Denitrification Potential and Carbon Quality of Four Aquatic Plants in Wetland Microcosms

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

ABSTRACT

The C quality of four aquatic plants found in wetlands explains the differences in their relative capacity to fuel denitrification. In replicated (n = 4) flow-through microcosm experiments we compared nitrate removal using equal C additions of two emergent plants (*Scirpus acutus*, *Typha latifolia*) and two floating plants (*Hydrocotyle umbellata*, *Lemna minor*). As expected, plant litter with the highest initial C/N and lignin content (*Scirpus acutus*), required significantly (p < 0.05) higher dry matter addition to achieve the nitrate removals of the other plants studied. For all plants, losses in C content and significantly (p < 0.05) higher C/N ratios in leached litter is consistent with preferential hydrolysis of low molecular weight (MW) carbohydrates. Interestingly, plant-specific differences in this denitrification potential were removed when nitrate removal data was compared on an acid-soluble carbohydrate (ASC) basis. As an efficiency measure, high ratios of applied C to NO$_3$–N decreased denitrification potentials, whereas increasing NO$_3$–N loading at equal C additions increased denitrification potentials. Differences in denitrification potential resulting from carbohydrate and lignin content of the plants studied were generally smaller than relative differences in reported productivity. That much of the plant C left the microcosms before oxidation is consistent with overall electron acceptor limitation in wetlands and suggests observable differences in field-scale denitrification performance may still arise with the presence or absence of highly productive reed (e.g., *Scirpus sp.*, *Typha sp.*) stands.

Providing easily oxidized sources of C to bacteria in wetland soils and litter is one of the few alternatives to increasing the removal efficiency of residual nitrates from waters that receive agricultural runoff and treated municipal effluent. Combining a large available surface area for bacterial attachment on aquatic plant litter with a rich organic matter supply, wetlands are important natural sites for denitrification. Denitrification is generally carried out by facultative anaerobes, since the respiratory electron transfer between plant C and NO$_3$ yields only slightly less free energy than the C/O couple. Potentially, this electron transfer would oxidize available C at a ratio of 5:4 for every molecule of NO$_3$ reduced to N$_2$ gas. However, this ratio is rarely achieved since the slow diffusive mass transfer of dissolved NO$_3$ and the lack of a continuing supply of sediment- or litter-bound C to attached bacteria often limits denitrification in many aquatic ecosystems (Seitzinger, 1988).

Compilations of both short-term wetland studies presented by Hammer (1989) and longer term ones by Kadlec (1995) show that denitrification in wetland litter is dependent upon the supply of organic C, relatively low levels of dissolved oxygen, variations in temperature, and pH. In wetlands, the economy of this C supply is considered to be largely dependent upon differences in plant productivity between floating and emergent aquatic plants (Westlake et al., 1998). However, less attention has been given to the relative differences in the composition of the decomposing litter of wetland plants. The chemical composition, or C quality of litter has long been considered a critical factor in determining the rate of mass loss through decay (Melillo et al., 1982). It has been suggested that the presence of wind and gravity places similar structural and excretory requirements on emergent aquatic plants as terrestrial angiosperms and gymnosperms (Gifford and Foster, 1989), resulting in the presence of poorly degradable, intercellular lignins (Freudenberg and Neish, 1968). The energy requirements to break down ligno-cellulose in plant litter are large and in general higher lignin content is associated with lower litter decay rates (Melillo et al., 1982; Hobbie, 1996). In contrast, high initial N content (low C/N) and high ASC content have both been used to explain high litter decay rates in wetlands (Westlake et al., 1998). Early terrestrial soil amendment studies identified differences in denitrification activity between various cereal grains in both the relative amounts of dry matter addition and on a total C addition basis (Bremner and Shaw, 1957). In wetland microcosm studies, the ratio of applied C to N required to achieve complete denitrification ranges between 4 and 10 (Ingersoll and Baker, 1998; Hume et al., 2002). At still higher C loading, N immobilization by bacteria (Bowden, 1987) may compete with denitrification as the dominant NO$_3$ removal mechanism from wetland litter. However, less attention has been given to how C quality of wetland plants affects their ability to fuel denitrification (Bachand and Horne, 2000) at similar ratios of applied C/N. All other factors being equal questions remain as to which wetland plants reduce more NO$_3$ per unit mass of dry plant matter addition and which plants have the highest denitrification potential?

