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The influence of ammonium, nitrate, and dissolved oxygen concentrations on uptake, nitrification, and denitrification rates associated with prairie stream substrata

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Abstract

Substrata samples were collected from Kings Creek on Konza Prairie Biological Station (Manhattan, Kansas) and incubated with varying levels of ammonium (NH_4^+), nitrate (NO_3^-), and dissolved oxygen (O_2) to examine the response of nitrogen (N) uptake and transformation rates. Substrata collected were fine benthic organic matter (FBOM), coarse benthic organic matter, filamentous green algae, bryophytes, suspended particulate organic matter, and epilithic diatoms. Nitrification and denitrification were estimated by use of the nitrapyrin and acetylene inhibition methods, respectively. Ammonium uptake demonstrated Michaelis-Menten kinetics, with the highest maximum rates (V_{max}) associated with filamentous green algae (5.90 mg N gdm^{-1} d^{-1}) and epilithic diatoms (4.96 mg N gdm^{-1} d^{-1}). Nitrate uptake did not saturate at the highest NO_3^- addition (25 μg N L^{-1}) above ambient when associated with FBOM. Overall, maximum uptake rates of NH_4^+ were 10-fold higher than for NO_3^-. Nitrification response to increasing NH_4^+ concentrations was highly variable, depending on the substrata type. Nitrification was lowest under low O_2 conditions, being undetectable when NO_3^- was added but not when NH_4^+ was added. Denitrification increased linearly with NO_3^- concentration when associated with epilithic diatoms and FBOM but became saturated at ~20 μg N L^{-1} above ambient concentrations when associated with filamentous green algae. Samples purged with N_2 gas had the highest rates of denitrification. We predicted stream ecosystem rates using equations derived from the experimental data and substrata mass estimates measured in the field. Substantial temporal variability was predicted in uptake (0–1,300 mg NH_4^+-N m^{-2} d^{-1}; 0–5.2 mg NO_3^--N m^{-2} d^{-1}), nitrification (0–35 mg NH_4^+-N m^{-2} d^{-1}), and denitrification (0–130 μg N_2O-N m^{-2} d^{-1}) as due to natural variation in water column NH_4^+, NO_3^-, and O_2 concentrations.

Streams are naturally subject to high spatial and temporal variability in nutrient concentrations, and this variation is likely to affect distribution of organisms and rates of ecosystem processes (Munn and Meyer 1990; Dent and Grimm 1999; Kemp and Dodds 2001*a*). General fluxes of the nitrogen (N) cycle have been studied in many aquatic systems, but factors controlling rates of specific processes and how rates may respond or contribute to fluctuating nutrient concentrations are not well described for streams. Determining the rates at which N is taken up and transformed and how variability in substrate concentrations affects these rates may aid in assessing how much nutrient loading an ecosystem can absorb before its integrity is negatively affected.

In streams, the benthos represents a potentially important biotic sink for nutrients because uptake and transformation rates within the benthos may control movement and removal of N in the stream channel (DeLaune et al. 1991; Henriksen et al. 1993; Peterson et al. 2001). Possible fates of ammonium (NH_4^+) within the benthos include biotic uptake, nitrification, volatilization, and adsorption. Possible fates of nitrate (NO_3^-) include biotic uptake, denitrification, and adsorption. At ambient nutrient concentrations, uptake rates may be limited by the capacity of stream biota and, in some cases, may not saturate with short-term nutrient pulses up to several times ambient concentrations (Dodds et al. in press). However, plant and microbial pools can become saturated with N, which results in a decline in their N absorbing capacity over time (Aber et al. 1989; Dodds et al. in press) that potentially exceeds their ability to sequester additional nutrients (Mulholland et al. 1990). Streams may be especially vulnerable to N saturation relative to other systems (e.g., forests) because of low biomass and limited organic matter storage.

Factors that regulate nitrification are integral to ecosystem function and, thus, to eutrophication and health concerns related to elevated NO_3^- concentrations in freshwaters because nitrification is central to the accumulation and loss of NO_3^- (DeLaune et al. 1991; Henriksen et al. 1993). Recent studies have also identified nitrification as an important regulator for N retention due to coupling with denitrification (DeLaune et al. 1991; An and Joye 2001). Despite the importance of nitrification and denitrification, few studies have explored the factors regulating these processes in streams, and no single set of factors has emerged consistently as the key regulator.

The rates of uptake, nitrification, and denitrification are controlled primarily by the availability of substrates, which are supplied largely by diffusion along concentration gradients that vary over space and time (Seitzinger 1988). The availability of N regulates many ecological processes, particularly if it is the limiting nutrient. Changes in the rates of ecological processes due to variability in substrate concentrations may result in alterations of stream community structure and downstream export (Dent and Grimm 1999). We

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experimentally tested the influence of variable NO_3^-, NH_4^+, and dissolved oxygen (O_2) concentrations on a variety of microbial assemblages taken from a prairie stream, to examine the response of N uptake and transformation rates to substrate variability. Experimental data were combined with long-term data on water column N concentrations to develop a model that examins stream ecosystem N uptake and transformation response to variability in substrate concentrations.

Materials and methods

Study site characteristics—Substrata samples were collected from Kings Creek watershed (N04d), which is entirely encompassed by Konza Prairie Biological Station and ~10 km southeast of Manhattan, Kansas. The site is tallgrass prairie characterized by low NH_4^+ and NO_3^- concentrations and little riparian cover in the upstream reaches. Detailed descriptions of the geology, hydrology, ecology, N dynamics, and long-term sample collection and analysis methods at this site have been published elsewhere (Gray and Dodds 1999; Gray et al. 1999; Oviatt 1999; Dodds et al. 2000; Kemp and Dodds 2001*b*).

Characteristics of this site are measured regularly, and data from 1999 and 2000 were used for analyses in this study. Grab samples of stream water have been collected for chemical analyses three times per week since 1986. Samples are collected from the center of the stream above a concrete flume and several centimeters below the surface in acid-washed bottles. Filtered water samples are refrigerated immediately and analyzed within 48 h on a Technicon AutoAnalyzer II for NO_3^- concentrations ($NO_3^- + NO_2^-$), by diazo dye formation after cadmium reduction, and NH_4^+ concentrations ($NH_4^+ + NH_3$), by the indo-phenol blue method (APHA 1995). The limit of detection was 1 μg N L^{-1} for both NO_3^- and NH_4^+.

Stream-water temperature is measured continuously by use of a thermocouple wire placed in the channel and is monitored by a Campbell Scientific data logger (21×). Water temperatures ranged 1.2–25.9°C over the sampling periods, with spring, summer, fall, and winter means being 12.7°C, 19.1°C, 13.3°C, and 8.8°C, respectively. Daytime water column O_2 concentrations were measured once a month from January 1999 to December 2000 between 0900 and 1100 h by use of a conventional YSI O_2 meter. Detailed diurnal measurements of O_2 at this site published elsewhere (Mulholland et al. in press) have indicated a diurnal variation of ~30% (9–12 mg L^{-1}), similar to daytime water column variation observed in the monthly measurements of O_2 concentrations (Fig. 1C).

