Nitrate Depletion in a Second-Order Mountain Stream[1]

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ABSTRACT

The amount of NO_3-N exported in a second-order mountain stream draining a clearcut and logged mixed hardwood forest was studied over a 4-year period. Calculations based on measurements of stream chemistry and discharge rates indicated a within-stream depletion of NO_3 from the upper reaches of the stream to the watershed outlet. Within-stream depletion the first year of treatment was 127% of total NO_3-N discharged from the watershed outlet and declined in succeeding years after treatment to 99, 42, and 5%. Assays of the quantities of denitrifying enzymes in stream sediment samples suggested 1.7 kg N year^{-1} were lost via this pathway, compared with 3.9 kg N year^{-1} calculated from within-stream depletion for the same time period. This study suggests sediment denitrification is a major pathway by which NO_3-N is lost. Within-stream gaseous transformations are important when accounting for changes in N dynamics associated with forest management practices, and the measurement of only hydrologic discharge of NO_3 could result in underestimation of N losses.

Additional Index Words: denitrification, nitrogen losses, stream export, stream nutrient cycling.

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Research on N cycling has been emphasized during the past decade as part of the interdisciplinary research effort on nutrient cycling in forest ecosystems at the Coweeta Hydrologic Laboratory in western North Carolina (Monk et al., 1977). The cycling of this element is especially important in forest productivity and is subject to substantial alteration with forest disturbance (Swank and Waide, 1980; Vitousek and Melillo, 1979). During a recent watershed clearcutting experiment, indirect evidence based on stream chemistry was collected that suggested sediment denitrification was occurring where NO_3-N concentrations in stream water did not exceed 200 µg L^{-1}. Our objective is to present quantitative evidence of stream NO_3 dynamics and denitrifying activity in the sediments of a second-order stream draining a mixed hardwood forest that was clearcut and commercially logged.

Denitrification in sediments of well-oxygenated streams has been implicated as a potentially significant route of N disappearance in flowing water (Kaushik et al., 1975; Kaushik and Robinson, 1976; Van Kessel, 1977; Hill, 1979). These studies were conducted in aquatic systems with NO_3-N concentrations above 1,000 µg L^{-1} and were based on measurements of NO_3 disappearance. Denitrifying activity measured as N_2O evolution has also been documented in sediments of two eutrophic lakes, but amendments of 500 µg L^{-1} NO_3-N were used in the study (Chan and Knowles, 1979).

MATERIALS AND METHODS

Site Description and Management

This study was conducted within the Coweeta Hydrologic Laboratory, a 2,270-ha facility of the USDA Forest Service, located in the southern Appalachian Mountains of North Carolina. Land-use history, climate, and physical characteristics of the experimental basin have been previously described (Johnson and Swank, 1973). Watershed 7, a 59-ha south-facing catchment with elevations from 720 to 1,065 m and slopes of 20–80%, is drained by a second-order stream. The dominant soil series at lower elevations is the Tusquitee, a member of the fine-loamy, mixed mesic family of Humic Hapludults. Ridge and slope soils are dominated by the Chandler series, a member of the coarse-loamy, micaceous, mesic family of Typic Dystrochrepts. The forest cover is the oak-hickory (*Quercus-Carya*) community with dominant associates being yellow-poplar (*Liriodendron*), hemlock (*Tsuga*), maple (*Acer*), and pine (*Pinus*) species.

Management of the watershed can be separated into three major operations: (i) road construction and stabilization, (ii) logging, and (iii) site preparation. Three logging roads totaling 2.95 km were constructed (Fig. 1) between mid-April and mid-June 1976. Road cuts and fills were stabilized by seeding grass and applying commercial fertilizer (10-10-10) and lime immediately after construction (Table 1). This work was completed by the end of June 1976. Seed, fertilizer, and lime were again applied to cuts and fills in July 1977, and to the running surface of the road in June 1978.

Logging began in January 1977, and was completed in June. Most of the logging was conducted from the roads with a mobile cable system; tractor skidding was used on the gentle slopes on a small portion of the catchment. Compaction of the surface soil was minimal and the forest floor generally remained intact. In a separate operation completed in October 1977, the site was prepared by felling the stems remaining after logging.

