

## Nitrification rates in a headwater stream: influences of seasonal variation in C and N supply

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**Abstract.** Nitrification, the chemoautotrophic process by which  $\text{NH}_4\text{-N}$  is converted to  $\text{NO}_3\text{-N}$ , is an integral biogeochemical transformation in stream ecosystems. Previous research has shown that experimental addition of dissolved organic C inhibits rates of nitrification, and that  $\text{NH}_4\text{-N}$  addition stimulates rates of nitrification. In many streams, large amounts of C and N may be present in particulate and sorbed forms. Hugh White Creek, a headwater stream located in the southern Appalachian mountains of North Carolina, USA, has very low concentrations of dissolved N and receives large inputs of allochthonous particulate organic matter (POM) each autumn. We conducted a seasonal survey of organic matter (OM) standing stocks and nitrification rates, and we experimentally manipulated dissolved C and N supplies in stream-sediment microcosms to determine: 1) how rates of nitrification compare across seasons, and 2) to what extent nitrification rates are influenced by seasonal changes in standing stocks and relative abundances of particulate, sorbed, and dissolved forms of C and N. Rates of nitrification were closely and positively related to rates of ammonification which, in turn, were negatively related to C:N ratios of fine benthic organic matter (FBOM). Uniform additions of dissolved C and N had varying effects on sediment N-transformation rates during different seasons. Variable responses to experimental additions probably reflected the changing relative importance of C and N as sediment OM stocks were depleted and underwent changes in quality. Slow rates of nitrification for much of the year may be attributed to colder temperatures and large quantities of particulate C relative to N. To the extent that changes in POM stocks dictate changes in C and N availability, seasonal OM dynamics are closely linked to rates of nitrification.

**Key words:** nitrification, nitrogen, carbon, particulate, dissolved, sorbed, C:N, FBOM, headwater streams, bacteria, microbial stream ecology.

Human alteration of the N cycle has doubled the rate at which fixed N is supplied to the biosphere (Vitousek 1994) and has changed the balance between supply and demand for plant-available N, a condition that has led to N saturation of terrestrial ecosystems in many parts of the world (Aber et al. 1998, Tietema et al. 1998). One consequence of N saturation is increased delivery of N to streams (Aber et al. 1998).

Agricultural land use also has enhanced N delivery to streams (David and Gentry 2000) with implications for nutrient loads to large rivers (Seitzinger et al. 2002) and estuarine systems

(Rabalais et al. 2002). Recent work has suggested that N processing by headwater streams may alter N load to downstream systems (Alexander et al. 2000, Peterson et al. 2001, Seitzinger et al. 2002). Consequently, an increasing amount of work has addressed the balance between N uptake and transformation in headwater streams (Mulholland et al. 2000, Kemp and Dodds 2001).

Nitrification is of particular interest because the process transforms N in the form of  $\text{NH}_4\text{-N}$  to N in the form of  $\text{NO}_3\text{-N}$ , a more mobile chemical species. Likens et al. (1969) emphasized that a marked increase in nitrification resulted in massive loss of N following clear-cutting of a northern hardwood forest. Variation across biomes (Vitousek et al. 1979), together with disturbance history (Ollinger et al. 2002), emphasize the significance of nitrification as a fundamental process regulating N loss following dis-

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turbance. Stream ecologists have recently increased efforts to address the relevance of nitrification to streams (Peterson et al. 2001, Bernhardt et al. 2002).

Studies investigating factors influencing  $\text{NH}_4\text{-N}$  dynamics at the stream-reach scale have found negligible uptake by nitrifying bacteria relative to other processes such as instream heterotrophic assimilation (Webster et al. 2003). However, other studies addressing stream function have described nitrification as quantitatively important (Mulholland et al. 2000, Findlay and Sinsabaugh 2003). Relatively few studies have addressed potential variation in rates of nitrification and associated control variables (but see Bernhardt and Likens 2002, Strauss et al. 2002).

Numerous environmental variables including temperature, dissolved  $\text{O}_2$  (DO),  $\text{NH}_4\text{-N}$  availability, and pH represent physiological constraints known to influence nitrification rates in controlled environments (Richardson 1985). Of 13 variables potentially associated with sediment nitrification from 36 streams in northern Wisconsin and Michigan,  $\text{NH}_4\text{-N}$  availability and pH best predicted nitrification rates (Strauss et al. 2002). Nitrification rates increased with greater  $\text{NH}_4\text{-N}$  availability and declined with lower pH (Strauss et al. 2002). Significant positive relationships between nitrification rates and DO availability also have been demonstrated (Kemp and Dodds 2001).

The tendency for elevated C to slow or inhibit nitrification has been observed for agricultural fields (Venterea and Rolston 2000), wastewater treatment facilities (Richardson 1985), forests (Montagnini et al. 1989a, b, Ollinger et al. 2002), and streams (Bernhardt and Likens 2002). C influences on nitrification may be direct via chemical toxicity (Strauss and Lamberti 2000) or indirect via changes in microbial dynamics (Verhagen and Laanbroek 1991, Strauss and Lamberti 2000). The latter is found where C is abundant (i.e., high atomic C:N ratio) and heterotrophic microbes out-compete autotrophic nitrifying bacteria for  $\text{NH}_4\text{-N}$  because of more efficient metabolic sequestration.

These influences of C and N on nitrification rates typically have been demonstrated using sediment microcosms and manipulation of dissolved organic C (DOC) and inorganic N in stream water (Strauss et al. 2002). However, far less is known about how particulate C and N

influence rates of nitrification. Both the N and C that make up biofilms and detrital substrates of particulate organic matter (POM) may be available to microbes (Lock 1981). At the same time, a large supply of DOC and  $\text{NH}_4\text{-N}$  may be sorbed to stream sediments and may become available to stream microflora (Triska et al. 1994, Baker et al. 2000).

In the southern Appalachians, C and N availability in headwater streams is largely influenced by allochthonous inputs of organic matter (OM), such as leaves and wood (Meyer et al. 1998). This terrestrial-aquatic linkage produces cyclical patterns of C and N availability (Meyer et al. 1998) that may influence rates of nitrification. We address how seasonal C and N variation associated with particulate, sorbed, and dissolved sources influences rates of nitrification in Hugh White Creek (HWC), a southern Appalachian headwater stream characterized by low DOC ( $\sim 0.5$  mg/L) and low dissolved N (mean  $\text{NO}_3\text{-N} = 0.010$  mg/L). We conducted a seasonal survey of OM standing stocks and used experimental manipulation of dissolved C and N supplies in stream sediment microcosms to determine: 1) how rates of nitrification compare across seasons, and 2) to what extent nitrification rates are influenced by seasonal changes in standing stocks and relative abundances of particulate, sorbed, and dissolved forms of C and N.

Our research addressed 3 main predictions: 1) given the low concentrations of dissolved C and N characteristic of HWC, rates of nitrification are tied to particulate sources of C and N, 2) seasonal differences in standing stock result in lowest nitrification during autumn when particulate C standing stock is greatest and highest rates in summer when particulate C is lower and temperatures are warmer, and 3) if rates of nitrification are tied to particulate resources, then experimental augmentation of dissolved C or N resources should have different influences on nitrification rates depending upon season and particulate supply.

## Methods

### *Site description*

Our study was conducted in HWC, a 2<sup>nd</sup>-order stream within the Blue Ridge Physiographic Province at the Coweeta Hydrologic Laboratory,

North Carolina. Granitic catchments support mesic hardwood forests dominated by large oak-hickory stands with cove hardwoods common along the stream channel. An extensive perennial understory of mountain laurel (*Kalmia latifolia* L.) and rosebay rhododendron (*Rhododendron maximum* L.) decreases light to very low levels (2–5% of incident; Webster et al. 1983) resulting in very low instream primary production despite seasonal variation in the extent of overstory canopy. The stream drains a 61.1-ha catchment with an average gradient of 23% along the 1125-m main-channel length. Study site elevation was ~820 m. At the study site, the stream had an average width of 2.0 m and an average depth of 3.8 cm. Annual precipitation (200 cm) is distributed evenly among months, and average annual discharge in HWC from 1972 to 2001 was 19.6 L/s (J. Vose, USDA Forest Service, Coweeta Hydrologic Laboratory, unpublished data). Channel units in the stream can be distinguished as depositional (i.e., sand-gravel) runs, cobble riffles, and bedrock cascades making up 26, 64, and 10% of the study reach, respectively (HNV, unpublished data). Channel water conductivity is low (~10–15  $\mu\text{S}/\text{cm}$ ) with constant pH near 6.8 (Swank and Crossley 1988).