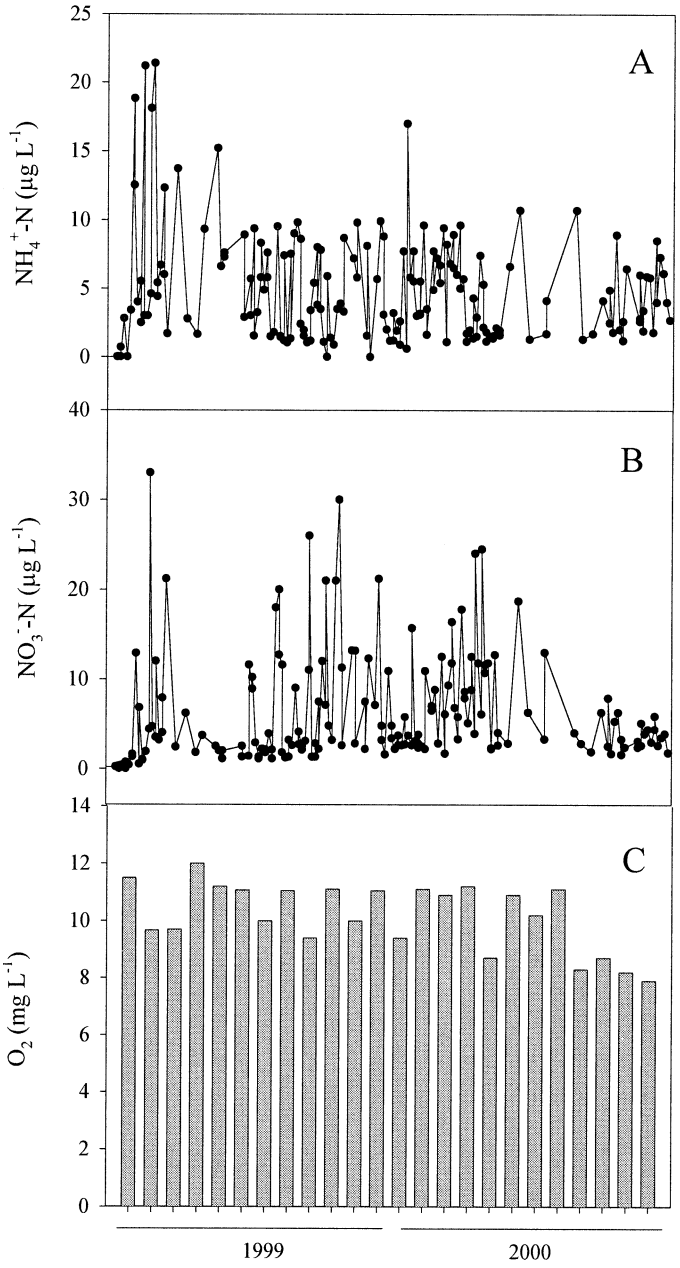

Fig. 1. Variation in water column (A) NH_4^+, (B) NO_3^-, and (C) daytime O_2 concentrations in a prairie stream between January 1999 and December 2000. NH_4^+ and NO_3^- concentrations are weekly means, and daytime O_2 concentrations are monthly estimates.

Substrata collection—In this article, ''substratum'' and ''substrata'' refer to materials on the stream bottom, and ''substrate'' and ''substrates'' refer to chemicals used by cellular processes. The substrata collected for experimental analyses in the laboratory were epilithic diatoms, filamentous green algae, coarse benthic organic matter (leaves and wood; CBOM), fine benthic organic matter (FBOM), bryophytes, and suspended particulate organic matter (SPOM). All substrata were collected without bias where available within the stream reach, and composite samples of several collections were used for experiments. Four replicates of each substrata type were used for all experiments and treatments.

Laboratory experiments were conducted between August and December 2000 with three substrata collection periods. All experiments were performed at three temperatures representative of mean ambient winter (10°C), spring/fall (15°C), and summer (20°C) temperatures. All process experiments for each temperature were conducted by use of the same pool of substrata from one of the collection periods. Average ambient stream water NH_4^+ and NO_3^- concentrations

during these collection periods were 4.1 (standard error [SE] 0.8) and 6 (SE 2.3) μg N L^{-1}, respectively. Substrata and stream water were acclimated to changes in temperature in environmental chambers for 2–3 d prior to the start of the experiments.

Nitrification rates— Two experiments were performed to examine nitrification response to variable substrate concentrations. The first experiment, which examined the nitrification response to increasing NH_4^+ addition, was done with use of 10 cm^3 of substratum and 30 ml of stream water in a 50-ml test tube with one of six treatments applied. The treatments were a 0 (control), 5, 10, 15, 20, or 25 μg L^{-1} of NH_4^+-N addition above the ambient stream water concentration in the form of ammonium chloride (NH_4Cl).

The second experiment, which examined the nitrification response to variable O_2, NH_4^+, and NO_3^- concentrations, used 25 cm^3 of substratum with 75 ml of stream water in 125-ml flasks. Each flask was equipped with plastic tubing attached to an air bubbler or nitrogen gas (N_2) tank, to allow treatment gases to be continuously bubbled into the flask during the incubation period. Air-bubbled treatments are hereafter referred to as O_2 additions, and N_2-bubbled treatments are hereafter referred to as N_2 additions. Treatments were a control (ambient N concentration), NH_4^+ addition at 15 μg N L^{-1} above ambient, NH_4^+ and O_2 addition, NH_4^+ and N_2 addition, NO_3^- addition at 15 μg N L^{-1} above ambient in the form of potassium nitrate (KNO_3), NO_3^- and O_2 addition, NO_3^- and N_2 addition, O_2 addition only, and N_2 addition only. Cathode-type O_2 microelectrodes were used to measure concentrations within the substrata samples during the experiments to establish the effectiveness of the bubbled gas treatments. Electrodes were 5–10 μm in diameter encased in a 16-mm-gauge hypodermic needle. Detailed methods of this procedure have been published elsewhere (Kemp and Dodds 2001*a*). Treatments with the O_2 addition had mean O_2 concentrations at 1 cm depth into the substratum of 10.9 mg O_2 L^{-1} (SE 2.1), whereas treatments with N_2 bubbled in had 0–5 mg O_2 L^{-1} at 1 cm depth in the substrata.

For all nitrification experiments, replicate samples were randomly paired (a total of eight samples per treatment and substrata type, four replicates); one paired sample received 50 μl (10 mg L^{-1} final concentration) of nitrapyrin (2-chloro-6-[trichloromethyl]-pyridine, Sigma Chemical Co.) dissolved in dimethyl sulfoxide (DMSO), and the other paired sample received 50 μl of DMSO for a control (Powell and Prosser 1985). Nitrapyrin inhibits the function of the enzyme ammonium monoxygenase and hence inhibits NH_4^+ oxidation (the first step of nitrification). Nitrapyrin does not appear to significantly affect the remaining microbial community because total microbial biomass and respiration are not altered (Bauhus et al. 1996).

Nitrification experiments were incubated for 3 d on an orbital shaker (175 rpm) at appropriate stream temperatures in environmental chambers on a 12 : 12 light : dark cycle. The duration of the incubations was determined in three preliminary experiments, with use of all substrata, where incubation times were varied (1, 3, or 5 d). Incubation periods of 3 d consistently provided the best results for this volume of substrata (i.e., shorter incubations did not allow for detection of nitrification in all samples and longer incubations led to a plateau in the NH_4^+ concentrations). After the incubations, a potassium chloride (KCl) extraction was performed by adding 5 ml of 1 N KCl into each sample and incubating on an orbital shaker (175 rpm) for 30 min to extract NH_4^+ from sorption sites on the surface of the substrata prior to NH_4^+ analysis (Solarzano 1969; Strauss and Lamberti 2000). The filtered extracts were analyzed for NH_4^+ by use of the phenol hypochlorite method (Solarzano 1969; Strauss and Lamberti 2000). A 5-cm path cell was used, and great care was taken to clean glassware and ensure that reagent blanks were low, which allowed a limit of detection of 0.1 μg L^{-1} NH_4^+-N. Standards were made by use of a matrix of DMSO/KCl/deionized water in appropriate proportions.

Gross nitrification rates in both experiments were calculated as the difference in the NH_4^+-N concentration between the paired nitrapyrin and control (DMSO only) samples according to the following equation:

$$\text{nitrification} = \frac{\left(\frac{Cn - Cc}{g}\right)}{t} \quad (1)$$

where Cn is the final NH_4^+-N concentration of the nitrapyrin-treated sample, Cc is the final NH_4^+-N concentration of the control sample (DMSO only), g is grams dry mass (gdm) of substratum, and t is time.

Ammonium uptake—Ammonium uptake rates were measured in conjunction with the first experiment described above to examine the nitrification response to increasing NH_4^+ concentrations. Only the control samples (DMSO only) were used for uptake calculations. Uptake rates of NH_4^+ were calculated according to the method of Steinman and Mulholland (1996):

$$V = \frac{(Cf - Co)\cdot L}{t} \quad (2)$$

where V is uptake, Co is the initial NH_4^+ concentration (ambient + addition), Cf is the final NH_4^+ concentration after the incubation period, and L is volume. Uptake rates reported hereafter represent net uptake measurements. Gross uptake was not measured because of the inability to measure remineralization in these experiments. Substrata within the samples were dried and weighed after analysis, and mass-specific rates were used to scale up to stream ecosystem estimates (see calculations below). For all experiments and substrata samples, NH_4^+ concentrations were within detection limits.

Denitrification rates—Two experiments to estimate denitrification rates in response to variable substrate concentrations were similar to those described for nitrification rates. Preliminary experiments yielded no measurable rates of denitrification associated with SPOM or bryophytes; therefore, these substrata were not included in the denitrification experiments. The first experiment examined denitrification response to increasing NO_3^- concentrations. Experiments were performed with use of 10 cm^3 substratum and 30 ml of stream water added to tubes sealed with rubber septa. Treat-

ments were an addition of 0 (control), 5, 10, 15, 20, or 25 μg L^{-1} above ambient levels of NO_3^--N.

The second experiment examined denitrification response to variable O_2, NH_4^+, and NO_3^- concentrations with use of 25 cm^3 substrata with 75 ml of stream water in 125-ml flasks purged with air or N_2 (5 min) prior to start of the incubation period. Samples purged with air are hereafter referred to as O_2 addition, and samples purged with N_2 gas are hereafter referred to as N_2 addition. Treatments were a control (ambient N concentration), NH_4^+ addition at 15 μg N L^{-1} above ambient, NH_4^+ and O_2 addition, NH_4^+ and N_2 addition, NO_3^- addition at 15 μg N L^{-1} above ambient, NO_3^- and O_2 addition, NO_3^- and N_2 addition, O_2 addition only, and N_2 addition only. Prior to sealing the flasks, NH_4^+ or NO_3^- were added and samples were purged with air or N_2 gas according to the treatment. After sealing, 10–25 cm^3 ($\sim$1/3 the total volume of the container) of headspace remained for gas sampling. All experiments were carried out in the laboratory, and samples were kept at appropriate stream temperatures in environmental chambers.

