mg/l detection limit of the ion-specific electrode, with the highest removals corresponding to the highest $C_{\text{App}}:N_{\text{Red}}$ ratios. Although the steady state removal fractions ($C_{\text{out}}/C_{\text{in}}$) may be fitted to first-order kinetics ($r^2 = 0.86 \pm 0.02, p < 0.0001$) using a single mixed tank model [1], the highest $C_{\text{App}}:N_{\text{Red}}$ treatments had insufficient NO$_3^-$-N to estimate the residual chemical reduction capacity of the added plant materials. Excluding these data eliminated several rate constants over 1 d$^{-1}$, only improving the average first-order fit slightly ($r^2 = 0.88 \pm 0.02, p < 0.0001$), but increased the confidence in the remaining rate constants. The remaining rate constants (0.2–1.4 d$^{-1}$) varied both by plant type and weakly by applied NO$_3^-$-N concentration (Table 1).

The apparent first-order rate constants were not significantly correlated with the carbon quality measures (C : N ratio, lignin, acid-soluble carbohydrates) shown in Table 2. On a litter C : N basis, the two floating aquatics (Pennywort and Duckweed) had nearly three times the nitrogen content of the two emergent plants (Bulrush and Cattail), but only the bulrush litter exhibited low rate constants (Table 1) with characteristically high C : N ratios and lignin content. Although, the first-order rate constants were only weakly related to plant type and litter carbon quality measures at each $C_{\text{App}}:N_{\text{Red}}$ ratio, the bulrush rate constants were consistently lower than all other plant sources (0.01 < $p$ < 0.06) across all conditions.

The 17 denitrification rate constants shown in Table 1 vary significantly with $C_{\text{App}}:N_{\text{Red}}$ ratio ($r^2 = 0.58, p < 0.001$). However, including only the acid-soluble carbohydrate fraction of the total carbon content in this ratio, Fig. 4 shows the variation in the rate constants ($r^2 = 0.77, p < 0.0001$) is better explained by the ratio of applied acid-soluble carbohydrates to NO$_3^-$-N reduced ($CH_2O_{\text{App}}:N_{\text{Red}}$) than by the total carbon to NO$_3^-$-N ratio ($C_{\text{App}}:N_{\text{Red}}$). The apparent differences in the plant-specific rate constants at each total $C_{\text{App}}:N_{\text{Red}}$ ratio (increasing from bulrush to duckweed, cattail to pennywort) were largely eliminated when compared on a $CH_2O_{\text{App}}:N_{\text{Red}}$ basis. In addition to the apparent effect of carbon and NO$_3^-$-N co-limitation in these experiments, this suggests a preferential use of a common source of easily oxidized carbon across all plant species, acid-soluble carbohydrates.

6. Discussion

6.1. Regulation of denitrification activity by carbon quality of wetland litter

Although direct carbon quality measures (e.g., C : N, lignin) are often used to explain rates of litter decomposition [11], they do not explain the differences in the denitrification rate constants here as well as the acid soluble carbohydrate fraction of the litter. The characteristically low litter C : N of the two floating aquatics (marsh pennywort and duckweed) did not correspond to consistently higher rate constants, only the bulrush litter exhibited significantly lower denitrification rate constants at high lignin and C : N ratios. On a dry weight basis, the oxidation rates required to match the reported denitrification rates here (0.2–1.4 d$^{-1}$) are typically much faster than the rates of mass loss used in litter decomposition studies [18]. To supply readily oxidized plant carbon at these rates, only the most rapidly
These experiments proceeded from low to high nitrate levels and did not test whether the higher nitrate levels increased the hydrolysis rate of soluble carbohydrates. It is possible that this may have affected the carbon supplied for subsequent experimental conditions, since these materials appear to be very rapidly assimilated by attached bacteria in wetlands [5]. Other authors have shown senescent wetland plants and litter become rapidly depleted of their soluble carbohydrates and higher molecular weight compounds such as lignins with low hydrolysis rates [19] are selectively concentrated in the remaining litter [11]. For this reason, carbon supplied by the developing peat and litter arriving from the prior year’s plant productivity may contribute less to denitrification activity than the rapidly hydrolyzed carbohydrates in fresh and senescent litter.