#### *Particulate C and N standing stocks*

Benthic organic matter (BOM) was sampled from 4 evenly spaced sampling sites within a 100-m study reach in autumn (25 November 2002), winter (25 January 2003), spring (25 April 2003), and summer (13 July 2003). Each site included a transect traversing the stream perpendicular to flow. Slow-moving depositional areas (usually at the head of small riffles) were identified near each transect, and a circular pot sampler (30-cm diameter) was inserted into the stream bed at 3 to 5 random locations within each area.

Coarse benthic organic matter (CBOM) was collected from the pot sampler, dried (50°C) for  $\geq 24$  h, and separated into small wood (<1.5-cm diameter) and leaves. Wood >1.5-cm diameter was discarded. Dried small wood and leaf samples were weighed and ground, and subsamples were ashed (550°C for 45 min) to determine ash-free dry mass (AFDM). Atomic C:N ratios and % abundance of C and N were determined for additional small wood (wood C:N) and leaf

(leaf C:N) subsamples using a VarioMAX CNS Macro Elemental Analyzer (Elementar, Mt. Laurel, New Jersey). Total C and N as leaves (or wood) were calculated by multiplying the % abundance of C or N in each subsample by the total leaf (or wood) standing stock measured at each transect for each season.

After removal of CBOM, fine benthic organic matter (FBOM, <1-mm diameter) in the sampler was collected by measuring water depth (m), agitating the top 3 to 5 cm of stream sediment, and collecting a single 125-mL sample of slurry that was then passed through a 1-mm-mesh net. Slurry samples were filtered onto preweighed and pre-ashed glass-fiber filters (Whatman GFF, 0.70- $\mu\text{m}$  pore size). Filter contents were dried, weighed, and ashed to quantify FBOM concentration (g AFDM/ $\text{m}^3$  of known volume of subsample). FBOM concentration was multiplied by water depth within the sampler to determine areal standing stock (g AFDM/ $\text{m}^2$ ). The remainder of the subsample was dried (50°C) and ground for analysis of atomic C:N ratio; fine benthic organic C (FBOC) and fine benthic organic N (FBON) were determined as described above.

#### *Sediment microcosms*

One day after BOM sampling, a streambed-sediment sample was collected from slow-moving depositional areas ~5 m upstream from each transect. At each site, a single composite sample consisting of multiple sediment cores (taken to 10-cm depth) was collected, transported on ice, and processed in the laboratory within 6 to 10 h of sampling. In the lab, each sample was sieved (1-cm mesh) and further homogenized by stirring. Some sediment was set aside for experimental manipulations (see below).

Four replicate 50-cm<sup>3</sup> aliquots of the homogenized sediment from each site were placed into experimental microcosms (250-mL Erlenmeyer flasks) for a total of 16 microcosms (4 sites  $\times$  4 microcosms/site). One microcosm from each site was used to assess sorbed C and particulate C and N standing stocks (see below). A 2<sup>nd</sup> microcosm from each site was used to determine sorbed N (initial  $\text{NH}_4\text{-N}$  content) for calculating ammonification rates (see below). The 2 remaining microcosms, to which 100 mL of channel water from HWC were added, were used for nitrification assays. Assays specifically used

channel water rather than interstitial water to compare the relationship between nitrification rates and dissolved C and N supplied by stream water and the relationship between nitrification rates and sorbed or particulate forms of C and N.

#### *Sorbed C and N*

Sorbed C (i.e., water-soluble sediment organic C) was determined in the first microcosm from each site with H<sub>2</sub>O extraction. The sediment was amended with 100 mL of deionized water and extracted for 1 h at ambient stream temperature (sensu Baker et al. 2000). Thereafter, an ~50-mL water sample was removed, centrifuged at 4500 rpm, filtered through a glass-fiber filter (Whatman GFF, 0.70- $\mu$ m pore size), and analyzed for DOC. Microcosm sediments were passed through a 1-mm sieve, and FBOM collected was weighed and analyzed for C:N content for comparison with other microcosm observations.

Sorbed NH<sub>4</sub>-N was determined in the 2<sup>nd</sup> microcosm from each site with KCl extraction (Sparks 2003). The sediment was amended with a mixture of 100 mL of 2 N KCl and 100 mL of channel water and shaken at 100 rpm for ~1 h. Thereafter, an ~50-mL water sample was removed, centrifuged at 4500 rpm, filtered through a glass-fiber filter (Whatman GFF), and analyzed for NH<sub>4</sub>-N. The mass of NH<sub>4</sub>-N sorbed to the sediment was calculated as the total mass of NH<sub>4</sub>-N present minus the mass introduced by channel water added to the microcosm. Sorbed NH<sub>4</sub>-N determined in this manner was used as a measure of initial availability (i.e., [NH<sub>4</sub>-N]<sub>initial</sub>) in determination of nitrification rates (see below). Although methods of extraction of C and N were different (i.e., water vs KCl), we used sorbed C:N to describe relative availability of C and N assuming that NO<sub>3</sub>-N sorbed to sediment was negligible.

#### *Microcosm nitrification assay*

Benthic nitrification assays (Hall 1984, Strauss and Lamberti 2000) were carried out using the 2 remaining microcosms (hereafter referred to as reference and blocked microcosms) from each site. Blocked microcosms were amended with nitrapyrin (2-chloro-6-[trichloromethyl]-pyridine), a nitrification inhibitor, dissolved in dimethyl sulfoxide (DMSO). Reference microcosms were amended with DMSO alone. Micro-

cosms were incubated in the laboratory in the dark at ambient stream temperature on a shaker table agitated at 100 rpm for 12 h. Following incubation, 100 mL of 2 N KCl was added to each flask (final KCl concentration ~1 N) to terminate incubation and extract sediment NH<sub>4</sub>-N. A 50-mL water sample was removed from each flask, centrifuged at 4500 rpm, and filtered through a glass-fiber filter (Whatman GFF) for NH<sub>4</sub>-N analysis.

Rates of N transformations were determined by comparing changes in NH<sub>4</sub>-N extracted from microcosm sediments. N transformations in the reference microcosm included immobilization, ammonification, and nitrification. The addition of nitrapyrin eliminated nitrification in blocked microcosms. Gross rates of nitrification ( $\mu$ g N cm<sup>-3</sup> h<sup>-1</sup>) were calculated by subtracting NH<sub>4</sub>-N concentrations ( $\mu$ g/L) in reference microcosms ([NH<sub>4</sub>-N]<sub>ref</sub>) from NH<sub>4</sub>-N concentrations in blocked microcosms ([NH<sub>4</sub>-N]<sub>block</sub>) and adjusting for sediment volume and incubation time as follows:

$$\begin{aligned} & \text{gross nitrification} \\ &= \frac{0.2([\text{NH}_4\text{-N}]_{\text{block}} - [\text{NH}_4\text{-N}]_{\text{ref}})}{VT} \quad [1] \end{aligned}$$

where V is the volume of sediment (cm<sup>3</sup>) in each microcosm, T is the duration of the incubation (h), and 0.2 (L) is a correction factor to account for total extract volume. Net heterotrophic ammonification ( $\mu$ g N cm<sup>-3</sup> h<sup>-1</sup>) was calculated by subtracting [NH<sub>4</sub>-N]<sub>initial</sub> from [NH<sub>4</sub>-N]<sub>block</sub> and adjusting for time and volume as follows:

$$\begin{aligned} & \text{net ammonification} \\ &= \frac{0.2([\text{NH}_4\text{-N}]_{\text{block}} - [\text{NH}_4\text{-N}]_{\text{initial}})}{VT} \quad [2] \end{aligned}$$

Negative values from equation 2 represent net immobilization. Areal rates for N transformations were obtained by multiplying volumetric rates by sediment depth (i.e., 5 cm).

