Factors regulating nitrification in aquatic sediments: effects of organic carbon, nitrogen availability, and pH

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Abstract: We investigated the response in nitrification to organic carbon (C) availability, the interactive effects of the C: nitrogen (N) ratio and organic N availability, and differing pH in sediments from several streams in the upper midwestern United States. In addition, we surveyed 36 streams to assess variability in sediment nitrification rates. Labile dissolved organic carbon (DOC) additions of 30 mg $C \cdot L^{-1}$ (as acetate) to stream sediments reduced nitrification rates ($P < 0.003$), but lower concentration additions or dilution of ambient DOC concentration had no effect on nitrification. C:N and organic N availability strongly interacted to affect nitrification ($P < 0.0001$), with N availability increasing nitrification most at lower C:N. Nitrification was also strongly influenced by pH ($P < 0.002$), with maximum rates occurring at pH 7.5. A multiple regression model developed from the stream survey consisted of five variables (stream temperature, pH, conductivity, DOC concentration, and total extractable NH_4^+) and explained 60% of the variation observed in nitrification. Our results suggest that nitrification is regulated by several variables, with NH_4^+ availability and pH being the most important. Organic C is likely important at regulating nitrification only under high environmental C:N conditions and if most available C is relatively labile.

Résumé : Nous avons étudié la nitrification en fonction de la disponibilité du carbone organique (C), les effets interactifs du rapport C:N et de la disponibilité de l'azote organique (N) ainsi que les différences de pH dans les sédiments de plusieurs cours d'eau du Midwest supérieur des États-Unis. De plus, nous avons inventorié 36 cours d'eau pour déterminer la variabilité des taux de nitrification dans les sédiments. L'addition de carbone organique dissous (DOC) labile à raison de 30 mg $C \cdot L^{-1}$ (sous forme d'acétate) aux sédiments des cours d'eau réduit les taux de nitrification ($P < 0{,}003$), mais l'addition de concentrations moindres ou la dilution des concentrations ambiantes de DOC restent sans effet sur la nitrification. Le rapport C:N et la disponibilité de N organique interagissent fortement pour affecter la nitrification ($P < 0{,}0001$) et c'est aux valeurs inférieures du rapport C:N que la disponibilité de N agit le plus sur la nitrification. La nitrification est aussi fortement influencée par le pH ($P < 0{,}002$), avec un taux maximal qui se situe à pH 7,5. Un modèle de régression multiple élaboré à partir des inventaires des cours d'eau contient cinq variables (température du cours d'eau, pH, conductivité, concentration de DOC et NH_4^+ total extractible) et explique 60% de la variation observée dans la nitrification. Il apparaît donc que la nitrification est contrôlée par plusieurs variables, dont les plus importantes sont la disponibilité de NH_4^+ et le pH. Le C organique n'a probablement de rôle important à jouer dans le contrôle de la nitrification que lorsque le rapport C:N dans l'environnement est élevé et que la plus grande partie du C disponible est relativement labile.

[Traduit par la Rédaction]

Introduction

Understanding the natural cycling of nitrogen (N) in freshwater ecosystems is of key importance, because N is an essential component of proteins, enzymes, and other biologically important compounds. Furthermore, low N availability can limit primary productivity, whereas excess N has been linked to eutrophication and health concerns (Carpenter et al. 1998). Great progress has been made in understanding N cycling in aquatic ecosystems (reviewed by Duff and Triska 2000), but many gaps in our knowledge persist. For example, a firm understanding of the factors regulating certain transformations, such as nitrification, remains elusive.

Nitrification, the chemoautotrophic conversion of NH_4^+ to NO_3^-, is a key transformation in the N cycle because it is the only natural process by which NH_4^+ can be converted to NO_3^-. Excess NO_3^- is an aquatic pollutant and a potential health risk. Furthermore, NO_3^- is an initial substrate for denitrification, the reduction of oxidized N forms (NO_3^-, NO_2^-, NO, or N_2O) to N_2. Because of the common link (NO_3^-) between nitrification and denitrification, these processes may be coupled (i.e., localized denitrification rates are dependent upon the NO_3^- produced by nearby nitrifying bacteria) (Duff and Triska 2000). Thus, a better understand-

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ing of the spatial and temporal patterns of nitrification in aquatic sediments can provide insight into observed denitrification patterns and eventual losses of N from ecosystems.

Previous studies in freshwater systems have suggested that nitrifying bacteria and nitrification rates in general may be regulated by several factors including NH_4^+ availability (Triska et al. 1990) or ammonification of organic matter (Jones et al. 1995), oxygen concentration (Triska et al. 1990; Kemp and Dodds 2001), and organic carbon (C) availability (Strauss and Dodds 1997; Strauss and Lamberti 2000). Nitrifying bacteria are autotrophic organisms, i.e., they utilize CO_2 (not organic C) as a C source; however, our previous research has resulted in the hypothesis that increases in organic C levels could reduce or even inhibit nitrification depending on the C concentration and source (Strauss and Lamberti 2000). Heterotrophic bacteria apparently can outcompete nitrifying bacteria for NH_4^+ when its availability is low and organic C is not limiting. Labile organic C also may be able to elicit a stronger negative effect on nitrification than more refractory C (Strauss and Lamberti 2002). To date, the only natural system in which the relationship between organic C and nitrification has been examined is for a northern Indiana stream (Strauss and Lamberti 2000, 2002). It is unknown whether or not such a relationship exists in other aquatic systems. Many studies have identified organic matter as a mineralizable source of NH_4^+ for nitrification (Duff and Triska 2000), but it is unclear if C content, N content, and C:N (total organic C : total N) of the organic matter can interact to affect nitrification rates.

Furthermore, few studies have examined how various factors regulate nitrification over a large spatial scale. One study of several sites in the Rhone River estuary (Mediterranean Sea) found that NH_4^+ availability alone accounted for 74% of the variability in nitrification rates (Bianchi et al. 1999). However, we are unaware of any study that has examined variables regulating nitrification rates across several distinct aquatic systems.

