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Relationships between DOC bioavailability and nitrate removal in an upland stream: An experimental approach

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Abstract. The Catskill Mountains of southeastern New York State have among the highest rates of atmospheric nitrogen deposition in the United States. Some streams draining Catskill catchments have shown dramatic increases in nitrate concentrations while others have maintained low nitrate concentrations. Streams in which exchange occurs between surface and subsurface (i.e. hyporheic) waters are thought to be conducive to nitrate removal via microbial assimilation and/or denitrification. Hyporheic exchange was documented in the Neversink River in the southern Catskill Mountains, but dissolved organic carbon (DOC) and nitrate (NO_3^-) losses along hyporheic flowpaths were negligible. In this study, Neversink River water was amended with natural, bioavailable dissolved organic carbon (BDOC) (leaf leachate) in a series of experimental mesocosms that simulated hyporheic flowpaths. DOC and N dynamics were examined before and throughout a three week BDOC amendment. In addition, bacterial production, dissolved oxygen demand, denitrification, and six extracellular enzyme activities were measured to arrive at a mechanistic understanding of potential DOC and NO_3^- removal along hyporheic flowpaths. There were marked declines in DOC and complete removal of nitrate in the BDOC amended mesocosms. Independent approaches were used to partition NO_3^- loss into two fractions: denitrification and assimilation. Microbial assimilation appears to be the predominant process explaining N loss. These results suggest that variability in BDOC may contribute to temporal differences in NO_3^- export from streams in the Catskill Mountains.

Introduction

NO_3^- is a major pollutant in rivers, reservoirs, groundwaters, and coastal waters in much of the northern hemisphere (NRC 2000). Major sources of NO_3^- in large rivers and coastal waters include atmospheric deposition, agricultural run-off, and sewage effluent (Howarth et al. 1996). High rates of atmospheric deposition of nitrogen result in elevated NO_3^- concentrations even in flowing waters that do not receive agricultural or sewage inputs (Lovett 1994; Likens and Bormann 1995). Although atmospheric deposition contains an array of N-compounds, catchment loss is frequently dominated by NO_3^- due to its high mobility.

The potential for N-retention in forested ecosystems is presumably high since temperate forest growth is frequently N-limited (Aber et al. (1989); see Aber and Melillo (1991)) and soil microbes can function as a N-sink (Groffman et al. 1999). However, NO_3^- losses from forested catchments can be appreciable and variable among streams (Lovett et al. 2000). In relatively pristine forested catchments receiving nominal N-deposition, NO_3^- contributed a smaller fraction of total N in streams (Hedin et al. 1995). These findings, and those of others (e.g., Likens et al. (1996)), have resulted in a critical examination of previous conceptual models (e.g., Vitousek and Reiners (1975)) explaining N-retention in forested ecosystems as a function of net ecosystem production. As N accumulates in forested catchments, N supply may exceed biotic demand and result in increased concentrations of NO_3^- and dissolved organic nitrogen (DON) in stream water regardless of rates of net ecosystem production.

Although N-inputs are elevated in much of the northern hemisphere, high N-concentrations in natural waters cannot be understood simply by a mass-balance approach because N undergoes a variety of biotic transformations. In anoxic environments, such as soil porewaters, denitrification results in N-loss to the atmosphere, and is subject to multiple potential controlling factors (Groffman et al. 1999). NO_3^- in transport from upland forests to streams can be partially removed by microbial assimilation and denitrification in riparian soils (Groffman et al. 1992; Lowrance 1992) and soil-stream interfaces (McClain et al. 1994; Hedin et al. 1998; Hill et al. 2000). In general, small streams seem to be particularly effective at modifying dissolved inorganic nitrogen concentrations during transport to coastal waters (Alexander et al. 2000; Peterson et al. 2001), however high NO_3^- concentrations persist in many streams and rivers (Howarth et al. 1996). These findings suggest that assimilative and dissimilative N-sinks do not completely deplete the flux of N to downstream ecosystems in many catchments receiving large N inputs.

The Catskill Mountains of New York State receive some of the highest amounts of N-deposition in the US (Lovett 1994). Although catchments in this region are predominantly forested, there is large export of N in the form of NO_3^- in streamwater draining many of these catchments (Lovett et al. 2000). NO_3^- concentrations in some of these streams have increased dramatically during the past 30 years suggesting that N inputs have exceeded catchment-level controls on N loss (Murdoch and Stoddard (1993); see Lovett et al. (2000)), while NO_3^- concentrations in other streams remain low (Lovett et al. 2000). NO_3^- loss from Catskill Mountain catchments has received acute attention because Catskill Mountain streams supply the vast majority (90%) of New York City drinking water (Iwan 1987), are sensitive to acidification (Murdoch and Stoddard 1992), and drain into major estuaries (New York, Delaware, and Chesapeake Bays) that are sensitive to eutrophication (Howarth et al. 1996).

Streams that have significant exchange between surface and subsurface waters can be active areas of N removal and may further mediate the flux of NO_3^- to downstream ecosystems (Triska et al. 1989; Jones and Holmes 1996; Duff and Triska 2000). Subsurface flow (i.e. hyporheic flow) is known to be appreciable in the Neversink River, a fourth-order river that drains the southern Catskill Moun-