#### *Experimental assessment of C and N influence on nitrification and ammonification rates*

The reserved sediment from each site was used to assess the effects of C and N availability on rates of nitrification in Hugh White Creek during each season. As above, 50-mL aliquots of sediments were combined with 100 mL of chan-

nel water in 250-mL Erlenmeyer flasks. The experimental unit was one set of paired flasks (one blocked with nitrapyrin and one reference without nitrapyrin). One set of paired flasks from each site was used in each treatment (i.e.,  $n = 4$  replicates/treatment). Four flasks were used to determine initial sorbed  $\text{NH}_4\text{-N}$  for ammonification calculations (see above). Glucose was chosen as the source of labile dissolved C, and N was added in the form of  $\text{NH}_4\text{-N}$ . A randomized complete block design was used to measure the effects of 4 treatments: +0 (control, no addition, determined from reference flasks described above), +C (glucose addition, final concentration 10 mg/L above background), +N ( $\text{NH}_4\text{-N}$  addition, final concentration 300  $\mu\text{g/L}$  above background), and +CN (combined  $\text{NH}_4\text{-N}$  and glucose addition). Nitrification rates were determined for each treatment by the same method used to assay seasonal rates (described above).

#### *Channel water*

Temperature ( $^{\circ}\text{C}$ ), DO (mg/L), and specific conductance ( $\mu\text{S/cm}$ ) of stream water were measured using YSI meters (Model 55, Yellow Springs Instruments, Yellow Springs, Ohio). One water sample was collected at each of 4 transects along the 100 m reach, filtered through a glass-fiber filter (Whatman GFF) in the field, frozen, and analyzed (see below) within 1 mo of collection for  $\text{NH}_4\text{-N}$ ,  $\text{NO}_3\text{-N}$ , and DOC. Channel water C:N ratios were determined from DOC and dissolved inorganic N (DIN).

#### *Water-chemistry analysis*

Concentrations of  $\text{NO}_3\text{-N}$  were determined using a Cd-reduction method on a Technicon Autoanalyser II (Technicon, Saskatoon, Saskatchewan, Canada), according to Technicon (1973) Industrial Method 100-70W; the reagents are similar to, but more dilute than, those specified by USEPA (1979). Concentrations of  $\text{NH}_4\text{-N}$  were measured by phenol hypochlorite analysis on a Technicon Autoanalyser (USEPA 1997). DIN was calculated as the sum of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  concentrations. Minimum detection limits for both N forms were 1  $\mu\text{g/L}$ . For samples from nitrification assays, standards were made using a matrix of DMSO, nitrapyrin, KCl, and deionized water in appropriate proportions to ad-

dress matrix effects on  $\text{NH}_4\text{-N}$  concentrations. DOC was analyzed using an Oceanography International Model 700 Total Carbon Analyzer (Oceanographic International, College Station, Texas) by wet persulfate digestion (Menzel and Vaccaro 1964).

#### *Statistical analysis*

Differences in stream chemistry, OM stocks, mineralization, and nitrification rates among seasons were assessed by 1-way analysis of variance (ANOVA) (season = main effect) followed by Tukey's multiple comparisons test (MCT). Effects of experimental amendments of dissolved C and N on rates of nitrification and ammonification within seasons also were assessed by 1-way ANOVA followed by Tukey's MCT. A 2-way ANOVA was used to assess the season  $\times$  amendment interaction (season and amendment = main effects). Sediment samples from different seasons were considered independent because samples were collected at 3-mo intervals, never collected from exactly the same location, and presumed to reflect ambient conditions in time and space. Relationships between nitrification and potential control variables were assessed with Pearson's product moment correlation and linear regression analyses. Rates of nitrification and ammonification in +0 microcosms were regressed against microcosm conditions (e.g., FPOM, C:N, sorbed C, etc). All statistical analyses were conducted using SAS V9.1 (SAS Institute, Cary, North Carolina). The significance level for statistical assessment was set at  $\alpha = 0.05$ .

## **Results**

Stream water temperatures varied seasonally (ANOVA,  $p < 0.0001$ ; Table 1) with minimum temperature in winter ( $5.0^{\circ}\text{C}$ ) and maximum temperature in summer ( $15.2^{\circ}\text{C}$ ). The stream was well oxygenated throughout the year; minimum DO in the winter was 63% of saturation while DO concentration in other seasons was significantly greater (ANOVA,  $p < 0.05$ ; Table 1) and nearly 100% saturated. Average specific conductance varied significantly (ANOVA,  $p < 0.05$ ; Table 1) among seasons but ranged only 1.5  $\mu\text{S/cm}$  (12–13.5  $\mu\text{S/cm}$ ).

TABLE 1. Mean ( $\pm 1$  SE) seasonal water characteristics in Hugh White Creek, North Carolina. Significant differences among seasons for each variable are denoted by different superscripts. Dissolved inorganic N (DIN) is the sum of  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  concentrations.  $n = 4$  for all means. NA = not available, DOC = dissolved organic C.

|   | Autumn                       | Winter                      | Spring                       | Summer                        |
|---|------------------------------|-----------------------------|------------------------------|-------------------------------|
| Temperature ( $^{\circ}\text{C}$ )                          | 9.3 $\pm$ 0.09 <sup>a</sup>  | 5.0 $\pm$ 0.1 <sup>b</sup>  | 12.7 $\pm$ 0.1 <sup>c</sup>  | 15.2 $\pm$ 0.09 <sup>d</sup>  |
| Dissolved $\text{O}_2$ (mg/L)                               | 10.2 $\pm$ 0.1 <sup>a</sup>  | 7.4 $\pm$ 0.12 <sup>c</sup> | 9.9 $\pm$ 0.1 <sup>b</sup>   | 9.64 $\pm$ 0.05 <sup>ab</sup> |
| Specific conductance ( $\mu\text{S}/\text{cm}$ )            | 13.5 $\pm$ 0.01 <sup>a</sup> | NA                          | 12.1 $\pm$ 0.01 <sup>b</sup> | 12.0 $\pm$ 0.11 <sup>b</sup>  |
| DOC (mg/L)  | 1.4 $\pm$ 0.5 <sup>a</sup>   | 0.4 $\pm$ 0.1 <sup>b</sup>  | 0.8 $\pm$ 0.1 <sup>b</sup>   | 0.7 $\pm$ 0.2 <sup>b</sup>    |
| Sorbed C ( $\mu\text{g}/\text{cm}^3$ )                      | 2.3 $\pm$ 0.1                | 2.8 $\pm$ 0.7               | 3.5 $\pm$ 0.9                | 1.2 $\pm$ 0.2                 |
| $\text{NO}_3\text{-N}$ ( $\mu\text{g}/\text{L}$ )           | 2 $\pm$ 1 <sup>cb</sup>      | 1 $\pm$ 1 <sup>c</sup>      | 6 $\pm$ 1 <sup>ab</sup>      | 8 $\pm$ 1 <sup>a</sup>        |
| $\text{NH}_4\text{-N}$ ( $\mu\text{g}/\text{L}$ )           | 1 $\pm$ 1                    | 2 $\pm$ 1                   | 3 $\pm$ 1                    | 3 $\pm$ 3                     |
| DIN ( $\mu\text{g}/\text{L}$ )                              | 3 $\pm$ 2 <sup>a</sup>       | 3 $\pm$ 1 <sup>a</sup>      | 9 $\pm$ 1 <sup>ab</sup>      | 11 $\pm$ 2 <sup>b</sup>       |
| Sorbed $\text{NH}_4\text{-N}$ ( $\mu\text{g}/\text{cm}^3$ ) | 0.2 $\pm$ 0.1                | 0.2 $\pm$ 0.1               | 0.1 $\pm$ 0.1                | 0.2 $\pm$ 0.3                 |
| Dissolved C:N   | 451 $\pm$ 260 <sup>a</sup>   | 148 $\pm$ 26 <sup>b</sup>   | 74 $\pm$ 30 <sup>b</sup>     | 78 $\pm$ 15 <sup>b</sup>      |
| Sorbed C:N  | 13 $\pm$ 4                   | 17 $\pm$ 8                  | 30 $\pm$ 8                   | 5 $\pm$ 1                     |