Two major storms occurred after road construction and prior to logging. On 15 May and 28–30 May 1976 the experimental area received 17 and 21 cm precipitation, respectively. These two storms produced peak flows of 368 and 1,302 L s^{-1} from the watershed, which represent recurrence intervals of 7 and 100 years (Douglass, 1974). During these storms, large amounts of sediments were delivered to the streams from road sections.

Streamflow and Nutrient Measurements

Proportional stream samplers were located below and above roads and at the watershed outlet to characterize sediment and nutrient loads for pre- and post-treatment periods (Fig. 1). Stream discharge records and water samples in the headwater portion of the catchment were collected with a 30-cm H-flume in combination with a 60-cm Coshocton wheel and a 10:1 splitter (Douglass and Swift, 1977). This configuration provided a composited water sample proportional to streamflow for periodic collections taken over intervals of 3–7 d. The watershed outlet has been continuously gauged since 1935 with a conventional weir, and water samples collected since 1974 with a flow-proportional sampler (Fredriksen, 1969). Analyses of flow records (Hibbert and Cunningham, 1967) and water chemistry samples[3] followed procedures routinely used at the Coweeta Hydrologic Laboratory. Periodic output of nutrients per unit of watershed area (kg ha^{-1}) was obtained by multiplying concentrations by the appropriate volume.

Sediment Sites and Analysis

Ten areas of significant sediment accumulation were designated as denitrification sampling sites and sequentially numbered proceeding upstream (Fig. 1). Volume of sediment in each area was calculated from depth and surface area measurements.

Four cores (4.8-cm diam for the total sediment depth) were collected at each site in alternate months beginning in June 1979 and
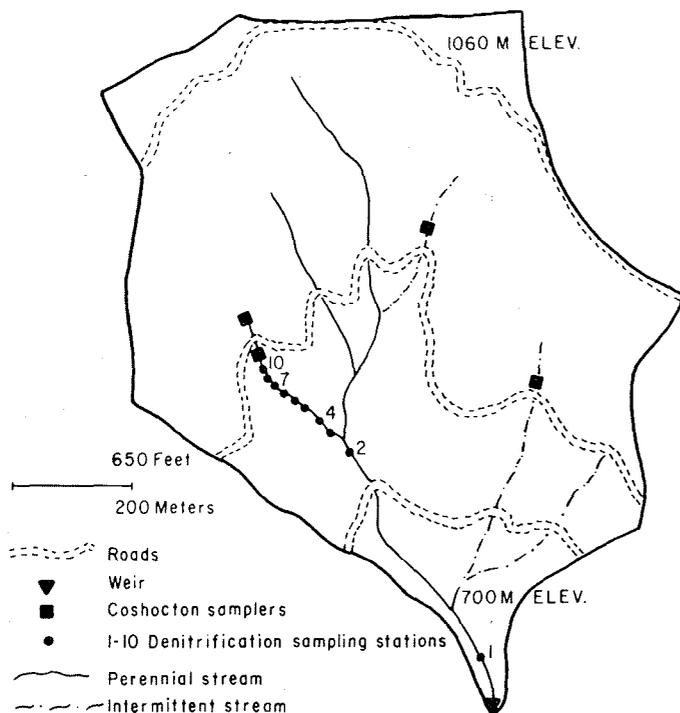
Fig. 1—Locations of stream chemistry and denitrification sampling sites on a 59-ha catchment at Coweeta Hydrologic Laboratory in western North Carolina.

ending April 1980. The cores were pooled in plastic bags, mixed thoroughly, and stored at 4°C. The sediment samples were extracted with 2N KCl and analyzed for NO₃-N and NH₄-N. Total Kjeldahl N was analyzed using the cyanurate-salicylate reaction with auto-analysis, and organic matter was analyzed using the Walkley-Black rapid titration method.[3]