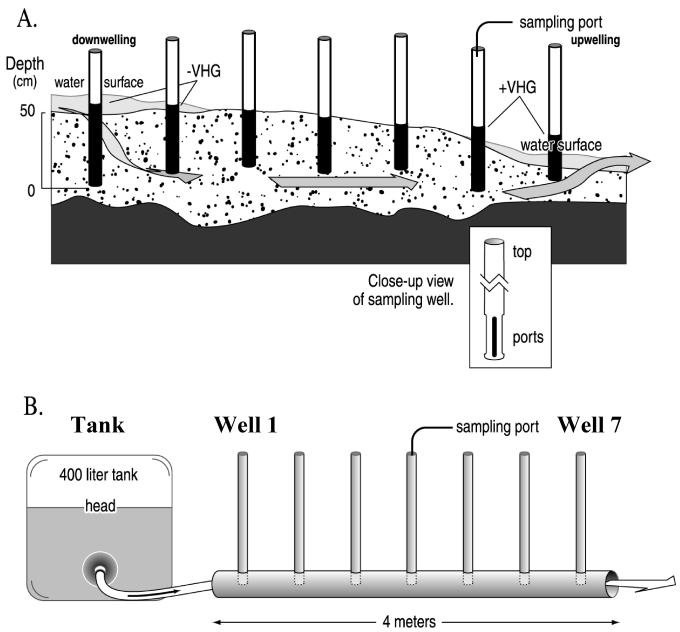

Figure 1. A) Cross-sectional view of exposed-gravel bar highlighting the hydrological characteristics of the hyporheic flowpaths that were studied in the Neversink River. B) Schematic of one of six mesocosms that were constructed to simulate natural-hyporheic flowpaths.

tains (Burns 1998; Sobczak and Findlay 2002 (in press)). NO_3^- concentrations in small streams draining into the Neversink River are relatively high and vary seasonally (Murdoch and Stoddard (1993); see Lovett et al. (2000)), but the Neversink River's potential for controlling downstream loss of NO_3^- may be variable (Burns 1998). Burns (1998) documented some in-stream retention using a mass-balance approach, but retention mechanisms could not be quantified. There was some evidence for modest NO_3^- declines during daylight hours which suggested biotic uptake (Burns 1998). Using a separate approach, Sobczak and Findlay (2002) (in press) documented conservative DOC, dissolved oxygen, and NO_3^- transport along two hyporheic flowpaths during separate field years. These patterns were observed along hyporheic flowpaths through gravel bars that were not inundated at base flow, hence infiltration of surface waters could not account for the observed conservative transport of solutes (Figure 1A). These findings suggest that the Neversink River has low bioavailable DOC (BDOC) and, as a result, low microbial activity and low potential for NO_3^- removal.

The objective of this study was to examine the potential linkage between low BDOC and low NO_3^- retention in Neversink River sediments. To experimentally test the hypothesis that NO_3^- removal is limited by BDOC, leaf leachate was added to hyporheic flowpaths in a series of experimental mesocosms that simulated *in situ* hyporheic flowpaths (Figure 1B). Experimental mesocosms provided replication of treatments, controlled for hydrologic dispersion, and enabled calculation of DOC

and N inputs and outputs. Bacterial production, dissolved oxygen demand, denitrification, and six extracellular enzyme activities were examined to help explain DOC and N removal mechanisms.

Study area

The Catskill Mountain Region is located west of the Hudson River Valley in New York USA, approximately 150 km north of New York City. The Catskill Mountains consist predominantly of second growth mixed oak forests, although composition and stand-age vary among catchments (Lovett et al. 2000). For example, the Neversink River catchment contains more sugar maple, American beech, and yellow birch, and less red oak than adjacent catchments. The Catskill Mountains consist of 35 peaks between 1067–1274 m in elevation, resulting from erosional forces during post-glaciation. Atmospheric N deposition has been some of the highest in the US (total N deposition exceeds 10 kg ha^{-1} y^{-1}) (Ollinger et al. 1993; Lovett 1994). Catskill Mountain water quality varies among streams, but can be generalized as having low pH (mean = 6.5; n = 38 streams), NH_4^+ (mean = 31 μg/L; n = 39), DON (mean = 69 μg/L; n = 39) and DOC (0.87 mg/L; n = 39). Unlike other chemical attributes, NO_3^- (mean = 1.4 mg/L) varied 17-fold and the ratio of DON: NO_3^- varied $\sim$50-fold among catchments (n = 39) (as detailed in Lovett et al. (2000)). Furthermore, standard hydrologic normalization procedures (e.g. slope, area) did not remove variation in N concentrations among catchments (Lovett et al. 2000).

This study focused on the West Branch of the Neversink River (henceforth Neversink River) which is the main tributary entering the Neversink Reservoir and is characteristic of many other Catskill Mountain streams in both catchment and water quality attributes (see Lovett et al. (2000)). Neversink River DOC concentrations (mean = 0.96 mg/L ± 0.08 SE; n = 32 dates) are comparable to other Catskill Mountain streams, but BDOC concentrations are low (undetectable on most dates) relative to streams in the Hudson River Valley (BDOC range = 0–50% of surface water DOC) (Sobczak and Findlay 2002 (in press)). NO_3^- concentrations in the Neversink River's tributaries (mean = 1.3 mg/L ± 0.12 SE; n = 4 streams) and main-stem (mean = 1.4 mg/L ± 0.02 SE; n = 16 dates) are similar to those found across Catskill Mountain streams (as described above) (Lovett et al. 2000), although seasonal and storm-flow variance can be appreciable (Murdoch and Stoddard 1992). Noteworthy to this study, NO_3^- concentrations in streams within and adjacent to the Neversink River's catchment decrease during autumn with the most dramatic declines occurring after leaf fall (Murdoch and Stoddard (1992, 1993)). In addition, NO_3^- mass balance calculations suggest modest diurnal variation in the Neversink River with declines occurring during daylight hours (Burns 1998). The Neversink River is underlain by sandstone and conglomerate and overlain by till deposits and alluvium derived from the last glaciation (see Burns (1998)). Alluvium width ranges

from a few meters in the headwaters to hundreds of meters at downstream reaches and the potential for exchange of surface water and subsurface water appears high.