Our overall goal in this study was to quantify spatial variability in nitrification in stream sediments and explore factors potentially responsible for that variability. Specifically, we first tested if organic C, C:N, N availability, and pH regulate lotic nitrification by conducting a series of laboratory experiments using sediments and water from a variety of streams. Second, we measured sediment nitrification rates for 36 streams in Wisconsin and Michigan, and related rates to other physical and chemical variables that could influence nitrification.

Materials and methods

Dissolved organic carbon addition and reduction experiments

In July 1998, we tested the effect of dissolved organic carbon (DOC) on nitrification for six "focal" streams (Mill Creek, Pelton Creek, Scott and Howe Creek, Star Creek, Tamarack Creek, and Tenderfoot Creek) located in northern Wisconsin or the Upper Peninsula of Michigan (Fig. 1), which had different physical and chemical characteristics (Table 1). Three streams (Mill, Star, and Tenderfoot) were lake outlets and were sampled within 3 km of their source. The other three streams were sampled in forested areas and either drained wetlands (Pelton and Tamarack) or upland forest (Scott and Howe). One characteristic that all six streams shared was a relatively high DOC concentration (DOC > 10 mg $C \cdot L^{-1}$, Table 1) when compared with a global average of approximately 5 mg $C \cdot L^{-1}$ (Sedell and Dahm 1990). However, high DOC is typical of streams, rivers, and lakes in this geographic region (Gergel et al. 1999).

Initial nitrification rates were determined for each stream using the nitrapyrin method previously described in detail by Strauss and Lamberti (2000). Unfiltered stream water and sieved (0.64 cm) sediment were collected at each site and returned to the laboratory and stored in the refrigerator at 4°C. Sediments were collected in the mid-channel region of each stream and consisted of either a silt–sand (Pelton and Tenderfoot) or a sand–gravel mixture (Mill, Star, Tamarack, Scott, and Howe). Particulate organic matter was not abundant in any of the streams at the time of sampling. Within 24 h, nitrification incubations were started on four replicate samples for each stream. Each replicate consisted of two flasks, each containing 25 mL sediment and 85 mL stream water. Nitrification was inhibited in one flask by adding 10 mg $\cdot L^{-1}$ nitrapyrin (C-1930, Sigma Chemical Co. St. Louis, Mo.). Because nitrapyrin is not soluble in water, it was dissolved in dimethyl sulfoxide (DMSO) before addition. The other flask received DMSO only as a control. Neither flask received an NH_4^+ amendment. The flasks were then incubated at 23°C (mean temperature of streams) in the dark for three days on an orbital shaker (175 rpm). Dissolved oxygen (DO) and pH were measured for each replicate at the beginning and end of the incubation to identify potential shifts in environmental conditions. Flask contents were homogenized with vigorous shaking and subsamples removed at the beginning and end of the incubation, from which NH_4^+ was extracted using 1 M KCl. Nitrification rates over the incubation period were calculated by subtracting the observed mass change of total extractable NH_4^+ in the flasks containing only DMSO from the NH_4^+ increase in the flasks that contained nitrapyrin, which blocked ammonium oxidation.

In the DOC addition experiment, we added a labile C source, sodium acetate, at four C concentrations (0 (control), 2, 10, and 30 mg $C \cdot L^{-1}$) to flasks containing stream sediment (25 mL, sieved to < 0.64 cm) and unfiltered stream water (85 mL). Nitrification rates were measured as described above for four replicates of each of 24 treatment combinations (6 streams × 4 C concentrations).

In the DOC reduction experiment, we added 85 mL of undiluted or diluted (10%, 50%, or 80% diluted) stream water to flasks containing sediments (25 mL, sieved to <0.64 cm). Stream water was diluted with reverse osmosis treated water and micro- and macro-nutrients were re-amended by adding 100 μL of sterile NH_4^+-oxidizer media to each flask. The added media contained no organic C and was made according to Schmidt and Belser (1994). The undiluted treatment also received 100 μL of the NH_4^+ oxidizer media as a control. Nitrification rates were determined on four replicates of each treatment as described earlier. Results from both experiments were analyzed as a two-way analysis of variance (ANOVA) with stream and DOC treatment as factors.

Fig. 1. Map showing the location of stream sites sampled in Wisconsin and Michigan. Site numbers correspond to stream numbers listed in Table 2. The location of Juday Creek in northern Indiana is shown on the inset map.