Denitrifying activity was measured during phase I of denitrification (Smith and Tiedje, 1979) as N_2O production in acetylene-inhibited slurries prepared by adding 50 mL of a solution containing 0.20 g NO₃-N, 1.0 g glucose, and 0.25 g chloramphenicol per L to 50 g (fresh wt) of sediment contained in a 125-mL Erlenmeyer flask. Chloramphenicol inhibits protein synthesis in procaryotic cells; thus, rates reflect the maximum activity of preexisting denitrifying enzymes present in the cells at the initiation of the experiment. The slurries were sparged with O₂-free Ar and capped with a rubber stopper pierced by a glass tube sealed with a serum stopper. Acetylene (0.1 atm) was added and the slurries were incubated for 2 h at room temperature (21-22°C) on a rotary shaker at 18 g. Samples of the headspace gases were removed for analysis using a 1.0-mL tuberculin syringe fitted with a Mininert (Supelco Inc., Ann Arbor, Mich.) Teflon valve. Nitrous oxide was measured using a Perkin-Elmer Model 900 gas chromatograph (Perkin-Elmer Corp., Norwalk, Conn.) equipped with a ⁶³Ni electron capture detector operated at 300°C. Separation of N_2O was achieved using a Porapak Q (Supelco Inc.) column (3 mm by 2.0 m) at 50°C. The carrier gas was 5% CH₄ in Ar at a flow rate of 40 mL min⁻¹.[4]

RESULTS

Stream Chemistry Response

During 1976 and early 1977, monthly weighted concentration values of NO₃-N at the watershed outlet

[3] Procedures for chemical analysis at the Coweeta Hydrologic Laboratory. Unpublished manual, USDA-FS, Otto, N.C.

[4] Mention of trade, firm, or corporation names is for the information and convenience of the reader only. Such use does not imply endorsement by USDA over similar products of other companies not mentioned.

Table 1—Total nutrients contained in fertilizer and lime applied to roads within Coweeta Watershed 7.

	Date				
Nutrient	23 Apr. 1976	30 June 1976	25 July 1977	22 June 1978	Total
	kg				
Total N	45	220	230	20	515
Ca	360	1,760	1,850	165	4,135
K	45	230	240	20	535
Mg	4	18	20	2	44
PO₄-P	20	95	100	8	223

(weir) remained near baseline levels; i.e., below 10 μg L⁻¹ (Fig. 2). In contrast, elevated NO₃-N concentrations were observed in headwater stream samples collected below the middle logging road, apparently in response to N leached from the cuts and fills of the newly constructed roads. Following road stabilization in July 1977, NO₃-N increased at the weir, and mean monthly values fluctuated around 50 μg L⁻¹ through November 1978. However, substantially higher concentrations were observed in the headwater stream and monthly values frequently exceeded 100 μg L⁻¹. The magnitude of concentration differences between the headwater and weir sites was maintained throughout 1979 and then declined the following year, primarily because of reduced headwater concentrations. In contrast to NO₃, data for other nutrients contained in lime and fertilizer (Ca, K, Mg) showed little difference in concentrations between headwater and watershed outlet locations (Table 2) over the same time period (Fig. 2). Concentrations of SiO₂, a more biologically inert dissolved constituent, also remained relatively constant between locations. Taken collectively, we interpret these data as evidence for within-stream NO₃ depletion rather than dilution over the length of the stream.

An estimate of stream NO₃ depletion was calculated from export differences between the headwater stream and the watershed outlet (Table 3). During the year following road construction and logging, depletion represented 127% of the NO₃-N discharged from the watershed at the weir. During each of the next 2 years, the absolute amount of NO₃-N lost within the stream increased, but because of increased NO₃ discharge at the weir, the percentage of the total decreased to 99 and 42%, respectively. The stream depletion of 3.9 kg N during the year beginning June 1979 represented only 5% of the total NO₃-N discharged from the watershed.

Denitrification Activity

Spatial dimensions of the 10 sampling sites were measured twice (June 1979 and April 1980) and N and organic matter in the sediments were measured from December 1979 through April 1980 (Table 4). No consistent trends in sediment chemistry from the headwater region (sites 9 and 10) toward the lower portion of the stream (sites 1 and 2) were observed.

