

Biophysicochemical process coupling controls nitrate use by benthic biofilms

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Abstract

A combination of macroscale and microscale observations was used to show that the interplay between hydrodynamic transport, local chemical conditions, and microbial metabolism controls nitrate use by benthic microbial communities. While it is usually assumed that interfacial transport and nutrient removal both increase monotonically with the velocity of the overlying flow, in laboratory flume experiments we observed substantially greater bulk nitrate removal under slower velocities (0.05 and 0.5 cm s⁻¹) than under a faster velocity (5 cm s⁻¹), with the greatest rate of nitrate removal occurring under the intermediate flow condition. These results demonstrate that hydrodynamic control of solute transport, specifically here the flux of oxygen and nitrate from the water column to the benthic microbial community, causes facultative bacteria to shift between anaerobic and aerobic metabolism under different flow conditions. Aerobic metabolism is promoted by more rapid hydrodynamic transport conditions, while anaerobic metabolism is favored under low-transport conditions. This type of coupling is expected to regulate microbial activity in all surficial sedimentary environments and must be parameterized in order to forecast long-term average biochemical transformation rates in rivers and other dynamic aquatic systems.

Excess nitrogen in river networks is a global problem leading to eutrophication and hypoxia of coastal waters (Turner and Rabalais 1994; Alexander et al. 2000; Diaz 2001). Improved estimates of in-stream denitrification are needed to protect and restore these aquatic ecosystems (Peterson et al. 2001; Alexander et al. 2002; Mulholland et al. 2004). Unfortunately, poor understanding of the coupling between physical transport and benthic microbial processes greatly hinders the estimation of average biochemical transformation rates under the spatially and temporally variable flow conditions found in rivers (Alexander et al. 2000; Battin et al. 2003).

Relating nitrogen transformation rates to system structure is particularly important in assessing causes of and solutions for coastal eutrophication because of the extensive modification of lowland stream channels for navigation, agriculture, and urban development. For example, in the Mississippi River system, not only is there substantial nitrogen input from fertilizers and municipal wastewater, but there has also been extensive drainage of riparian wetlands and deepening and straightening of headwater stream channels (Sparks 1995; Alexander et al. 2000; Knox 2001). The role of this widespread system modification in nitrogen dynamics is not known, and, while it is often assumed that stream restoration efforts will provide better conditions for natural denitrification in headwater stream

channels, it is not clear how the restored channel geometries and flow regimes will affect whole-stream average nitrate transformation rates and downstream nitrate export.

Solute mass transfer from the water column through the diffusive boundary layer to the underlying sediments is often the rate-limiting process for nutrient use in aquatic systems and is therefore an important regulator of ecosystem-level metabolism (Duff and Triska 2000; Larned et al. 2004). While flow conditions have been observed to play a critical role in nutrient cycling in both marine and freshwater systems (Nielsen et al. 1990; Duff and Triska 2000), no clear trends between overlying velocity and nitrate removal have been identified (Leu et al. 1998; Alexander et al. 2000; Eriksson 2001). Moreover, the link between processes occurring at different spatial scales remains unexplored, despite the fact that interscale connections are expected to become very important when process interactions are complex and competition between alternative transformation pathways occurs within the microbial community. For example, denitrification depends on delivery of nitrate and organic carbon to microbial populations within sedimentary biofilms, but it can be suppressed by oxygen because most denitrifying organisms are facultative anaerobes (Tiedje 1988). Thus, it is particularly difficult to estimate denitrification rates because this requires consideration of the hydrodynamic delivery of nitrate and other important chemical species to microorganisms within benthic, hyporheic, and epiphytic biofilms, as well as the analysis of local nitrogen transformation rates within these microbial communities. To demonstrate the way in which coupling between bulk flow conditions, microscale transport processes, and microbial activity influences nitrogen dynamics, we observed bulk removal of nitrate and microscale distributions of oxygen under different flow conditions in a laboratory flume packed with a sediment bed composed of silica sand hosting a mixed photoautotrophic–heterotrophic biofilm.

