

Denitrification potential in sediments of headwater streams in the southern Appalachian Mountains, USA

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Abstract. We investigated variations in resource availability ($\text{NO}_3\text{-N}$ and labile organic C [LOC]) as determinants of potential denitrification in stream sediments in the southern Appalachian Mountains, USA. Stream water and sediments were sampled seasonally in 2 streams of contrasting $\text{NO}_3\text{-N}$ availability, Noland Creek (high $\text{NO}_3\text{-N}$) and Walker Branch (low $\text{NO}_3\text{-N}$). Eight additional streams with varying $\text{NO}_3\text{-N}$ levels were sampled once during summer. Stream sediments were incubated at ambient stream temperatures, and nitrous oxide accumulation was quantified following acetylene inhibition of nitrous oxide reduction. Denitrification potential was greater in Noland Creek than Walker Branch. In autumn and spring, $\text{NO}_3\text{-N}$ and LOC amendments indicated that denitrification potential in Walker Branch sediments was $\text{NO}_3\text{-N}$ limited, whereas temperature had no effect on rates. Denitrification potential in Noland Creek sediments was not limited by $\text{NO}_3\text{-N}$ or LOC, but was significantly affected by season and temperature. However, no differences in denitrification potential were detected when Noland Creek seasonal data were adjusted to a common temperature. $\text{NO}_3\text{-N}$ in the 10 surveyed streams ranged from 10 to 549 $\mu\text{g/L}$, with the highest $\text{NO}_3\text{-N}$ levels and denitrification rates generally occurring in the higher-elevation streams of Great Smoky Mountains National Park. Our results suggest that $\text{NO}_3\text{-N}$ availability is the primary factor limiting potential denitrification in Southern Appalachian streams. Despite the ideal conditions of slurry studies, extrapolation of potential rates to estimate denitrification loss in the catchment channels indicates that the process is an insignificant N sink (1.7% of stream N export in Walker Branch and 1.5% of N export in the Noland Divide Watershed).

Key words: denitrification, sediments, nutrient limitations, temporal variability, nitrate, dissolved organic carbon, nitrogen deposition, stream ecology.

Several mass-balance studies have investigated N dynamics at the catchment scale (Vitousek and Reiners 1975, Bormann and Likens 1979, Hedin and Brown 1994, Swank and Vose 1997), yet few studies of this type have been conducted in streams (Triska et al. 1984, Webster and Swank 1985, Duff and Triska 1990, Burns 1998). Stream studies have led to the conclusion that stream export of N is often less than catchment inputs, suggesting significant N-retention in catchments. Sinks for N in catchments are considered to be primarily terrestrial (e.g., vegetation uptake, incorporation into soil organic matter, etc.), whereas instream sinks have been largely ignored. Several recent studies have sug-

gested that instream processes may be important N sinks, exerting significant controls on N outputs from catchments (Holmes et al. 1996, Mulholland and Hill 1997).

Denitrification may be a significant mechanism for N loss in streams (Swank and Caskey 1982, Cooke and White 1987, Triska et al. 1993, Bradley et al. 1995). Denitrification is a microbially mediated process in which facultative bacteria oxidize organic C while using $\text{NO}_3\text{-N}$ as an alternative electron acceptor (Rosswall 1981), often producing N gases such as nitrous oxide (N_2O) and dinitrogen gas (N_2). In contrast to the assimilatory pathway of $\text{NO}_3\text{-N}$ reduction, which produces $\text{NH}_4\text{-N}$ for biosynthesis under aerobic conditions, denitrification ultimately removes available N from an ecosystem.

Denitrification may improve surface-water quality and thereby help offset the negative ef-

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fects of N enrichment in streams characterized by high concentrations of $\text{NO}_3\text{-N}$ (Ventullo and Rowe 1982, Holmes et al. 1996, Jordan and Weller 1996). Additional loss of $\text{NO}_3\text{-N}$ through denitrification can negatively affect ecosystem production in streams with low concentrations of $\text{NO}_3\text{-N}$ and/or where N is limiting to algae and microbes (Neilsen et al. 1990, Rysgaard et al. 1994). Yet, our understanding of N loss from streams via denitrification is limited (Sinsabaugh 1994) because denitrification is potentially regulated by complex hydrological, chemical, geological, and biological factors.

We investigated the patterns of seasonal variation and role of resource availability on denitrification potential in the sediments of headwater streams in the southern Appalachian Mountains. Two streams of contrasting $\text{NO}_3\text{-N}$ availability (West Fork of Walker Branch and Noland Creek) were selected as primary sites for the investigation. Denitrification potential was also surveyed in 8 additional streams with different $\text{NO}_3\text{-N}$ concentrations located near the 2 primary study streams. Specific objectives of our study were: 1) to characterize the denitrification potential in the sediments of 2 headwater streams of contrasting $\text{NO}_3\text{-N}$ concentration, 2) to identify temporal variation in denitrification potential, and 3) to determine whether $\text{NO}_3\text{-N}$ or labile organic C (LOC) limit denitrification potential.

Study Sites

The West Fork of Walker Branch is located within the Ridge and Valley Province in eastern Tennessee (lat 35°58'N, long 84°17'W) and drains a 38.4-ha catchment at an elevation of 265 m located on the US Department of Energy's Oak Ridge National Laboratory Reserve (Fig. 1). It arises from 4 perennial springs, resulting in base flows of generally 5 to 10 L/s (Mulholland 1992). The width and depth of the West Fork of Walker Branch during base flow are ~1.5 to 3.0 m and ~5.0 to 10.0 cm, respectively. The streambed consists of heterogeneous cobble, gravel, and fine-grained organic-rich sediments, with sections of exposed dolomitic bedrock. Baseflow alkalinity and pH are moderately high, with values of 2 to 3 meq/L and 7.8 to 8.2, respectively. Riparian vegetation is primarily oaks, red maple, yellow poplar, and other mesophytic hardwoods.

Noland Creek drains a 17.4-ha catchment located in the Great Smoky Mountains National Park (GSMNP) (lat 35°34'N, long 83°28'W) (Fig. 1), situated at 1692 m elevation along the Tennessee–North Carolina border (Shubzda et al. 1995). Noland Creek is a poorly buffered high-elevation stream with high $\text{NO}_3\text{-N}$ concentrations as a result of high rates of atmospheric deposition and low rates of uptake by vegetation in the catchment (Nodvin et al. 1995). The soils of the Noland Creek catchment are poorly buffered inceptisols formed from the underlying Thunderhead Sandstone (King et al. 1968, Johnson et al. 1991). The overstory vegetation of the catchment is dominated by mature stands of red spruce, yellow birch, beech, and hemlock. The riparian understory consists of dense rhododendron, contributing to stream shading (Johnson et al. 1991). The streambed of Noland Creek consists of large boulders and woody debris dams along riffle/run reaches.

Four additional streams on the Oak Ridge reservation (East Fork Walker Branch, Upper White Oak Creek, Pinhook Branch, and Gum Hollow Branch) and 4 additional streams in the GSMNP (Rattlebox Creek, Mossy Rock Creek, Hickory King Branch, and Beech Flats Prong) were studied. Stream elevations ranged from 244 to 1646 m. The streams on the Oak Ridge reservation were expected to have relatively low $\text{NO}_3\text{-N}$ concentrations, whereas concentrations in the GSMNP streams were expected to vary greatly depending on elevation.

Methods

Sampling and analyses

Seasonal sampling in Walker Branch and Noland Creek included measurements of stream temperature, conductivity, $\text{NO}_3\text{-N}$, and dissolved organic C (DOC) concentrations, and collection of sediments. Sampling was conducted seasonally in 1998 through 1999 (Table 1). The survey of the additional 8 streams was conducted in summer 1999, at which time stream water pH was also measured.