#### Dissolved C and N

DIN ( $\text{NO}_3\text{-N} + \text{NH}_4\text{-N}$ ) in stream water varied from 3 to 11  $\mu\text{g}/\text{L}$  across seasons and was dominated by  $\text{NO}_3\text{-N}$ . Average  $\text{NO}_3\text{-N}$  concentrations were significantly greater in summer than in autumn and winter, when they dropped to near detection levels (MCT,  $p < 0.05$ ; Table 1). Across seasons,  $\text{NH}_4\text{-N}$  concentrations were very low (1–3  $\mu\text{g}/\text{L}$ ), varied little, and did not differ significantly among seasons (ANOVA,  $p > 0.05$ ; Table 1). Average DOC concentration was significantly greater in autumn (1.4 mg/L) than in other seasons (0.4 to 0.8 mg/L) (MCT,  $p < 0.05$ ; Table 1). Concentrations of DOC and DIN in channel water generated C:N ratios that varied from 74 to 451 (Table 1). C:N ratios were nearly identical in spring and summer (74 and 78, respectively), and higher in winter (148). C:N ratios in autumn (451) were significantly greater than in the other seasons (MCT,  $p < 0.05$ ; Table 1).

#### Sorbed C and N

Sorbed C and N concentrations were more constant than channel-water concentrations (Table 1). Sorbed  $\text{NH}_4\text{-N}$  associated with microcosm sediments varied from 0.1 to 0.2  $\mu\text{g N}/\text{cm}^3$  and did not differ between seasons (ANOVA,  $p > 0.05$ ; Table 1). Sorbed C concentrations were higher and more variable, but did not differ significantly among seasons (MCT,  $p > 0.05$ ; Table 1). Maximum sorbed C concentrations, which occurred in spring (3.5  $\mu\text{g}/\text{cm}^3$ ), were

nearly 3 $\times$  the minimum concentrations, which occurred in summer (1.2  $\mu\text{g}/\text{cm}^3$ ). Thus, sorbed C:N ratios were lowest in summer (5), 6 $\times$  higher in spring (30), and intermediate in autumn and winter (Table 1).

#### Benthic particulate C and N

Streambed particulate C averaged 83 g C/m<sup>2</sup> in autumn when standing stocks were greatest, and declined continuously to 16 g/m<sup>2</sup> by summer when standing stocks were significantly lower (MCT,  $p < 0.05$ ; Table 2). Streambed particulate N also was highest in autumn (1.83 g/m<sup>2</sup>) and significantly lower in summer (0.61 g/m<sup>2</sup>) (MCT,  $p < 0.05$ ; Table 2). Mean C:N ratio of total POM did not differ significantly among seasons (ANOVA,  $p > 0.05$ ); however, the C:N ratio was substantially lower in summer (30) compared to other seasons (48–53) (Table 2).

Leaf C standing stock was significantly greater in autumn than in all other seasons and declined throughout the year (MCT,  $p < 0.05$ ; Table 2). Leaf N stocks also declined steadily from autumn to summer, and summer standing stock (0.05 g N/m<sup>2</sup>) was only 5% of autumn standing stock (1.03 g N/m<sup>2</sup>) (MCT,  $p = 0.01$ ; Table 2). C:N ratios of leaves declined from 62 in autumn to 50 in summer, but values did not differ significantly among seasons (ANOVA,  $p > 0.05$ ; Table 2).

Maximum FBOC standing stocks occurred in winter (21 g/m<sup>2</sup>) rather than autumn, and minimum standing stock occurred in summer (7 g

TABLE 2. Mean ( $\pm 1$  SE) seasonal particulate C and N standing stocks ( $\text{g}/\text{m}^2$ ) and C:N ratios in Hugh White Creek, North Carolina. Significant differences among seasons for each variable are denoted by different superscripts. FBOC = fine benthic organic C, FBON = fine benthic organic N, FBOM = fine benthic organic matter.

|                       | Autumn                       | Winter                        | Spring                        | Summer                       |
|-----------------------|------------------------------|-------------------------------|-------------------------------|------------------------------|
| <b>C</b>              |                              |                               |                               |                              |
| FBOC                  | 12 $\pm$ 3                   | 21 $\pm$ 9                    | 11 $\pm$ 6                    | 7 $\pm$ 3                    |
| C leaves              | 55 $\pm$ 9 <sup>a</sup>      | 29 $\pm$ 2 <sup>b</sup>       | 10 $\pm$ 1 <sup>b</sup>       | 2 $\pm$ 1 <sup>b</sup>       |
| C wood                | 16 $\pm$ 6                   | 29 $\pm$ 13                   | 19 $\pm$ 6                    | 4 $\pm$ 16                   |
| Total particulate C   | 83 $\pm$ 8 <sup>a</sup>      | 70 $\pm$ 19 <sup>ab</sup>     | 51 $\pm$ 4 <sup>ab</sup>      | 16 $\pm$ 20 <sup>b</sup>     |
| <b>N</b>              |                              |                               |                               |                              |
| FBON                  | 0.62 $\pm$ 0.14              | 1.09 $\pm$ 0.11               | 0.56 $\pm$ 0.31               | 0.4 $\pm$ 0.13               |
| N leaves              | 1.03 $\pm$ 0.25 <sup>a</sup> | 0.58 $\pm$ 0.17 <sup>ab</sup> | 0.23 $\pm$ 0.01 <sup>b</sup>  | 0.05 $\pm$ 0.02 <sup>b</sup> |
| N wood                | 0.02 $\pm$ 0.16              | 0.24 $\pm$ 0.10               | 0.23 $\pm$ 0.07               | 0.04 $\pm$ 0.01              |
| Total particulate N   | 1.83 $\pm$ 0.37 <sup>a</sup> | 1.71 $\pm$ 0.18 <sup>a</sup>  | 1.14 $\pm$ 0.29 <sup>ab</sup> | 0.61 $\pm$ 0.12 <sup>b</sup> |
| <b>C:N ratio</b>      |                              |                               |                               |                              |
| C:N FBOM              | 22 $\pm$ 6                   | 22 $\pm$ 3                    | 23 $\pm$ 13                   | 21 $\pm$ 8                   |
| C:N leaves            | 62 $\pm$ 17                  | 58 $\pm$ 26                   | 52 $\pm$ 12                   | 50 $\pm$ 18                  |
| C:N wood              | 97 $\pm$ 35                  | 139 $\pm$ 79                  | 94 $\pm$ 34                   | 112 $\pm$ 45                 |
| Total particulate C:N | 53 $\pm$ 10                  | 48 $\pm$ 10                   | 50 $\pm$ 5                    | 30 $\pm$ 6                   |

C/ $\text{m}^2$ ). Mean FBOC did not differ significantly among seasons (ANOVA,  $p > 0.05$ ; Table 2). Similar to FBOC, FBON was highest in winter (1.09 g N/ $\text{m}^2$ ) and lowest in summer (0.4 g N/ $\text{m}^2$ ) (Table 2). Within seasons, mean C:N ratio of FBOM was highly variable (CV = 28–113%). Mean C:N ratios did not differ significantly among seasons (ANOVA,  $p > 0.05$ ; Table 2), but average C:N for FBOM in summer was the lowest of any season.