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Methods and materials

Cultivation of benthic biofilm—The effects of overlying flow conditions on nitrate removal and oxygen consumption by benthic biofilms were studied using a 250 cm long and 20 cm wide recirculating flume (Ren and Packman 2004) packed with natural silica sand (500 μm average diameter) to form a 5 cm deep sand bed with a flat surface. An initial benthic microbial seed was obtained in November 2004 from Mill Creek near Wadsworth, Illinois. Biofilm was scraped from bed material that was taken from the sediment–water interface, mixed in 2 liters of stream water, filtered at 250 μm to remove large grazers and particles, and evenly spread over the sand bed in the flume under a water column of 7 cm depth. After a 24-h settling period, flow was initiated and maintained at 5 cm s^{-1} for 2 months to allow biofilm growth. The water in the flume was continuously treated in a secondary loop by filtering at 20 μm to remove particulates and using a chiller (DS-3, Aqualogic) to maintain the temperature at $15 \pm 1^\circ\text{C}$. Nitrate–nitrogen ($\text{NO}_3\text{-N}$) concentrations were maintained between 6 and 9 mg L^{-1} during the growth period (using KNO_3), and other nutrients were supplied every 2 weeks following the protocol of Beakes et al. (1988). Addition of KNO_3 was accompanied by KH_2PO_4 to maintain a N:P ratio of 16:1. Irradiance was supplied at 140 ± 10 (photosynthetic active radiation [PAR]) by means of 400 W metal-halide lamps, in a light:dark cycle of 8:16 h to support algal growth. Dissolved organic carbon was produced continuously in situ by periphytic autotrophs and maintained at $12 \pm 2 \text{ mg L}^{-1}$ by diluting flume water with deionized water (Apollo 9000 total organic carbon [TOC] analyzer, Teledyne Tekmar).

Surface–subsurface solute exchange—Solute exchange between the overlying free surface flow and a sand bed was measured in the recirculating flume at five different stream velocities following the methods of Packman et al. (2004). Dissolved sodium chloride was used as a conservative tracer, and its concentration was measured as electrical conductivity (Horiba ES-10 conductivity meter). The experiments were conducted by introducing the salt solution to the recirculating water, while the streambed was initially salt free, and then monitoring the reduction in the in-stream tracer concentration over time.

Nitrate transformation—Nitrate transformation measurements were conducted after 2 months of biofilm growth. Net nitrate removal from the water column was measured under three stream velocities, starting at 5 cm s^{-1} and then proceeding to 0.5 cm s^{-1} and 0.05 cm s^{-1} . The Reynolds numbers ($\text{Re} = VL/\nu$) of these flows were 1,833, 183.3, and 18.3, respectively (Chaudhry 1993), where V is the mean velocity of the overlying flow (cm s^{-1}), L is a characteristic length (cm) defined here as the hydraulic radius, and ν is the kinematic viscosity of the fluid ($\text{cm}^2 \text{ s}^{-1}$). Nitrate removal was evaluated as the reduction in the in-stream nitrate concentration over time. At the beginning of each nitrate removal experiment, the in-stream nitrate concentration was amended to $8.8 \pm 0.1 \text{ mg}$

$\text{NO}_3\text{-N L}^{-1}$. Nitrate concentrations were then measured in the water column on a daily basis, at the end of the dark cycle, for 1 week at each velocity. $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$, $\text{NH}_4\text{-N}$, and total nitrogen (TN) concentrations were measured on a daily basis according to standard methods (APHA 1998).

Evaluation of benthic bacterial biomass—Bacterial biomass was measured twice: once before reducing the flow from 5 to 0.5 cm s^{-1} and once after the nitrate transformation measurements were completed. Samples were taken at three locations along the flume (60, 120, and 180 cm from the flume inlet). Each sample included all bed material contained within an area of 5.73 cm^2 to a depth of 1.5 cm, including both the benthic biofilm and the underlying sand. Preserved samples (0.5% glutaraldehyde) were mechanically ground, sonicated (2 min), and vortexed (30 s) twice to break down flocks and suspend bacteria before staining with 4',6-diamidino-2-phenylindole (DAPI). Bacteria were then enumerated via epifluorescence microscopy (Zeiss, Axiophot) (Kepner and Pratt 1994).