Following measurement of stream temperature and conductivity, water for chemical analyses was filtered (0.45- μm membrane filters), placed on ice, and then frozen upon return to the laboratory. $\text{NO}_2\text{-N}$ plus $\text{NO}_3\text{-N}$ concentrations were determined by the Cd-reduction

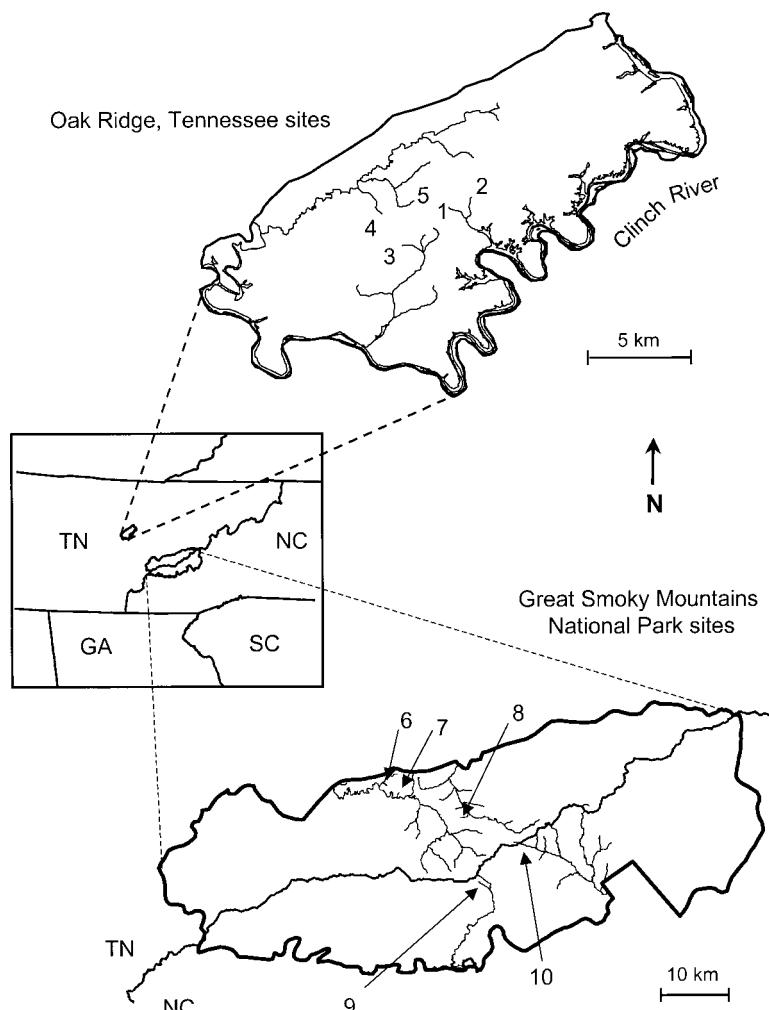


FIG. 1. Oak Ridge and Great Smoky Mountains National Park (GSMNP) sites sampled in this study. Oak Ridge: 1 = West Fork Walker Branch, 2 = East Fork Walker Branch, 3 = Upper White Oak Creek, 4 = Pinhook Branch, 5 = Gum Hollow Branch. GSMNP: 6 = Rattlebox Creek, 7 = Mossy Rock Creek, 8 = Hickory King Branch, 9 = Noland Creek, and 10 = Beech Flats Prong.

method using a Bran Luebbe TRAACS 800 auto-analyzer (reported as $\text{NO}_3\text{-N}$ because $\text{NO}_2\text{-N}$ is presumed minimal in these streams with dissolved oxygen concentrations near saturation). DOC was determined using a Shimadzu TOC-5000 total organic C analyzer. Stream water pH was measured on unfiltered samples in the laboratory using an Orion pH meter. An additional liter of unfiltered water was collected for use in the denitrification assays.

Approximately 1 kg (wet mass) of sediment from each of 12 depositional areas ($\sim 4\text{--}10\text{ cm}$ deep) was collected from each stream on each

sampling date using a 385-cm³ aluminum corer, then transferred to 1.6-L Whirlpak® bags. Sediment samples were stored at $\sim 4^\circ\text{C}$ until each denitrification assay was initiated (4–24 h following sample collection).

Denitrification assays

Sediment denitrification rates were estimated based on the accumulation of N_2O after addition of acetylene (C_2H_2) to inhibit N_2O reduction (Tiedje et al. 1989, Holmes et al. 1996). Approximately 150 g (wet mass) of each sediment sam-

TABLE 1. Temporal variation in stream water biogeochemical data measured quarterly (1998–1999) in Walker Branch and Noland Creek. Annual means and SEs were calculated for each site's parameters using seasonal data. m.d. = missing data. DOC = dissolved organic C, OM = organic matter.

Stream	Date	Season	NO ₃ -N ($\mu\text{g/L}$) ^a	DOC (mg/L) ^a	Stream temperature ($^{\circ}\text{C}$)	% OM in sediments ^a
Walker Branch	18 Nov 98	Autumn	7.4	0.54	11.3	2.11
	18 Feb 99	Winter	20.0	m.d.	11.8	2.28
	21 Apr 99	Spring	19.4	0.32	13.0	1.74
	21 Jun 99	Summer	50.0	0.11	15.3	2.29
		Mean	24.2	0.32	12.9	2.11
		SE	9.1	0.11	0.9	0.0013
Noland Creek	21 Oct 98	Autumn	620	1.25	12.3	2.30
	2 Mar 99	Winter	682	0.55	4.7	3.34
	9 May 99	Spring	634	0.63	8.4	2.08
	8 Jul 99	Summer	549	0.71	11.8	3.14
		Mean	621	0.78	9.3	2.72
		SE	27.5	0.16	1.8	0.0031

^a Significant differences exist between the means of each site in paired *t*-test, *p* < 0.05

ple was transferred to an ashed 250-mL media bottle and unfiltered stream water was added to bring the total volume to 125 mL. The microcosms were capped and sealed with septa-fitted screw-top lids.

Anoxia was induced by purging the microcosms with He for 15 min. C₂H₂ was generated through the dissolution of calcium carbide in water and was collected in a gas sampling bag for each experiment. C₂H₂ was added to each microcosm to yield 10% by total volume. Following vigorous mixing, microcosms were equilibrated for a few minutes and then excess pressure was released, bringing the internal pressure to ~1 atm. Microcosms were incubated (unshaken) at approximate ambient stream temperature for 12 h.

Gas headspace samples (8 mL) were collected using gas-tight syringes after 1, 6, and 12 h. Prior to headspace sampling, microcosms were shaken vigorously. The headspace samples were injected into pre-evacuated vacutainers, sealed with silicone, and stored at room temperature until analyzed for N₂O (within 8 wk). Following the last N₂O sampling, the microcosms were opened and the sediments dried at 60°C for ~96 h. Sediment was weighed, combusted at 500°C for 5 h, and reweighed to obtain percent organic matter (% OM) and ash-free dry mass (AFDM).

N₂O concentrations were determined using a Perkin Elmer gas chromatograph fitted with a ⁶³Ni electron capture detector. The P10 argon/

methane carrier gas flow averaged 30 mL/min. Column and detector temperatures were 50°C and 300°C, respectively. Autumn, winter, and spring headspace samples were analyzed using a backflush-to-vent system (Tiedje et al. 1989) requiring a 3-mL sample injection. The gas chromatograph was subsequently reconfigured to eliminate the need for back-flushing and headspace samples from the summer assays were analyzed using a direct injection method (J. Duff, US Geological Survey, Menlo Park, California, personal communication) requiring a sample injection of only 100 μL . N₂O standards ranging from 0.015 to 0.36 $\mu\text{g/L}$ were made from 99.8% pure N₂O and 100% He stock gases using a standard dilution series.