The C:N ratio of small wood (94–139) was noticeably higher than the C:N ratios of other forms of POM and more variable (Table 2). Wood C was highest in the winter (29 g/ $\text{m}^2$ ). This value was  $\sim 7\times$  greater than summer standing stocks (4 g/ $\text{m}^2$ ), but this difference was not statistically significant (ANOVA,  $p = 0.25$ ; Table 2). Wood N stocks were  $\geq 6\times$  greater in winter and spring (0.24 and 0.23 g/ $\text{m}^2$ , respectively) than in autumn and summer (0.02 and 0.04 g/ $\text{m}^2$ , respectively) (Table 2).

#### Seasonal variation in rates of nitrification

Rates of nitrification differed significantly among seasons (ANOVA,  $p < 0.05$ ; Fig. 1). Nitrification rates in autumn were highly variable and were not significantly different from rates measured in the other seasons (MCT,  $p > 0.05$ ). Lowest average nitrification rates occurred in winter (0.19  $\mu\text{g N cm}^{-3} \text{d}^{-1}$ ), when rates were

similar to those in spring (0.29  $\mu\text{g N cm}^{-3} \text{d}^{-1}$ ). The average rate in summer (0.95  $\mu\text{g N cm}^{-3} \text{d}^{-1}$ ) was significantly greater than spring and winter rates and  $2\times$  to  $3\times$  higher than rates measured in all other seasons (MCT,  $p < 0.05$ ; Fig. 1).

#### Linking nitrification to C and N supplies

In +0 microcosms, nitrification rates were positively correlated with rates of ammonification ( $r^2 = 0.45$ ,  $p = 0.004$ ; Fig. 2A) along a nearly 1:1 regression line (slope = 0.87). FBOM C:N ratios varied from 15 to 20 in microcosm sediments. Both ammonification ( $r^2 = 0.48$ ,  $p = 0.004$ ; Fig. 2B) and nitrification rates ( $r^2 = 0.18$ ,  $p = 0.10$ ; Fig. 2C) were negatively correlated with FBOM C:N, but a few very low values for nitrification rates resulted in a nonsignificant correlation.

In +0 microcosms, average rates of nitrification were related to factors measured in the field, including water temperature and supplies of both dissolved and particulate C and N. Nitrification rates increased at higher temperatures (Fig. 3A) and declined with increasing FBOM C:N (Fig. 3B) and sorbed C (Fig. 3C). Rates of nitrification were positively related to sorbed  $\text{NH}_4\text{-N}$  (Fig. 3D).

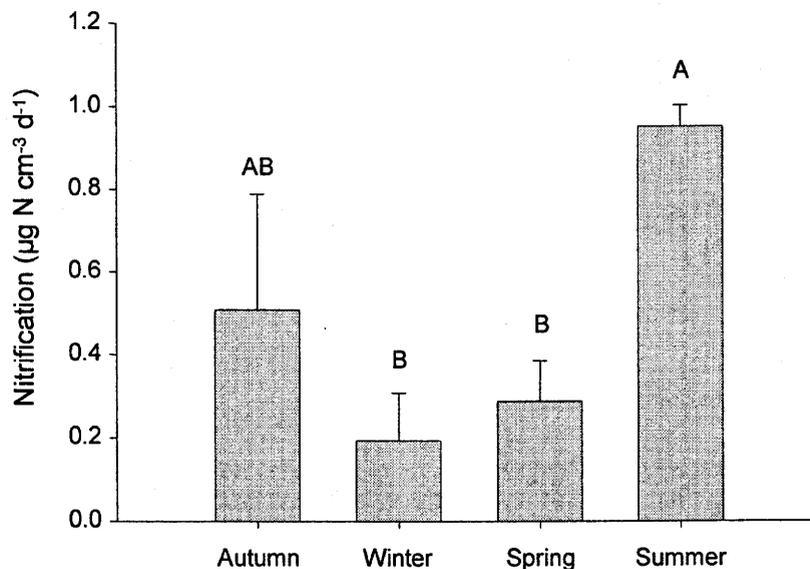


FIG. 1. Mean (+1 SE) seasonal nitrification rates in Hugh White Creek. Significant differences in seasonal rates are denoted by different superscripts.

#### *Influences of C and N manipulation on nitrification and ammonification*

Across seasons, C and N amendment significantly influenced rates of nitrification (2-way ANOVA, treatment main effect,  $p = 0.04$ ). Nitrification rate differed with season (season main effect,  $p < 0.001$ ), and the influence of C and N amendment on rates of nitrification varied significantly with season (significant treatment  $\times$  season interaction,  $p = 0.01$ ).

Nitrification rates in +N, +C, and +CN treatment groups did not differ from rates in the +0 treatment group in autumn, winter, or spring (ANOVA,  $p > 0.05$ ; Fig. 4A). In autumn, mean nitrification rates in +0 and +N treatments were  $>0.5 \mu\text{g N cm}^{-3} \text{d}^{-1}$ . In winter and spring, nitrification rates were low in all treatment groups, and rates did not differ significantly (MCT,  $p > 0.05$ ; Fig. 4A). In summer, nitrification rates in the +N treatment group were significantly greater than rates in the +C treatment group, and rates in the +0 and +CN treatment groups were intermediate (MCT,  $p < 0.05$ ; Fig. 4A).

Overall, dissolved C and N amendments did not significantly affect rates of ammonification (2-way ANOVA, treatment main effect,  $p = 0.24$ , Fig. 4B), nor was there a significant treatment  $\times$  season interaction (ANOVA,  $p = 0.13$ ). However, ammonification rates did differ among seasons

(ANOVA,  $p < 0.0001$ ) with highest rates evident during summer.

#### Discussion

##### *Standing stocks of OM, C, and N*

Temporal variation in ambient nitrification rates and the relative influences of dissolved C and N amendments suggest that controls over rates of nitrification vary seasonally in HWC. Further, correlations between particulate C and N and rates of nitrification suggest that the temporal changes are related to seasonal variation in particulate standing stocks.

Sorbed C content was greater in cooler seasons (autumn, winter, and spring) and was lowest in summer. An identical pattern in sorbed C content was reported for a different Coweeta stream, and the pattern was linked to variation in dissolved terrestrial inputs (Crocker and Meyer 1987). During autumn, elevated channel DOC concentrations are attributable to leaf litter leaching (Meyer et al. 1998) and may be an important source reestablishing sorbed C following summer decline. Sorbed N does not follow the same pattern.

Sorbed N may be an important N source for stream processes (Triska et al. 1994). In HWC, large quantities of mica in the stream bed may play an important role in  $\text{NH}_4\text{-N}$  availability be-