Oxygen profiling—Oxygen microprofiles were measured during the nitrate removal experiments using a Clark-type oxygen electrode mounted on micromanipulator with computerized depth control and data acquisition (Unisense). The microelectrode had a tip diameter of 50 μm , a stirring sensitivity <2%, and a 90% response time <5 s.

Data analysis—We evaluated the rate of nitrate removal from the water column in terms of first-order rate constants, as is commonly done in modeling nitrogen dynamics (e.g., Alexander et al. 2000). First-order nitrate removal rate constants were estimated by least-squares regression of the natural logarithm of the measured in-stream nitrate concentrations versus time (Thomas et al. 2000). Differences between the nitrate removal rates at the different velocities were compared using linear regression coefficients (Sokal and Rohlf 1981). Periodic observations of bacterial numbers were compared using a t -test. The rates of stream–subsurface exchange under the different flow conditions were evaluated as effective diffusion coefficients (D_{eff}) obtained from the salt injection experiments. The effective diffusion coefficient is used as a measure of the interfacial exchange because we do not know the exact transport processes that control solute flux between the water column and sediments. An effective diffusion coefficient, representing the net effects of all stream–subsurface exchange processes, can be found from the initial slope of a plot of the in-stream tracer concentration versus the square root of the time using the following relationship (Packman et al. 2004):

$$D_{\text{eff}} = \left[d \frac{\pi}{2} \frac{dC^*}{d(t^{1/2})} \right] \quad (1)$$

where D_{eff} is the effective diffusion coefficient ($\text{cm}^2 \text{ s}^{-1}$), d is the effective stream depth (volume per unit bed area, cm), t is time (s), and C^* is the in-stream tracer concentration normalized by the initial concentration.

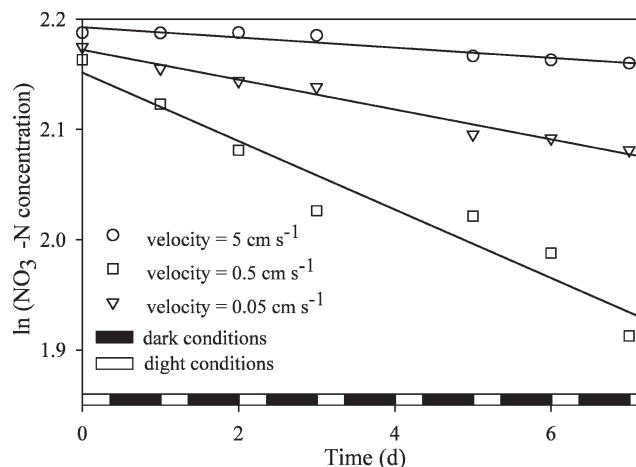


Fig. 1. Long-term observations of in-stream nitrate concentrations under different stream velocities and alternating light/dark conditions show that overlying flow conditions strongly influence average nitrate consumption rates. Nitrate concentrations were measured at the end of the dark cycle. The resulting first-order rate constants for nitrate removal are 0.0135 ($r^2 = 0.9$), 0.0308 ($r^2 = 0.93$), and 0.0045 d^{-1} ($r^2 = 0.93$), for velocities of 0.05, 0.5, and 5 cm s^{-1} , respectively. The rate constants at the different velocities are significantly different from each other ($p < 0.001$), and the rate constant at 5 cm s^{-1} is also significantly greater than zero ($p < 0.01$). Error bars are smaller than the symbol size.