Potential denitrification rates were calculated based on differences in N₂O concentrations measured at 6 and 12 h. Total mass of N₂O in the headspace was calculated using the headspace N₂O concentrations and total microcosm volumes following correction for reduced N₂O solubility in the aqueous phase with an appropriate temperature-dependent Bunsen coefficient (Knowles 1979). Denitrification rates were calculated per gram AFDM (gAFDM) and per gram dry mass (gDM). Denitrification rates determined in these assays represent maximum potential denitrification rates in these streams under anoxic conditions. In situ stream denitrification rates likely would be lower because these sediments are probably not completely an-

oxic, and natural diffusion of $\text{NO}_3\text{-N}$ across the sediment–water interface would be enhanced in the slurried conditions in these assays.

Differences in denitrification rates between streams were compared using a *t*-test for each sampling date. Seasonal variation in denitrification rates for each stream was investigated using nonparametric ANOVAs on ranked rates (Kruskal–Wallis) because of the skewed distribution and lack of normality among rates for each season. These analyses were conducted on rates normalized to gAFDM as well as gDM when appropriate. Percent OM data were arcsin-square root transformed for ANOVA between sites. All statistical analyses were conducted using SAS (Windows version 4.10.2222, release 7.00, SAS Institute, Inc., Cary, North Carolina) with a significance level of $p < 0.05$.

Nutrient limitations

To identify how $\text{NO}_3\text{-N}$ or LOC availability may limit rates of denitrification, microcosms similar to those described above were set up with nutrient-amended stream water during autumn 1998 and spring 1999. Unfiltered stream water was amended with 200 mg $\text{NO}_3\text{-N/L}$ (as KNO_3), 1 g $\text{C}_{12}\text{H}_6\text{O}_{12}\text{-C/L}$ (as dextrose), or both. Six microcosms were set up for each of the 3 amendments and all were run concurrently with the 12 unamended microcosms described above. Gas headspace samples for N_2O were collected, stored, and analyzed as above.

Treatment effects were investigated using a 1-way ANOVA for each stream and each sampling date. The Dunnett's post hoc multiple comparisons test (MCT) was used to identify significant differences between samples of a respective treatment as compared to the unamended samples. Scheffe's post hoc MCT was also conducted to identify significant differences among samples of the 3 treatments.

Temperature effects

Relationships between denitrification potential and temperature were determined for sediments collected in winter and summer 1999 in Walker Branch and Noland Creek. For this experiment, 12 sediment samples collected from each stream were pooled and homogenized. For each stream, 5 microcosms were set up with unamended stream water and incubated at each of

4 temperatures (4, 9, 12, and $18^\circ\text{C} \pm 1^\circ\text{C}$). Gas headspace samples were collected, stored, and analyzed as described above. Q_{10} coefficients were calculated for each stream for winter and summer samplings as e^{10k} , where k is the slope of the regression between the ln-transformed denitrification rates and temperature (Tank 1992).

Stream survey

The stream survey was conducted over a 3-wk period during the summer 1999 sampling season. The Oak Ridge streams were sampled on 17 June 1999 and the GSMNP streams were sampled on 28 June 1999. Eight 1-kg sediment samples were collected from areas of fine-sediment accumulation in each stream. Unfiltered stream water was collected and used in the C_2H_2 inhibition experiments for each stream, as described above. Samples were incubated at the average ambient stream temperatures of each site group (16°C for all Oak Ridge streams and 14°C for all GSMNP streams). The Walker Branch and Noland Creek samples obtained in this survey were also used as summer samples in the seasonal investigation of denitrification potential. Stream water $\text{NO}_3\text{-N}$ and DOC concentrations, pH, conductivity, temperature, sediment % OM, and elevation values obtained during the stream survey were entered into a stepwise multiple regression analysis to identify predictors of denitrification potential (default significance level for variable entry = 0.15).

Results

Stream characteristics

Characteristics of the study streams and their sediments are summarized in Table 1. The average $\text{NO}_3\text{-N}$ concentration observed in the surface water of Noland Creek was significantly different (~25 times greater) from Walker Branch (paired *t*-test, $p = 0.0004$). Minimum and maximum $\text{NO}_3\text{-N}$ concentrations in Walker Branch were observed during autumn (7.4 $\mu\text{g/L}$) and summer (50.0 $\mu\text{g/L}$) sampling, respectively. In contrast, seasonal variation in $\text{NO}_3\text{-N}$ concentrations in Noland Creek was small (620–682 $\mu\text{g/L}$). DOC concentrations in the surface water of Noland Creek (mean of 0.78 mg/L) were significantly greater than in Walker Branch

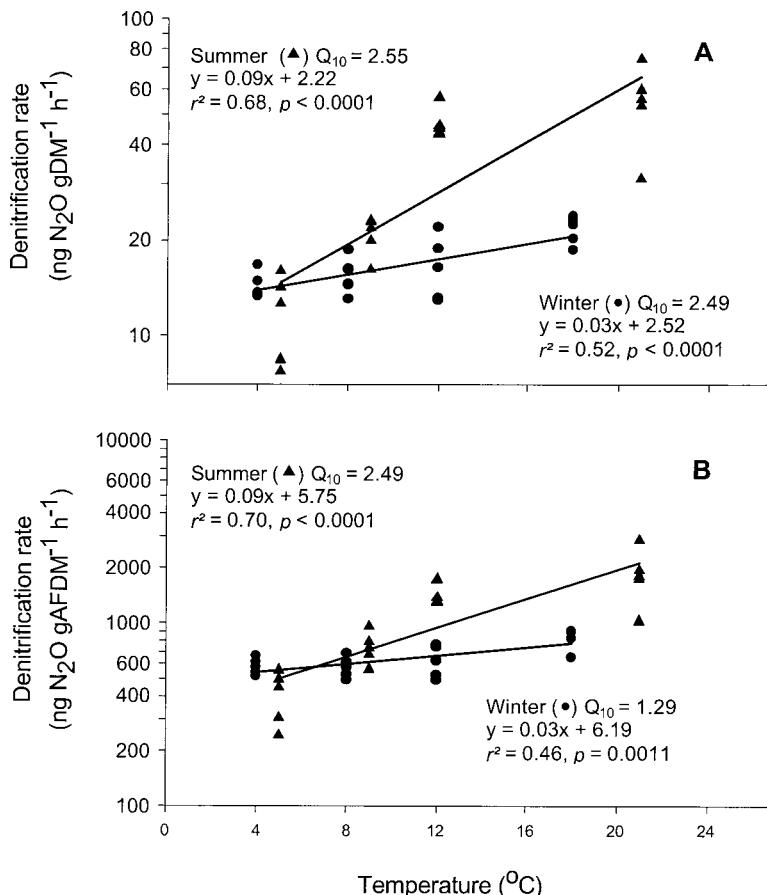


FIG. 2. Effect of temperature on denitrification rates in Noland Creek sediments in winter and summer 1999 normalized to gDM (A) and gAFDM (B). Temperature effect on Walker Branch denitrification rates was insignificant ($p > 0.05$).

(mean of 0.32 mg/L) (paired t -test, $p = 0.0427$). Maximum DOC concentrations were observed in autumn in both streams. Noland Creek water temperatures (4.7–12.3°C) were more variable and generally lower than those in Walker Branch (11.3–15.3°C).

The % OM in sediments from the depositional areas sampled in Walker Branch and Noland Creek did not vary significantly among seasons (ANOVA, $p > 0.05$). However, the mean % OM content in Noland Creek sediments was significantly greater than that of Walker Branch (paired t -test, $p = 0.0261$).