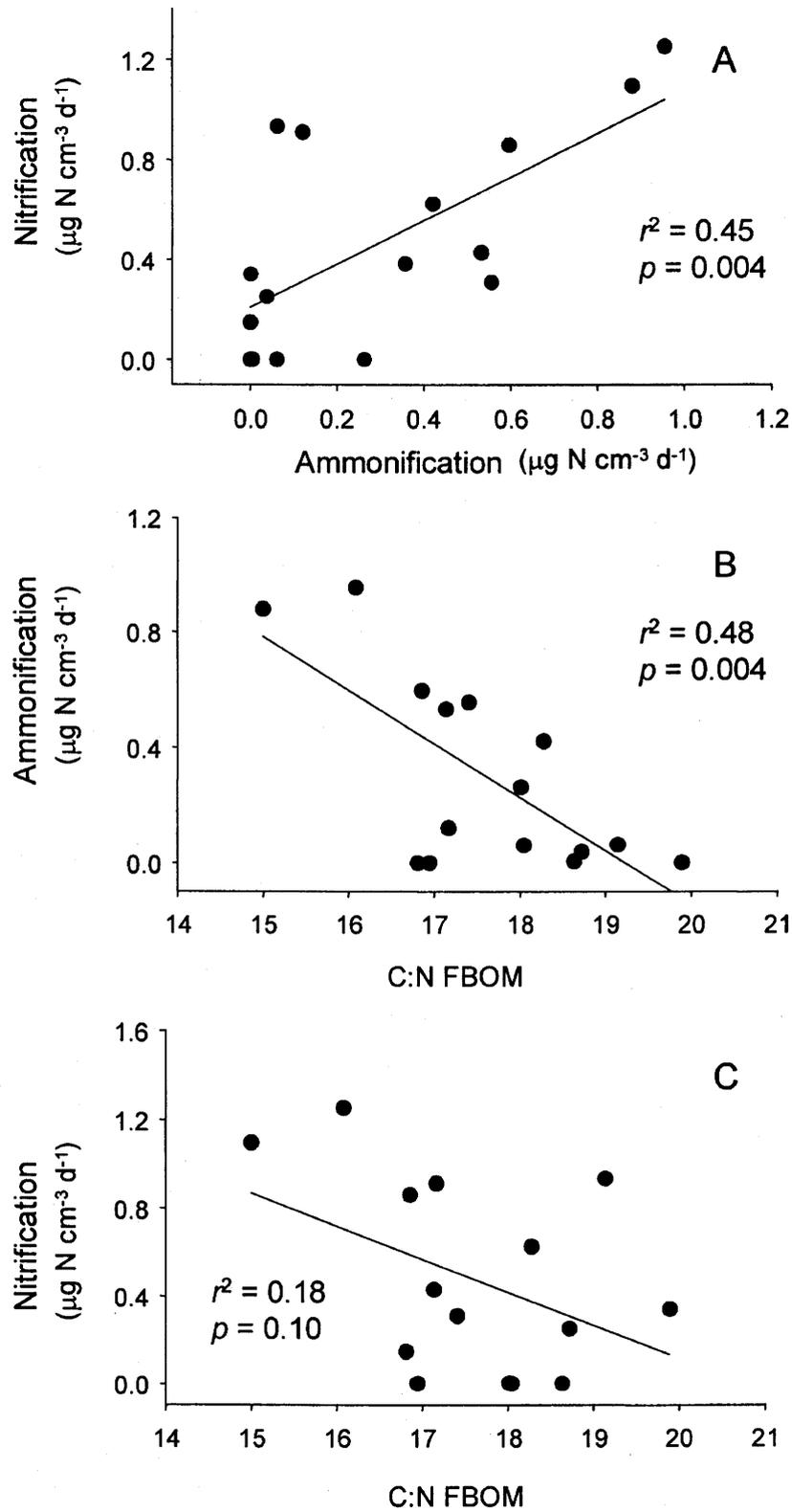


FIG. 2. Linear relationships between nitrification and ammonification rates (A), ammonification rate and C:N ratio of sediment fine benthic organic matter (FBOM) (B), and nitrification rate and C:N ratio of sediment FBOM (C). Rates were determined from +0 microcosms.

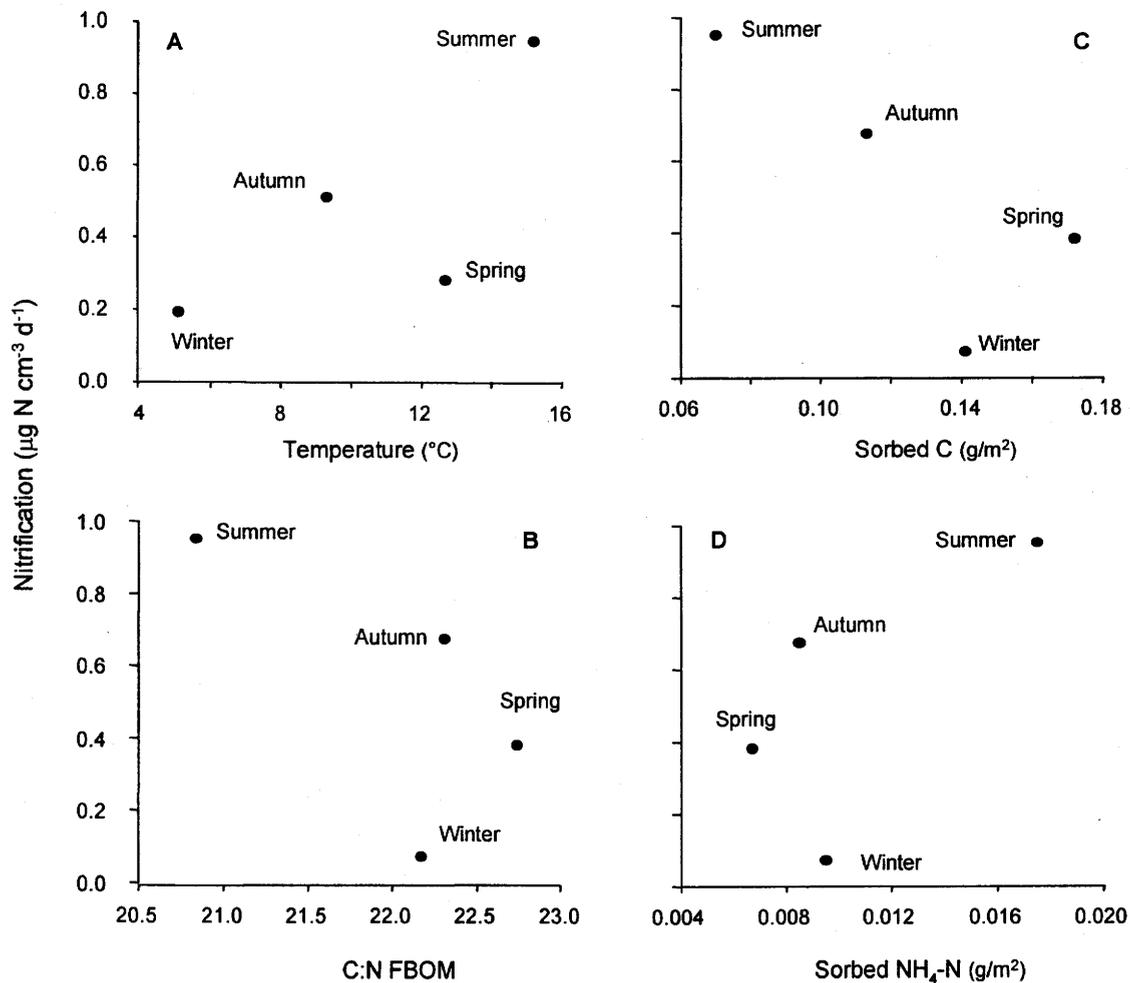


FIG. 3. Relationships between average seasonal rates of nitrification derived from laboratory assays on stream sediments and mean stream water temperature (A), C:N ratio of fine benthic organic matter (FBOM) (B), sorbed C (C), and sorbed  $\text{NH}_4\text{-N}$  (D) measured from field samples.

cause mica is a clay mineral with great sorption potential (Sparks 2003). However, average exchangeable  $\text{NH}_4\text{-N/cm}^3$  sediment (determined using a 2:1 sediment mass/volume ratio and data from Table 1) is  $\sim 0.10 \mu\text{g N/g}$  sediment at HWC. This estimate is orders of magnitude lower than values reported for streams with similar channel water  $\text{NH}_4\text{-N}$  concentrations such as Little Lost Man Creek, California ( $0.4\text{--}1.7 \mu\text{g N/g}$  sediment; Triska et al. 1994) and Bear Brook, New Hampshire ( $1\text{--}9 \mu\text{g N/g}$  sediment; Richey et al. 1985). Low channel concentrations documented in our study and low standing stocks of sorbed N suggest that N is in short supply at HWC, and the ecological significance of this sorbed source should not be underestimated.