### Table 1
Nitrate removal in continuous flow, anoxic microcosms using weekly additions of milled plant litter. Rate constants determined by log-linear regression of NO$_3$–N removal by residence time (4–6 day) assuming single mixed reactor hydraulics shown in Fig. 2

<table>
<thead>
<tr>
<th>NO$_3$–N (mg/l)</th>
<th>Applied carbon and nitrate reduced</th>
<th>First-order kinetics assuming single mixed reactor ($n = 4$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Feed tank</td>
<td>Outlet</td>
</tr>
<tr>
<td>Pennywort ($Hydrocotyle umbellata$)</td>
<td>21.6</td>
<td>8.6</td>
</tr>
<tr>
<td></td>
<td>57.7</td>
<td>17.9</td>
</tr>
<tr>
<td></td>
<td>19.2</td>
<td>3.4</td>
</tr>
<tr>
<td>Duckweed ($Lemna minor$)</td>
<td>21.6</td>
<td>7.3</td>
</tr>
<tr>
<td></td>
<td>57.7</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>33.0</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>19.2</td>
<td>3.1</td>
</tr>
<tr>
<td>Bulrush ($Scirpus acutus$)</td>
<td>21.6</td>
<td>10.8</td>
</tr>
<tr>
<td></td>
<td>57.7</td>
<td>33.7</td>
</tr>
<tr>
<td></td>
<td>11.3</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>33.0</td>
<td>12.8</td>
</tr>
<tr>
<td></td>
<td>19.2</td>
<td>4.5</td>
</tr>
<tr>
<td>Cattail ($Typha latifolia$)</td>
<td>21.6</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>57.7</td>
<td>21.4</td>
</tr>
<tr>
<td></td>
<td>33.0</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>19.2</td>
<td>2.9</td>
</tr>
</tbody>
</table>

### Table 2
Dried litter carbon quality of floating and emergent aquatic plants. Litter C : N ratios and lignin contents are significantly lower for floating aquatic plants than for emergents. Reported uncertainties are ±1 SE ($n = 3$). For complete description of carbon quality changes in leached and microbially colonized litter, see Ref. [12]

<table>
<thead>
<tr>
<th>Plant type</th>
<th>Floating aquatics</th>
<th>Emergent aquatics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant species</td>
<td>Marsh pennywort ($Hydrocotyle umbellata$)</td>
<td>Duck weed ($Lemna minor$)</td>
</tr>
<tr>
<td>Carbon (mgC/gDW)</td>
<td>380 ± 2</td>
<td>372 ± 1</td>
</tr>
<tr>
<td>Nitrogen (mgN/gDW)</td>
<td>44 ± 0.4</td>
<td>45 ± 0.4</td>
</tr>
<tr>
<td>C : N (gC/gN)</td>
<td>8.6 ± 0.1</td>
<td>8.3 ± 0.1</td>
</tr>
<tr>
<td>Klason lignin (mg/gDW)</td>
<td>4 ± 4</td>
<td>59 ± 23</td>
</tr>
<tr>
<td>Carbohydrate (mgC/gDW)</td>
<td>215 ± 3</td>
<td>194 ± 10</td>
</tr>
<tr>
<td>Ash (mg/gDW)</td>
<td>178 ± 1</td>
<td>193 ± 1</td>
</tr>
</tbody>
</table>
Although lignin content of wetland plant litter exerted less influence on denitrification activity here than acid soluble carbohydrates, the role of lignins may become increasingly important in controlling the rate of carbon supply to support denitrification in aged litter. As aged litter will become increasingly concentrated in lignins, testing of this hypothesis would require a series of mono-specific microcosm experiments conducted at equal carbon (or carbohydrate) mass loading but at increasing litter age (i.e., higher lignin content).