Denitrification rates for all experiments were estimated by use of the acetylene (C_2H_2) inhibition technique (Chan and Knowles 1976; Raymond et al. 1992; Hallin and Pell 1994). An initial sampling of the headspace gas for background nitrous oxide (N_2O) concentration was performed by taking a 1 ml gas sample with a gas-tight syringe and placing it into a sealed, preevacuated vial. An equal volume of air was returned to the flask to avoid changing pressure. Acetylene gas (C_2H_2, 10–15 kPa) was then added to the flasks by use of a gas-tight syringe and pumped several times for even distribution in the substrata, water, and headspace. The pumping was vigorous enough that mixing of the water column in the incubation tubes was observed. We did not shake substrata samples at the beginning of the incubation, to minimize disruption. Preliminary tests were performed to determine the most effective method for estimating rates. If C_2H_2 had not been evenly distributed, we would have expected to see increasing rates of N_2O accumulation as the acetylene diffused into the sample, but rates were linear throughout the incubation period. All denitrification experiments had 2-h incubation periods, and flasks remained aerobic throughout (i.e., similar to field conditions). Gas samples were taken every 15 min during preliminary experiments to determine the appropriate incubation period, and subsequent incubations required only an initial and final gas sampling for steady linear accumulation of N_2O.

Gas samples were analyzed for N_2O on a Packard model 427 gas chromatograph equipped with a ^{63}Ni electron capture detector (320°C). The components were separated on an 80/100 mesh Porapak Q column (2 m long and 3.2 mm wide) at a temperature of 60°C with Me/Ar as the carrier gas (21 ml min^{-1}; Mosier and Mack 1980). A correction for dissolved N_2O was made at the extraction temperature with Bunsen solubility coefficients. Control samples without C_2H_2 addition never showed significant N_2O accumulation.

Denitrification rates were calculated as the linear increase in N_2O concentration over time multiplied by the Bunsen solubility coefficient and expressed per unit mass:

$$\text{denitrification} = \frac{\left[\frac{(F_N - I_N)\cdot c}{g}\right]}{t} \quad (3)$$

where F_N is the final N_2O concentration, I_N is the initial N_2O concentration, and c is the Bunsen solubility coefficient.

Nitrate uptake—Net NO_3^- uptake was estimated in conjunction with the first denitrification experiment described above that examined the response to increasing NO_3^- concentrations. Nitrate in the water column after the 2 h incubation was measured and compared with initial concentrations prior to the incubation period (*see* the NH_4^+ uptake calculation above). The filtered water samples were refrigerated immediately after collection and analyzed within 48 h on a Technicon AutoAnalyzer II for NO_3^- concentrations ($NO_3^- + NO_2^-$) by diazo dye formation after cadmium reduction. For all experiments and all substrata samples, NO_3^- concentrations were within detection limits.

Substrata mass estimates—Substrata abundance was measured in the field for all substrata types examined experimentally. Mass estimates were determined seasonally (January 1999–December 2000; total of eight sampling periods) by use of a 200 m stream reach within the Kings Creek watershed, and seasonal means were used for calculations. The reach was $\sim$100 m upstream from where substrata samples for experiments were collected.

Epilithic diatoms were sampled quantitatively by scrubbing and washing all material within a 174 cm^2 area from six replicate rocks. Each replicate sample was filtered onto Whatman GF/F filters of known weight, dried, and weighed. Mass of filamentous green algae and bryophytes was estimated by collecting all the substratum within six 1 m^2 quadrants randomly placed within the stream reach. The total wet weight was recorded, and subsamples were taken to determine dry mass. Six samples of leaves and small wood (CBOM), as well as FBOM, were collected from inside a 313 cm^2 corer placed randomly within the stream reach. All small wood and leaves were removed carefully from the isolated stream bottom, then FBOM was collected by stirring the sediment vigorously and taking a subsample of known volume that was filtered, dried, and weighed. One liter of stream water was collected from the center of the stream channel and subsequently filtered onto a GF/F Whatman filter for estimates of SPOM. This collection was repeated six times, moving from downstream to upstream. Detailed descriptions of these methods and data collected have been published elsewhere (Dodds et al. 2000; Kemp and Dodds 2001*b*, in press).

The substrata mass estimates were used to scale substrata uptake, nitrification, and denitrification rates, which had been determined experimentally, up to stream ecosystem rates by use of the following equation:

$$\text{stream ecosystem rate} = \sum (\text{individual substratum rate} \times \text{individual substratum mass}) \quad (4)$$

where the stream ecosystem rate (mg N m^{-2} d^{-1}) is equal to the sum of all six substrata types calculated by use of the individual substratum rates (mg N gdm^{-1} d^{-1}) multiplied by the individual substratum mass (gdm m^{-2}). Average seasonal mass estimates (summer, spring, fall, and winter) for the 2-yr sampling period were used in the calculations and combined with the appropriate experimental results according to representative incubation temperatures. These data have been published and analyzed elsewhere (Kemp and Dodds in press).

Statistics—A one-way analysis of variance (ANOVA) was used to identify significant differences in nitrification, denitrification, and uptake rates for the experiments examining response to increasing concentrations of NH_4^+ and NO_3^-. A three-way unbalanced ANOVA was performed to identify differences in rates due to varying O_2, NH_4^+, and NO_3^- concentrations for each substrata type. Linear and nonlinear regression analyses were used to fit the best line to the data for uptake, nitrification, and denitrification rates with increasing N concentrations. The nonlinear regression procedure used (PROC NLIN, SAS/STAT 4th edition; SAS Institute, Inc.) computes least-squares estimates of a given nonlinear model by use of the multivariate secant iterative method. All linear and nonlinear models were forced through the origin because there can be no net transformation when substrate concentration is zero. Because of the N present in stream water at ambient conditions, the substrate concentration never was zero experimentally. The equations derived were used to predict stream ecosystem uptake and nitrification response to increasing NH_4^+ concentration or stream ecosystem uptake and denitrification response to increasing NO_3^- concentrations. A multiple regression model that used a backward elimination procedure was developed for examining nitrification and denitrification response to changes in NO_3^-, NH_4^+, and O_2 concentrations. The equation derived from this model was used to predict stream ecosystem nitrification and denitrification rates over time with use of known water column NH_4^+, NO_3^-, and O_2 concentrations from long-term data sets (Fig. 1).

Results

Substrate variability—Concentrations of stream water NH_4^+ demonstrated a >10-fold variation over time, ranging from below detection limits (1 μg N L^{-1}) to 22 μg N L^{-1} (Fig. 1A). Nitrate concentrations also varied >10-fold, ranging from below detection to 34 μg N L^{-1} (Fig. 1B). Peaks in NH_4^+ concentrations were not always associated with peaks in NO_3^- concentrations. Daytime water column O_2 concentrations also varied over time, ranging from 8 to 12 mg O_2 L^{-1} (Fig. 1C). Variability in substrata biomass over time have been published elsewhere for this site (Kemp and Dodds in press).

NH_4^+ uptake and nitrification rates—No significant differences between NH_4^+ uptake or nitrification rates at the different temperatures tested were observed; therefore, data for all temperatures were combined for all analyses (data not shown). Ammonium uptake demonstrated Michaelis-Menten kinetics when associated with all substrata, but the magnitude of uptake varied among the substrata types (Fig. 2, Table 1). The maximum uptake rates (V_{max}) were associated with filamentous green algae (5.90 mg NH_4^+-N gdm^{-1} d^{-1}) and epilithic diatoms (4.96 mg NH_4^+-N gdm^{-1} d^{-1}), and the lowest V_{max} was associated with bryophytes (1.95 mg NH_4^+-N gdm^{-1} d^{-1}). Uptake associated with epilithon, filamentous green algae, and CBOM was saturated even at the lowest concentrations (ambient).