#### *Seasonal and spatial variation in rates of nitrification*

We applied nitrification rates derived from +0 microcosms to a sediment depth of 5 cm and generated areal rates of nitrification that varied from  $9.5$  to  $47.5 \text{ mg N m}^{-2} \text{ d}^{-1}$  (Table 3). In a concurrent study of N cycling in HWC, Brookshire et al. (2005) used solute addition experiments and calculated nitrification rates that varied from  $7$  to  $47 \text{ mg N m}^{-2} \text{ d}^{-1}$ , rates very similar to those derived by scaling up our microcosms results. These measures of nitrification rates in HWC sediments contribute to a growing number of estimates for stream ecosystems (Table 3). Rates vary across time and space (Table 3) but appear highest in late spring and

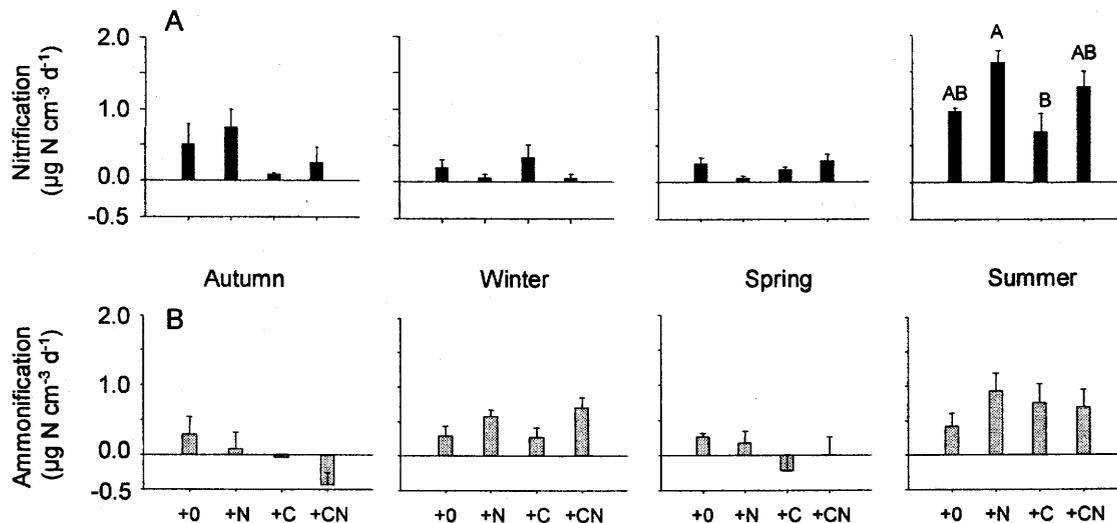


FIG. 4. Mean (+1 SE) rates of nitrification (A) and ammonification (B) in sediments from Hugh White Creek in response to amendments of the water column with nothing (+0),  $\text{NH}_4\text{-N}$  (+N), dissolved organic C (DOC) (+C), and DOC and  $\text{NH}_4\text{-N}$  (+CN). In vitro microcosm experiments were conducted seasonally. Significant differences within seasons for each treatment are denoted by different superscripts.

summer. Summer nitrification rate in HWC was 62% of that measured in late spring in an upland prairie stream (Kings Creek, Kansas; Dodds et al. 2000) and 74% of the summer rate recorded for a desert stream (Sycamore Creek, Arizona; Grimm et al. 1991).

Using a 10-y record of discharge and  $\text{NO}_3\text{-N}$  concentration for HWC (J. Vose, unpublished data), we determined that the average seasonal

$\text{NO}_3\text{-N}$  loads for HWC are 11, 14, 25, and 34 g N/d for autumn, winter, spring, and summer, respectively. Applying our areal nitrification rates to a total stream area of 8085  $\text{m}^2$  (Golladay et al. 1989), we calculated the stream-wide  $\text{NO}_3\text{-N}$  load from nitrification as 206, 77, 113, and 384 g N/d for autumn, winter, spring, and summer, respectively. Stream-wide nitrification, therefore, overestimated  $\text{NO}_3\text{-N}$  output by factors of

TABLE 3. Nitrification rates in lotic ecosystems. All rates were determined using nitrification blockers in sediment microcosms and were corrected for sample depth. Areal rates were determined as the production of volumetric rates assuming a sediment depth of 5 cm. NA = not available.

| Stream name and location         | Stream/catchment characteristics                           | Nitrification ( $\text{mg N m}^{-2} \text{d}^{-1}$ ) | Sampling period | Reference                |
|----------------------------------|--|--|-----------------|--------------------------|
| Kings Creek, Kansas              | Upland prairie stream                                      | 77   | April–May       | Dodds et al. 2000        |
| Sycamore Creek, Arizona          | Intermittent desert stream                                 | 64.5   | NA              | Grimm et al. 1991        |
| Hugh White Creek, North Carolina | Appalachian headwater stream                               | 47.5   | August          | This study               |
| Juday Creek, Indiana             | 3 <sup>rd</sup> -order stream; watershed of mixed land use | 45   | Summer          | Straus and Lamberti 2000 |
| Hugh White Creek, North Carolina | Appalachian headwater stream                               | 25.5   | November        | This study               |
| Hugh White Creek, North Carolina | Appalachian headwater stream                               | 14   | April           | This study               |
| Hugh White Creek, North Carolina | Appalachian headwater stream                               | 9.5  | January         | This study               |

19, 5, 5, and 11 in autumn, winter, spring, and summer, respectively. These are reasonable errors given that microcosms contained only fine-grained material from depositional areas that may make up only 1 to 10% of total streambed area. Further, these calculations assumed no  $\text{NO}_3\text{-N}$  consumption despite documentation of efficient uptake in this study reach (Brookshire et al. 2005). In any case, instream  $\text{NO}_3\text{-N}$  production rates appear to be high enough to account for a large % of  $\text{NO}_3\text{-N}$  exported from the HWC watershed.

Nitrification rates for stream sediments of HWC are greater than those determined for the surrounding forest, but they display seasonal patterns similar to those of forest soils. Estimates of nitrification in various soil horizons of the HWC watershed varied from 0.14 to 2.0 mg N kg soil<sup>-1</sup> 28 d<sup>-1</sup>, with greatest rates in the summer and no measurable nitrification in winter (Montagnini et al. 1989a, b, Knoepp and Swank 1998). Conversion of our volumetric measures yielded nitrification rates of 2.8 mg N kg soil<sup>-1</sup> 28 d<sup>-1</sup> in winter and 12.6 mg N kg soil<sup>-1</sup> 28 d<sup>-1</sup> in summer. In comparison to terrestrial soils, depositional sediments of HWC occupy a very small % of total watershed area, but on a mass-specific basis, sediment rates are similar to the highest terrestrial rates recorded in the HWC watershed.

#### *Factors influencing rates of nitrification*

Controls of ecosystem function commonly associated with microbial processes include temperature and DO availability (Richardson 1985), both of which varied significantly with season in HWC and were positively related to rates of nitrification. However, these relationships were not statistically significant in our study because of small sample sizes and low statistical power. DO varied significantly with season, but it was never <7 mg/L, and samples from hyporheic wells along the study reach showed that interstitial water was well oxygenated (Brookshire et al. 2005). Nitrification rates also increased with temperature, and rates may have been thermally limited during colder seasons (Richardson 1985, Sheibley et al. 2003). Thus, temperature probably interacted with other variables to influence nitrification rates.

Relationships between rates of nitrification and control variables in experimental micro-

cosms are consistent with the hypothesis that increased C availability stimulates heterotrophic metabolism and reduces the ability of nitrifiers to compete for  $\text{NH}_4\text{-N}$  (Verhagen and Laanbroek 1991, Strauss and Lamberti 2000). The positive relationship between nitrification and ammonification suggests that nitrification is primarily controlled by net heterotrophic demand for  $\text{NH}_4\text{-N}$ . Nitrification often is correlated with ammonification in studies of N transformation in forest soils (Holmes and Zak 1999, Ollinger et al. 2002). Ammonification, in turn, has been linked to foliage litter quality (Scott and Binkley 1997). In our study, the correlation between ammonification and FBOM quality (i.e., low C:N ratio) suggests similar relationships among  $\text{NH}_4\text{-N}$  production, nitrification, and sediment detrital quality in stream ecosystems. Ollinger et al. (2002) proposed that rates of nitrification increase rapidly in forest soils when the C:N ratio is less than the critical value of 22. Field measures of FBOM C:N were very similar to this value. Further, as C:N ratios of FBOM in sediment microcosms declined from 20 to 15, rates of nitrification increased by nearly an order of magnitude. Thus, FBOM may exist in a state of resource quality that is pivotal in determining the fates of N and rates of ammonification and nitrification in HWC.