Methods

Experimental design

Neversink River surface water was transported via a tanker truck (volume = 24,000 L) on October 5, 1997 and stored for the duration of the experiment. Water retrieval was timed to coincide with the beginning of leaf senescence. Neversink River water was gravity fed to six 400-L holding tanks associated with independent mesocosms. Each mesocosm consisted of a 400-L reservoir attached to an opaque PVC pipe (4 m length × 15 cm diameter) filled with 1/4-inch gravel from a local quarry. Gravel was thoroughly washed in a concrete-mixer before being vertically packed into mesocosms and re-washed with $\sim$1000-L of Neversink River water after packing. Mesocosms were outside and susceptible to daily temperature fluctuations, but surface water was not exposed to sunlight. A series of seven wells allowed interstitial water to be sampled along each experimental flowpath (Figure 1B). Neversink River water was supplied to sediments for two weeks before manipulation to allow microbial colonization of the washed sediments. Water samples were analyzed at the end of this period (details below) and sterile ceramic tiles were added to reservoirs (i.e. tanks) and wells at the head (Well 1) and tail (Well 7) of mesocosm flowpaths. The experimental period was initiated with the addition of BDOC (i.e. leaf leachate) to three of the six mesocosms on Oct 21. Reservoir DOC concentrations were elevated 0.5 mg/L (relative to ambient Neversink River DOC concentration) in the treatment mesocosms during the first two weeks of the experiment and elevated to +1.0 mg/L above ambient DOC during the third week of the experiment. Leaf leachate was obtained by soaking a mix of pre-abscission leaves from sugar maple and red oak trees that represent potential catchment and regional leaf leachate. Flow rate was maintained at $\sim$10 cm/h by adjusting an outlet valve and height of outlet hose.

Sampling regime and laboratory procedures

Water samples were taken in duplicate at the head and tail of all mesocosms weekly and analyzed for DOC, dissolved oxygen, NO_3^-, and soluble reactive phosphorus (SRP). Total organic nitrogen (TON) was measured on the final sampling date. Additional water samples were taken from all wells only on the final sampling date. DOC was measured using a Shimadzu 5000 TIC/TOC Analyzer following acidification and sparging (minimum detection limit = 0.1 mg/L). Dissolved oxygen was measured in the field using a YSI Model 57 portable oxygen probe. NO_3^- and SRP were measured using an Alpkem Flow Solution III autoanalyzer with continuous flow (detection limit = 0.02 mg/L). Total organic nitrogen (TON) was estimated by

digesting water samples in potassium persulfate reagent, measuring total N, and correcting for original NO_3^- (see Lovett et al. (2000)).

Ceramic tiles (n = 6; size = 2.5 × 2.5 cm) were used as artificial substrates for microbial colonization. Tiles were combusted at 450 °C for 5 h, soaked 24 h in low-DOC water, enclosed in mesh cases to aid retrieval, and inserted into wells. Tiles were incubated for three weeks within tanks and respective wells before being removed. Bacterial productivity was estimated by measuring the rate ^{3}H-thymidine was incorporated into bacterial DNA (Findlay et al. 1984; Findlay 1993) following the procedure described in Sobczak and Findlay (2002) (in press).

Six extracellular enzyme activities were examined on the final sampling date at the head and tail of experimental flowpaths. Enzyme activities can be used to examine microbial activity, nutrient limitation, and organic carbon sources. Methods used by Sinsabaugh et al. (1997) and Findlay et al. (1998) were modified. Biofilms were removed from incubated tiles by scrubbing with autoclaved brushes and resuspended in representative tank or hyporheic water. The following fluorescently-labeled substrates were used in conjunction with the enzyme they assay: 4-MUF-acetate: esterase; 4-MUF-phosphate: phosphatase; L-leucine 7-amido-4-methylcoumarin: leucine aminopeptidase; 4-MUF-β-D-glucoside: β-glucosidase; 4-MUF-β-xyloside: β-xylosidase; 4-MUF-N-acetyl-β-glucosaminide: β-N-acetylglucosaminidase. Changes in fluorescence were monitored during short incubation periods and related to enzyme activity. Measurements were made using a Perkin-Elmer fluorometer after 0, 5, 10, 15, 60, 120, 180 minutes to ensure linear response. Activity corresponds to differences in fluorescence between time 0 and time x and is expressed as nmol substrate cleaved per ml of biofilm suspension per hour.

Potential denitrification was estimated using the acetylene-block method in which N_2O becomes the predominant gaseous form of N. Sediments were removed from the head and tail of leaf-leachate amended and control flowpaths following the final sampling date, placed in triplicate flasks, saturated with source water, sealed, de-gassed, and injected with acetylene following the general procedures of Groffman et al. (1999) for soils. Transfer of sediment from mesocosms to flasks resulted in a disturbance, but sediments from both treatments were handled the same. N_2O in the headspace of each flask was sampled at time = 0 and following a 24 h incubation period at room temperature and measured using a gas chromatograph. A 24 h incubation period was used to allow measurable accumulation of N_2O from these low-activity samples. N_2O concentrations were converted into the amount of N denitrified per gram wet-sediment per day following the calculations of Groffman et al. (1999). Incubations using sediments from the leaf-leachate amended and control mesocosms were compared with and without additional glucose additions. Glucose-amended flasks received source water with DOC concentrations raised 1 mg/L.

Bottle experiment

A bottle experiment examining effects of a broader range of carbon and nutrient additions on DOC and N removal was conducted in conjunction with the mesocosm experiment during the final week of the study. Freshly collected, unfiltered Neversink River water (without sediment) received the following independent additions: low glucose (1 mg/L), high glucose (3 mg/L), low leaf leachate (1 mg/L), high leaf leachate (3 mg/L), N ($NaNO_3^-$-N 1 mg/L), P ($NaPO_4$+-P 0.1 mg/L), N + P, N + low glucose, and P + low glucose. All treatments were replicated in five independent 125 mL bottles and compared with unamended controls. DOC was measured immediately following amendments and after one, three and seven days. DOC-loss over time was analyzed in treatments receiving carbon additions. DOC loss relative to unamended Neversink River water was analyzed in treatments receiving inorganic nutrient additions. DOC loss relative to low glucose treatment was analyzed in treatments receiving inorganic nutrients and low glucose additions.