The denitrifying activity at each site varied through the year (Table 5). No detectable denitrification occurred during winter for most sites. Sites 9 and 10, in the upper reaches of the stream, were most active and remained active throughout the year (Table 5).
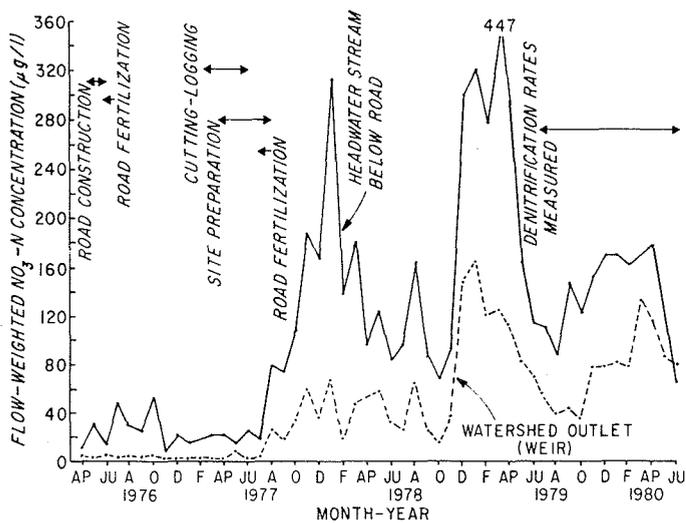
Fig. 2—Mean monthly weighted NO_3-N concentrations in the headwater stream and at the weir of Coweeta Watershed 7.

Rates of denitrification varied considerably but analysis showed that the mean activity at each site for the study period was positively correlated with TKN (r = 0.73) and organic matter (r = 0.52), and negatively correlated with sediment NO_3 concentrations (r = 0.64). The measured activities were projected to monthly rates of denitrification for each site based on volumes of the different deposits, and potential annual losses of N were calculated by summing the monthly rates for the year (1979-1980). Thus, a loss of 1.7 kg N from the system could be explained as a result of sediment denitrification. This amount is probably an underestimate of the total loss because small areas of sediment accumulation were not measured. Mass-balance calculations of stream chemistry and flow data for the period revealed a deficit of 3.9 kg N (Table 3).

DISCUSSION

Results suggest that sediment denitrification can be a major pathway of N loss from a harvested forest ecosystem. Concurrent measurements of denitrifying activity and depletion were available only in the fourth year following harvest, a period when depletion was 3.9 kg. However, in the second and third years after harvest, depletion was 28 and 32 kg. Although denitrifying activity data were unavailable for these years, the magnitude of other potential processes resulting in NO_3 disappearance over the 4-year study period was probably relatively constant. Aquatic studies showed some increases in the normally low levels of primary production found in this stream.[5] Therefore, some N removal by algae could have occurred. Another possible source of loss might be N_2O production by nitrifying bacteria (Bremner and Blackmer, 1979), particularly since marine NH_3-oxidizing bacteria have been shown to produce increased amounts of N_2O under reduced O_2 concentrations (Goreau et al., 1980). Uptake of NO_3-N by heterotrophic bacteria is another potential route of disappearance. Ammonium concentrations in the sedi-

[5] J. J. Hains, Jr. 1981. The response of stream flora to watershed perturbation. M.S. Thesis. Clemson University, Clemson, S.C.

Table 2—Mean annual weighted concentrations of dissolved constituents in stream water at two locations over a 3-year period on Coweeta Watershed 7.

	Concentration of dissolved constituent			
Water year	Ca	K	Mg	SiO_2
	mg L^{-1}			
Headwater				
1977	0.94	0.73	0.40	8.05
1978	0.93	0.64	0.39	7.73
1979	0.89	0.71	0.37	8.09
Watershed outlet				
1977	0.96	0.52	0.42	8.03
1978	0.99	0.53	0.40	7.91
1979	0.82	0.56	0.38	7.39

Table 3—Nitrate-N export and within-stream depletion during the period of system disturbance and recovery.

	NO_3-N exported			Proportion of total
Year (June–May)	Below road†	Watershed outlet (weir)	NO_3-N depletion‡	NO_3-N discharge at weir
	kg			%
1976-1977	5.9	2.6	3.3	127
1977-1978	55.9	28.0	27.9	99
1978-1979	108.7	76.3	32.4	43
1979-1980	83.9	80.0	3.9	5

† Based on accumulation of N for all drainages below the road.
‡ Calculated from the difference between export from contributing areas below the road and export at the weir.

Table 4—Average spatial dimensions and concentrations of N and organic matter for the sediment deposits in Coweeta Watershed 7 stream.