Effects of temperature

Temperature significantly affected denitrification potential in Noland Creek sediments dur-

ing both winter and summer when rates were expressed as both gAFDM and gDM (Fig. 2). Q_{10} coefficients of 1.29 and 2.49 for gAFDM were determined for winter and summer, respectively. No significant effect of temperature on denitrification was detected for Walker Branch sediments because no denitrification activity was observed in any samples during the temperature experiments.

Denitrification in streams of contrasting NO₃-N availability

Denitrification potential in Noland Creek was high, averaging ~ 891 ng N₂O gAFDM⁻¹ h⁻¹, whereas denitrification rates in Walker Branch were much lower and more variable, averaging ~ 61 ng N₂O gAFDM⁻¹ h⁻¹ (Table 2). Eighty per-

TABLE 2. Denitrification rates ($\text{ng N}_2\text{O gAFDM}^{-1} \text{h}^{-1}$) in sediments from streams of contrasting $\text{NO}_3\text{-N}$ availability. SEs are indicated in parentheses. Rates were significantly different between sites each season (t -test, $p < 0.05$).

Season	Walker Branch	Noland Creek
Autumn	12.1 (4)	1004 (290)
Winter	50.1 (14)	300 (86)
Spring	152 (44)	1231 (355)
Summer	30.9 (9)	1041 (301)
Annual mean	61.3 (23)	891 (133)

cent of the incubations of Walker Branch sediments had no N_2O production. Denitrification rates were significantly greater in Noland Creek sediments than in sediments from Walker Branch in all sampling seasons (t -tests, $p < 0.05$, Table 2). No significant seasonal variation in Walker Branch denitrification rates was observed, regardless of data normalization ($p > 0.05$, Fig. 3). Nonparametric ANOVA of denitrification rates normalized to gDM and gAFDM in Noland Creek sediments among seasons detected significant seasonal variation ($p = 0.01$ and 0.02, respectively, Fig. 3A, B). However, seasonal variation was no longer significant upon adjustment of these denitrification rates to a

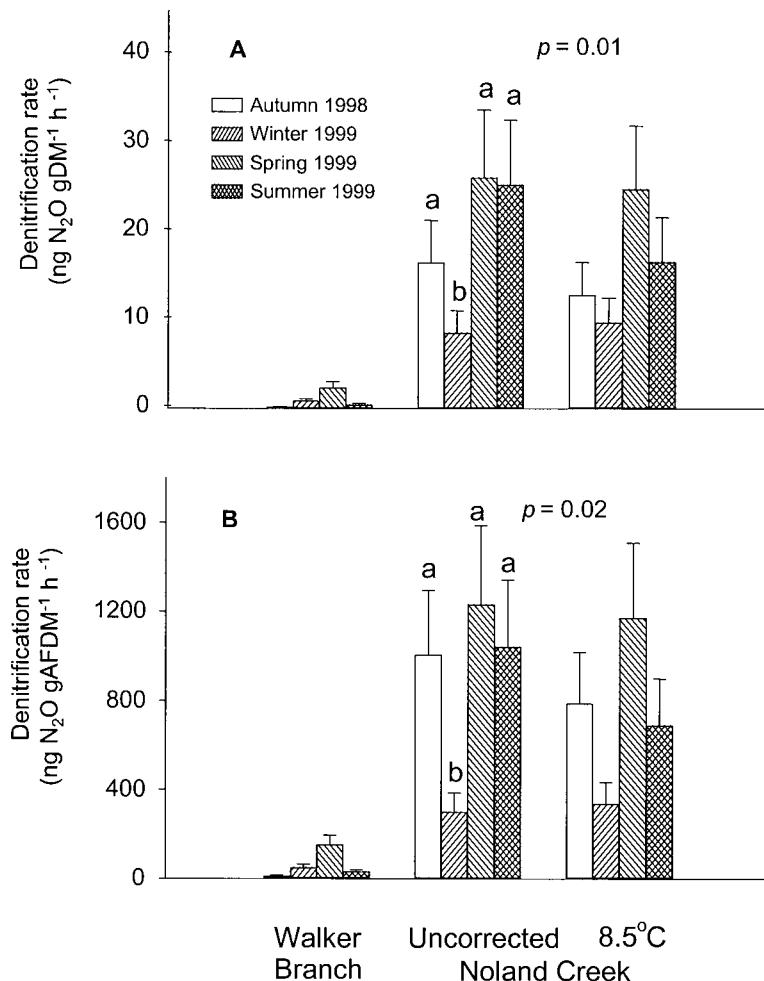


FIG. 3. Seasonal variation in denitrification rates (± 1 SE) in sediments from Walker Branch and Noland Creek normalized to gDM (A) and gAFDM (B). Bars with the same letters and unlettered bars are not significantly different ($p > 0.05$).

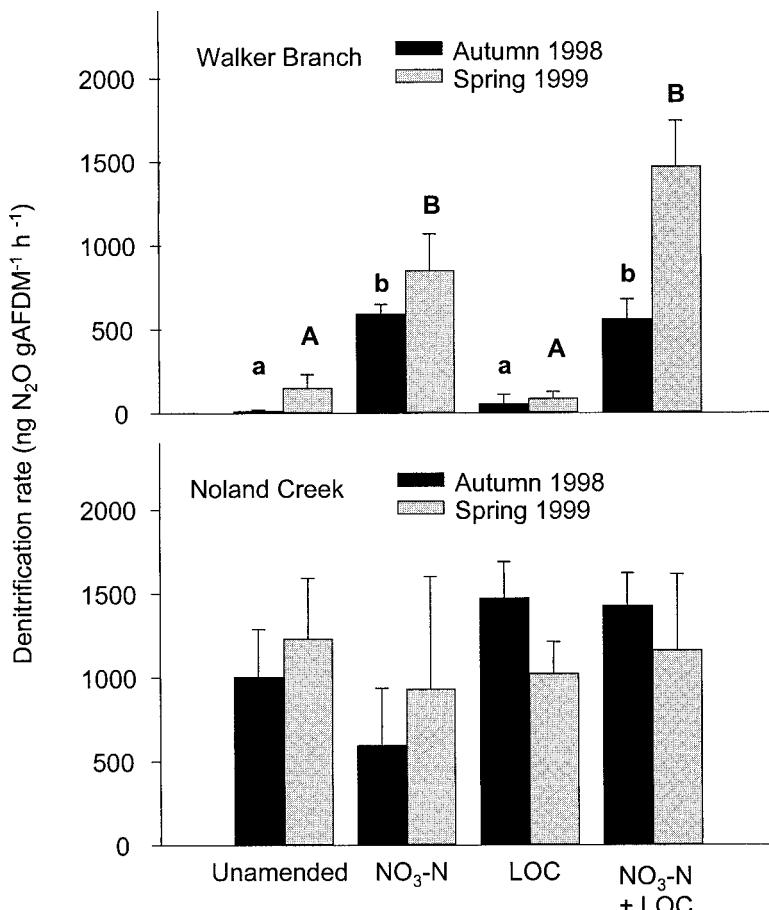


FIG. 4. Effect of nutrient amendments on denitrification rates (± 1 SE) in Walker Branch and Noland Creek sediments. Amendments yielding denitrification rates significantly different from those of the unamended controls are indicated by a letter different from that of the unamended sample set (ANOVA followed by Dunnett's multiple comparison test, $p < 0.0001$) (letters for the autumn 1998 experiment are lower case and those for spring 1999 are upper case).

common temperature (8.5°C) using each season's corresponding Q_{10} value (a mean Q_{10} value of $1.94/\text{gDM}$ and $1.89/\text{gAFDM}$ was used for autumn and spring denitrification rates). Seasonal denitrification rates in each stream normalized to either gDM or gAFDM were not significantly correlated with any of the individual stream characteristics presented in Table 1.