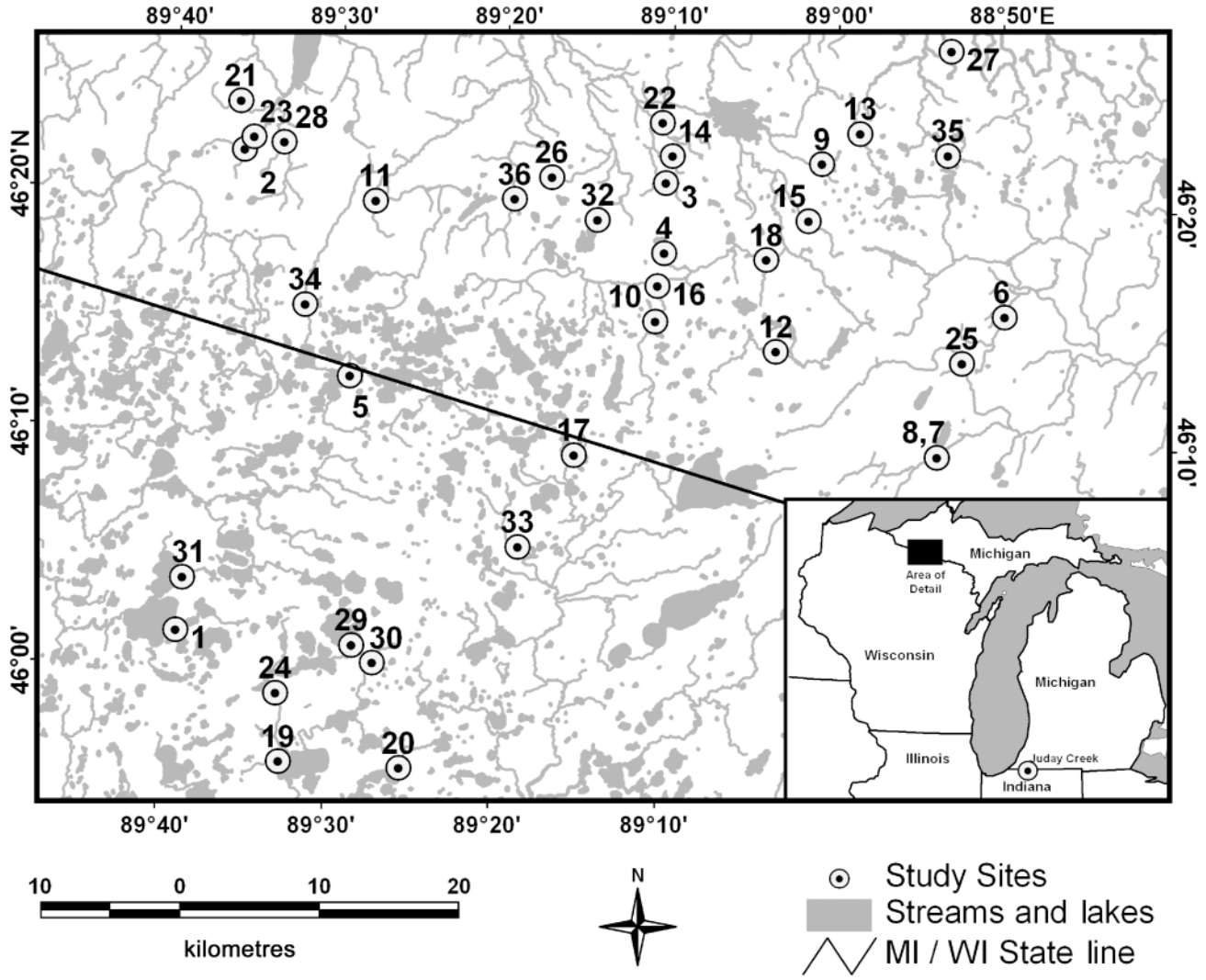


Table 1. Surface water characteristics of the six focal streams used in the dissolved organic carbon (DOC) addition and removal experiments.

Stream	Stream No.	Temperature (°C)	DO ($mg \cdot L^{-1}$)	pH	Velocity ($m \cdot s^{-1}$)	NH_4^+–N ($\mu g \cdot L^{-1}$)	DOC ($mg \cdot L^{-1}$)
Mill Creek	17	27.1	6.8	7.4	0.49	175	16.4
Pelton Creek	23	19.5	6.3	6.6	<0.03	54	33.0
Scott and Howe Creek	26	20.8	7.5	7.0	0.24	2	12.1
Star Creek	29	25.6	7.9	7.7	0.30	5	10.3
Tamarack Creek	33	22.6	7.4	6.7	0.79	<1	20.4
Tenderfoot Creek	34	25.9	4.1	7.0	0.27	<1	13.8

Note: Values shown are from samples collected at the time of the DOC addition experiment (13 July 1998). Stream No. corresponds to those shown in Fig. 1. DO, dissolved oxygen.

C:N and N availability experiment

To test the interactive effects of C:N and N availability on nitrification, we used stream water and sediments collected from a forested reach of Juday Creek, a third-order stream in north-central Indiana (Fig. 1). A detailed description of Juday Creek can be found in Lamberti and Berg (1995). Earlier studies on this stream have shown that nitrification is NH_4^+ limited and can be inhibited with organic C amendments (Strauss and Lamberti 2000). We amended stream sediments and water with organic C and N at three ratios (based on moles) as follows: 7:1, 23:1, and 70:1. In addition, three levels of organic N were tested within each C:N: 0.1, 1.0, and 10 mg $N \cdot L^{-1}$. All N (and some C) was added in organic form as L-lysine acetate, an organic amino salt with a C:N of 4.0. To achieve targeted C:N, additional C was added as sodium acetate. Nitrification rates were determined as before for nine replicates of each of the nine treatment combinations (3 C:N × 3 N levels) and a control (no C or N amendment). The C:N molar ratio of the sediments in Juday Creek has been previously determined to be around 21:1. (Strauss

Table 2. Stream water temperature, conductivity, and dissolved organic carbon (DOC) concentration of 36 streams sampled during the 1998 field survey.

Stream	No.	Date sampled	Temperature (°C)	Conductivity ($\mu mhos \cdot cm^{-1}$)	DOC ($mg\ C \cdot L^{-1}$)
Allequash Creek	1	26 July	20.7	92	14.8
Banner Creek	2	25 July	14.3	52	50.3
Bluff Creek	3	1 August	15.1	228	15.3
Boniface Creek	4	29 July	21.6	88	19.2
Brown Creek	5	11 August	21.0	191	13.4
Bush Creek	6	11 August	24.3	169	6.8
Cooks Run (A)	7	10 August	16.0	163	3.8
Cooks Run (B)	8	11 August	15.1	170	28.0
Deadman Creek	9	10 August	11.4	189	6.3
Duck Creek	10	21 July	22.4	185	7.4
Grosbeck Creek	11	19 July	20.8	158	26.0
Imp Creek	12	11 August	20.5	171	16.5
Jumbo River	13	10 August	22.9	225	8.5
Matheson Creek	14	21 July	26.1	92	17.0
McGinty Creek	15	10 August	20.3	215	24.7
Mid. Br. Ontanogan River	16	21 July	23.3	193	21.2
Mill Creek	17	6 August	21.8	98	19.4
Morrison Creek	18	10 August	20.8	233	9.2
Mud Creek	19	6 August	17.8	102	19.1
Muskellunge Creek	20	6 August	17.0	99	44.7
Nelson Creek	21	29 July	15.3	108	37.7
Paulding Creek	22	2 August	14.6	242	11.7
Pelton Creek	23	6 August	16.5	175	25.1
Plum Creek	24	2 August	24.3	108	10.6
S. Branch Paint River	25	11 August	22.1	193	8.7
Scott and Howe Creek	26	6 August	16.9	151	16.9
Shane Creek	27	10 August	16.4	133	22.3
Slate River	28	29 July	15.5	213	18.0
Star Creek	29	6 August	21.4	84	20.7
Stella Creek	30	6 August	19.4	112	10.7
Stevenson Creek	31	26 July	16.7	107	14.1
Sucker Creek	32	19 July	27.9	153	71.3
Tamarack Creek	33	6 August	16.0	109	18.6
Tenderfoot Creek	34	13 July	22.3	134	18.7
Tepee Creek	35	10 August	26.1	159	13.0
Twomile Creek	36	19 July	23.8	138	35.5