		Depth						
Site	Surface area	Maximum	Minimum	Volume	NH_4-N	NO_3-N	TKN†	Organic matter
	m²	cm		m³	µg g^{-1}		mg g^{-1}	%
1	12.3	20.3	4.1	1.3	1.88	2.11	0.72	0.39
2	9.6	40.0	10.1	2.8	1.07	1.42	0.76	0.56
3	1.0	27.9	5.1	0.2	3.91	1.47	1.21	1.23
4	9.8	24.1	8.9	1.4	1.25	1.38	1.52	0.95
5	2.5	31.0	13.0	0.5	1.74	1.67	1.20	0.67
6	2.6	21.6	10.2	0.4	1.81	1.57	0.90	0.58
7	4.8	15.9	5.1	0.2	3.79	1.40	1.44	0.94
8	5.3	29.2	10.2	0.9	3.32	1.08	1.34	0.63
9	2.3	29.2	5.1	0.4	2.25	0.86	1.76	0.94
10	4.4	31.8	8.9	0.7	2.76	1.01	2.02	1.29

† Total Kjeldahl N.

Table 5—Denitrification rates measured with sediment slurries as N_2O evolution in the presence of C_2H_2.

	N_2O production†					
Site	June 1979	Aug. 1979	Oct. 1979	Dec. 1979	Feb. 1980	Apr. 1980
	nL h^{-1} g^{-1}					
1	--	7.3(0.6)‡	18.3(2.3)	0	0	7.6(0.5)
2	4.3(0.3)	18.8(1.3)	2.4(0.3)	0	0	20.4(2.1)
3	--	9.8(1.0)	3.7(0.1)	0	0	5.4(0.2)
4	14.7(0.7)	17.9(1.2)	16.7(2.0)	0	0	14.8(0.9)
5	6.0(0.1)	11.9(0.6)	4.7(0.1)	0	0	9.5(1.0)
6	5.8	27.2(0.6)	3.0(0.3)	0	0	11.0(0.9)
7	14.1(0.2)	0.1(0.1)	11.2(1.0)	0	0	9.0(0.8)
8	5.3(0.1)	0.1(0.1)	--	0	0	2.5(0.2)
9	43.2(1.1)	35.0(1.3)	74.9(4.2)	47.9(1.6)	8.9(0.1)	58.4(1.5)
10	--	34.0(1.0)	35.0(2.3)	64.8(3.6)	16.3(0.2)	55.3(3.9)

† N_2O corrected for solubility of N_2O in water.
‡ One standard error.

ments were essentially equal to the NO_3-N; hence, the bacteria seem unlikely to be using NO_3-N as a source of N.

Sediment depths exceeded 5 cm and contained an abundance of organic N (Table 4), factors ideal for the growth of denitrifying microorganisms (Van Kessel, 1978; Sain et al., 1977). Furthermore, the ability to detect effects of denitrification on surface-water N concentrations is related to the ratio of water volume to wet bottom area (Huang and Wozniak, 1981). When this ratio is small, as in our study (< 2.0), sediment denitrification could have a large effect upon the NO_3 concentration in the water.

Some of the variation in denitrifying activity from site to site probably resulted from the instability of the deposits. The sediments have a high mica-schist content and considerable movement occurs in the streambed during stormflow periods. Scouring and aeration of the sediments result. Sites 1, 9, and 10 were the most stable, occurring on relatively flat reaches. A second factor contributing to intersite variability was the existence of intermittently flowing seeps along the stream, which locally increased stream NO_3-N and organic matter concentrations. The measured rates of denitrification up to 93.7 ng N h^{-1} are consistent with NO_3-N losses reported for other streams (Hill, 1979; Chatarpaul et al., 1980), but is almost two orders of magnitude greater than activities measured in eutrophic lake sediments (Chan and Knowles, 1979).

In previous studies of N cycling in watersheds, budgets have been constructed with hydrologic losses based on NO_3-N concentrations at the weir. Our findings suggest such budgets can significantly underestimate N losses from the system and that future studies should be designed to measure within-stream depletion of N. Furthermore, denitrification losses are related to the quantity of stream sediment (Van Kessel, 1977). In our study, forest floor disturbance and density of logging roads were minimal. Given the observed precipitation amounts and patterns, sediment yields to the stream were less in this clearcutting experiment than would have occurred from conventional logging methods. Tractor and rubber-tired skidders are typically used in logging operations in this region. These machines are usually associated with a much greater road density and produce more surface disturbance and sedimentation than occurred in this study. Increased sediment inputs may enhance anaerobic conditions by reducing advective flow through sediments or increasing organic matter. Therefore, stream N loss via denitrification could quantitatively be of even greater significance where ecosystem disturbance is more severe.

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