Results and discussion

The benthic biofilm that developed in the flume consisted primarily of the green algae *Spirogyra* and *Mougeotia*. These two species represented more than 99% of the algal biovolume. Net nitrate removal was evaluated by monitoring the decrease in nitrate concentration in the bulk water under alternating light and dark conditions (Fig. 1). The highest rate of nitrate removal (88.16 $\text{mg NO}_3\text{-N m}^{-2} \text{d}^{-1}$) occurred at the intermediate velocity, 0.5 cm s^{-1} , and the lowest rate (10.53 $\text{mg NO}_3\text{-N m}^{-2} \text{d}^{-1}$) was found at the fastest velocity, 5 cm s^{-1} . An intermediate rate of nitrate removal (38.55 $\text{mg NO}_3\text{-N m}^{-2} \text{d}^{-1}$) was measured at the slowest velocity, 0.05 cm s^{-1} . Independent measurements indicated that denitrification was the primary mechanism of bulk nitrate removal. The benthic cell density was $(6.15 \pm 5.44) \times 10^7$ cells cm^{-2} after the nitrate removal measurements at 5 cm s^{-1} , and $(3.18 \pm 1.76) \times 10^7$ cells cm^{-2} at the end of the experiment. These observations indicate that there was no significant net growth or decay of bacterial cells (t -test, $p = 0.52$), suggesting that there was little net nitrate assimilation over the course of the experiments. The low rate of nitrate removal observed under the fastest velocity (Fig. 1) also places an upper bound on nitrate assimilation, since these types of green algae normally grow most rapidly under this condition (Hondzo and Wang 2002). Alternative nitrate transformation pathways (other than denitrification) also did not play a significant role, since ammonium and nitrite were $<1.5\%$ of the total nitrogen mass in the system at all times, with $\text{NH}_4^+\text{-N}$ concentrations ($<0.1 \text{ mg L}^{-1}$) two

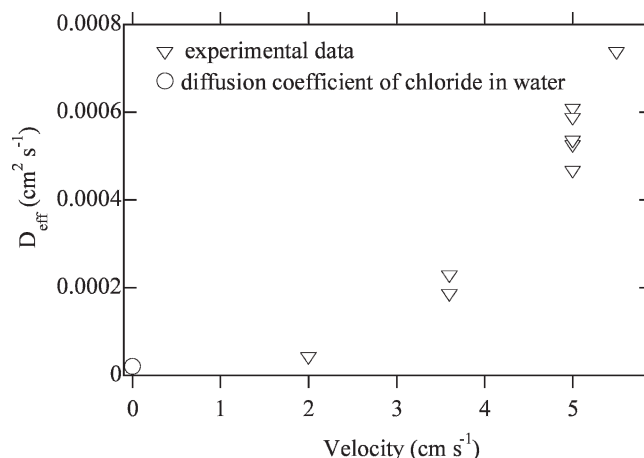


Fig. 2. Observations of conservative solute transport between the water column and sediment bed demonstrate that the interfacial flux increases substantially with stream velocity. These results are compared against the diffusion coefficient for chloride in water, determined at 25°C ($2.03 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$, Li and Gregory 1974).

orders of magnitude lower than $\text{NO}_3^-\text{-N}$ ($\sim 10 \text{ mg L}^{-1}$). Further, $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ concentrations did not display any consistent trend during the nitrate removal experiments, while total dissolved nitrogen followed the decline of nitrate (data not shown). Production of nitrate by nitrification is expected to be insignificant in this system because it is commonly shown that the ammonium produced from organic material decomposition is quickly reused by microorganisms (Allan 1995). No detectable nitrate removal occurred in the flume after the sediments were removed at the end of the experiment, which verified that there was no nitrate transformation anywhere outside of the sediment bed.

Our results indicate that the relationship between flow conditions and denitrification is complex and should not be expected to be linear or even necessarily monotonic when evaluated over a wide range of conditions. Instead, interplay between competing biological and physicochemical processes controls overall nitrate use in this interfacial system. Interfacial solute transport increases substantially with overlying velocity, as shown in Fig. 2. If the local denitrification rate within the benthic biofilm depends only on the availability of nitrate to denitrifying bacteria, as is typically assumed, then the bulk nitrate removal rate would increase monotonically with stream velocity because of enhanced delivery of nitrate from the water column to sedimentary biofilms under higher velocity conditions. The fact that in-stream nitrate consumption initially increases with velocity and then decreases at a higher velocity requires an additional inverse relationship between stream velocity and denitrification not considered in current models for nitrogen dynamics. A ready explanation for the observed patterns of bulk nitrate use can be found by considering the interplay of the physical transport environment, local chemical conditions, and microbial metabolism. Increasing stream velocities enhance the interfacial transport of oxygen as well as nitrogen, facilitating aerobic