Note: Stream locations are shown in Fig. 1.

and Lamberti 2000). Data were analyzed using two-way ANOVA.

pH experiment

We determined the effect of pH on sediment nitrification rates in the laboratory. Stream sediments consisted of a sand–silt mixture collected from Juday Creek. Nitrification rates were determined in flasks containing 10 mL of stream sediment and 100 mL of 60 mM phosphate buffer (K_2HPO_4 and KH_2PO_4 with pH adjusted according to treatment) using the nitrapyrin method as before. Each flask also received an initial NH_4^+ addition of 0.5 mg $N \cdot L^{-1}$ (as NH_4Cl). Eight pH treatments were used: pH 5.9, 6.1, 6.5, 7.0, 7.5, 7.9, 8.4, and 8.7 (five replicates per treatment). Data were analyzed using one-way ANOVA.

Stream survey

During summer 1998, we sampled 36 "survey" streams in northern Wisconsin and the upper peninsula of Michigan, (Fig. 1; Table 2) to identify factors that potentially influence nitrification rates. Nitrification rates were measured (as described above) together with 12 additional variables on the same stream water or sediments: temperature, pH, dissolved oxygen, conductivity, DOC concentration, redox potential, salinity, sediment ash-free dry mass (AFDM), specific ultraviolet absorbance (SUVA), stream water NH_4^+ concentration, soluble reactive phosphorus concentration (SRP), and total extractable NH_4^+. Environmental variables were correlated (Spearman's *r*) with nitrification rates to search for significant associations. In addition, we used stepwise multiple regression to best predict nitrification rates based on the environmental variables. All statistical analyses were done using SAS v. 6.12 (SAS Institute Inc. 1990).

Analytical methods

Total extractable NH_4^+ (water column NH_4^+ plus sediment

extracted NH_4^+) and surface water NH_4^+ concentrations were determined colorimetrically on filtered samples (Whatman GF/F) using the phenol–hypochlorite method (Solorzano 1969). DOC concentration was determined on filtered (preashed Whatman GF/F) samples using a Shimadzu Model TOC-5000A high-temperature combustion carbon analyzer (Shimadzu Scientific Instruments, Inc., Columbia, Md.). SUVA, a measure of DOC quality, was determined by dividing the measured DOC concentration of a water sample by its ultraviolet absorbance measured at 254 nm (Ravichandran et al. 1998). SRP was measured colorimetrically on filtered (Whatman GF/F) water samples using the acid molybdate method (Strickland and Parsons 1972). All spectrophotometer measurements were conducted on a Spectronic Genesys 2 spectrophotometer (Thermo Spectronic, Rochester, N.Y.).

Results

Ambient nitrification rates for the six focal streams ranged from 0.08 to 1.1 μg N·mL sediment^{-1}·day^{-1} (Fig. 2*a*), with Mill and Tenderfoot Creeks exhibiting the highest nitrification rates and Star and Pelton Creeks having the lowest. DOC additions (as acetate) to sediments and water from the six streams revealed that DOC significantly reduced ($P \leq 0.003$) nitrification rates only when added at the highest level of 30 mg·L^{-1} (Fig. 2*b*). Nitrification reduction was not caused by the formation of anaerobic conditions or environmental pH shifts, because DO and pH were not appreciably different from control flasks that received no DOC amendments (e.g., post-incubation conditions for 30 mg C·L^{-1} addition treatment and control: $\bar{x}$ O_2 for treatment = 7.8 mg·L^{-1}, $\bar{x}$ O_2 for control = 7.3 mg·L^{-1}; $\bar{x}$ pH for treatment = 7.7, $\bar{x}$ pH for control = 7.5). Dilution of stream water to reduce DOC concentrations had no significant effect on nitrification (Fig. 2*c*). Nitrification rates observed in the DOC reduction experiment, in general, were higher than those initially measured and those in the DOC addition experiment, but this was likely a result of adding nitrifier media to counteract the effect of nutrient removal by dilutions. Neither experiment revealed statistically significant interaction between stream and DOC treatment. In both experiments, stream-specific mean nitrification rates exhibited patterns similar to those measured initially (i.e., Mill and Tenderfoot consistently had the highest nitrification rates, whereas Pelton always had the lowest).

The experiment examining the interactive effects of C:N and N availability on nitrification rates (Fig. 3) suggested that both factors were important in regulating nitrification (significant C:N and N availability interaction; $P < 0.0001$). However, this significant interaction was likely a result of the large nitrification response to the 10 mg N·L^{-1} addition at the 7:1 C:N. The C:N factor also was significant, with each of the three ratios being significantly different ($P < 0.0001$) from one another. The highest C:N generally decreased nitrification, whereas N concentration affected nitrification only at the lowest C:N. Environmental pH had a strong effect on nitrification (Fig. 4). Nitrification rates were highest at pH 7.5 ($P < 0.002$) and decreased more gradually with declining pH than with increasing pH.

Fig. 2. Results from (*a*) the initial nitrification measurements, (*b*) the dissolved organic caron (DOC) addition experiment, and (*c*) the DOC dilution experiment. Error bars ± 1 SE are shown. Lowercase letter combinations over bars in *a* and *b* denote significance among treatments; treatments containing the same lowercase letter were not significantly different ($P > 0.05$, LS means procedure). *F* statistic in *b* represents ANOVA results for DOC addition factor. Significant treatment differences were not observed in the DOC dilution experiment. Asterisk indicates that nitrification rate was below detection.