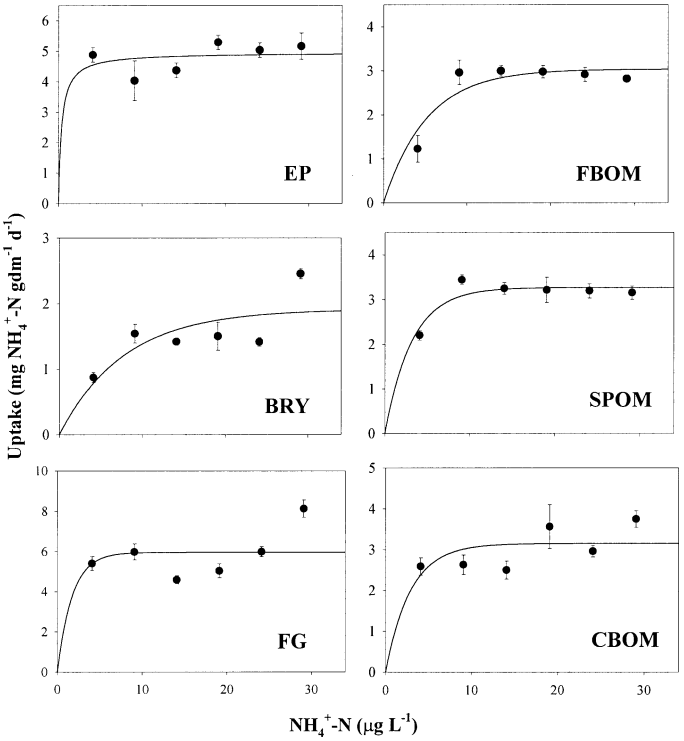

Fig. 2. Mean uptake response after 3 d incubation to increasing NH_4^+ concentration above ambient in prairie stream substrata. Graphs are not on the same *y* scale. Bars, ±1 SE. BRY, bryophytes; CBOM, coarse benthic organic matter; EP, epilithic diatoms; FBOM, fine benthic organic matter; FG, filamentous green algae; SPOM, suspended particulate organic matter. See Table 1 and text for equations and statistics.

Nitrification rates responded variably to NH_4^+ additions above ambient concentrations, depending on the substratum being observed (Fig. 3, Table 1). The highest rates of increase in nitrification with NH_4^+ addition were associated with filamentous green algae and epilithic diatoms, but nitrification associated with filamentous green algae increased only up to 20 μg N L^{-1} NH_4^+ addition, after which significant declines in nitrification relative to the control (0 NH_4^+ addition above ambient; $P < 0.001$) were observed. Nitrification rates associated with SPOM and CBOM increased at the lowest NH_4^+ addition above ambient but demonstrated saturation at high NH_4^+ additions. No significant increases in nitrification rates were observed in FBOM or bryophytes at low NH_4^+ addition (5 and 10 μg N L^{-1} above ambient), although significant increases and subsequent saturation when associated with FBOM were observed at the higher additions.

Table 1. Equations for uptake and transformation rates with increasing N concentrations. $[x]$ = NH_4^+–N concentration in μg L^{-1}, $[y]$ = NO_3^-–N concentration in μg L^{-1}. Ammonium uptake and nitrification rates in mg NH_4^+–N gdm^{-1} d^{-1}, except for stream ecosystem rates in mg NH_4^+–N m^{-2} d^{-1}. Nitrate uptake and denitrification rates in μg NO_3^-–N gdm^{-1} d^{-1} except for stream ecosystem rates, in μg NO_3^-–N m^{-2} d^{-1}.

Source	Rate (N m^2 d^{-1})	P	r^2	Type
NH_4^+–N uptake				
Filamentous green algae	$5.90 \cdot \frac{[x]}{2.4 + [x]}$	0.051	0.836	Michaelis-Menten
Epilithic diatoms	$4.96 \cdot \frac{[x]}{0.6 + [x]}$	0.037	0.942	Michaelis-Menten
Fine benthic organic matter	$3.10 \cdot \frac{[x]}{6.2 + [x]}$	0.032	0.953	Michaelis-Menten
Coarse benthic organic matter	$3.29 \cdot \frac{[x]}{3.4 + [x]}$	0.005	0.878	Michaelis-Menten
Bryophytes	$1.95 \cdot \frac{[x]}{7.6 + [x]}$	0.042	0.798	Michaelis-Menten
Suspended particulate organic matter	$3.4 \cdot \frac{[x]}{5.6 + [x]}$	0.022	0.980	Michaelis-Menten
Stream ecosystem	$1372 \cdot \frac{[x]}{6.7 + [x]}$	0.002	0.998	Michaelis-Menten
Nitrification				
Filamentous green algae	0.96*	*	*	*
Epilithic diatoms	$0.08[x]$	<0.0001	0.815	Linear
Fine benthic organic matter	$\frac{0.49}{1 + e^{\{([x]-2.22)/1.39\}}}$	<0.0001	0.989	Sigmoidal
Coarse benthic organic matter	$0.28 \cdot \frac{[x]}{19 + [x]}$	0.0005	0.978	Michaelis-Menten
Bryophytes	$\frac{0.27}{1 + e^{\{([x]-1.17)/0.80\}}}$	0.0002	0.995	Sigmoidal
Suspended particulate organic matter	$0.21 \cdot \frac{[x]}{15 + [x]}$	0.001	0.861	Michaelis-Menten
Stream ecosystem	$0.95[x]$	0.001	0.780	Linear
NO_3^-–N uptake				
Filamentous green algae	$6.9 \cdot \frac{[y]}{5.1 + [y]}$	0.1346	0.331	Michaelis-Menten
Epilithic diatoms	$2.8 \cdot \frac{[y]}{4.9 + [y]}$	0.034	0.643	Michaelis-Menten
Fine benthic organic matter	$\frac{30.9}{1 + e^{\{([y]-16.4)/3.58\}}}$	0.006	0.944	Sigmoidal
Coarse benthic organic matter	$6.4 \cdot \frac{[y]}{0.7 + [y]}$	0.0005	0.913	Michaelis-Menten
Stream ecosystem	$5690 \cdot \frac{[y]}{12.3 + [y]}$	0.006	0.951	Michaelis-Menten
Denitrification				
Filamentous green algae	$2.32 \cdot \frac{[y]}{1.72 + [y]}$	<0.0001	0.988	Michaelis-Menten
Epilithic diatoms	$0.02[y]$	0.03	0.477	Linear
Fine benthic organic matter	$0.18[y]$	<0.0001	0.869	Linear
Coarse benthic organic matter	$\frac{40.6}{1 + e^{\{([y]-78.46)/11.6\}}}$	0.0009	0.984	Sigmoidal
Stream ecosystem	$4.30[y]$	0.001	0.81	Linear

* = Not significant ($P > 0.15$).

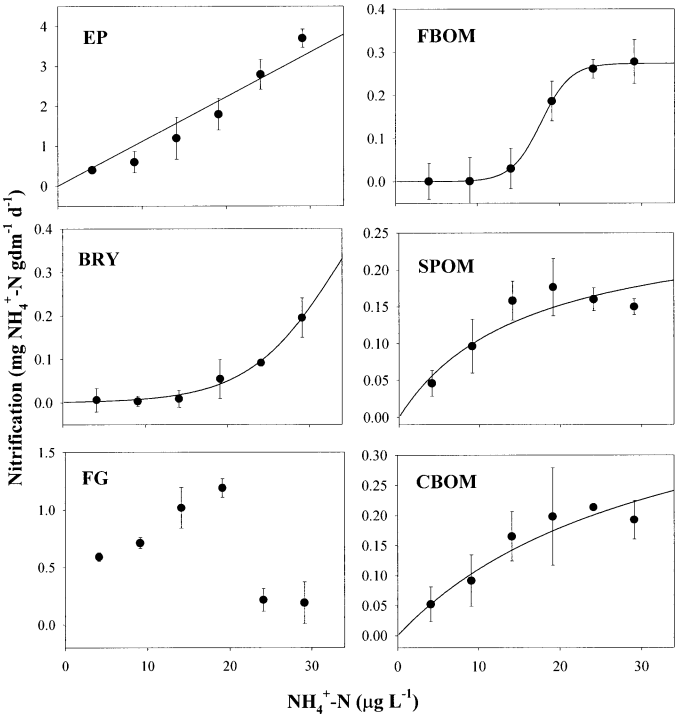

Fig. 3. Mean nitrification response to increasing NH_4^+ concentration above ambient in prairie stream substrata after 3 d incubation. Graphs vary in scale. Bars, ±1 SE; some cannot be seen when smaller than the symbol. Abbreviations as in Fig. 1. See Table 1 and text for equations and statistics.

Changes in nitrification when exposed to varying O_2, NH_4^+, and NO_3^- concentrations also varied with substrata type (Fig. 4). Nitrification rates associated with SPOM and bryophytes were minimal relative to the other substrata but demonstrated proportionally larger increases (>300%) on addition of NH_4^+ relative to control treatments of the same substrata (Fig. 5). Bryophytes, CBOM, FBOM, and epilithic diatoms demonstrated a cumulative response of increasing nitrification rates when both NH_4^+ and O_2 were added (Fig. 4). Rates of nitrification increased only slightly in NO_3^- treatments and decreased significantly ($P < 0.0001$) for all substrata in the N_2 treatments (Figs. 4, 5). All substrata demonstrated a 100% decline in nitrification rates in the NO_3^- + N_2. However, CBOM, bryophytes, and FBOM demonstrated a smaller decline in nitrification rates in the NH_4^+ + N_2 treatment (Fig. 5).