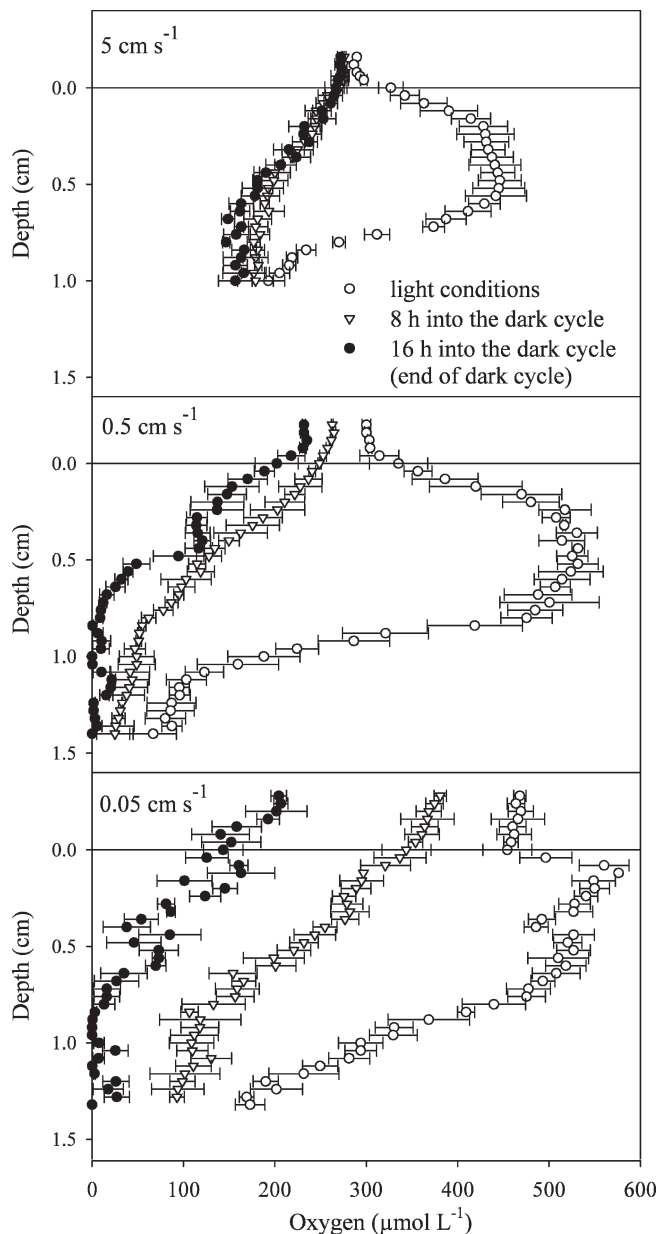


Fig. 3. Microelectrode measurements show the effects of overlying flow conditions on the temporal evolution of benthic oxygen distributions under an imposed light/dark cycle. Denitrifying conditions are found in the sediment bed toward the end of the dark cycle at velocities of 0.05 and 0.5 cm s^{-1} , but not at 5 cm s^{-1} . The origin is defined as the location of the sediment–water interface (depth = 0). Error bars indicate ± 1 standard deviation of three consecutive measurements at each depth.

metabolism and reducing denitrification in the benthic microbial community.

In order to quantify the link between physical transport, local redox conditions, and bulk nitrate removal, we measured oxygen microprofiles within the sediments concurrently with our monitoring of in-stream nitrate concentrations. Oxygen microprofiles obtained at different times during the light/dark cycle are shown in Fig. 3. Aerobic conditions occurred throughout the active biofilm

layer when the bed was illuminated. Photosynthetic production of oxygen caused oxygen supersaturation in the upper layer of the sediments. Oxygen concentrations decreased with depth below around 0.7 cm because of consumption by heterotrophic bacteria residing in the benthic biofilm. Under dark conditions, ongoing heterotrophic bacterial activity caused oxygen concentrations to decrease throughout the sediment bed.