**Abbreviations:** AFDW, ash-free dry weight; ASC, acid soluble Carbohydrates; DW, dry weight; MW, molecular weight.
MATERIALS AND METHODS

Experimental Design

Hypotheses

Because both litter decay and respiratory denitrification proceed by heterotrophic metabolism, C quality may also be used to explain differences in nitrate removal in wetlands. The differences in physiology between emergent reeds and floating aquatic plants and their C quality may explain differences in plant-specific denitrification potential. More specifically, differences in soluble-plant carbohydrates are more important for optimum denitrification performance than other plant-specific differences in C quality.

Design

Using two floating and two emergent aquatic plants commonly found in constructed wetlands, these experiments examine how denitrification potential is affected by the composition and allocation of plant C in common plants found in wetland C. A total of 32 treatments were preselected using replicated (n = 4) microcosms. Litter samples of the four plant species were added at two C loadings (500 and 2000 g m⁻² yr⁻¹) and four nitrate loading (2–56 mg N L⁻¹) conditions. After 4 to 8 wk startup, each C loading and nitrate condition was allowed to reach near-steady state nitrate concentrations over 2 wk prior to changing to the subsequent nitrate concentration. We chose high nitrate and applied C levels to minimize disturbance from litter subsampling and also to improve measurement precision of denitrification potential. Variations in nitrate removal performance of the added plant materials were assessed on a dry matter, total C, and an ASC basis.

Materials

Microcosms

Sixteen flow-through microcosms were constructed to represent the low oxygen, organic slurry that defines the sediment-water interface of denitrification wetlands (Fig. 1). Seven grams (on a C basis) of plant litter were added to 4 L PET (polyethylene terephthalate) plastic storage containers filled with 2.5 L each of water and nutrients. Temperature was uncontrolled during these experiments but ranged from 19 to 22°C within the laboratory fume hoods. To ensure the added NO₃⁻ was the primary electron acceptor available for plant C oxidation in the microcosms, we excluded O₂ by sparging with N₂ gas and limited Fe³⁺, Mn⁴⁺, and SO₄ additions in the nutrient makeup water. Although atmospheric O₂ contamination was limited to below 2 mg d⁻¹ by water seals, the highest O₂ leakage rates in preliminary clean water tests were equivalent to a feed concentration of 4 mg L⁻¹ NO₃⁻N. This exceeded the lowest two NO₃⁻ feed concentrations (2 and 4 mg N L⁻¹), but fell to <10% of the NO₃⁻ feed at 56 mg N L⁻¹ and the microcosms remained anoxic (<1 mg L⁻¹) throughout the 8-wk experiments.

Plant Carbon

Live, aboveground samples of hard stem bulrush (Scirpus acutus) and broad-leaved cattail (Typha latifolia) were collected in June 1996 along with floating duckweed (Lemma minor) from agricultural drainage canals on Sherman Island, CA, in the Sacramento/San Joaquin river delta. Marsh pennywort (Hydrocotyle umbellata) was collected in May 1997 from the margins of the treatment marsh at the city of Arcata, CA.

Although drying and milling techniques have been shown to affect litter decomposition rates (Moorhead et al., 1988), all plant samples were dried at 45°C and milled to pass a 2-mm screen. Despite different growth forms of submerged and floating aquatic plants, milled samples were passed through standard sieves and weighed to show similar specific surface area (0.3 ± 0.1 m² g⁻¹ dry weight [DW]) across all plant materials.

Bulk plant litter additions of the four plants were adjusted by ash content (Table 1) to an equal C basis at amounts (170–690 mg C wk⁻¹) simulating litter accumulation in low and high productivity wetlands (500 and 2000 g C m⁻² yr⁻¹). The microcosms were fed between 0.4 and 1.8 g DW wk⁻¹ of dried and milled litter for 12 to 16 wk. Accumulated litter was sampled every 4 to 6 wk (1–3 g DW) to maintain near steady-state C supply in the microcosms.