Nutrient limitations

Nutrient additions to the sediment microcosms yielded consistent results for both the autumn 1998 and spring 1999 experiments for each site (Fig. 4). In Walker Branch sediments,

addition of $\text{NO}_3\text{-N}$ or $\text{NO}_3\text{-N} + \text{LOC}$ significantly increased denitrification rates 5- to 50-fold for spring and autumn, respectively ($p < 0.0001$). The addition of LOC alone did not significantly change denitrification potential relative to unamended controls. Denitrification in Walker Branch was not statistically different between microcosms amended with $\text{NO}_3\text{-N}$ or $\text{NO}_3\text{-N} + \text{LOC}$ during either season (Scheffe's MCT).

Survey of denitrification potential in streams along a $\text{NO}_3\text{-N}$ gradient

Surface water $\text{NO}_3\text{-N}$ in the 10 streams sampled in the $\text{NO}_3\text{-N}$ gradient study ranged from

TABLE 3. Mean physical and biogeochemical characteristics for stream sites sampled during summer 1999. Elevations were estimated across the sampling reach. DOC = dissolved organic C, OM = organic matter.

Site	Elevation (m)	[NO ₃ -N] (μ g/L)	[DOC] (mg/L)	% OM	Conduc- tivity (μ S/cm)	Stream water pH	Stream temp. (°C)	Denitrification (ng N ₂ O gAFDM ⁻¹ h ⁻¹)	
								Mean	SE
Oak Ridge (17 Jun 99)									
East Fork Walker Branch	270	43.0	0.27	2.47	242.0	8.37	14.4	78.2	27.6
West Fork Walker Branch	265	50.0	0.11	3.79	282.0	7.76	15.3	30.9	8.9
Upper White Oak Creek	271	77.4	0.28	1.83	246.0	7.67	15.0	197.4	69.8
Gum Hollow Branch	250	85.7	0.63	3.05	276.0	7.93	17.0	118.2	41.8
Pinhook Branch	244	97.6	0.75	4.11	253.0	7.89	18.6	87.0	30.8
Great Smoky Mountain National Park (8 Jul 99)									
Rattlebox Creek	610	10.2	1.38	2.14	12.6	6.30	18.2	422.3	149.3
Mossy Rock Creek	668	63.0	1.08	3.06	25.6	6.84	18.8	125.8	44.5
Hickory King Branch	521	376	0.46	3.42	20.9	6.24	13.9	1348.6	476.8
Beech Flats Prong	1250	532	0.31	2.84	41.0	5.99	11.6	894.1	316.1
Noland Creek	1646	549	0.71	1.52	14.0	5.76	11.8	1041.5	300.7

10 to 549 μ g/L, with the highest NO₃-N levels generally occurring in the higher-elevation streams in the GSMNP (Table 3). Surface water DOC concentrations in these streams ranged from 0.11 to 1.38 mg/L and sediment OM content varied from 1.52 to 4.11%. Conductivity and pH ranged from 12.6 to 282 μ S/cm and 5.76 to 8.37, respectively, and were consistently lower in the streams of the GSMNP compared to streams on the Oak Ridge reservation.

Sediment denitrification potentials were most strongly related to stream water NO₃-N concentrations ($r^2 = 0.74$, $p = 0.001$) (Fig. 5). Denitrification was generally higher in sediments from the higher elevation streams ($r^2 = 0.45$), and this relationship was stronger when data were normalized to grams dry sediment ($r^2 = 0.77$). Denitrification rates were negatively related to stream water pH ($r^2 = 0.68$, $p = 0.003$), conductivity ($r^2 = 0.53$, $p = 0.02$), and temperature ($r^2 = 0.43$, $p = 0.04$), but were not significantly related to % OM and DOC concentration.

A stepwise multiple regression analysis (Table 4) identified NO₃-N concentration as the primary predictor of denitrification potential normalized by gAFDM (model $R^2 = 0.74$, $p = 0.001$). The stepwise incorporation of conductivity (model $R^2 = 0.83$) into the model, followed by elevation ($R^2 = 0.93$) explained an additional 19% of variation in denitrification potential ($p = 0.0008$). Elevation was identified as the sole sig-

nificant predictor ($R^2 = 0.77$, $p = 0.0009$) of denitrification potential normalized to gDM.

Discussion

Factors influencing denitrification potential

In our 2-stream comparison, Noland Creek denitrification potential in unamended slurries were an order of magnitude greater than those of Walker Branch. Denitrification potentials in sediments from Walker Branch were lower than those observed in most studies, but they were similar to denitrification rates observed in sediments from the upwelling hyporheic zones of Sycamore Creek (Holmes et al. 1996) using similar techniques. They were also similar to rates observed 1 to 5 m into the parafluvial flowpaths of this N-limited Sonoran Desert stream. In contrast, the average annual denitrification potential in sediments from Noland Creek was >2 times the maximum denitrification potential observed by Holmes et al. (1996), and only approached rates observed in other aquatic sediments (Seitzinger et al. 1993).

Noland Creek had considerably higher levels of NO₃-N and organic C availability (both as DOC and as % OM in sediments) than the West Fork of Walker Branch. Until now, the degree to which these factors potentially affect denitrification in stream sediments in the southern Ap-