The rate at which oxygen concentrations decreased under dark conditions varied substantially with stream velocity because of differences in hydrodynamic delivery of oxygen from the water column to the sediment bed. The water column remained oxygenated at all times, as can be seen from the uppermost microelectrode measurements in Fig. 3, while interfacial solute exchange increased substantially with stream velocity, as shown in Fig. 2. As a result, the high rate of hydrodynamic transport at the greatest velocity tested (5 cm s^{-1}) produced conditions where oxygen penetration into the sediments ultimately balanced bacterial consumption, supporting aerobic metabolism throughout the active biofilm layer and suppressing denitrification. Conversely, oxygen consumption exceeded influx at the lower flow velocities (0.5 cm s^{-1} and 0.05 cm s^{-1}), ultimately depleting oxygen throughout most of the biofilm and producing a metabolic shift to denitrification. Conditions favorable for denitrification were found below ~ 0.7 cm under the two lower velocity flow conditions for much of the dark cycle, as shown in Fig. 3.

Until the point where denitrification was suppressed by high oxygen concentrations in the sediments, bulk nitrate removal did increase with increasing nitrate flux across the sediment–water interface. The direct forcing provided by enhanced delivery of nitrate to denitrifying bacteria readily explains the observation of greater nitrate removal at 0.5 cm s^{-1} than at 0.05 cm s^{-1} (Fig. 1). However, the significant decrease in nitrate removal observed at the higher velocity of 5 cm s^{-1} can only be explained by the shift to aerobic metabolism mediated by the system structure and the transport environment. Essentially, these results show that the defining physical–chemical structural features of this system are an oxygen- and nitrate-rich water column underlain by carbon- and biomass-rich sediments, with rapid transport in the water column, slow transport in the sediments, and interfacial transport coupled to overlying flow conditions. This structure produces a sedimentary microbial habitat highly prone to metabolic shifts induced by the transport-limited availability of alternate electron acceptors at different stream velocities.

This study, while exploring a simplified system, clearly illustrates the processes that influence nitrate reduction in surficial sediments. These observations provide the basis for an improved conceptual model for coupled nitrogen and oxygen dynamics in nitrate-enriched systems, as illustrated schematically in Fig. 4. At high stream velocities, hydrodynamic transport of oxygen can exceed metabolic consumption, leading to bulk oxic conditions throughout the bed even under dark conditions. Conversely, under low-transport conditions there can be little nitrate