Nutrients

Micronutrient additions were based upon a low ionic strength (0.01 M) modification of standard methanogenic culture media (Tanner, 1997). Between 400 and 600 mL d⁻¹ of fresh nutrients were supplied from a 120-L N₂-sparged feed tank using 16 drip feeders (250–500 µL min⁻¹) mounted to a constant pressure, 0.2-µm filtered, recirculating header pipe (Fig. 1). To ensure that nitrate and plant C were the only limiting resources for denitrifiers, we prepared the nutrient feed tank using NaNO₃ added to deionized water (dissolve organic C [DOC] < 0.2 mg L⁻¹) along with trace metals (e.g., Fe, SeO₄, etc.), mineral salts (e.g., Ca, K, Mg, etc.) and vitamins (Kit V-1, Sigma Chemical Co., St. Louis, MO). We substituted pH 6.5 phosphate buffer (9.5 KH₂PO₄/K₂HPO₄) for organic acid buffers (e.g., BIS-TRIS, MOPS) to ensure plant C was the only useable substrate for denitrification. Potassium salts were substituted for ammonium salts to avoid interferences from coupled nitrification and denitrification within the microcosms. Lastly, we reduced ion-specific electrode measurement interferences by substituting chloride for sulfate salts.

Sample Preparations

Daily 500-mL samples were collected and filtered through glass fiber filters (Gelman GF/C, Pall Life Sciences, Ann Arbor, MI) prior to electrochemical measurements (i.e., pH,
Table 1. Carbon quality of four common floating and emergent aquatic plants found in denitrification wetlands. Reported uncertainties are ±1 standard errors (S.E.) (n = 4).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Floating aquatics</th>
<th>Emergent aquatics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant analysis†</td>
<td>Marsh pennywort (Hydrocotyle umbellata)</td>
<td>Duck weed (Lemna minor)</td>
</tr>
<tr>
<td>C/N, g C g N⁻¹</td>
<td>8.61 ± 0.05</td>
<td>8.25 ± 0.07</td>
</tr>
<tr>
<td>Lignin, g kg⁻¹ AFDW</td>
<td>4 ± 4</td>
<td>73 ± 31</td>
</tr>
<tr>
<td>C, g kg⁻¹ AFDW</td>
<td>458 ± 3</td>
<td>457 ± 1</td>
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<tr>
<td>Ash, g kg⁻¹ DW</td>
<td>178 ± 1</td>
<td>193 ± 1</td>
</tr>
<tr>
<td>Leached Litter After 8 wk</td>
<td>8.91 ± 0.17</td>
<td>9.07 ± 0.14</td>
</tr>
<tr>
<td>C/N, g C g N⁻¹</td>
<td>146 ± 31</td>
<td>163 ± 8</td>
</tr>
<tr>
<td>Lignin, g kg⁻¹ AFDW</td>
<td>482 ± 15</td>
<td>454 ± 3</td>
</tr>
<tr>
<td>C, g kg⁻¹ AFDW</td>
<td>56 ± 2</td>
<td>84 ± 1</td>
</tr>
<tr>
<td>Ash, g kg⁻¹ DW</td>
<td>57 ± 4</td>
<td>79 ± 6</td>
</tr>
<tr>
<td>Bacterially Colonized Litter After 16 wk at 500 g C m⁻² yr⁻¹</td>
<td>8.93 ± 0.18</td>
<td>7.04 ± 0.06</td>
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<tr>
<td>C/N, g C g N⁻¹</td>
<td>310 ± 2</td>
<td>180 ± 10</td>
</tr>
<tr>
<td>Lignin, g kg⁻¹ AFDW</td>
<td>525 ± 3</td>
<td>480 ± 1</td>
</tr>
<tr>
<td>C, g kg⁻¹ AFDW</td>
<td>57 ± 4</td>
<td>79 ± 6</td>
</tr>
<tr>
<td>Ash, g kg⁻¹ DW</td>
<td>57 ± 4</td>
<td>79 ± 6</td>
</tr>
<tr>
<td>Bacterially Colonized Litter After 12 wk at 2000 g C m⁻² yr⁻¹</td>
<td>8.03 ± 0.27</td>
<td>7.00 ± 0.01</td>
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<tr>
<td>C/N, g C g N⁻¹</td>
<td>274 ± 22</td>
<td>162 ± 13</td>
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<tr>
<td>Lignin, g kg⁻¹ AFDW</td>
<td>522 ± 2</td>
<td>476 ± 3</td>
</tr>
<tr>
<td>C, g kg⁻¹ AFDW</td>
<td>72 ± 1</td>
<td>104 ± 1</td>
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</table>

† AFDW, ash free dry weight; DW, Dry weight.