#### *Comparison of the influence of channel-water and other forms of C and N*

Studies comparing nitrification rates to field and microcosm variables suggest that both dissolved and particulate C and N can stimulate microbial activity (Sobczak et al. 1998). In our study, rates of nitrification decreased during periods of increased sorbed and particulate C availability and increased with enhanced sorbed and particulate N availability. In contrast, measures of dissolved C or N failed to predict microcosm nitrification rates. Variables that best predicted average nitrification rates included temperature, FBOM C:N ratio, and sorbed C and N. These findings suggest that channel-water C:N is related less to rates of nitrification in HWC than to other variables such as C and N in sorbed and particulate (i.e., FBOM) forms. This latter perspective may prove useful in understanding the effects of dissolved C and N addition on rates of nitrification observed in our study.

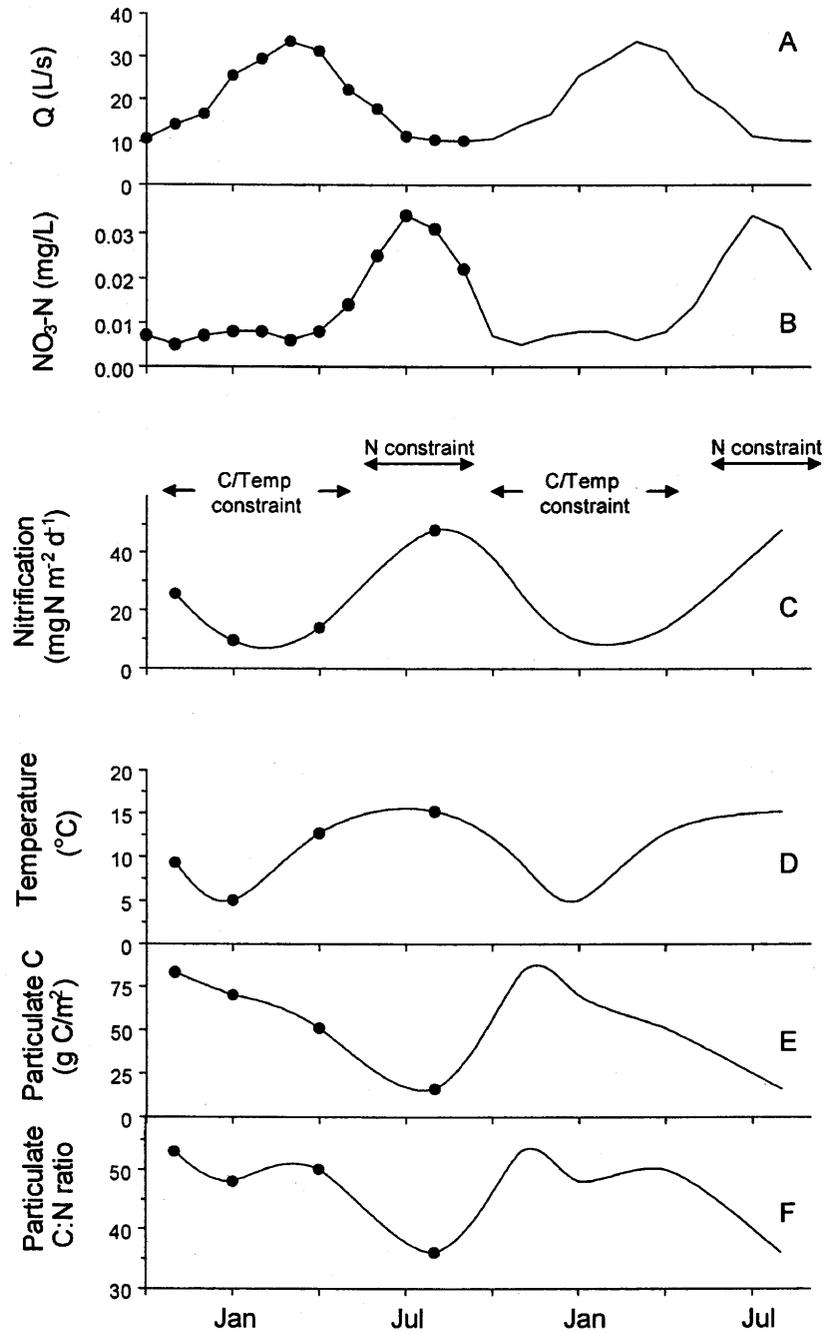


FIG. 5. Conceptual model of seasonal controls on nitrification in forested headwater streams. In all panels, seasonal patterns are repeated for an additional year for illustrative purposes. A.—Monthly mean stream discharge ( $Q$ ) in Hugh White Creek from 1991 to 2001 (J. Vose, USDA Forest Service, Coweeta Hydrologic Laboratory, unpublished data). B.—Monthly mean  $\text{NO}_3\text{-N}$  concentrations in Hugh White Creek from 1991 to 2001 (J. Vose, unpublished data). C.—Smoothed fit of nitrification rate derived from our study to the seasonal data. D.—Smoothed fit of temperature derived from our study to the seasonal data. E.—Smoothed fit of particulate C derived from our study to the seasonal data. F.—Smoothed fit of particulate C:N ratio derived from our study to the seasonal data. See text for explanation of periods of N, C, or temperature (Temp) constraints on rates of nitrification.

Rates of nitrification in microcosms amended with dissolved C and N were not significantly different from ambient rates in any season. The extent of N enrichment used in our study (i.e., 300  $\mu\text{g/L}$   $\text{NH}_4\text{-N}$ ) was high relative to channel N, but low compared to treatments used by other investigators (cf. Strauss and Lamberti 2000). Nevertheless, trends of C inhibition and N stimulation of nitrification observed by Strauss and Lamberti (2000) were evident in autumn and summer in HWC, and the trends were most pronounced in summer. In the winter and spring, C and N amendment did not influence rates of nitrification in any observable way. Instead, rates were low and varied little among treatments. We contend that during spring, ambient rates of nitrification were at seasonal lows as a result of relatively cold temperatures, low-quality FBOM, and high C:N of sorbed materials. Further support for this contention is provided by observations of net immobilization during spring in response to C amendment, indicating the possibility of nitrification inhibition by heterotrophic  $\text{NH}_4\text{-N}$  consumption. In winter, inhibition of nitrification by C may be less efficient, as is indicated by net ammonification in +C microcosms. Net immobilization did not occur in summer, and N amendment was a more effective stimulant for nitrification, suggesting reduced C constraint.

#### *Conceptual model*

Together, our results support a conceptual model (Fig. 5) of how the influence of particulate and interstitial forms of C and N on nitrification rates may vary seasonally in forested headwater streams. A 10-y historical record (J. Vose, unpublished data) illustrates the seasonal change in discharge and  $\text{NO}_3\text{-N}$  concentration in HWC (Fig. 5A, B). Seasonal changes in stream water  $\text{NO}_3\text{-N}$  concentration and load (g N/d, data not shown) correspond to periods of lower and higher rates of nitrification (Fig. 5C). Our study suggests that seasonal changes in C and N availability interact with temperature (Fig. 5D) to create periods of C constraint that alternate with shorter periods of N constraint. As OM stocks of C (Fig. 5E) and N are depleted and undergo annual changes in quality (Fig. 5F), rates of nitrification are influenced by dissolved N and C, changing temperature, and variation in C and N supply from other sources

such as FBOM and sorbed C. The areas labeled "N constraint" in Fig. 5 represent times when C:N ratios of usable OM sources decline to a critical value below which particulate and interstitial C availability no longer constrain rates of nitrification. N availability in sorbed or dissolved form is more likely to affect nitrification during this time of minimized C constraint than when C is constraining. Autumnal leaf fall then resets organic matter standing stocks and reestablishes C constraint. Instream processes then alter the relative abundances of particulate and sorbed C and N until they again approach a critical threshold where nitrification rates may be stimulated by N availability. Thus, to the extent that fluctuations in OM stocks dictate change in C and N availability, seasonal OM dynamics represent changes in ecosystem structure relevant to rates of nitrification.

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