NO_3^- uptake and denitrification rates—No significant differences between NO_3^- uptake or denitrification rates at the different temperatures tested were observed; therefore, data for all temperatures were combined for all analyses (data not shown). Overall, uptake rates for NO_3^- at all concentrations were 1,000-fold lower than rates of NH_4^+ uptake (Figs. 2, 6). Nitrate uptake associated with CBOM, filamentous green algae, and epilithic diatoms demonstrated Michaelis-Menten kinetics with increasing NO_3^- concentrations. Uptake associated with filamentous green algae had the highest V_{max} (6.90 μg N gdm^{-1} d^{-1}), and epilithic diatoms had the lowest associated V_{max} (2.80 μg N gdm^{-1} d^{-1}; Table 1). Uptake associated with FBOM did not saturate even at the highest concentration of NO_3^- addition tested (25 μg N L^{-1} above ambient).

The denitrification response to increasing NO_3^- concentrations varied with substrata type (Fig. 7). The largest absolute increase in denitrification was associated with FBOM, with rates responding linearly to increasing NO_3^-. Denitrification associated with epilithic diatoms also demonstrated a linear increase with NO_3^- concentration but started to become saturated when associated with filamentous green algae at higher concentrations (20 and 25 μg N L^{-1}). Denitrification increased significantly in all substrata when NO_3^- was added and anoxic conditions were present (Fig. 8, $P < 0.001$) and when associated with CBOM and FBOM in the NH_4^+ + N_2

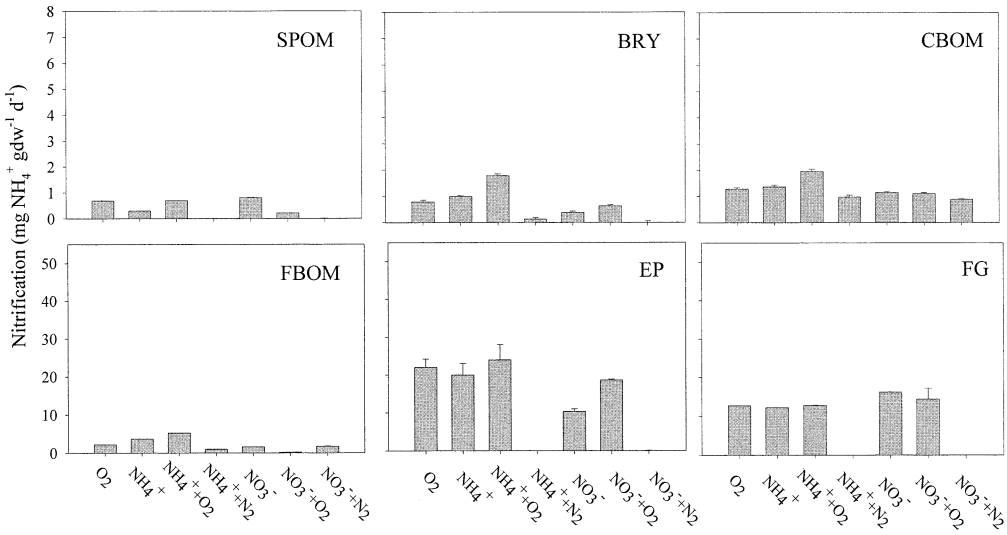

Fig. 4. Mean nitrification response to variable O_2, NH_4^+, and NO_3^- concentrations in prairie stream substrata. Bars (treatment rate − control rate), ±1 SE; some cannot be seen when smaller than the symbol. Graphs vary in scale. Abbreviations as in Fig. 1. See text for treatment explanation.

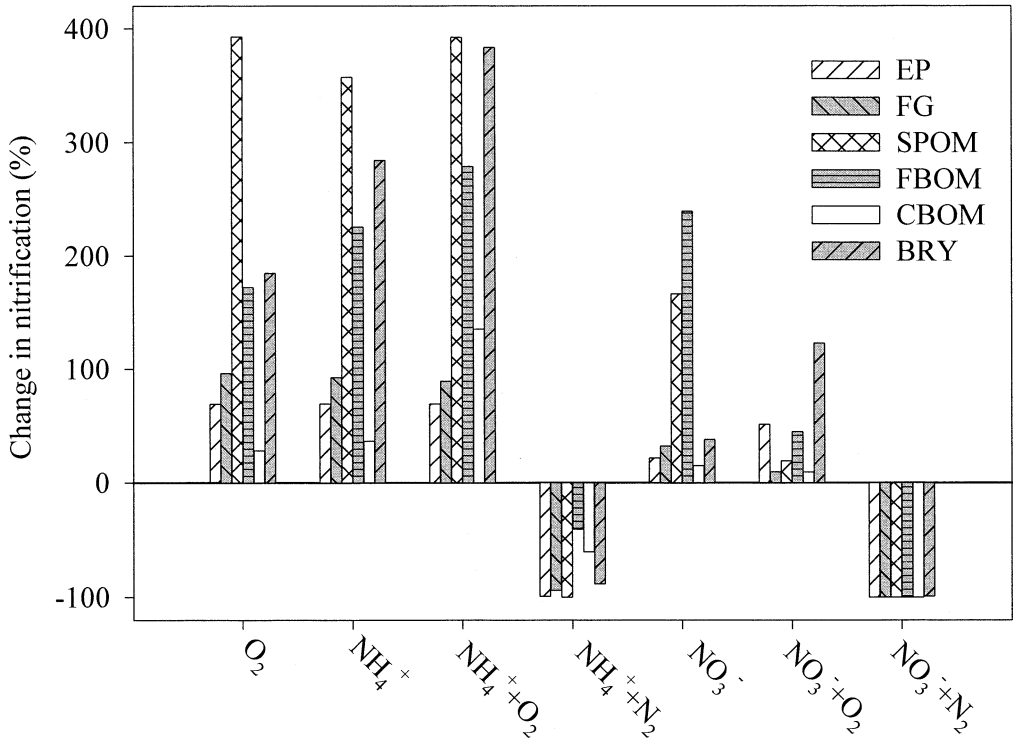

Fig. 5. Mean percentage change in nitrification rates associated with prairie stream substrata in response to O_2, NH_4^+, and NO_3^- treatments. Abbreviations as in Fig. 1. See text for treatment explanation.

treatment (Fig. 8, $P < 0.001$). All substrata demonstrated a large percentage increase in denitrification rates when the habitat was made anoxic (N_2 addition; Fig. 9). Denitrification associated with FBOM and CBOM demonstrated the largest changes overall (>200% increase) in the N_2 treatments.

Model results—Mass-weighted rates of stream ecosystem uptake, nitrification, and denitrification were estimated by use of the linear and nonlinear equations derived from the experimental data (Table 1, Figs. 10, 11). The predicted total stream ecosystem NH_4^+ uptake demonstrated Michaelis-Menten kinetics ($V_{max} \sim$ 1,300 mg N m^{-2} d^{-1}) dominated by uptake associated with FBOM and epilithic diatoms. The predicted total stream ecosystem nitrification increased linearly with N added and was primarily dominated by nitrification associated with FBOM and epilithic diatoms (Fig. 10). The predicted stream ecosystem NO_3^- uptake also demonstrated Michaelis-Menten kinetics ($V_{max} \sim$ 5.2 mg N m^{-2} d^{-1}) primarily associated with filamentous green algae, but predicted stream ecosystem denitrification was driven by rates associated with FBOM, increasing linearly with NO_3^- concentration (Fig. 11).

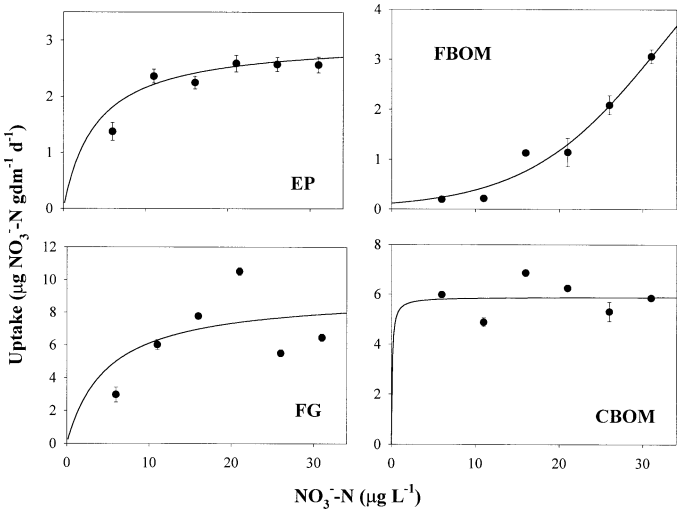

Fig. 6. Mean uptake response to increasing NO_3^- concentration. Graphs vary in scale. Bars, ±1 SE. Abbreviations as in Fig. 1. See Table 1 for equations and statistics.