Nitrogen Species

Nitrate was measured by ion-specific electrode (Orion 93-07, Thermo Orion, Beverly, MA) using standard method NO₃-D (APHA 1998) and linear regression from known standards. Electrode response near the manufacturer’s method detection limit of 1 mg N L⁻¹ was improved by adding 15 mL of acid buffered alum and Ag₂SO₄ solution to 10 mL of sample, thereby precipitating excess sulfides and chlorides. To assure denitrification was the dominant nitrate reduction pathway, we measured ammonia and nitrite samples spectrophotometrically by standard methods NH₃-F (640 nm) and NO₂-B (543 nm), respectively (APHA 1998).

Carbon Quality

Here, C quality includes C and N content, nonpolar resins, polyphenolic compounds such as lignins, and ASC such as hemicellulose, cellulose, simple sugars, and starch. All litter samples were prepared for analysis of total C content, N, and Klason lignin by oven drying at 45°C and milling to pass a 40-mesh screen. Nitrogen and C content of plant litter was determined using a C/N elemental organic analyzer (Carlo Erba, Milan, Italy) by high temperature CrO/CoO-catalyzed flash combustion, followed by Cu-catalyzed reduction of N oxides to N₂, chromatographic separation, and detection using a thermal conductivity detector (Pella, 1990). We measured Klason lignin gravimetrically by the 72% H₂SO₄ acid-insoluble residue (TAPPI, 1996, Method 222) after benzene and ethanol extraction of oils and resins from the litter samples. Total lignin was estimated by the sum of the Klason lignin and the acid soluble lignin fractions (TAPPI, 1996, Method 202).

Because the lignins were determined gravimetrically, rather than by chromatographic techniques, we corrected the mass of retained solids for the nonlignin components (Sarkanen and Ludwig, 1971). Total lignins were corrected for coprecipitated proteins by subtraction of sample protein contents. To account for the presence of nonprotein N in the plant samples, protein contents were estimated by multiplying N contents by 43.7 ± 3.9 g kg⁻¹ (Yeoh and Wee, 1994). Acid-soluble carbohydrates were calculated by difference, subtracting the nonprotein, acid-insoluble residue from the ash-free dry weight (AFDW) of the prepared lignin samples. After determination of ash content, the C content of proteins, resins, total lignins, and ASCs were estimated from published proximate analysis values for the AFDW fractions of these constituents (Sarkanen and Ludwig, 1971; Westlake, 1963).

Leaching Controls

To examine the effects of hydrolysis on the added litter, abiotic controls were prepared using 3 g DW of each plant material and 10 g CuSO₄ added to deionized water in 1-L amber glass bottles. The control litter was leached in the dark for 8 wk prior to C quality analysis. Approximately 90% of the water was decanted and exchanged every 2 wk.

Both the controls and the litter that accumulated in the microcosms were examined for microbial colonization by epifluorescent microscopy (Bhupathiraju and Alvarez-Cohen, 1998). There were 1 to 2 active cells in 20 fields of view in almost all of the controls, indicating some bacterial colonization. However, although expanded method uncertainty placed the upper 95% confidence limit of active bacterial counts at 1 × 10⁵ g DW⁻¹, this bacterial activity was still over three orders of magnitude below the nonsterile treatments.