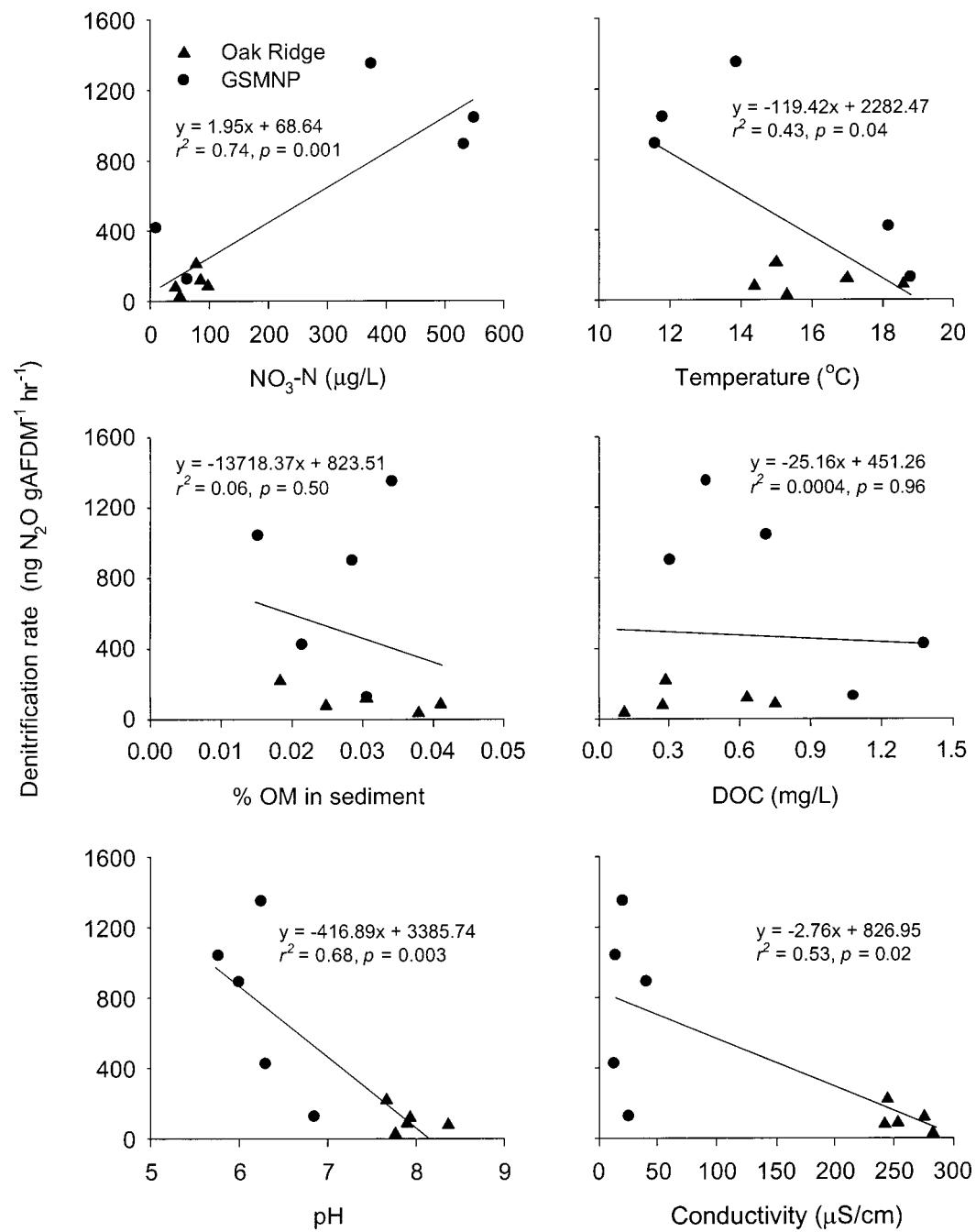


FIG. 5. Linear regressions between denitrification rates and stream characteristics measured during summer 1999 survey of Oak Ridge streams and Great Smoky Mountains National Park (GSMNP) streams. DOC = dissolved organic C, OM = organic matter.

TABLE 4. Summary of stepwise selection of predictor variables in multiple regression with denitrification rates normalized by either grams ash-free dry mass (gAFDM) or grams dry mass (gDM). Default significance level for variable entry = 0.15.

Dependent variable	Predictor variable	Model coefficient	Partial R^2	Model R^2	F	p
Denitrification rate ^a (ng N ₂ O gAFDM ⁻¹ h ⁻¹)	NO ₃ -N	25.76	0.74	0.74	22.96	0.001
	Conductivity	17.37	0.08	0.83	3.4	0.11
	Elevation	6.76	0.1	0.93	8.63	0.03
Denitrification rate (ng N ₂ O gDM ⁻¹ h ⁻¹)	Elevation	-1.89	0.77	0.77	26.6	0.0009

^a Overall model was significant at $p = 0.0008$

palachian mountains had not been investigated, although N loading via atmospheric deposition has received considerable attention (Johnson and Lindberg 1992, Flum and Nodvin 1995, Shubzda et al. 1995). Our results clearly showed neither NO₃-N nor LOC enrichment significantly enhanced denitrification potential in sediments collected from Noland Creek.

Unlike some studies that have demonstrated C-limited denitrification (Knowles 1982, Limmer and Steele 1982, Tiedje et al. 1982), we found that NO₃-N availability, rather than LOC availability, directly influenced the potential for denitrification in Walker Branch. Enrichment of the Walker Branch sediments with NO₃-N, or with NO₃-N + LOC, but not LOC alone, resulted in significant increases in denitrification rates. Similar results were reported for Sycamore Creek where NO₃-N addition alone generated a 2- to 10-fold increase in denitrification rates in hyporheic and parafluvial sediments and an ~30-fold increase in denitrification in the bank sediments (Holmes et al. 1996).

Denitrification potential in sediments from Noland Creek and Walker Branch was generally lower in autumn than other seasons, when DOC availability was maximum. The relatively low autumn denitrification rates and the lack of response when the LOC concentration was increased during the nutrient amendment experiments strongly suggests that C availability was not the primary limiting factor of denitrification potential in sediments from these streams. It is possible that the lack of response in denitrification following LOC additions was caused by a metabolic shift toward dissimilatory reduction of NO₃-N to NH₄-N as observed by Knowles (1982), although the relatively low ambient LOC

availability year-round likely precludes this pathway.

The results of our stream survey further emphasized that NO₃-N rather than C availability likely determines the potential for denitrification in sediments from these streams in the southern Appalachian Mountains. However, this conclusion may be biased by high particulate organic C (POC) accumulations in our study streams. C might more likely limit denitrification in streams with less POC. Where NO₃-N availability was limited, as in the lower-elevation Oak Ridge streams, denitrification potential was low regardless of C availability or ambient stream temperatures. Where NO₃-N concentration was high and neither NO₃-N nor LOC limitations were evident, as in Noland Creek, denitrification potential was high and primarily influenced by temperature.

Denitrification potential in sediments from Rattlebox Creek was similar to other GSMNP stream sediments at similar elevation, although it had the lowest NO₃-N concentration of all streams in the survey. There are several possible explanations for why the denitrification potential in Rattlebox Creek was higher than would be predicted based on background NO₃-N concentration. First, NO₃-N concentrations during the survey may have been higher than much of the year, and the observed activity of the denitrifier community may reflect higher NO₃-N concentrations. Second, the highest DOC concentration observed in this survey was measured in Rattlebox Creek. Although DOC did not significantly affect denitrification potential in Noland Creek and Walker Branch, it could have contributed to the higher denitrification activity of Rattlebox Creek sediments.

Our stream survey also suggested that additional stream characteristics may have influenced denitrification potential. Not surprisingly, elevation was a secondary predictor of denitrification potential normalized to gAFDM and the primary predictor for rates normalized to gDM, as identified in the stepwise regression. The higher elevation streams in the southern Appalachian Mountains receive among the highest atmospheric inputs of $\text{NO}_3\text{-N}$ in North America (Nodvin et al. 1995). Shubzda et al. (1995) demonstrated that N-deposition rates increased with increasing elevation in this region, and $\text{NO}_3\text{-N}$ concentrations were higher in the higher-elevation streams in the GSMNP (Flum and Nodvin 1995).

The significant correlation between denitrification and conductivity and the appearance of conductivity as a significant predictor in the model were likely the result of the contrast in bedrock type between the Oak Ridge and Smoky Mountains stream catchments. The high conductivity of the Oak Ridge streams is likely a result of relatively high rates of weathering of the underlying dolomite formations, whereas the highly resistant sandstone formations of the GSMNP typically produce streams with lower pH and total ionic strength. Thus, the effect of conductivity on denitrification rate was probably not causal.

Stream pH can indirectly influence denitrification in stream sediments. Several studies have shown that acidic conditions may reduce or even completely inhibit the process in stream sediments (Klemetsson et al. 1977, Knowles 1982, Davidson and Swank 1987), although bacterial numbers and activity in sediment are not necessarily correlated with the pH of overlying water (Palumbo et al. 1987). Our study suggested a significant negative relationship between pH and denitrification, a pattern opposite to most studies. $\text{NO}_3\text{-N}$ concentrations and pH were also inversely related in our study, with the high-elevation, high- $\text{NO}_3\text{-N}$ GSMNP streams (e.g., Noland Creek, Beech Flats Prong, and Hickory King Branch) exhibiting significantly greater denitrification potential than the lower-elevation, higher-pH streams in the Oak Ridge area. We suspect that the high $\text{NO}_3\text{-N}$ inputs from atmospheric deposition in high-elevation streams in the southern Appalachian Mountains sustain endogenous assemblages of

denitrifying organisms in otherwise suboptimal, mildly acidic conditions.