Multiple regression equations for nitrification and denitrification were also derived from the experimental data (Table 2) and combined with long-term data on water column O_2, NH_4^+, and NO_3^- concentrations within the stream channel (Fig. 1) to predict natural variability in stream ecosystem nitrification and denitrification rates over time. These models predicted a more than twofold annual variation in denitrification (2–6 mg N m^{-2} d^{-1}) and nitrification (30–70 mg N m^{-2} d^{-1}) rates over time. Denitrification rates were 10-fold lower than nitrification rates at the stream ecosystem level, and denitrification was only marginally correlated with nitrification (Fig. 12).

Discussion

N uptake—Uptake rates have previously been associated with substrata type and are highly variable among freshwater

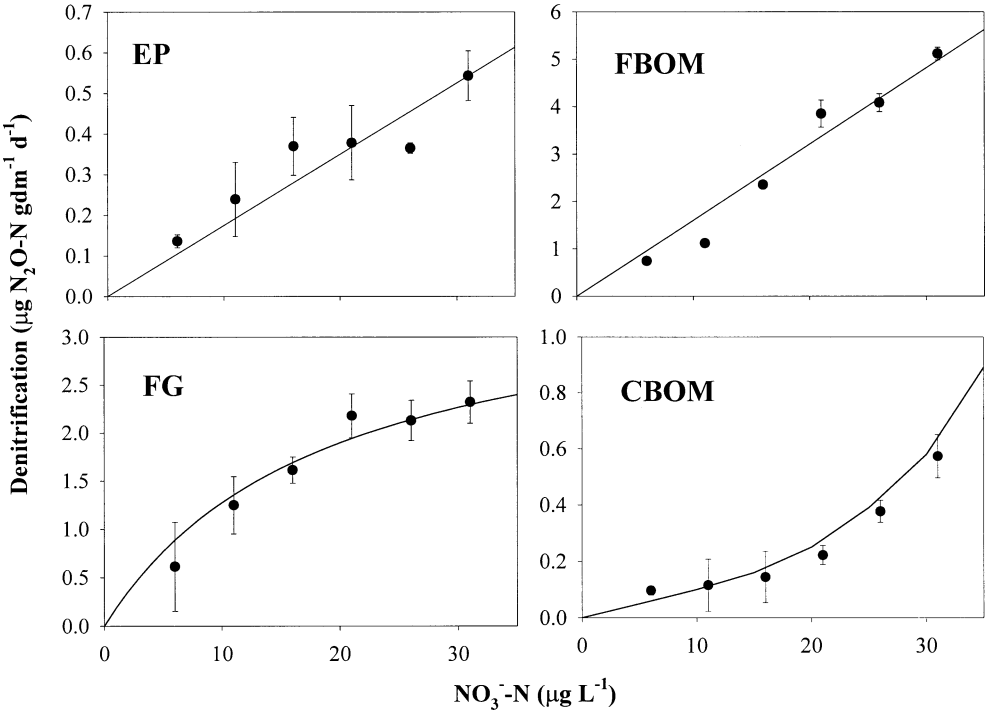

Fig. 7. Mean denitrification response to increasing NO_3^- concentration. Graphs are not all on the same *y* scale. Bars, ±1 SE; some cannot be seen when smaller than the symbol. Abbreviations as in Fig. 1. See Table 1 for equations and statistics.

systems (e.g., Munn and Meyer 1990; Peckol et al. 1994). Peckol et al. (1994) demonstrated that species of *Cladophora* had site-specific N uptake rates, which they indicated may be due to physiological strategies that take advantage of greater N availability and may aid endurance of the indirect effects of N loading (e.g., reduced irradiance and anoxia). Furthermore, Munn and Meyer (1990) demonstrated differences in N uptake between an eastern and western stream, speculating that in the western stream, strong biotic control of N uptake combined with strong N demand resulted in shorter uptake lengths (i.e., higher uptake rates).

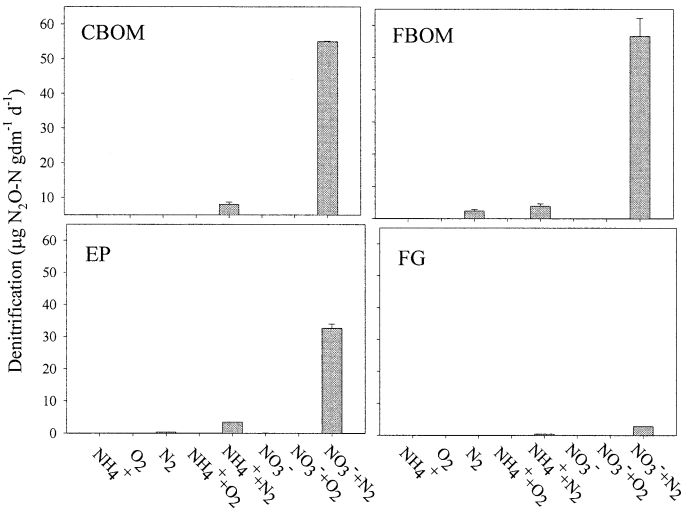

Fig. 8. Mean denitrification response to variable O_2, NH_4^+, and NO_3^- concentrations in prairie stream substrata. Bars (treatment rate − control rate), ±1 SE; when bars cannot be seen, it is smaller than the symbol. Abbreviations as in Fig. 1. See text for treatment explanation.

Results from our study demonstrated temporal and spatial (i.e., substrata type) variation of N uptake rates within the stream benthos that is likely due to both chemical and biological factors that influence overall rates. The relationship between dissolved inorganic N concentration and uptake varied among substrata types (Table 1, Figs. 2, 6). Ammonium uptake demonstrated Michaelis-Menten kinetics for all substrata, although the magnitude of uptake varied with the different types. Biomass-specific uptake of NH_4^+ was greatest when associated with filamentous green algae and epilithic diatoms, and NO_3^- uptake was also greatest when associated with filamentous green algae. Uptake of NO_3^- did not saturate when associated with FBOM, likely because of the relatively low NO_3^- concentrations used in these experimental additions.

The half-saturation constants (K_s) calculated for uptake (data not shown) were within the range of values published elsewhere. Dodds (2002) compiled several sources of data on algal N uptake and found a 10^3 range for K_s between 0.001 and 0.98 mg L^{-1} that included both NO_3^- and NH_4^+ uptake values. The K_s values estimated in this study were lower, albeit in the same range (0.6–7.6 μg L^{-1}; Figs. 2, 6). The larger uptake values observed for NH_4^+ are likely due to preferential uptake of NH_4^+, which, in contrast to NO_3^-, does not have to be reduced by organisms prior to incorporation and consequently requires less energy to assimilate.

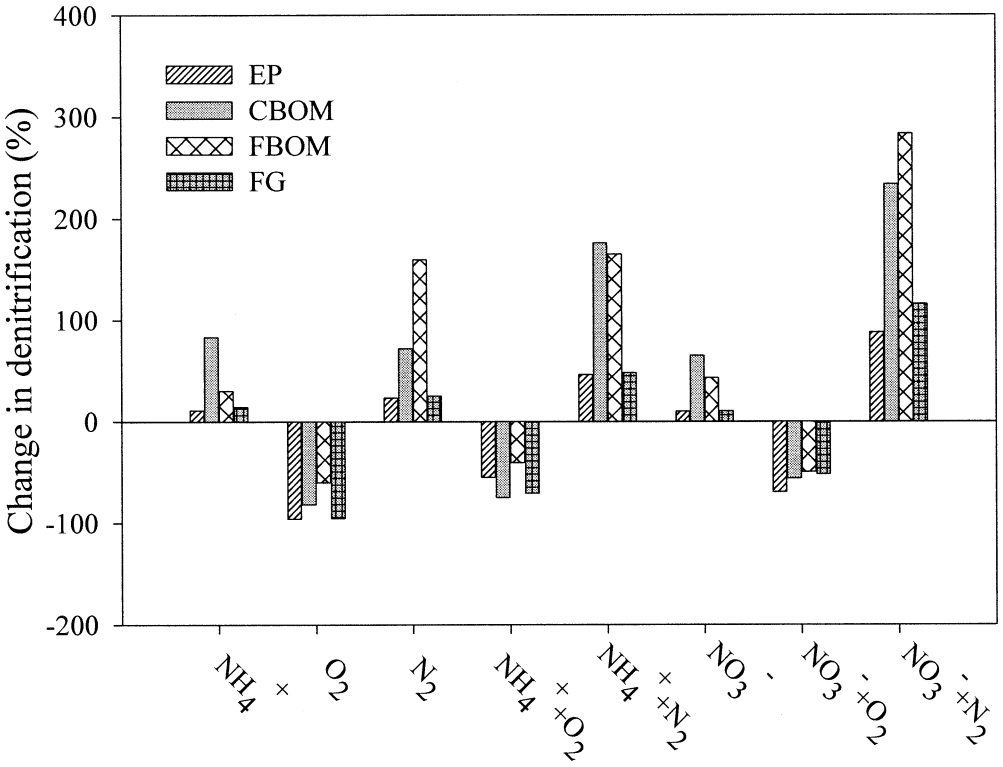

Fig. 9. Mean percentage change in denitrification rates associated with prairie stream substrata in response to variable O_2, NH_4^+, and NO_3^- treatments. Abbreviations as in Fig. 1. See text for treatment explanation.