Statistical analyses

Mesocosms provided replication of flowpaths in space and wells at fixed distances between treatments (i.e. leaf-leachate amended vs. control). Leaf-leachate additions were designed to increase DOC concentrations, hence DOC and DON declines were analyzed on the final sampling date using linear regressions. Dissolved oxygen demand, NO_3^-, and SRP were analyzed on individual dates using t-tests comparing mesocosm tanks and tails (i.e. well 7) for each treatment. Bacterial productivity and extracellular enzyme activities were analyzed on the final sampling date with two-way ANOVAs in which "treatment" and "distance" were considered fixed factors. Extracellular enzyme activities from NS-Well 7 were derived from pooled samples from replicate mesocosms, hence NS-Tank variances were used in ANOVA analyses. Laboratory bottle experiments were analyzed using selected paired comparisons (discussed above). Potential denitrification assays were pseudoreplicated since variance data were derived from triplicate flasks from the same mesocosm, hence variance data were reported but not compared statistically. Significance was attributed to statistical values in which the probability of a Type I error is $p \leqslant 0.05$. SYSTAT® version 6.1 (SYSTAT Corp. 1996) was used for all statistical analyses.

Results and discussion

DOC dynamics

DOC concentrations declined 38% (linear regression: $p < 0.001$) along leaf-leachate amended flowpaths (henceforth called LL) and did not decline along flowpaths receiving unamended Neversink River water (henceforth called NS) (Figure 2A).

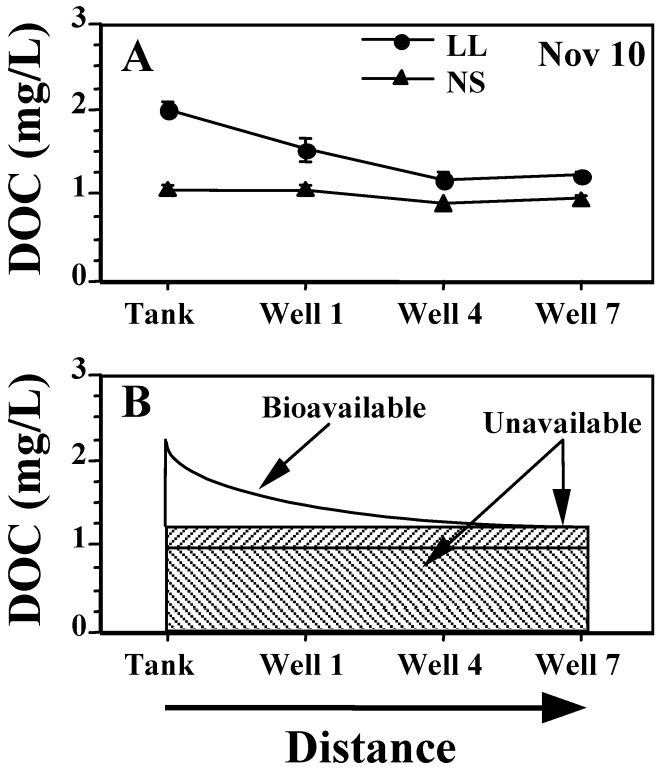

Figure 2. A) DOC (mg/L) along leaf-leachate amended (i.e. LL) and control (i.e. NS) flowpaths on the final sampling date (i.e. Nov 10). Points represent the mean of replicate mesocosms (n = 3) ± SE. B) DOC (mg/L) from (A) conceptualized as bioavailable (BDOC) and unavailable fractions.

These data provided the experimental framework for examining coupled DOC and N dynamics; specifically, they suggested that the LL treatment contained a large addition of bioavailable DOC (Figure 2B). DOC patterns on the final sampling date reflected DOC patterns throughout the experiment (Figure 3). DOC removal along LL flowpaths requires evidence that declines were the result of microbial metabolism to be considered a function of DOC bioavailability. For example, if declines in DOC concentration were mediated by microbial metabolism a concomitant loss of dissolved oxygen is predicted. Dissolved oxygen significantly decreased ($p < 0.001$) along LL flowpaths on all sampling dates (Figure 4). On Nov 4 dissolved oxygen declined 1.2 mg/L along LL flowpaths yielding a predicted DOC loss of 0.45 mg/L, which was almost identical to our measured DOC loss (i.e. BDOC) of 0.44 mg/L. On Nov 10 the expected and observed losses of DOC were 1.95 mg/L and 2.0 mg/L. These data suggest DOC removal was mediated by microbial metabolism rather than abiotic adsorption.

Previous work has demonstrated strong positive relationships between DOC loss and bacterial productivity along riparian flowpaths (Sobczak et al. 1998) and hy-