Within-site variability among denitrification rates

The variability observed in denitrification potential among sediment samples in each stream was relatively high, especially in Walker Branch. Such variability is consistent with observations of substantial patchiness of denitrification hot-spots in studies in other streams (Parkin 1987, Steinhart et al. 2000). We originally hypothesized that normalization of N_2O production data to gAFDM would reduce much of the anticipated variability associated with heterogeneous organic content of sediments and differential nutrient retention among samples. However, the OM content in the depositional zones sampled was not highly variable ($\text{SE} = 0.0013$ and 0.0031 for Walker Branch and Noland Creek, respectively). Hence, statistical analyses of denitrification potential in these streams were consistent regardless of data normalization to sediment OM content.

Our study did not address variability introduced by sediment-particle-size distribution. Several studies have shown that denitrification activity is higher in fine-textured sediments ($<100 \mu\text{m}$) where the probability of anaerobic conditions is greater (Groffman and Tiedje 1989, Garcia-Ruiz et al. 1998, Steinhart et al. 2000). In contrast, depositional areas characterized by more coarsely textured substrates are likely to be more oxic and support less active denitrification. Therefore, one might expect a reduced potential for denitrification in these areas (Rysgaard et al. 1994). The degree of sediment homogeneity in the denitrification assays might further explain the variability in denitrification potentials observed.

The preparation time allotted for experimental setup through the He-flushing step was presumed to be sufficient for sample warming and reacclimatization, yet a lag in denitrification activity was observed early in the incubations. Sudden shifts to anoxia in laboratory experiments through such treatments as He flushing do not permit denitrifiers to acclimatize to optimum denitrifying conditions as they would if oxygen were depleted gradually (i.e., through a natural cascade of metabolic processes, Knowles 1982). In addition, the time required for sample reacclimatization to ambient stream tempera-

ture following 4- to 24-h storage at 4°C is uncertain. Therefore, we calculated denitrification rates using N₂O accumulation between 6- and 12-h.

Denitrification in stream sediments may contribute to loss of NO₃-N from catchments in the southern Appalachian Mountains. Our study suggests that ~100 and 600 ng N gAFDM⁻¹ h⁻¹ could be lost via denitrification in the stream sediments of Walker Branch (East and West Forks) and Noland Creek, respectively. These potential denitrification rates assume the optimum conditions of complete anoxia in the sediments and interstitial NO₃-N concentrations equal to those of overlying stream water. The effect of C₂H₂ inhibiting nitrification and thus limiting NO₃-N availability has not been considered. Tiedje et al. (1982) proposed that in situ denitrification rates are probably only a few % of the potential rates observed *in vitro* (Tiedje et al. 1982), but the C₂H₂ inhibition technique for measuring denitrification *in vitro* bears many limitations and can underestimate in situ denitrification potential in some aquatic sediments (Seitzinger et al. 1993, Bollmann and Conrad 1997, Paerl 1998). The magnitude to which in situ denitrification serves as a mechanism of NO₃-N removal from these southern Appalachian Mountain streams appears low. Regardless, even modest rates of denitrification can significantly impact stream-water NO₃-N (Holmes et al. 1996), particularly in N-limited streams.

Catchment significance of denitrification in southern Appalachian Mountain streams

We attempted to estimate the catchment significance of stream denitrification using our measured rates of potential denitrification, catchment inputs and outputs of N, and streambed characteristics. The Walker Branch catchment receives ~10 kg N ha⁻¹ y⁻¹ (or 975 kg N/y) from atmospheric deposition, whereas ~0.2 kg N ha⁻¹ y⁻¹ (19.5 kg N/y) are lost as stream export, mostly as NO₃-N (PJM, unpublished data). Nodvin et al. (1995) reported that the catchment of Noland Creek receives ~20 kg N ha⁻¹ y⁻¹ (348 kg N/y), ~17 kg N ha⁻¹ y⁻¹ (296 kg N/y) of which is lost from the catchment via stream export. Our study indicates a denitrification rate of 100 and 600 ng N₂O-N gAFDM⁻¹ h⁻¹ in the stream sediments of Walker Branch (East and West Forks) and Noland Creek, respectively. As-

suming that 505 gAFDM/m² of benthic particulate OM, as reported for Walker Branch (Muholland 1997), is typical of streams in the southern Appalachian Mountains (Webster and Meyer 1997), denitrification in the stream sediments in Walker Branch and in Noland Creek produces 0.39 and 2.52 g N₂O-N m⁻² y⁻¹, respectively. The East and West Forks of Walker Branch constitute ~1800 m² of the entire 975,000-m² catchment (PJM, unpublished data). The streambed area of Noland Creek is unknown, but based on estimates of streambed area throughout the region (Webster and Meyer 1997), we estimate that Noland Creek constitutes 1%, or 1740 m², of the entire 174,000-m² catchment. Using these values, we calculate that 0.34 kg N/y and 4.38 kg N/y may be lost via denitrification from the stream sediments of Walker Branch (East and West Forks combined) and Noland Divide, respectively. Thus, denitrification in stream sediments is ~0.03% of total N inputs and ~1.7% of stream N export in the Walker Branch catchment, and ~1.3% of total N inputs and 1.5% of stream N export in the Noland Divide catchment.

Considering the ideal conditions for denitrification in the slurry studies, the proportion of N loss from the catchment via denitrification in these stream sediments appears insignificant. However, Holmes et al. (1996) proposed that even modest rates of denitrification have significant impacts on the fate of stream-water NO₃-N, particularly in N-limited streams. Future investigations in the southern Appalachian mountains should focus on the magnitude to which in situ denitrification serves as a mechanism of NO₃-N removal in streams draining areas of high atmospheric deposition, perhaps with a concentration of effort on porewaters of bank-side riparian habitats where conditions to support high denitrification rates may be present.