This result is consistent with observations elsewhere in which NH_4^+ was removed more quickly than NO_3^- (Dodds et al. 1991; Peterson et al. 2001).

Our results also demonstrate lower uptake rates of both NH_4^+ and NO_3^- associated with CBOM and higher rates associated with filamentous green algae and epilithic diatoms (Figs. 2, 6). This may be due to the dominance of autotrophy in this system with little woody vegetation along the stream channel and low allochthonous input. Primary producers dominate N uptake in these streams (Dodds et al. 2000), and they also have higher assimilation of NH_4^+ into the cells, relative to NO_3^-, even in forested systems (Tank et al. 2000).

Nitrification—Nitrification accounted for 9%–15% of NH_4^+ uptake in the stream substrata, consistent with documented values for this system (Dodds et al. 2000). In a comparative study that used ^{15}N tracers, Peterson et al. (2001) found that, on average, 20%–30% of NH_4^+ uptake within the stream channel was due to nitrification, ranging from <3% to 60% across systems. In our study, the highest percentages of NH_4^+ uptake due to nitrification were associated with primary producers (filamentous green algae and epilithic diatoms).

Nitrifying bacteria can survive as viable inactive cells during periods of low substrate concentrations or poor growing conditions (e.g., Verhagen et al. 1992). Thus, no correlation between the number of nitrifying bacteria and nitrification activity is typically observed. Dormant nitrifiers may explain why substrata that typically were not associated with high rates of nitrification (Kemp and Dodds 2001*a*) demonstrated >300% increases in associated rates in the presence of optimal growing conditions (e.g., SPOM and bryophytes, $NH_4^+ + O_2$ treatment; Fig. 5). In contrast, substrata typically associated with high rates of nitrification demonstrated much smaller increases in the presence of optimal conditions.

Estimated stream ecosystem nitrification rates suggested that increases with NH_4^+ concentrations are primarily driven by nitrification associated with epilithic diatoms or FBOM (Fig. 10). Nitrification associated with other substrata either had insufficient mass within the stream channel or associated nitrification rates were too low to substantially impact rates at the stream ecosystem level, despite increases in absolute rates with NH_4^+ addition. Thus, responses to increasing N in natural systems will likely vary depending on the available substrata within the stream channel.

Denitrification—Denitrification is an important N sink in aquatic environments, and there are two main sources of NO_3^- for substrata-associated denitrification: NO_3^- diffusing into the substrata from the water column and NO_3^- produced by nitrification in the oxic portion of the substrata (Seitzinger 1988). Nitrate diffusion from both these sources depends on the thickness of the oxic surface layer, which serves as a diffusional barrier for NO_3^- to the denitrification zone (Christensen et al. 1990). Epilithic diatoms and filamentous green algae have many supersaturated O_2 microsites within the substrata (e.g., Kemp and Dodds 2001*a*). Thus, we expected low rates of associated denitrification relative to other sub-

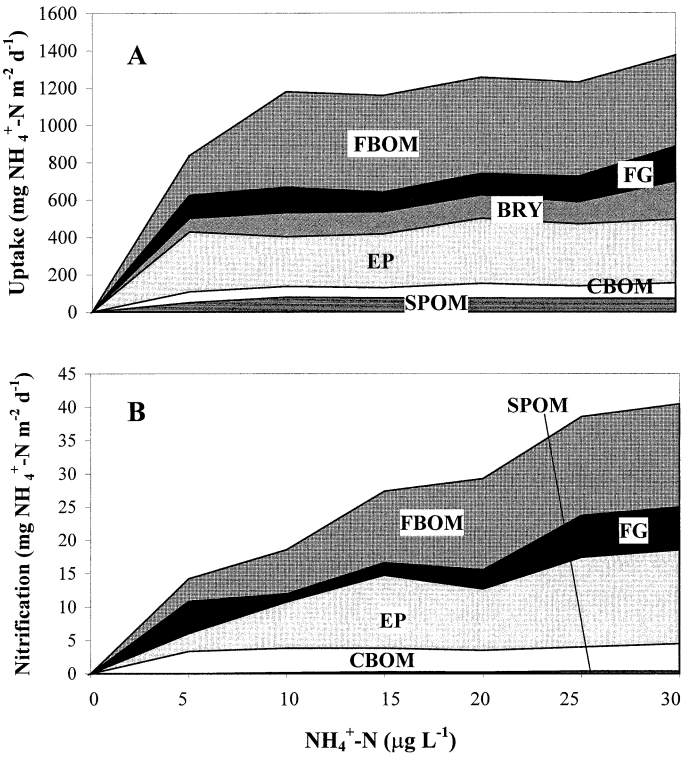

Fig. 10. Predicted stream ecosystem (A) NH_4^+ uptake and (B) nitrification rates as a function of increasing NH_4^+ concentration. Rates were calculated by use of mass-weighted rates of each substrata type. Error bars are 95% confidence intervals for stream ecosystem rates. See Table 1 and text for equations. Abbreviations as in Fig. 1.

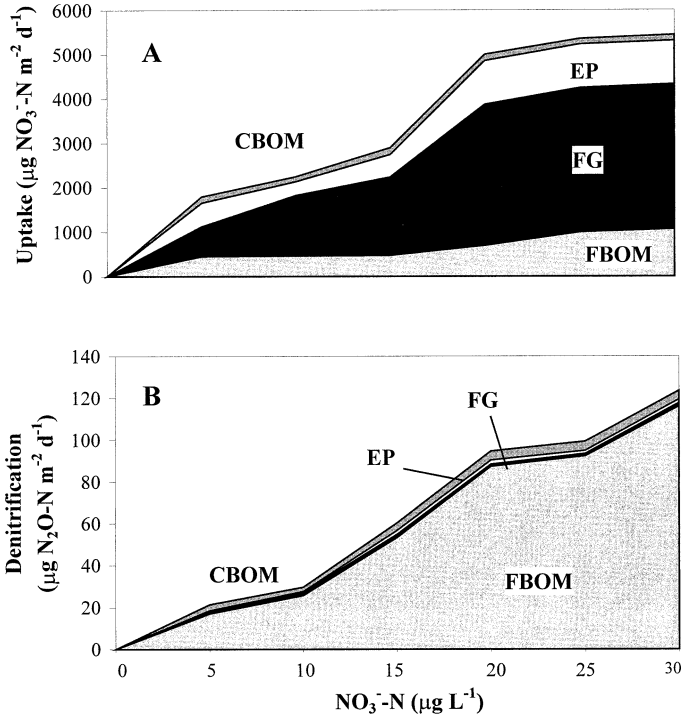

Fig. 11. Predicted stream ecosystem (A) NO_3^- uptake and (B) denitrification rates as a function of increasing NO_3^- concentration. Rates were calculated by use of mass-weighted rates of each substrata type. Error bars are 95% confidence intervals for stream ecosystem rates. See Table 1 and text for equations. Abbreviations as in Fig. 1.

strata as observed. We also expected a large increase in denitrification with increasing NO_3^- concentrations when associated with FBOM or CBOM. However, only FBOM had a significant increase in denitrification rates with increased NO_3^- concentrations (Figs. 7, 11). The particle density of FBOM reduces diffusion of O_2, providing abundant anoxic zones (Kemp and Dodds 2001*a*), and, if NO_3^- is also available, high rates of denitrification occur. The coupling of available anoxic zones and NO_3^- promotes high rates of denitrification. It may also be true that larger pools of labile organic carbon or denitrifying bacteria are associated with FBOM, which also promotes higher rates of denitrification.

All substrata yielded significant increases in denitrification in the presence of anoxic conditions and abundant NO_3^- ($P < 0.0001$; Figs. 8, 9). The largest responses were observed in FBOM. Much smaller responses were observed when denitrification was associated with primary producers (Fig. 9). Denitrification associated with FBOM was always significantly stimulated when purged with N_2, regardless of whether NO_3^- or NH_4^+ was added, which indicates that NO_3^- was

Table 2. Multiple regression results for oxygen (O_2), ammonium (NH_4), and nitrate (NO_3) concentrations. All values in mg L^{-1}.