Statistical Methods

Except where noted, reported measurement uncertainties are expressed as standard deviation and each treatment was evaluated using four replicates (n = 4). Uncertainty in quantities calculated by difference (i.e., ASCs) was estimated using Gaussian error propagation of the independent variable uncertainties (Kempthorne, 1986). Tests for equality of mean DW litter quality (e.g., C, ASC, or lignin content) between
differing litter treatments (i.e., dried fresh litter, leached controls, low, and high simulated productivity) were made using two-tailed Student’s t-tests at \((n_1 + n_2 - 2)\) degrees of freedom (Zar 1999). Linear regression of explanatory variables was performed using standard statistical software (JMP v.4, SAS Institute, Cary, NC) to iterate successive approximations of slope and intercept of the straight line that fits the data by minimizing the residual sum of squares around the mean of the dependent variable (Zar, 1999).

**RESULTS**

**Carbon Quality**

Table 1 shows the decrease in ash content and changes in C quality from fresh litter, after 8 wk of controlled leaching in deionized water, and after 12 to 16 wk accumulation in the denitrification microcosms at 500 and 2000 g C m\(^{-2}\) yr\(^{-1}\) simulated wetland productivity. Total lignin content (g kg\(^{-1}\)) of fresh litter as ash free DW was significantly \((p < 0.01)\) lower than leached controls and all but one \((p < 0.15)\) of the microbially colonized litter treatments at both low and high C loading. Total C content of leached controls (Table 1) was only slightly lower than fresh litter for all treatments except marsh pennywort. However, C content of microbially colonized litter was significantly \((p < 0.09)\) higher than in fresh litter.

Figure 2 shows C content of acid insoluble constituents (i.e., proteins, resins, and total lignins) and ASCs estimated individually from published proximate analysis values for the AFDW fractions of these constituents (Sarkanen and Ludwig, 1971; Westlake, 1963). Total C content of all treatments and controls \((n = 16)\) determined by summation of proximate composition of the AFDW fractions \((451 \pm 33 \text{ g C kg}^{-1} \text{AFDW})\) was significantly \((p < 0.001)\) lower than direct C analyzer measurement of the bulk samples \((476 \pm 22 \text{ g C kg}^{-1} \text{AFDW})\).

For all plant types, ASC content decreased only slightly with leaching and after bacterial colonization. There were significant decreases in ASC content \((0.002 < p < 0.06)\) and significant \((p < 0.01)\) increases in lignin content for the two floating aquatic plants, whereas the bulrush treatments had no significant changes in either parameter. Total litter C to N (C/N) ratios increased significantly \((p < 0.05)\) with deionized water leaching but decreased significantly \((p < 0.01)\) in all but one of the eight microbially colonized litter treatments of the microcosms. This was accompanied by slight increases \((p < 0.19)\) in protein content across all treatments with bacterial colonization.

**Nitrogen Removal**

Nitrate added in the nutrient supply was the only mineral N form added to the microcosms during these experiments. Competing nitrate removal pathways were assessed by direct measurement of nitrite and nitrate (Total Kjeldahl N was not measured) loss between the feed tanks and microcosms. Despite our use of low DOC make-up water in the feed tank, NO\(_3\) loss to attached bacteria in the feed tank and tubing was measured at \(0.4 \pm 0.3\) mg L\(^{-1}\) early in the experiments. We measured no loss in a second set of measurements at the end of the experiments. Nitrites were within \(2 \pm 2\)% of the inlet nitrate concentration. Conversion of added nitrates and plant N to free ammonia was measured at less than \(5 \pm 4\) of the inlet nitrate concentrations. Lastly, although temporary bacterial immobilization of total organic N (TON) has often been attributed to denitrification, increases in litter N content were not sufficient to explain more than a few percent of the observed nitrate removals.
Denitrification Potential

Differences in plant specific denitrification were determined by dividing the mass of NO₃ removed in each microcosm by the amount of plant matter added. Figure 3 shows the mass of NO₃ reduced in each microcosm by the amount of plant matter added at the only NO₃ concentration (19 mg N L⁻¹) that was used at both low and high simulated wetland productivity. This DW denitrification potential of the four plants was on the order of 2 to 4% of added litter.

On a DW basis (Fig. 3), marsh pennywort and cattail treatments reduced significantly \( p < 0.05 \) more NO₃ than either duckweed or bulrush. The apparent differences in the DW denitrification potential may also be expressed on the basis of the relative proportions of the acid soluble (i.e., hemicellulose, cellulose, and starch) and acid insoluble fractions (i.e., proteins, lignins, and resins) of the total C pool of each plant (Fig. 2).