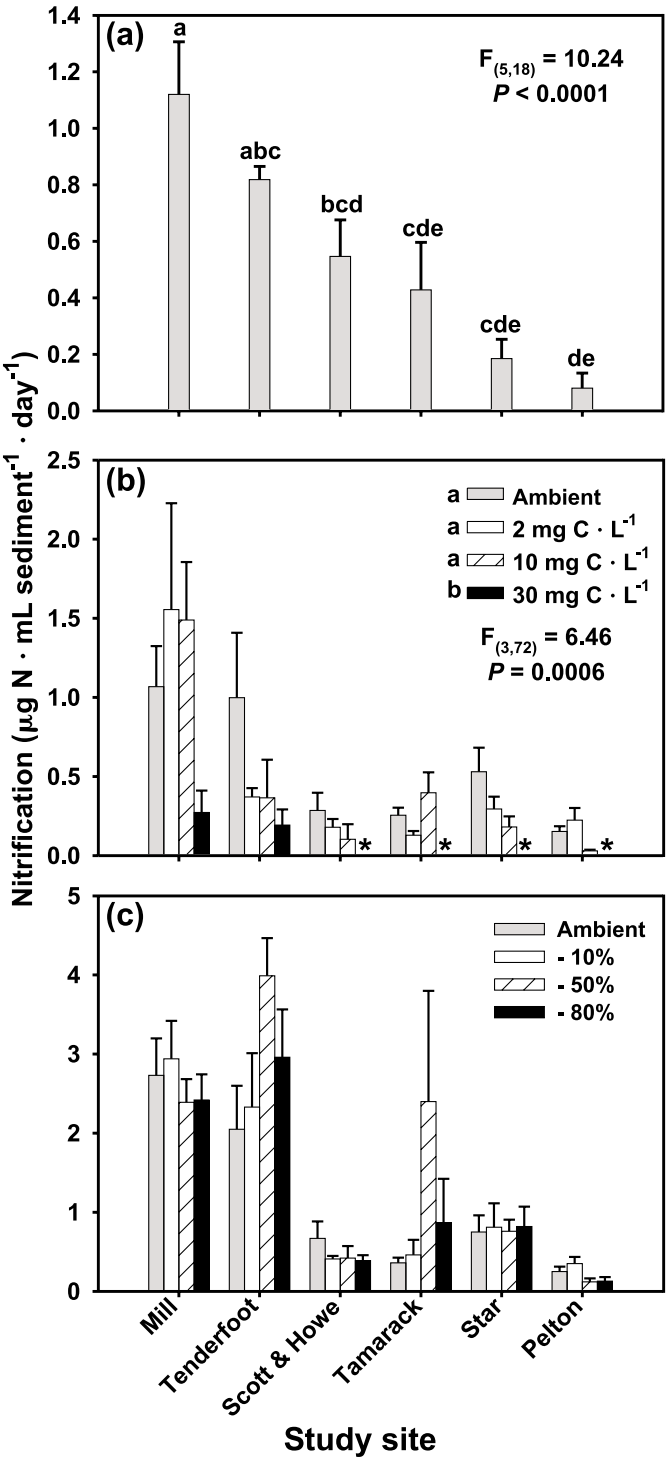


Fig. 3. Results from the experiment testing the interactive effects of C:N and N availability on nitrification rates in Juday Creek sediments. All N (and some C) was added in organic form as L-lysine acetate. Additional C was added as sodium acetate to reach the target C:N. The control was not amended with either N or C. Error bars ± 1 SE are shown. *F* statistic represents ANOVA results for the interaction among factors (C:N × N). Treatments containing the same lowercase letter were not significantly different ($P > 0.05$, LS means procedure).

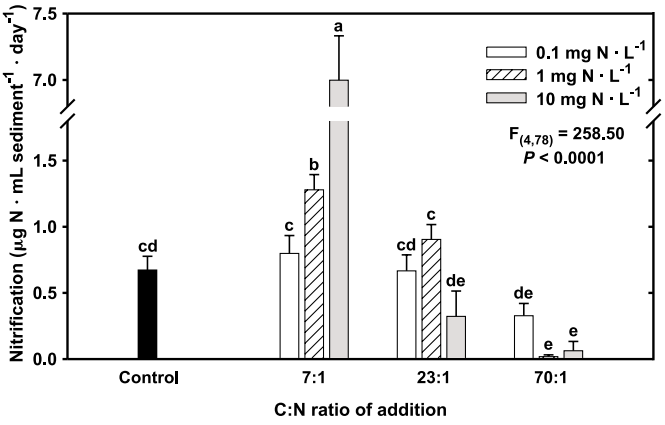
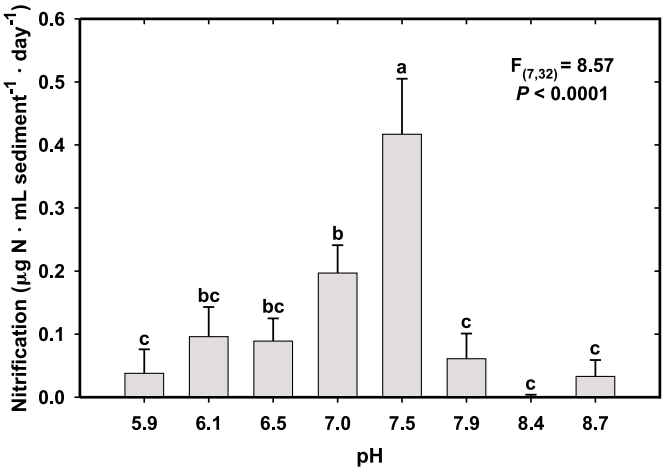
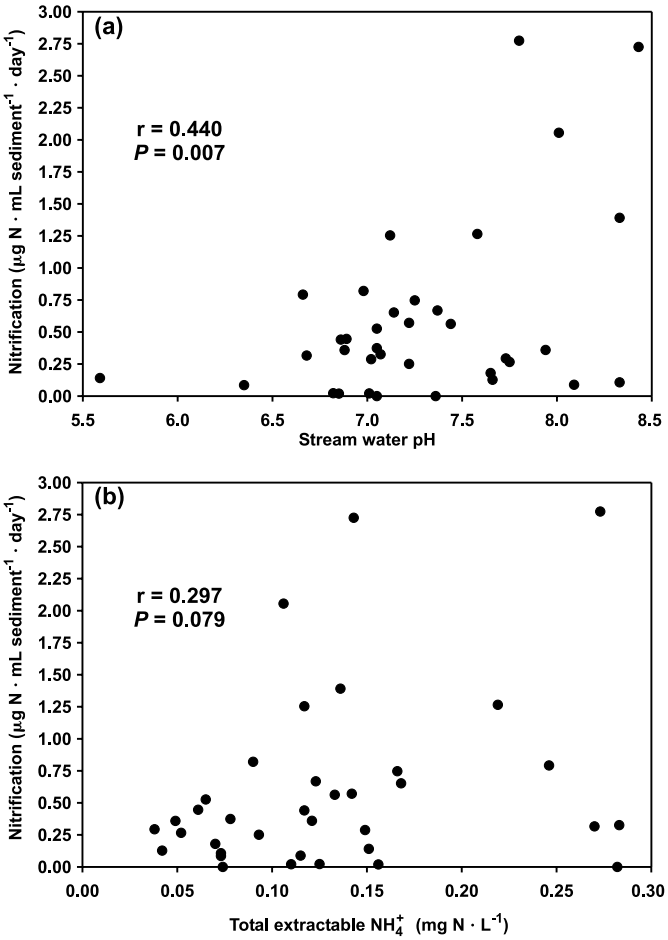