6.2. Total carbon limitation in constructed wetlands

In most real world systems such as constructed wetlands, the slow diffusion of NO\textsubscript{3}\textsuperscript{−} through sediment porewaters and the abundant plant litter produces a pseudo-first-order relationship and a NO\textsubscript{3}\textsuperscript{−} limitation on the overall reaction [20,21]. Many field studies have also shown zero-order kinetics with respect to NO\textsubscript{3}\textsuperscript{−} [22,23]. However, these studies typically do not address the characteristic oversupply of oxidizable plant carbon from the lack of other terminal electron acceptors in the strongly reducing environments of wetland soils [24].

The carbon supply in full-scale wetlands is largely dependent upon annual variations in plant productivity and inter-annual variations in plant species dominance in mixed communities [18]. Interestingly, when analyzed at time scales on the order of days, the carbon supply in these systems appears as near steady-state and may produce apparent zero and first-order denitrification kinetics. These experiments suggest that the typically large annual variations and site-to-site variations in apparent zero-order denitrification rate constants at the field scale [10] may in part reflect plant carbon co-limitation with NO\textsubscript{3}\textsuperscript{−}.

In full-scale wetlands that receive treated wastewater, high in dissolved NO\textsubscript{3}\textsuperscript{−} and low in DOC, the supply of plant carbon may limit denitrification rates [25]. In a study similar to this one, Ingersoll and Baker [26] found that increasing carbon additions of dried cattail litter in wetland microcosms increased NO\textsubscript{3}\textsuperscript{−} removal efficiencies, reaching near 100% above a C\textsubscript{App} : N\textsubscript{Red} ratio of 4–5 to 1. Although flushing from the microcosms also makes this ratio sensitive to variations in hydraulic loading, Fig. 3 shows a similar asymptotic approach in removal efficiency here at near the same ratios C\textsubscript{App} : N\textsubscript{Red}. However, the apparent differences between plant specific removal efficiencies are not well explained by the total C\textsubscript{App} : N\textsubscript{Red} ratio.

6.3. Effects of carbohydrate and nitrate co-limitation on observed kinetics

It is apparent from the carbohydrate dependency shown here that simplifications of denitrification kinetics that ignore carbon quantity and quality may not entirely explain observed NO\textsubscript{3}\textsuperscript{−}−N removals in wetlands. The strong relationship ($r^2 = 0.77$) between the apparent first-order rate constants and the CH\textsubscript{2}O\textsubscript{App} : N\textsubscript{Red} ratio suggests the dual resource limitation of acid-soluble carbohydrates and NO\textsubscript{3}\textsuperscript{−} may control the apparent denitrification rates in full scale treatment wetlands as well. Practically, few studies have shown apparent first-order denitrification rate constants above 2 d\textsuperscript{−}1 [21] and it is unclear whether the linear relationship observed in these experiments would be observed at rate constants of 2 d\textsuperscript{−}1 and the correspondingly higher CH\textsubscript{2}O\textsubscript{App} : N\textsubscript{Red} ratios than those that were observed here. However, it is apparent from these results that differences in the CH\textsubscript{2}O\textsubscript{App} : N\textsubscript{Red} ratio arising from annual cycles of primary productivity and NO\textsubscript{3}\textsuperscript{−} loading among wetlands and their plant assemblages may contribute to large differences in observed denitrification rates.

Although NO\textsubscript{3}\textsuperscript{−} limitation in most natural wetlands results in pseudo-first-order reductions and much higher CH\textsubscript{2}O\textsubscript{App} : N\textsubscript{Red} ratios than found in these studies, systems receiving a highly variable NO\textsubscript{3}\textsuperscript{−} loading will be subject to large variations in estimated zero-order (mg/m\textsuperscript{2}/d) or first-order (d\textsuperscript{−}1) rate constants. This may be either due to mass transport phenomenon [20] or a change in nitrogen utilization by switching from respiratory denitrification to nitrogen immobilization at or near applied C : N ratios of 16 : 1 [27,28].