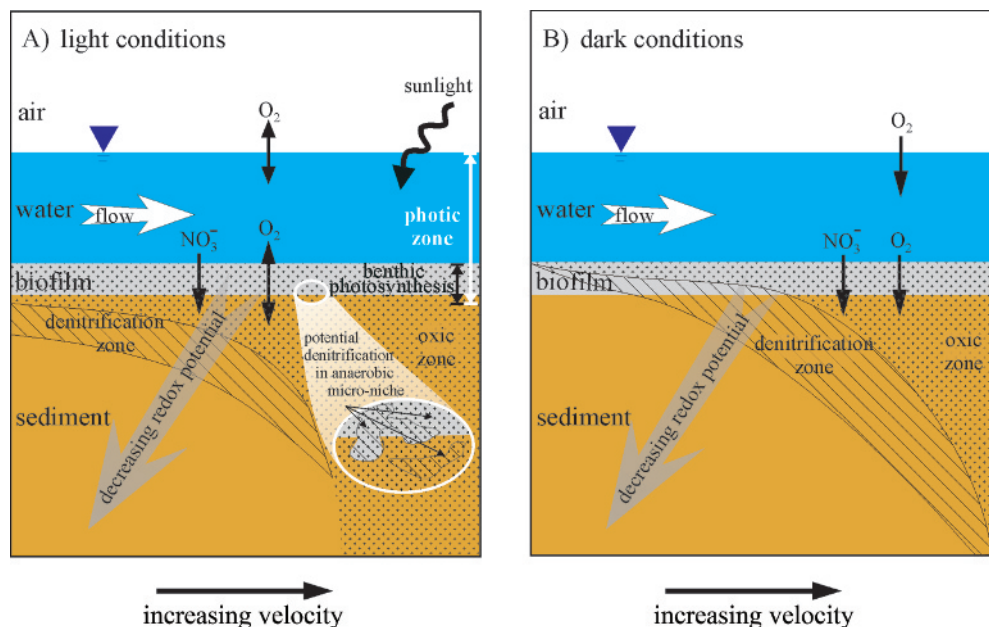


Fig. 4. Schematic illustration of the relationship between overlying velocity, benthic denitrification, and the physical-chemical structure of an aquatic system with oxygen- and nitrate-rich water column underlain by carbon- and biomass-rich sediments. Denitrification occurs only in regions where oxygen is depleted (hatched). Interfacial hydrodynamic transport increases with overlying velocity and enhances both nitrate and oxygen delivery to bacteria residing in the benthic biofilm and underlying sediments. (A) Under light conditions, benthic photosynthesis produces bulk oxic conditions in the near-surface sediments (dotted), so that denitrification occurs only in transport-limited anaerobic microniches or deeper regions of the benthic/hyporheic system. (B) Under dark conditions, residual photosynthetic oxygen is consumed by bacterial respiration, which can lead to formation of extensive regions with conditions favorable for denitrification. However, there is still mass transfer of oxygen from the atmosphere, through the water column, and into the sediments. When hydrodynamic transport rates are very high, benthic and hyporheic oxygen concentrations can remain high enough to suppress denitrification even under dark conditions.

delivery to anoxic regions, and therefore little opportunity for denitrification. Long-term average nitrate reduction rates, such as those shown in Fig. 1, reflect dynamic variation between the redox profiles that occur under alternating light and dark conditions.

This conceptual model suggests that a complex relationship exists between overlying flow conditions, interfacial solute transport, and the long-term average rate of nitrate reduction. Based on the results presented here, we hypothesize that hydrodynamic control of both nitrate and oxygen transport should result in optimal conditions for nitrate removal from the water column at intermediate velocities, as illustrated in Fig. 5. This relationship reflects the competing effects of two basic processes: hydrodynamic delivery of nitrate from the water column to denitrifying bacteria residing in benthic/hyporheic biofilms and suppression of denitrification when the biofilms become flushed with oxygen. Substantial reductions in whole-system nitrogen concentrations depend on denitrification and, ultimately, on the conversion of nitrogen to N_2 gas, which can exsolve from the system. While nitrogen dynamics can be very complex at the microscale and there can be considerable internal cycling of different nitrogen species within biofilms, removal of nitrate from the water column necessarily depends on transport to the benthic and

hyporheic microbial communities where most denitrification is known to occur (Duff and Triska 2000; Toet et al. 2003; Böhlke et al. 2004; Lefebvre et al. 2004). Therefore, bulk nitrate removal should normally be influenced by the hydrodynamic characteristics of the overlying flow. Further, because denitrification is a catabolic process and the organisms responsible for it are usually facultative anaerobes that preferentially perform aerobic metabolism when oxygen is available, nitrate reduction is also coupled to interfacial transport of oxygen as well as nitrate. When the benthic/hyporheic ecosystem becomes fully oxygenated, i.e., well flushed with oxygen because of high rates of transport from the water column, denitrification will be suppressed. The effect of these coupled processes on bulk nitrate removal from the water column is illustrated conceptually in Fig. 5.

This competition is expected to be important in shallow aquatic systems with high in-stream nitrate concentrations, which are of particular concern at the present time because of increasing nitrate inputs to surface waters worldwide (Alexander et al. 2000; Diaz 2001; Peterson et al. 2001). While denitrification rates have previously been observed to both increase and decrease with overlying velocity (Leu et al. 1998; Eriksson 2001), we believe that the complete relationship illustrated in Fig. 5 has not been observed