Fig. 4. Effect of pH on nitrification rate in stream sediments from Juday Creek. Error bars ± 1 SE are shown. Treatments containing the same lowercase letter were not significantly different ($P > 0.05$, LS means procedure).



Nitrification rates for the 36 survey streams spanned over two orders of magnitude and ranged from below detection (Stella and Tepee Creeks) to over 2.7 μg N·mL sediment^{-1}·day^{-1} (Mill Creek and Cooks Run B). Cooks Run B is a diversion from the main Cooks Run channel (Cooks Run A) and is used as a trout-rearing facility for a high density of trout fingerlings that receive regular inputs of commercial trout feed. The only significant correlation found between nitrification and the 12 environmental variables measured was with stream water pH (Fig. 5*a*, $r = 0.440$, $P = 0.007$). A weak positive correlation also was found between nitrification and total extractable NH_4^+ (Fig. 5*b*, $r = 0.297$, $P = 0.079$). Both relationships showed the highest variation in nitrification rates at higher pH or NH_4^+ concentration. A multiple regression

Fig. 5. Linear correlations (Spearman's *r*) between nitrification rate in stream sediments and (*a*) stream water pH, and (*b*) total extractable NH_4^+. Each point represents a different stream from the 36-stream survey.



model consisting of five environmental variables (stream temperature, pH, conductivity, DOC concentration, and total extractable NH_4^+) explained 60% of the variation in nitrification rates (Table 3). Observed nitrification rates in the 36 streams fit well with predicted rates based on the multiple regression model (Fig. 6).

Discussion

Nitrification appears to be a ubiquitous N transformation in stream sediments, but our study demonstrates that rates are highly variable. Furthermore, this variability may be related to a multitude of factors in natural ecosystems. Previous studies have suggested that organic C can regulate nitrification in streams (Strauss and Lamberti 2000), forest soils (Paavolainen et al. 1998), and during wastewater treatment (Cheng and Chen 1994). Our DOC addition experiment clearly demonstrated that high DOC (30 mg C·L^{-1}) could significantly reduce nitrification rates. However, lower DOC additions (2 mg C·L^{-1} and 10 mg C·L^{-1}) had a less profound effect on nitrification, likely because of the high background DOC (10.3–33 mg C·L^{-1}) of the water. The mechanism responsible for reducing nitrification at high DOC amendment

Table 3. Multiple regression model used to predict ambient nitrification rates in stream sediments explaining 60% of the variability observed in nitrification rates (overall model R^2 = 0.600).

(*a*)

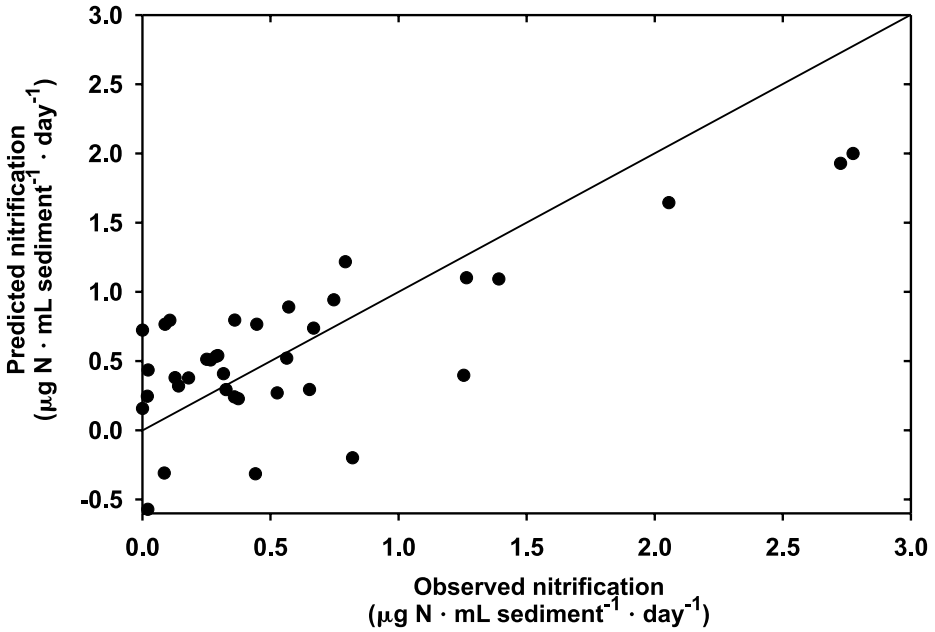
Source	df	Sum of squares	Mean square	*F* value	Prob. > *F*
Model	5	10.1081	2.0216	8.69	0.0001
Error	29	6.7464	0.2326		