Co-limitation of plant carbohydrates and NO\textsubscript{3}\textsuperscript{−}−N means that for many wetlands, either high plant productivity or low NO\textsubscript{3}\textsuperscript{−} loading (high CH\textsubscript{2}O\textsubscript{App} : N\textsubscript{Red})
should provide a higher apparent first order denitrification rate constant. In contrast, full scale wetlands that have low productivity or a high NO$_3^-$ loading (low CH$_2$O$_{App}$:N$_{Red}$) would exhibit a lower apparent rate constant. Since the first-order rate constants are determined from the ratio of concentrations, this co-limiting behavior suggests that intentionally manipulating wetland systems to achieve a higher CH$_2$O$_{App}$:N$_{Red}$ may be suitable to achieve low effluent concentrations. However, the US EPA and many state regulatory agencies have begun to manage receiving water quality on a mass-loading basis [29]. In the case of highly N-loaded systems, such as denitrification wetlands and estuaries that may be carbon limited, a higher NO$_3^-$ mass removal rate may be preferred at the expense of lower removal efficiency (i.e., higher effluent NO$_3^-$ concentration). In either case, it is apparent that more accurate inter-system comparisons of denitrification wetland performance may be possible by indexing rates to the ratio of applied carbon to nitrogen.

Although validating these results in field comparisons may be complicated by multiple sources of carbon and nitrogen, more accurate inter-system comparisons of wetland performance may be possible by indexing denitrification rates to the ratio of applied carbon to nitrogen. For highly N-loaded wetlands, carbon and nitrate co-limitation may necessitate not only higher order reaction kinetics (i.e., 1st or 2nd order) to better describe temporal variations in denitrification rates, but also estimation of the CH$_2$O$_{App}$:N$_{Red}$ ratio from a careful inventory of plant species, cover and seasonal productivity estimates. Lastly, in systems with greater amounts of open water, the daily carbon flux from algal productivity may approach or exceed the carbon supply by higher wetland plants.

7. Conclusions

In the microcosm studies presented here, the strong relationship ($r^2 = 0.77$) between the apparent first-order rate constants and the CH$_2$O$_{App}$:N$_{Red}$ ratio suggests this dual resource limitation between acid-soluble carbohydrates and NO$_3^-$ may control the apparent denitrification rates in full-scale treatment wetlands as well.

In most natural wetlands, the oversupply of oxidizable plant carbon and the slow diffusion of NO$_3^-$ through sediment porewaters produces a characteristic NO$_3^-$ limitation (high CH$_2$O$_{App}$:N$_{Red}$ ratio) and may contribute to observations of pseudo-first-order denitrification kinetics.

Although NO$_3^-$ limitation in most natural wetlands results in pseudo-first-order reductions and much higher CH$_2$O$_{App}$:N$_{Red}$ ratios than found in this study, systems receiving a highly variable NO$_3^-$ loading will be subject to large variations in estimated zero-order (mg/m$^2$/d) or first-order (d$^{-1}$) rate constants.

Co-limitation of plant carbohydrates and NO$_3^-$–N means that for many wetlands, either high plant productivity or low NO$_3^-$ loading (high CH$_2$O$_{App}$:N$_{Red}$) should provide a high effective denitrification rate.

Although carbon supply in full-scale wetlands follows annual variations in plant productivity and inter-annual variations in plant succession, when analyzed at time scales on the order of days, the carbon supply appears as near steady-state and obscures variations in the CH$_2$O$_{App}$:N$_{Red}$ ratio.

In addition to factors such as hydraulics, temperature, pH and NO$_3^-$ loading, extending these results to compare denitrification rates of larger scale wetland systems will require knowledge of the CH$_2$O$_{App}$:N$_{Red}$ ratio from a careful inventory of plant species, cover and productivity estimates.

References