Since the DW denitrification potential was expected to differ based upon C quality, all treatments were fed on an equal C basis. When expressed on a total C basis, significant \( p < 0.01 \) differences in denitrification potential remained between the plants studied, the bulrush treatment reducing the lowest amount of NO₃-N per gram of C added. However, plants with the highest ASC content also had the highest DW denitrification potential and the differences in denitrification potential were less significant \( 0.1 < p < 0.9 \) between the four plants when compared on an equal ASC basis.

Interestingly, while normalizing the plant-specific nitrate removal data to an ASC basis explained the differences in denitrification potential at any single condition, changing either nitrate or C loading strongly affected this stoichiometric efficiency. Figure 4 shows the differences in denitrification potential at high and low C loading while holding the inlet nitrate constant. At near 20 mg N L⁻¹, the high C loading (2000 mg C m² yr⁻¹) treatments reduced fewer nitrates per gram of ASC than the treatments simulating low wetland productivity (500 mg C m² yr⁻¹). The ASC denitrification potential was higher at the lower C loading, but was still only half of the stoichiometric ratio of 0.93 g N g⁻¹ C.

DISCUSSION

Proximate Analyses

Our use of proximate C contents to show the changes in the total C pool overestimated total C content of the litter by 4 ± 4%. The largest of these errors was associated with bulrush litter, where the average C content determined by direct C analysis was 11.2% below that estimated by proximate C content. These findings suggest we systematically recovered a portion of incompletely oxidized plant materials after the 72% H₂SO₄ acid digestion of the lignin analyses.

Carbon Quality Measures

Differences in cellulose and lignin content influence the rates of mass loss during litter degradation (DeBusk and Reddy, 1998) and high initial N content has been associated with higher rates of mineralization to CO₂ (Odum and Heywood, 1978). What is most interesting about this is that floating and submersed aquatic plants generally have higher initial N content and lower lignin content than their emergent and terrestrial counterparts (Godshalk and Wetzel, 1978; Westlake et al., 1998). The experiments conducted here do not entirely support...
hypotheses that low lignin and high N contents support high denitrification rates. Marsh pennywort and bulrush had the highest and lowest denitrification potential, consistent with lower and higher lignin contents and C/N ratios. In contrast, cattail and duckweed, which had high and low denitrification potentials at correspondingly high lignin contents and low C/N ratios (Table 1).

For all plants, losses in C content and significantly \( (p < 0.05) \) higher C/N ratios in leached litter (Table 1) is consistent with preferential hydrolysis of lower MW carbohydrates. Although not all of the ASC fraction is as water soluble as the simple sugars used in many laboratory denitrification studies, it is considered to be the most rapidly hydrolyzed pool of C available for microbial respiration (Hobbie, 1996). In contrast, higher MW plant constituents, such as lignins, are only slightly acid soluble and have characteristically lower hydrolysis rates (Sarkanen and Ludwig, 1971). Although proteins are generally less acid soluble than nonprotein N (Yeoh and Wee, 1994), plants with higher protein content have lower C/N ratios, lower lignin content, a greater proportion of ASCs and characteristically higher rates of hydrolysis than high C/N plants (Westlake et al., 1998). The C quality of the two floating aquatics used here (marsh pennywort and duckweed) follows this pattern, exhibiting greater mass loss from the litter pool than the emergent aquatics during the experiments (Table 1).

**Denitrification Potential**

In this study differences in C quality of the four plants were hypothesized to explain their relative ability to fuel denitrification (Fig. 3). Bulrush litter had the lowest N content and required significantly \( (p < 0.05) \) higher dry matter addition to achieve an equivalent NO\(_3^-\) reduction than the other plants used in the study. The plant with the highest C quality, both in terms of low C/N and low lignin content (marsh pennywort), also had the highest DW denitrification potential. However, the high lignin content in cattail litter provided a disproportionately high denitrification potential for what appears to be otherwise low quality litter.