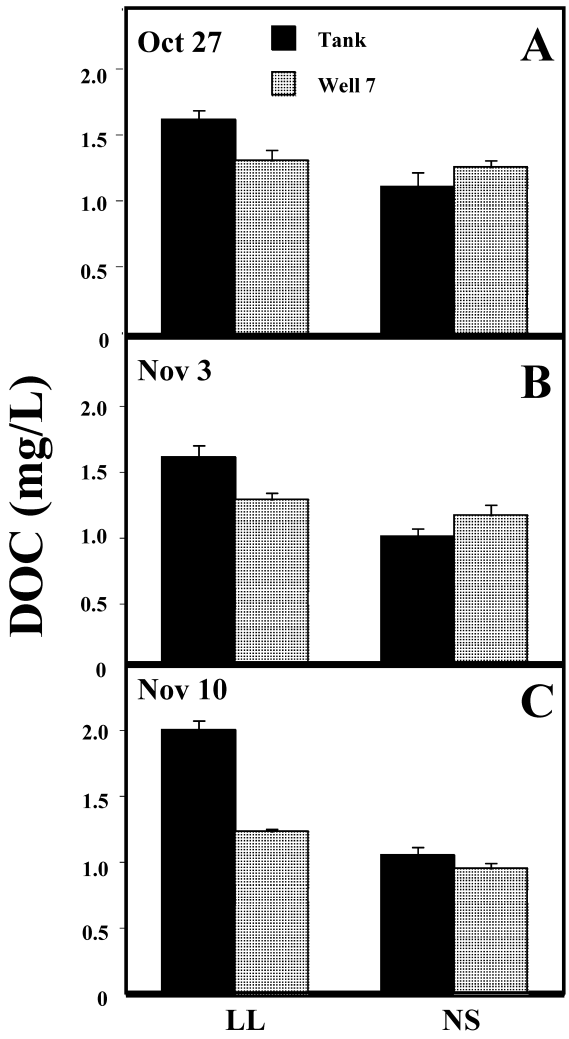

Figure 3. DOC (mg/L) at head (i.e. Tank) and tail (i.e. Well 7) of leaf-leachate amended (LL) and control (NS) flowpaths on three sampling dates. Bars represent the mean of replicate mesocosms (n = 3) ± SE.

porheic flowpaths (Sobczak and Findlay 2002 (in press)). In this study, bacterial productivity remained unchanged along NS flowpaths, presumably due to low BDOC. Conversely, productivity was significantly greater in LL treatments and declined along flowpaths ($p < 0.05$) (Figure 5). The significant difference between treatments was a function of higher rates of bacterial productivity in the LL tanks. The absence of a decline between well 1 and well 7 in the LL mesocosms suggests that DOC uptake was rapid. Correlation between patterns of DOC removal and independent measures of bacterial production supports the assertion that DOC re-

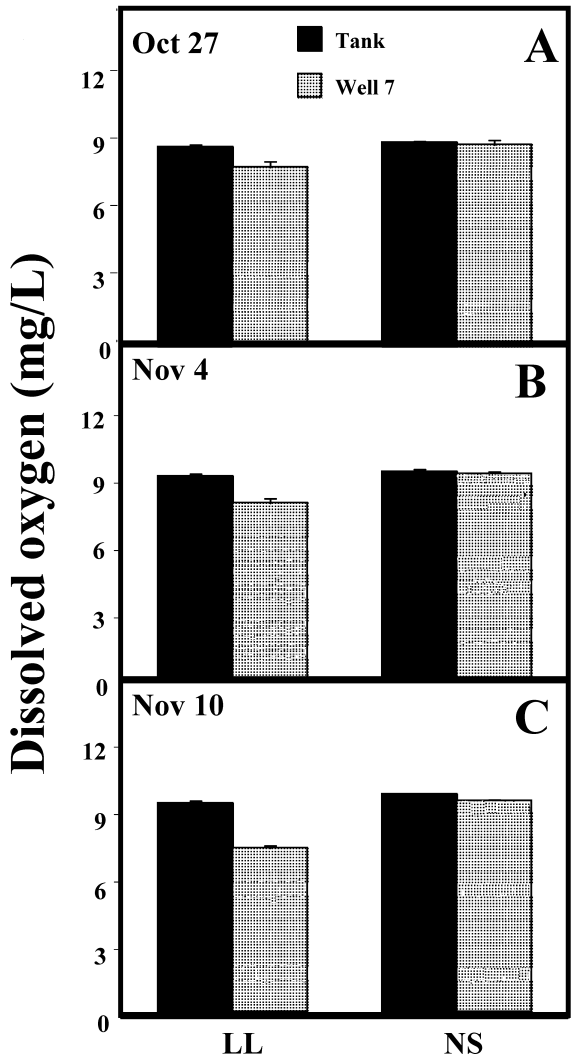

Figure 4. Dissolved oxygen at head (Tank) and tail (Well 7) of leaf-leachate amended (LL) and control (NS) flowpaths on three sampling dates. Bars represent the mean of replicate mesocosms (n = 3) ± SE.

moval was mediated by microbial metabolism. Hence, DOC removal in LL mesocosms (38% on the final sampling date) corresponds to the bioavailable fraction of the leaf-leachate amendment as conceptualized in Figure 2.

N-dynamics

Total N in Catskill Mountain streams is almost completely comprised of NO_3^- (82.0%) and DON (17.6%) (Lovett et al. 2000). Mesocosm NO_3^- concentrations

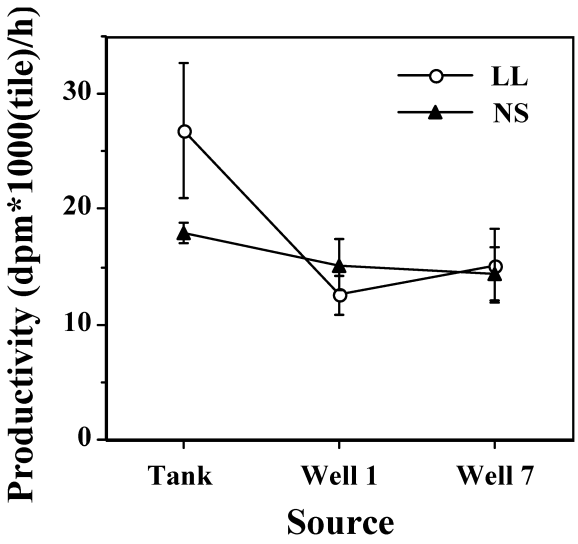

Figure 5. Bacterial productivity on tiles incubated within leaf-leachate amended (LL) and control (NS) flowpaths. Points represent the mean of replicate mesocosms (n = 3) ± SE.

were similar between treatments prior to the initiation of the experimental period (i.e. October 20) with a slight (6.7%), yet statistically significant decline along flowpaths ($p < 0.001$) (data not shown). One week after leachate amendments, NO_3^- throughout LL-mesocosms had declined to $\sim 50\%$ of values in NS mesocosms (Figure 6). After two weeks NO_3^- dropped to below detection limits throughout the LL-treatment mesocosms (Figure 6). NO_3^- declined more subtly, yet significantly ($p < 0.01$), on all dates in the NS treatment (Figure 6). DON concentrations, sampled on the final date, significantly declined along LL flowpaths ($p < 0.001$), but remained much higher than concentrations found along NS flowpaths (Figure 7). Interestingly, DON concentrations did not decrease following well 4 along LL flowpaths suggesting that the remaining DON was associated with unavailable DOC. NO_3^- and DON losses were striking considering that DOC was elevated to a realistic concentration found well within the range observed in the Neversink River.