(*b*)

Variable	df	Parameter estimate	Standard error	*t* for H_0: Param. = 0	Prob. > |*t*|	Partial R^2
Intercept	1	–5.7645	1.1476	–5.02	0.0001	—
pH	1	1.0148	0.1768	5.73	0.0001	0.1928
DOC	1	0.0181	0.0064	2.84	0.0081	0.1337
Total NH_4+	1	4.0952	1.1985	3.41	0.0019	0.1213
Temperature	1	–0.0591	0.0222	–2.66	0.0125	0.0584
Conductivity	1	–0.0052	0.0020	–2.60	0.0144	0.0935

Note: df, degrees of freedom; DOC, dissolved organic carbon; Param., parameter; Prob., probability.

Fig. 6. Relationship between nitrification rates measured in the 36 streams and those predicted by the multiple regression model presented in Table 3. The diagonal reference line plots a 1:1 relationship where observed rates would equal predicted rates.

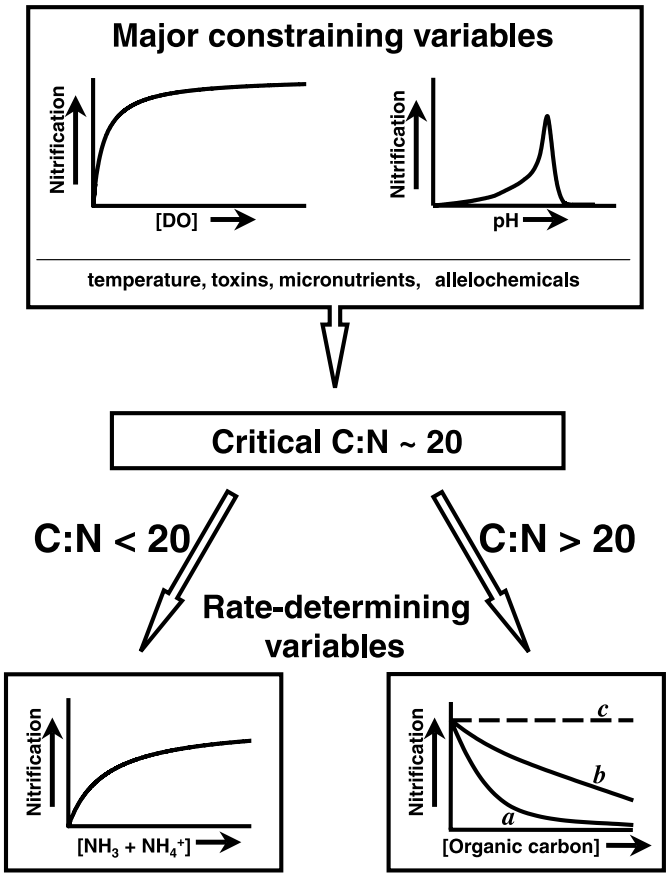


was likely stimulation of heterotrophic bacteria that outcompeted nitrifying bacteria for available NH_4^+ (Strauss and Lamberti 2000).

The results of the DOC removal experiment contrasted with those of the DOC addition experiment. If DOC availability regulated nitrification rates, nitrification rates should have increased with dilution. However, no significant effects were observed. Several possible explanations exist for these contrasting results. First, a large fraction of the organic C may have been particulate organic C or C bound to the sediments rather than DOC (Dahm 1981). Diluting stream water would not reduce these C pools, thereby limiting the effect on nitrification. Second, the positive effect from the inorganic nutrients in the nitrifying bacteria media (added to offset the loss of nutrients from dilution) may have offset any negative effect of higher DOC concentrations. Third, the quality or lability of the organic C reduced during dilution was not equal to that of the labile DOC (acetate) augmented during the addition. A recent study has shown that labile organic C has a stronger negative effect on nitrification than refractory organic C (Strauss and Lamberti 2002).

The C:N – organic N-availability experiment revealed two important relationships. First, nitrification was negatively affected by increasing the C:N. Low C:N (7:1) increased nitrification, whereas high C:N (70:1) inhibited nitrification, consistent with our organic C hypothesis (Strauss and Lamberti 2000). Under high environmental C:N, the metabolic requirement for N by bacteria is high, thus heterotrophic bacteria should out-compete nitrifying bacteria for mineralized organic N (NH_4^+), resulting in lower nitrification rates. Second, organic N availability influenced nitrification, but the effect depended on the C:N of the environment. The positive effect of organic N availability on nitrification was stronger at low C:N because heterotrophic bacteria were not favored. As the C:N of the environment increased, nitrifying bacteria did not effectively compete with

Fig. 7. Conceptual model of controls on nitrification in aquatic sediments. Certain physical and chemical variables of the environment (e.g., DO and pH) constrain nitrification (upper box). Under these constraints, regulation of nitrification depends on the environmental C:N and its relation to a critical value (C:N ~ 20) (middle box). Near and below this ratio (C:N < 20), maximum nitrification is determined by NH_4^+ availability (lower box, left) and follows Monod-type growth kinetics with reported half-saturation constants being as low as 600 μg $N \cdot L^{-1}$ (Denac et al. 1983). At C:N above the critical value (C:N > 20), nitrification rates are regulated by organic C quantity and quality (lower box, right). In general, nitrification decreases as organic C increases because of competition between heterotrophic and nitrifying bacteria for NH_3. High-quality C (line *a*) elicits a stronger negative effect than low-quality C (line *b*). Purely recalcitrant C would have no effect (line *c*).



heterotrophic bacteria for biologically available NH_4^+. The average global C:N in riverflow is around 11.1 (Schlesinger 1997). Based on our study, nitrification rates should be more strongly influenced by NH_4^+ availability at C:N near and below this global average, whereas at C:N above this average nitrification should be influenced more by C availability (Fig. 7).