Source	Rate (mg N m^{-2} d^{-1})	*P*	r^2
Nitrification			
Filamentous green algae	1.89 NH_4 + 0.046 $(O_2)^2$ − 2.54	0.003	0.76
Epilithic diatoms	1.69 NH_4 + 0.75 $(O_2)^2$ − 13.66	0.056	0.15
Fine benthic organic matter	0.53 NH_4 + 2.75 $(O_2)^2$ − 10.12	0.041	0.35
Coarse benthic organic matter	0.05 NH_4 + 0.13 $(O_2)^2$ − 0.03	0.018	0.58
Bryophytes	1.23 NH_4 + 0.6 $(O_2)^2$ − 0.66	0.008	0.60
Suspended particulate organic matter	2.03 NH_4 + 0.46 $(O_2)^2$ + 7.11	0.082	0.07
Denitrification			
Filamentous green algae	2.15 NO_3 − 0.32 $(O_2)^2$ + 4.61	0.007	0.59
Epilithic diatoms	9.2 NO_3 − 6.32 $(O_2)^2$ + 8.31	0.092	0.09
Fine benthic organic matter	1.56 NO_3 − 0.98 $(O_2)^2$ + 7.21	0.001	0.85
Coarse benthic organic matter	4.29 NO_3 − 2.72 $(O_2)^2$ + 14.61	0.065	0.27

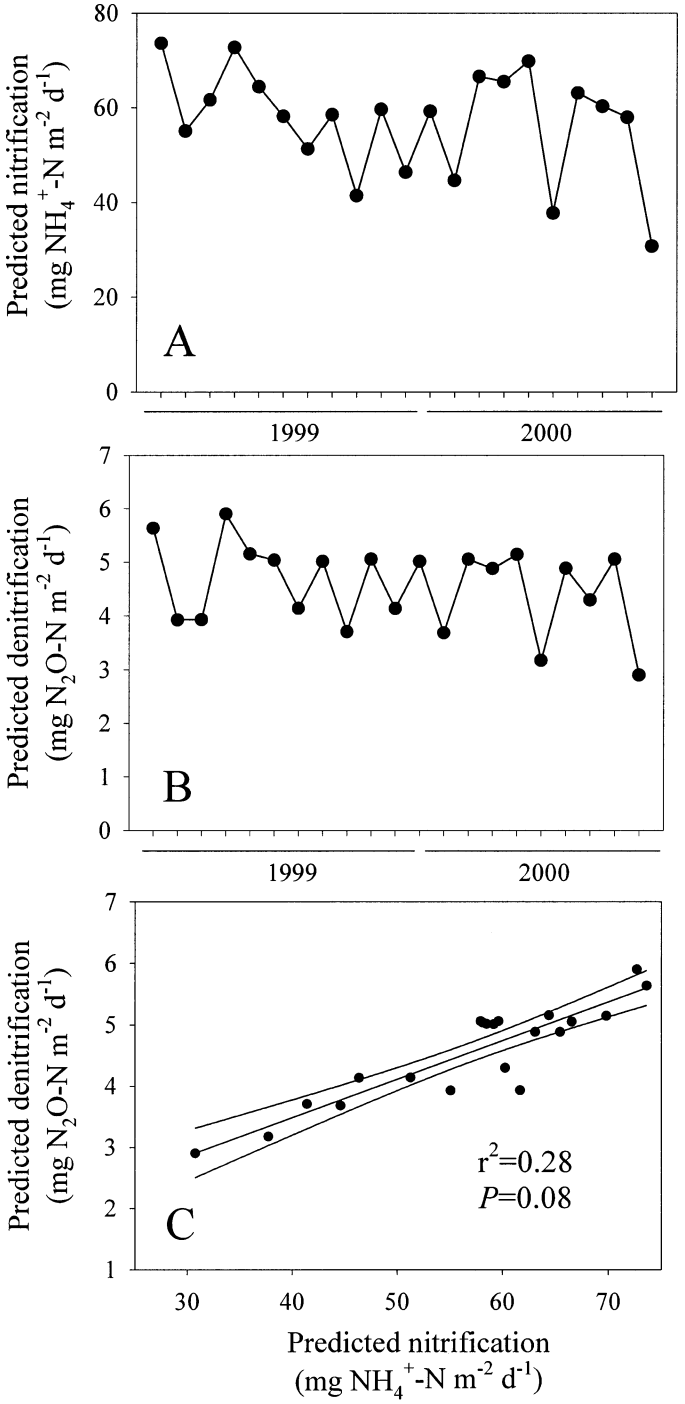

Fig. 12. Predicted stream ecosystem (A) nitrification and (B) denitrification rates in response to variable NH_4^+, NO_3^-, and O_2 concentrations and (C) relationship of predicted rates. Error bars are 95% confidence intervals for stream ecosystem rates. See Table 2 and text for equations. Values of NH_4^+, NO_3^-, and O_2 used in multiple regression models are monthly means of water column concentrations (Fig. 1).

abundant enough to promote denitrification under ambient conditions.

Substrate variability—The concentration of N at base flow supports production in streams, and fluctuations in substrate concentrations may have major affects on ecological processes; conversely, ecological processes may influence spatial and temporal patterns of substrate concentrations (e.g., Munn and Meyer 1990; Kemp and Dodds 2001*b*). We observed high temporal variations in water column NH_4^+, NO_3^-, and O_2 concentrations within the stream channel (Fig. 1) that likely affect rates of N uptake and transformation occurring in the benthos. Natural variation in O_2 and N concentrations within the stream channel can result in changes in N transformation rates either directly through altered uptake, mineralization, nitrification, or denitrification or indirectly through changes in nutrient and O_2 diffusion into the substratum (e.g., Christensen et al. 1990; Kemp and Dodds 2001*a*). Our models demonstrated both possible direct and indirect influences of substrate concentration on stream ecosystem uptake, nitrification, and denitrification rates. For example, NO_3^- additions moderately stimulated nitrification (Figs. 4, 5), and the increased nitrifying activity may alter abundance of O_2 within the substrata indirectly influencing denitrification rates.

Stream ecosystem nitrification rates responded linearly to increases in NH_4^+ concentrations, with an approximate doubling in rates for a doubling in concentration (Fig. 10). Denitrification also responded linearly to increasing NO_3^- concentrations but at a greater rate, with a 10-fold increase for every doubling in concentration (Fig. 11). Given the spatial and temporal variance of N and O_2 within the water column (Fig. 1), ecosystem rates of nitrification and denitrification were found to vary >30% over time because of natural variation in substrate concentrations alone (Fig. 12).

Variability of N uptake and transformation rates has a direct impact on stream ecosystem N cycling. In this study, rates of all processes were variable and depended not only on the substrate concentration but also on the substrata type with which the process was associated. Uptake of NH_4^+ and NO_3^- demonstrated Michaelis-Menton kinetics when associated with all substrata, although the magnitude of uptake varied with substrata type. In contrast, nitrification and denitrification rates responded variably, both in magnitude and in linearity, to changes in substrate concentration. These data indicate that the substrate concentration, the substrata types available, and the relative abundance of those substrata types within the stream channel are important components of stream ecosystem N cycling.

We make a number of assumptions when extrapolating laboratory measurements to field rates. These include the assumption that laboratory conditions approximated field conditions and that acclimation time to treatment conditions was minimal. Although laboratory incubations are inherently artificial, our preliminary time-course experiments suggested that optimal incubation times were used. We also assumed no temperature effects across season. Temperature did not significantly affect rates in experiments conducted at mean seasonal temperatures (10°C, 15°C, and 20°C; data not shown). It is possible that if more extreme temperatures

characteristic of summer and winter had been used, differences may have been detected. The temperatures used in the experiments more closely represented seasonal means and not the overall range, so we suggest that, on average, our results are indicative of rates in the stream. Because our modeling was done on monthly data, this approach seemed appropriate. Previous transplant experiments indicated that substrata acclimate to physical and chemical conditions in <6 d (Kemp and Dodds in press). Therefore, predicted rates on a 1-month temporal scale is warranted. Despite the assumptions we must make to extrapolate laboratory incubations to stream ecosystem rates, we demonstrate a potential range of variability in these rates that has not been described elsewhere.

Streams exert control over nutrient export to rivers, lakes, and estuaries. Streams with higher nitrification rates have higher NO_3^- concentrations, which suggests that nitrification within the stream channel as well external N inputs affect concentrations (Peterson et al. 2001). Marked spatial variation of N concentrations in streams, which is due to the many factors that regulate N uptake and transformation rates, has been documented at the micro- and macroscale (Rysgaard et al. 1994; Dent and Grimm 1999; Kemp and Dodds 2001*a*,*b*). Given the high variability of water column N and O_2 concentrations, we demonstrate how this variability may affect transformations within the stream and potentially the export of N. An important point that follows from our results is that uptake of NO_3^- and NH_4^+, nitrification, and denitrification are not necessarily associated with the same substrata types. Thus, N uptake and retention at the level of the stream ecosystem may be influenced by factors that reduce spatial variability in substrata such as flooding, channelization, or excessive sedimentation.

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