Normalizing the NO\(_3^-\) removal data to an ASC basis explains most of the plant-specific differences in denitrification potential (Fig. 3), suggesting this C fraction may be used preferentially by denitrifying bacteria. Interestingly, in one multiyear field study, lower productivity cattail wetlands demonstrated higher nitrate removal than higher productivity bulrush dominated wetlands (Bachand and Horne, 2000). This is consistent with the higher ASC fraction in cattail litter in these experiments and suggests the potential for field comparisons of wetland litter on an equal ASC basis as well.

**Applied Carbon to NO\(_3^-\)N Ratio**

These experiments clearly show that not all added plant C is available to fuel denitrification and sensitive to C loading. The ideally efficient (stoichiometric) reduction of 1 g of NO\(_3^-\)N requires the oxidation of 1.07 g of organic C. Based upon the average C content of the four plants studied here \( (476 \pm 22 \text{ g C kg}^{-1} \text{ AFDW}) \), the equivalent range in stoichiometric denitrification potential would be 424 to 465 g N kg\(^{-1}\) AFDW added. By comparison, the average denitrification potential in these experiments was \( 32 \pm 3 \text{ g N kg}^{-1} \text{ AFDW} \) added, over 10 times lower than ideal.

The lowest denitrification potentials occurred at the highest ratios of applied C/NO\(_3^-\)N, whereas higher denitrification potentials occurred at either low C or high NO\(_3^-\)N loading. This loss in efficiency may result from a combination of flushing out excess carbohydrates prior to oxidation or insufficient nitrate availability to oxidize this C fraction. That much of the plant C left the microcosms before oxidation is consistent with overall electron acceptor limitation in wetlands.

**Predicting Field Scale Denitrification Performance**

The absence of terminal electron acceptors, such as oxygen and NO\(_3^-\), in full-scale wetlands often limits oxidation of wetland litter (DeBusk and Reddy, 1998). Litter hydrolysis rates in mature wetlands generally exceed mineralization to CO\(_2\), resulting in elevated effluent DOC (Bachand, 1996) and rapid losses of nonlignocellulose components (Schipper and Reddy, 1995). The differences in carbohydrate, lignin content, and denitrification potential here were not as large as the differences in C supply characterized by the typically lower productivity of floating aquatic plants \( (150–500 \text{ g C m}^2 \text{ yr}^{-1}) \) as compared with emergent aquatics \( (1500–2500 \text{ g C m}^2 \text{ yr}^{-1}) \) (Westlake et al., 1998). While the C supplied by the accumulated soils in wetlands provides a relatively steady supply of useable C for denitrification, this laboratory study suggests the largest differences in fieldscale denitrification performance may still arise with the presence or absence of these highly productive reeds (e.g., bulrush, cattail) in wetlands.

The results here suggest two interesting points: First, based upon the DW denitrification potentials shown here, achieving reliably high average areal rates above 500 mg N m\(^{-2}\) d\(^{-1}\) would require extraordinarily high plant productivity, on the order of 17 g DW m\(^{-2}\) d\(^{-1}\). Second, although observations of high areal denitrification rates may be attributable to temporary bacterial immobilization rather than true denitrification, increases in litter N content here were insufficient to explain more than a few percentages of the observed nitrate removal.

**Future Investigations**

Practically speaking, if only 2 to 4% of dry matter participates in denitrification as bioavailable C, is the remaining material biochemically unusable or simply physically unavailable to the sites of microbial activity? Is this sequestered plant C released by sediment disturbance or by plant senescence in the autumn? It has been suggested in terrestrial ecology that N content explains litter degradation early after senescence (Odum and Heywood, 1978), but lignin content may exert a greater influence as the litter ages. If wetland litter becomes rapidly depleted of ASC upon senescence, intercellular...
lignins may become a limiting factor in denitrification by slowing the release of sequestered carbohydrates within the plant matrix.

It should be recognized that the plants used in these experiments were collected from only two locations at the same time of year and this may have affected the resulting C quality. That is, seasonal changes in productivity, protein and carbohydrate allocation, mixed stands with seasonal succession of various plant species might confound simple monospecific comparisons of denitrification performance. This suggests the need for further studies on plant-specific differences in denitrification potential and field-scale studies on the role of carbohydrate content of plants at differing growth stages.

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