Our multiple regression model to predict field nitrification rates explained 60% of the variability in nitrification, but had a tendency to underestimate higher nitrification rates. The 36 streams in our survey probably had different combinations of factors regulating nitrification, whereas a single system likely will have a more restrictive set of constraints on nitrification. For instance, in a one-year nitrification study of an estuarine area of the Rhone River (Bianchi et al. 1999), 74% of the variability in nitrification was explained by a single variable, NH_4^+ availability. Of the five variables in our model, total extractable NH_4^+, DOC, and stream water pH explained the most variation, but no single variable explained more than 20% of the total variation. NH_4^+ availability and nitrification rates have been positively associated in streams (Triska et al. 1990; Jones et al. 1995), lakes (Hall 1986), groundwater (Strauss and Dodds 1997), and soil systems (Davidson and Hackler 1994). The results from our C:N – N-availability experiment also demonstrated a positive relationship between N and nitrification rates at low C:N.

The effect of pH on nitrification has not been studied in natural aquatic systems. However, pH has received considerable attention in soils, wastewater treatment, and pure cultures. For example, Paavolainen and Smolander (1998) reported increased nitrification through a pH gradient of 4.2–6.2 in a spruce-forest soil. In wastewater treatment sludge, the optimum pH for nitrification was approximately 7.8 over a range of 6.4–8.2 (Antoniou et al. 1990). Watson et al. (1989) recommend a media pH of 7.5–7.8 for culturing pure strains of NH_4^+-oxidizing bacteria. In our study, we observed an optimum pH of 7.5 consistent with previous studies. The positive relationship between pH (up to 7.5) and nitrification is related to the increasing availability of NH_3, unprotonated ammonia, which is believed to be the true substrate for oxidation (Suzuki et al. 1974). Both NH_3 and NH_4^+ occur in the environment with relative proportions dependent upon pH and temperature. As pH increases, the relative NH_3 concentration increases by nearly a full order of magnitude for each pH unit (Emerson et al. 1975). Above the optimum pH for nitrification, the advantages of increased availability of free NH_3 may be counterbalanced by the energy required to maintain the cytoplasmic pH below that of the external environment (Wood 1988). As pH falls below the optimum, evidence exists that enzyme activity is negatively affected, thereby reducing nitrification (Prosser 1989).

In the 36 streams we surveyed, the relationship between nitrification and pH was significant but somewhat variable. Nitrification rates were low at low pH, but variable at higher pH. This suggests that nitrification is inhibited at low pH, but is enhanced at circumneutral pH values. However, optimal pH is not enough to ensure high nitrification and therefore additional environmental factors must be involved (Fig. 7).

The regression model also revealed a positive relationship between DOC and nitrification, in apparent disagreement with our organic C hypothesis. However, because high DOC has been associated with increased primary productivity (Kaplan and Bott 1989), we believe that this positive relationship may reflect increased nitrification in more eutrophic streams rather than a direct effect of DOC on nitrification. The better controlled DOC addition and the C:N – N-availability experiments indicate that additions of labile C can dramatically reduce nitrification rates in stream sediments. In addition, previous research has shown that a range of C sources (labile glucose to less-labile leaf leachates) can reduce nitrification rates in stream sediments (Strauss and Lamberti 2000). Our conceptual model proposes that ambient organic C can reduce nitrification in natural systems if the environmental C:N is high (>20) and a reasonable frac-

tion of the available organic C is relatively labile. Unfortunately, little is known about the factors that regulate C:N in aquatic ecosystems except that ratios generally increase with time following inputs of organic matter because N-containing amino and proteinaceous compounds are used more rapidly than carbohydrate-based compounds (Wetzel 2001). Regardless, stoichiometric ratios of important elements can exert profound influences on aquatic ecosystem structure and function (Elser et al. 1996), as we also suggest for nitrification.

Predicting specific conditions that could result in high environmental C:N with a large fraction of relatively labile organic C, a scenario where nitrification should be inhibited by organic C, is difficult because of the following: (*i*) inter- and intra-system variability is inherent to organic C dynamics, and (*ii*) the relative fraction of labile DOC depends on the DOC source. However, we can make a few generalizations. In temperate forested streams, we might expect to find these conditions during annual leaf fall when potentially very large inputs of high C:N material are deposited into the stream (Meyer et al. 1998). These conditions could easily be present in small, localized areas adjacent to leaf packs or debris jams where the particulate matter is concentrated. In desert and other nonforested streams where organic C is primarily of autochthonous origin, periodic floods and subsequent periphyton sloughing often result in increased organic C export (Jones et al. 1996). During these periods, the quantity and quality of organic C may be high enough to initiate a temporary system-wide reduction in nitrification. In lakes, wetlands, and large rivers, high C:N conditions may occur in vegetated littoral zones, sloughs, or in backwater areas after macrophyte senescence and its microbial decomposition. Also, waterways that receive C-rich effluent (e.g., paper pulp processing) may exhibit these conditions. In general, conditions conducive to organic C inhibition of nitrification are possible at several spatial and temporal scales, and may help explain N cycling patterns when these conditions exist.

Our study demonstrates that nitrification is a complex transformation in the aquatic N cycle that is regulated by a suite of environmental variables. Clearly, however, NH_4^+ availability, pH, and organic C exert strong influences on nitrification rates. In aerobic environments, nitrification is positively associated with NH_4^+ availability, but the importance of NH_4^+ declines with increasing C:N as heterotrophic bacteria dominate N-uptake dynamics. Nitrification is maximized at a pH of 7.5–7.8, but rates of nitrification are likely determined by other environmental factors. If organic C is labile and the C:N is high, organic C may be a primary determinant of nitrification rates in aquatic ecosystems.

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