Declines in NO_3^- concentrations along a stream reach or hyporheic flowpath may be the result of: 1) dilution from additional water sources (e.g. tributaries, groundwaters), 2) assimilation by microbes, and 3) denitrification. Mesocosms excluded dilution and dispersion as potential mechanisms for loss in transport; hence, NO_3^- loss along LL flowpaths, and subsequent loss in LL tanks, can potentially be attributed to either denitrification or microbial assimilation. These two potential NO_3^- sinks are difficult to separate. At the cellular level denitrification requires anoxic conditions, however porewater dissolved oxygen concentrations did not approach anoxia in this experiment (Figure 4). It is well established that denitrification can occur in anoxic biofilms or microsites, hence the absence of anoxic conditions does not preclude denitrification (Groffman et al. 1999).

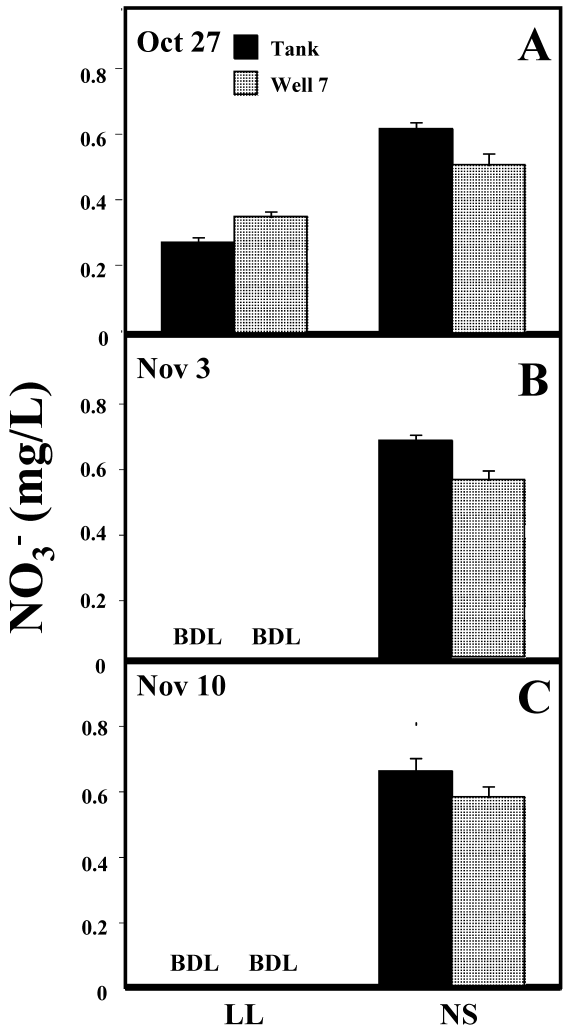

Figure 6. NO_3^- (mg/L) at head (Tank) and tail (Well 7) of leaf-leachate amended (LL) and control (NS) flowpaths on three weekly sampling dates. Bars represent the mean of replicate mesocosms (n = 3) ± SE. BDL = Below Detection Limit.

Potential rates of denitrification were examined at the head and tail of LL and NS flowpaths in the absence of *in vitro* carbon amendments and compared with glucose amended sediments. While variance among samples was low, true replication of experimental units was absent; hence, results must be discussed with caution. Sediments from the head of the LL flowpath had the highest rates of denitrification among flasks with and without glucose amendments (Figure 8). Despite measurable denitrification, rates were low and not sufficient to account for a significant fraction of NO_3^- removal. The maximum denitrification rate observed, 1.2

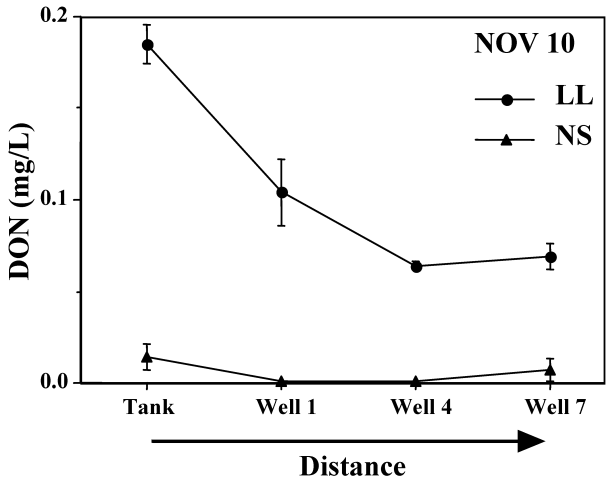

Figure 7. Dissolved organic nitrogen (DON) (mg/L) at the head (Tank) and tail (Well 7) of leaf-leachate amended (LL) and control (NS) flowpaths on the final sampling date (Nov 10). Points represent the mean of replicate mesocosms (n = 3) ± SE.

