Woody Debris: Denitrification Hotspots and N₂O Production in Fluvial Systems

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Research Questions

• What is the effect of fresh inputs of woody debris on nitrogen (N) cycling in streams?

• What is the effect of biofilm on N cycling in streams of differing N enrichments?
Only 20-25% of N added to biosphere is exported from rivers to ocean

Howarth et al., 1996
Watershed mass balance studies suggest considerable disappearance of N in landscape sinks; fluvial systems appear to have hotspots of N removal.
Denitrification

\[
\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2
\]

- Anaerobic
- Heterotrophic (requires organic C)
- Microbes, C, and N must mix
Hot Spot Hypothesis
(Tiedje et al., 1984; McClain et al., 2004; Groffman et al., 2009)

- Denitrification focused in select, localized settings and thought to be a function of:
  - Pools of labile C
  - Extended residence time
  - Anoxia microsite
<table>
<thead>
<tr>
<th>Big Spring Run, PA</th>
<th>Mawney Brook, RI</th>
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</thead>
<tbody>
<tr>
<td><strong>NO$_3$-N</strong>: 8.93 mg/L (agricultural)</td>
<td><strong>NO$_3$-N</strong>: 0.05 mg/L (suburban)</td>
</tr>
<tr>
<td><strong>sun</strong></td>
<td><strong>shade</strong></td>
</tr>
<tr>
<td><strong>No riparian buffer</strong></td>
<td><strong>Forested riparian buffer</strong></td>
</tr>
<tr>
<td><strong>$A_T$: 224 ppm</strong></td>
<td><strong>$A_T$: 7 ppm</strong></td>
</tr>
</tbody>
</table>
Big Spring Run, Lancaster County, PA

Mawney Brook, East Greenwich, RI
Evaluated denitrification rates from 3 different substrates in two locations

- Fresh Red Maple wood blocks
- Woody debris naturally found in stream
- Artificial stones

- All substrates placed in streams for 9 weeks;
- Biofilms developed over that period
Mesocosm Methods

- **$\text{NO}_3^- \ {}^{15}\text{N}$ tracer, 20 atom\% (Jenkins and Kemp, 1984)**
  - Heavier isotope used to trace DeN that occurs in mesocosm

- Incubation samples at time 0, 1.5 hrs, 3 hrs, and 18 hrs

- Rates expressed in ug N/m$^-2$ hr$^-1$

- Biofilm mass obtained

- Nitrate analyzed at time 0 and 18 hours later
At both sites extant wood and wood blocks generated high denitrification rates.
Wood substrates at forested site had higher denitrification than the agricultural site ($p \leq 0.05$, t-test)
Forest wood had higher denitrification than artificial stones
Agricultural site had no significant differences between wood and stone.
Denitrification rates of control wood blocks (no biofilm) were much greater than artificial stones without biofilms.
N₂O Flux

• N₂ gas accounted for 99% of the total denitrification; denitrification went to completion

• Compared to many other ecosystems woody substrates in these two stream ecosystems are not a substantial source for N₂O generation
## Biofilm Characteristics

<table>
<thead>
<tr>
<th>Site</th>
<th>Substrate</th>
<th>Mean Biomass (g)#</th>
<th>Stnd Dev.</th>
<th>Biomass C</th>
<th>Color</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forest</td>
<td>wood block</td>
<td>0.530&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.32</td>
<td>531 µg C cm&lt;sup&gt;-2&lt;/sup&gt;</td>
<td>dark brown</td>
<td>matted</td>
</tr>
<tr>
<td></td>
<td>artificial stone</td>
<td>0.068&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.05</td>
<td></td>
<td>dark brown</td>
<td>matted</td>
</tr>
<tr>
<td>Agric</td>
<td>wood block</td>
<td>0.304&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.20</td>
<td>213 µg C cm&lt;sup&gt;-2&lt;/sup&gt;</td>
<td>bright green</td>
<td>filamentous</td>
</tr>
<tr>
<td></td>
<td>artificial stone</td>
<td>0.133&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.17</td>
<td></td>
<td>bright green</td>
<td>filamentous</td>
</tr>
</tbody>
</table>

Significant differences are noted by different subscript letters, \( p \leq 0.05 \) using a Tukey post hoc test.
Biofilm Findings

• Nitrate-N removal was correlated to biomass ($r=0.69$, $p \leq 0.01$), however no correlation found between biomass and denitrification rates

• Note: In-stream wood blocks (with developed biofilms) had higher denitrification than wood block controls (no biofilm) emphasizing importance of **biofilm for N cycling**

• Low levels of carbon in the agricultural biofilm may be due to grazers that prefer algal biomass or to enhanced rates for microbial degradation (Hillebrant and Kahlert, 2001)
Denitrification Findings

• Wood substrates promote denitrification to completion
  – Occurs in starkly contrasting sites with different levels of riparian forest cover, ambient nutrient enrichment, and alkalinity

• Regardless of the extent of biofilm development, wood substrates tend to generate higher denitrification than biofilm on artificial stones

• Negligible N$_2$O production
Implications

This study supports the importance of woody debris for promoting conditions that stimulate N cycling within streams and further emphasizes the value of restoring mature forest cover in riparian settings for nitrogen management.
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