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Literature Cited

- BOLLMANN, A., AND R. CONRAD. 1997. Acetylene blockage technique leads to underestimation of denitrification rates in oxic soils due to scavenging of intermediate nitric oxide. *Soil Biology and Biochemistry* 29:1067–1077.
- BORMANN, F. H., AND G. E. LIKENS. 1979. Pattern and process in a forested ecosystem. Springer-Verlag, New York.
- BRADLEY, P. M., P. B. McMAHON, AND F. H. CHAPELLE. 1995. Effects of carbon and nitrate on denitrification in bottom sediments of an effluent-dominated river. *Water Resources Research* 31:1063–1068.
- BURNS, D. A. 1998. Retention of NO_3^- in an upland stream environment: a mass balance approach. *Biogeochemistry* 40:73–96.
- COOKE, J. G., AND R. E. WHITE. 1987. The effect of nitrate in stream water on the relationship between denitrification and nitrification in a stream-sediment microcosm. *Freshwater Biology* 18:213–226.
- DAVIDSON, E. A., AND W. T. SWANK. 1987. Factors limiting denitrification in soils from mature and disturbed southeastern hardwood forest. *Forest Science* 33:135–144.
- DUFF, J. H., AND F. J. TRISKA. 1990. Denitrification in sediments from the hyporheic zone adjacent to a small forested stream. *Canadian Journal of Fisheries and Aquatic Sciences* 47:1140–1147.
- FLUM, T., AND S. C. NODVIN. 1995. Factors affecting streamwater chemistry in the Great Smoky Mountains, USA. *Water, Air and Soil Pollution* 85:1707–1712.
- GARCIA-RUIZ, R., S. N. PATTINSON, AND B. A. WHITTON. 1998. Denitrification in river sediments: relationship between process rate and properties of water and sediment. *Freshwater Biology* 39:467–476.
- GROFFMAN, P. M., AND J. M. TIEDJE. 1989. Denitrification in north temperate forest soils: spatial and temporal patterns at the landscape and seasonal scales. *Soil Biology and Biochemistry* 21:613–620.
- HEDIN, L. O., AND M. G. BROWN. 1994. Watershed-level coupling of nitrogen and sulfur in a heterogeneous landscape. *Verhandlungen der Internation-* alen Vereinigung für theoretische und angewandte Limnologie 25:1477–1482.
- HOLMES, R. M., J. B. JONES, S. G. FISHER, AND N. B. GRIMM. 1996. Denitrification in a nitrogen-limited stream ecosystem. *Biogeochemistry* 33:125–146.
- JOHNSON, D. W., AND S. E. LINDBERG (EDITORS). 1992. *Atmospheric deposition and forest nutrient cycling: a synthesis of the integrated forest study*. Springer-Verlag, New York.
- JOHNSON, D. W., H. VAN MIEGROET, S. E. LINDBERG, D. E. TODD, AND R. B. HARRISON. 1991. Nutrient cycling in red spruce forests of the Great Smoky Mountains (North Carolina, USA). *Canadian Journal of Forest Research* 21:769–787.
- JORDAN, T. E., AND D. E. WELLER. 1996. Human contributions to terrestrial nitrogen fluxes. *BioScience* 46:655–664.
- KING, P. B., R. B. NEUMAN, AND J. B. HADLEY. 1968. *Geology of the Great Smoky Mountains National Park, Tennessee and North Carolina*. Professional Paper No. P587. US Geological Survey, Washington, DC.
- KLEMEDTSSON, L., B. H. SVENSSON, T. LINDBERG, AND T. ROSSWALL. 1977. The use of acetylene inhibition of nitrous oxide reductase in quantifying denitrification in soils. *Swedish Journal of Agricultural Resources* 7:179–185.
- KNOWLES, R. 1979. Denitrification, acetylene reduction, and methane metabolism in lake sediment exposed to acetylene. *Applied and Environmental Microbiology* 38:486–493.
- KNOWLES, R. 1982. Denitrification. *Microbiological Reviews* 46:43–70.
- LIMMER, A. W., AND K. W. STEELE. 1982. Denitrification potentials: measurement of seasonal variation using a short-term anaerobic incubation technique. *Soil Biology and Biochemistry* 14:179–182.
- MULHOLLAND, P. J. 1992. Regulation of nutrient concentrations in a temperate forest stream: roles of upland, riparian, and instream processes. *Limnology and Oceanography* 37:1512–1526.
- MULHOLLAND, P. J. 1997. Organic matter dynamics in the west fork of Walker Branch, Tennessee, USA. *Journal of the North American Benthological Society* 16:61–67.
- MULHOLLAND, P. J., AND W. R. HILL. 1997. Seasonal patterns in stream water nutrient and dissolved organic carbon concentrations: separating catchment flow path and in-stream effects. *Water Resources Research* 33:1297–1306.
- NEILSEN, L. P., P. B. CHRISTENSEN, N. P. REVSBECH, AND J. SØRENSEN. 1990. Denitrification and photosynthesis in stream sediment studied with microsensor and whole-core techniques. *Limnology and Oceanography* 35:1135–1144.
- NODVIN, S. C., H. VAN MIEGROET, S. E. LINDBERG, N. S. NICHOLAS, AND D. W. JOHNSON. 1995. Acidic deposition, ecosystem processes, and nitrogen

- saturation in a high elevation southern Appalachian watershed. *Water, Air and Soil Pollution* 85: 1647–1652.
- PAERL, H. W. 1998. Microbially mediated nitrogen cycling. Pages 3–30 in R. S. Burlage (editor). *Techniques in microbial ecology*. Oxford University Press, New York.
- PALUMBO, A. V., M. A. BOGLE, R. R. TURNER, J. W. ELWOOD, AND P. J. MULHOLLAND. 1987. Bacterial communities in acidic and circumneutral streams. *Applied and Environmental Microbiology* 53: 337–344.
- PARKIN, T. B. 1987. Soil microsites as a source of denitrification variability. *Soil Science Society of America Journal* 51:1194–1199.
- ROSSWALL, T. 1981. Ch. 2: The biogeochemical nitrogen cycle. Pages 25–49 in G. E. Likens (editor). *Some perspectives of the major biogeochemical cycles*. Wiley, Chichester, UK.
- RYSGAARD, S., N. RYSGAARD-PETERSEN, N. P. SLOTH, K. JENSEN, AND L. P. NIELSEN. 1994. Oxygen regulation of nitrification and denitrification in sediments. *Limnology and Oceanography* 39:1643–1652.
- SEITZINGER, S. P., L. P. NIELSEN, J. CAFFREY, AND P. B. CHRISTENSEN. 1993. Denitrification measurements in aquatic sediments: a comparison of three methods. *Biogeochemistry* 23:147–167.
- SHUBZDA, J., S. E. LINDBERG, C. T. GARTEN, AND S. C. NODVIN. 1995. Elevational trends in the fluxes of sulfur and nitrogen in throughfall in the southern Appalachian Mountains: some surprising results. *Water, Air, and Soil Pollution* 85:2265–2270.
- SINSABAUGH, R. L. 1994. Enzymatic analysis of microbial pattern and process. *Biology and Fertility of Soils* 17:69–74.
- STEINHART, G. S., G. E. LIKENS, AND P. M. GROFFMAN. 2000. Denitrification in stream sediments in five north-eastern (USA) streams. *Verhandlungen der Internationalen Vereinigung für theoretische und angewandte Limnologie* 27:1331–1336.
- SWANK, W. T., AND W. H. CASKEY. 1982. Nitrate depletion in a second-order mountain stream. *Journal of Environmental Quality* 11:581–584.
- SWANK, W. T., AND J. M. VOSE. 1997. Long-term nitro- gen dynamics of Coweeta forested watersheds in the southeastern United States of America. *Global Biogeochemical Cycles* 11:657–671.
- TANK, J. L. 1992. Microbial respiration on decaying leaves and sticks along an elevational gradient of a southern Appalachian stream. MS Thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.
- TIEDJE, J. M., A. J. SEXSTONE, D. D. MYROLD, AND J. A. ROBINSON. 1982. Denitrification: ecological niches, competition and survival. *Antonie van Leeuwenhoek* 48:569–583.
- TIEDJE, J. M., S. SIMKINS, AND P. M. GROFFMAN. 1989. Perspectives on measurement of denitrification in the field including recommended protocols for acetylene based methods. *Plant and Soil* 114:261–284.
- TRISKA, F. J., J. H. DUFF, AND R. J. AVANZINO. 1993. The role of water exchange between a stream channel and its hyporheic zone in nitrogen cycling at the terrestrial-aquatic interface. *Hydrobiologia* 251:167–184.
- TRISKA, F. J., J. R. SEDELL, K. CROMACK, S. V. GREGORY, AND F. M. MCCORISON. 1984. Nitrogen budget for a small coniferous forest stream. *Ecological Monographs* 54:119–140.
- VENTULLO, R. M., AND J. J. ROWE. 1982. Denitrification potential of epilithic communities in a lotic environment. *Current Microbiology* 7:29–33.
- VITUZEK, P. M., AND W. A. REINERS. 1975. Ecosystem succession and nutrient retention: a hypothesis. *BioScience* 25:376–381.
- WEBSTER, J. R., AND J. L. MEYER (EDITORS). 1997. Stream organic matter budgets. *Journal of the North American Benthological Society* 16:3–161.
- WEBSTER, J. R., AND W. T. SWANK. 1985. Within-stream factors affecting nutrient transport from forested and logged watersheds. Pages 18–41 in *Forestry and Water Quality: a Mid-South Symposium*, 8–9 May 1985, Little Rock, Arkansas. Department of Forest Resources, Cooperative Extension Service, University of Arkansas, Monticello, Arkansas.

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