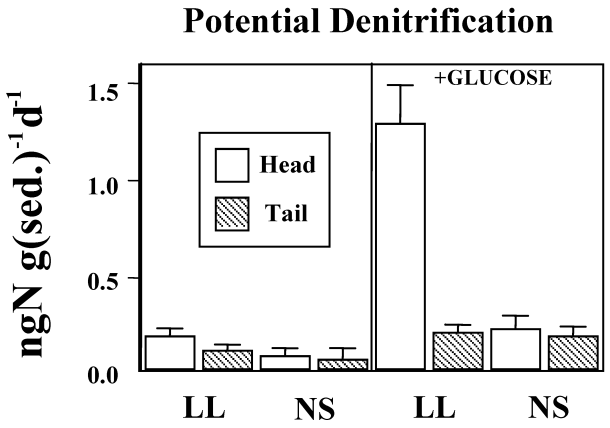

Figure 8. Potential denitrification (ng N g(sed.)$^{-1}$ d^{-1}) using sediments incubated at the head and tail of mesocosms. LL = leaf-leachate amended and NS = control flowpaths. Points represent the mean of triplicate sediment samples ± SE from either one LL mesocosm or one NS mesocosm. Incubations with and without additional glucose additions were compared. See Methods for details.

ng N g-sediment^{-1} d^{-1}, would correspond to 0.18 mg N per mesocosm per day and would account for < 5% of the daily NO_3^- removal in LL tanks (~ 3.75 mg/L). This calculation assumed: 1) 100 g wet-sediment accounted for 50 cm^3 of mesocosm space, 2) 1 L of sediment corresponded to 2,000 g and resulted in N-loss rates of 2.4 μg N L-sediment^{-1} d^{-1}, 3) each mesocosm contained 75 L of porewater, 4) initial NO_3^- = 0.15 mg/L, and 5) daily discharge = 25 L.

Bacterial assimilation may have accounted for the remainder of the reported NO_3^- removal. Assuming DOC loss = 1 mg/L, bacterial growth efficiency = 50%,

bacterial C:N = 7:1 then 0.5 mg C/L and 0.08 mg N/L could be assimilated into bacterial biomass. These calculations suggest microbial assimilation may account for $\sim$60% of NO_3^- loss in LL mesocosms, hence part of the NO_3^- remains unaccounted for. Additional NO_3^- may be associated with interstitial biofilms, although the ultimate fate of this NO_3^- is unknown.

Carbon limitation

Leaf leachate provided a complex and natural DOC addition but also contained inorganic nutrients that may have confounded comparisons of DOC and NO_3^- loss between amended and unamended flowpaths. Potential inorganic nutrient limitation was addressed with three independent methods:

1. stoichiometry analysis in which we predicted biological demand for C:N:P
2. a laboratory bottle experiment in which Neversink River water was amended with various combinations of C, N, and P (see Methods), and
3. examination of C, N, and P acquiring extracellular enzymes.

Neversink River NO_3^- concentrations were high relative to BDOC; hence, N limitation was highly unlikely. However SRP concentrations were relatively low and may have been limiting. SRP in Neversink River water was 8.4 μg/L and did not decline along NS flowpaths (data not shown). SRP concentrations throughout the study in NS tanks were sufficient to convert > 33% of total DOC into bacterial biomass assuming a Redfield C:P ratio of 106:1. Considering that only a fraction of total DOC in the NS was bioavailable and BDOC removal could occur at low bacterial growth efficiencies, P limitation seems unlikely in the NS mesocosms. Leaf leachate amendments increased SRP to $\sim$0.1 mg/L and SRP significantly decreased along LL flowpaths on all dates ($p < 0.001$) (data not shown). The magnitude of the loss was greater than would be predicted from DOC-loss, suggesting abiotic adsorption contributed to SRP removal. Overall, water chemistry stoichiometry does not support P-limitation.

Bottle experiment results were more definitive, although bottle experiments may not adequately represent sediment-bound biofilm activity. Dramatic and rapid DOC declines were observed in both glucose and leaf leachate amended bottles over time (t-test: $p < 0.001$) (Figure 9). Conversely, DOC did not significantly decline ($p > 0.05$) in ambient DOC treatments receiving N, P, and N and P relative to the ambient control. In summary, this experiment provided strong evidence that organic carbon quality was the predominant control on Neversink River DOC metabolism.

Extracellular enzyme activities were used to examine microbial C, N, and P demand and inorganic nutrient limitation. Overall, enzyme activities varied between treatments (Figure 10). Fatty-acid esterase and phosphatase activities declined along flowpaths in both NS and LL mesocosms ($p < 0.05$). Leucine-aminopeptidase, xylosidase, and β-glucosidase were significantly higher in the LL treatment and declined along flowpaths ($p < 0.001$). High DON concentrations in LL tanks likely resulted in high leu-aminopeptidase activities. Overall, enzyme activities at flow-

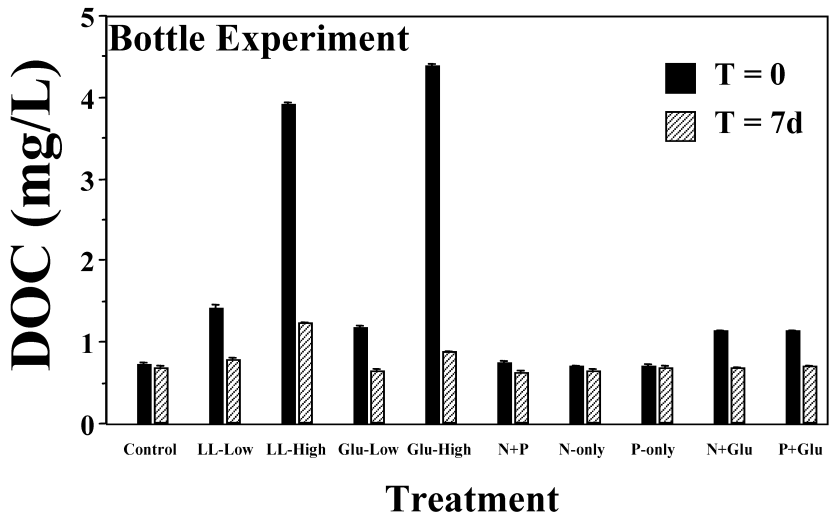

Figure 9. DOC (mg/L) in Neversink River water amended with various organic and inorganic nutrients during a one -week incubation period. Treatments include: control = unamended Neversink River water, LL-Low = low leaf-leachate, LL-High = high leaf-leachate, Glu-Low = low glucose, Glu-High = high glucose, N = $NaNO_3^-$ + P = $NaPO^4$+, N-only, P-only, N + Glu-Low, and P + Glu-Low. Bars represent the mean of replicate bottles (n = 5) ± SE. See Methods for details.

path tails were similar suggesting convergence in DOC composition. Hence, elevated DOC concentrations at the tails of treatment mesocosms appear to be less susceptible to degradation. Stimulation of extracellular enzymes by the LL amendment suggests there were polymers present in the leachate susceptible to bacterial degradation and the stimulation was not just due to monomers in the leachate.

Catchment-level consequences

Land-water interfaces are thought to be critical zones of metabolic regulation in many aquatic ecosystems (Likens 1984; Wetzel 1990). The flux of organic matter through stream ecosystems is greatly influenced by the hydrologic vectors through which DOC enters streams. DOC in soil-porewaters and riparian sediments can decrease markedly before entering stream surface water indicating the potential for metabolic regulation at terrestrial-lotic interfaces (McDowell and Likens 1988; Sobczak et al. 1998; Hill et al. 2000). Surface water DOC can be further influenced by continued interaction with surface sediments (Battin 2000) and hyporheic sediments (Sobczak and Findlay 2002 (in press)).

Results conclusively show that NO_3^- can be removed from oxic surface waters and porewaters with a realistic, natural BDOC addition. Delineation of assimilatory and dissimilatory losses was not definitive, but potential denitrification and microbial assimilation were both clearly greater in BDOC-amended sediments. Results suggest that microbial assimilation may partially account for declines in NO_3^- concentrations during autumn in some regional streams (Murdoch and Stoddard 1993).

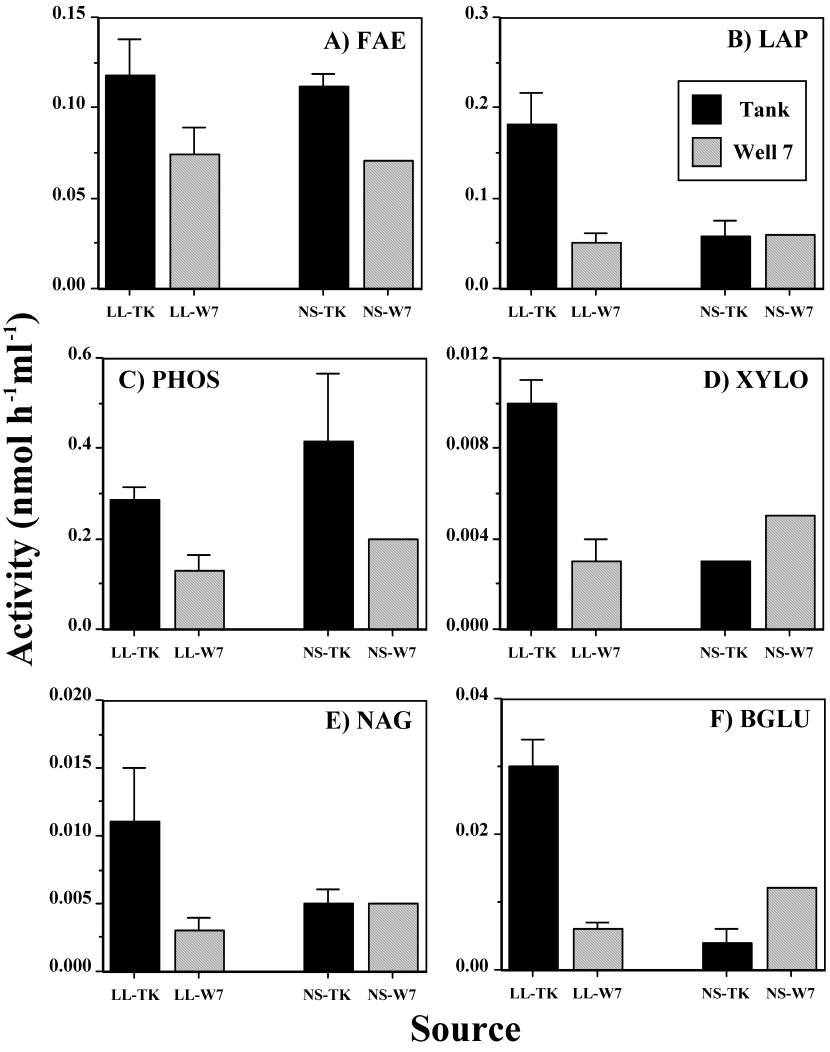

Figure 10. Extracellular enzyme activities of microbial communities colonized at the head (TK) and tail (W7) of leaf-leachate amended (LL) and control (NS) flowpaths. The following enzymes were assayed: A) FAE = fatty-acid esterase, B) LAP = leucine-aminopeptidase, C) PHOS = phosphatase, D) XYLO = β-xylosidase, E) NAG = β-N-acetylglucosaminidase, and F) BGLU = β-glucosidase. Bars represent the mean of replicate mesocosms (n = 3) ± SE, except for NS-W7, which is a composite value from pooled samples.

In addition, these results may partially explain modest diurnal NO_3^- declines during the spring and summer, however previous research suggests that photoautotroph assimilation could also explain these findings (Burns 1998). Daytime photoautotrophic production and subsequent release of labile exudates may provide BDOC for modest microbial assimilation of NO_3^- (sensu Kaplan and Bott (1989)). Overall,

findings suggest that the Neversink River may retain significant amounts of N when BDOC concentrations are elevated, yet the potential for N-removal appears low in the absence of increased BDOC.

Our findings contribute to the rapidly growing body of research suggesting that land-water interfaces can be metabolic and chemical regulators of the downstream flux of nutrients (sensu Wetzel (1990); see Boulton et al. (1998) and Hedin et al. (1998), Hill et al. (2000)), but our results also demonstrate that microbial activity at land-water interfaces can be variable. The potential for microbially-mediated control on the downstream flux of N in Catskill Mountain streams may be greatest in the fall immediately following autumn leaf-fall and in the spring following benthic-algal blooms due to episodic additions of BDOC.

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