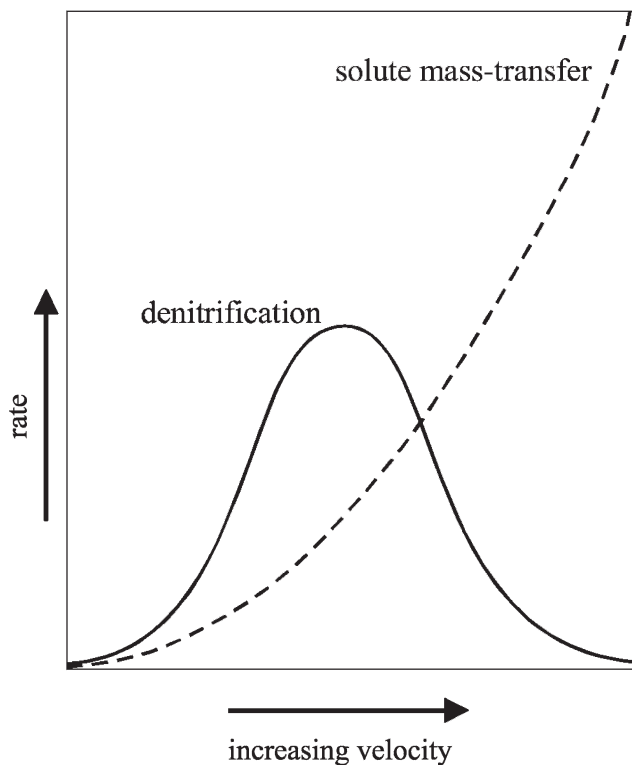


Fig. 5. Proposed relationship between overlying flow conditions, surface–subsurface solute exchange, and long-term average rate of bulk nitrate removal from the water column based on the conceptual model illustrated in Fig. 4. The maximum rate of nitrate removal is expected to occur at an intermediate velocity because benthic/hyporheic denitrification is limited by interfacial mass transfer of nitrate at low velocities, while denitrification is suppressed by the shift to aerobic metabolism induced by rapid interfacial transport of oxygen at high velocities. The exact form of the relationship between flow conditions and total nitrate reduction is not known and should depend on a wide range of factors including the composition of the microbial community, interactions with higher trophic levels (e.g., grazing), temporal variations in light levels and temperature, and disruption of the benthic/hyporheic system by episodic high-flow events. Nonetheless, the competing effects illustrated here are very basic and should be expected to be generally important in relatively shallow, slow-flow aquatic systems.

previously because nitrogen dynamics are normally investigated either under a relatively narrow range of overlying velocities under controlled conditions or over a very wide range of velocities under field conditions where other factors such as variability in the microbial community tend to obscure the basic process relationships. The coupling between oxygen and nitrogen dynamics found here is expected to be important in many aquatic systems because of the generality and robustness of bacterial metabolism of nitrate (Tiedje 1988; Christensen et al. 1990) but can, of course, be highly modulated or even overwhelmed by a wide variety of factors including the exact composition of the benthic microbial community, broader site conditions including channel morphology and the broader aquatic ecosystem, and temporal variation in temperature, light, and oxygen and nitrogen levels over

a wide variety of time scales. The results presented here are expected to be particularly relevant to many low-gradient streams as well as wetlands with relatively high velocities (e.g., riparian systems, and also constructed wetlands designed for nutrient removal). A considerable amount of additional study is required to better define the general relationship we suggest in Fig. 5, to determine the range of aquatic systems where this competition is important, and to assess the conditions under which other processes begin to dominate nitrogen dynamics.

The conceptual understanding of benthic/hyporheic biophysicochemical process coupling we have developed here can serve as the basis for improved quantitative modeling of nutrient dynamics. Because of current concerns regarding the effects of excess nitrogen introduced to river networks from urban centers or agriculture, there is a particular need for improved estimation of net nitrate removal within stream channels and riparian wetlands by denitrification. A comparison of several types of models for nitrogen transport in streams revealed that more detailed representations of nitrogen transformation processes and water flow paths produce predictions with considerably lower bias and higher precision (Alexander et al. 2002). However, modeling nutrient dynamics at regional scales, such as nitrate transport from headwater source areas through large watersheds to the coastal ocean, must rely on macroscale parameterization of variables such as channel length, water depth, and discharge (Alexander et al. 2000; Peterson et al. 2001; Alexander et al. 2002). Successful estimation of the effects of system modification on nitrate export from river systems will therefore require identification and parameterization of explicit relationships between macroscale physical state variables and microscale biogeochemical transformation rates. The pronounced coupling between physical, chemical, and microbial processes in the benthic and hyporheic regions requires that the critical structural features of these habitats be considered when analyzing bulk nitrogen dynamics in aquatic systems. It is particularly important to assess macroscale forcing of microscale chemical conditions, and the resulting regulation of microbial metabolism, in order to account for the way in which local process rates vary with larger system conditions. The results presented here specifically demonstrate that bulk nitrate removal rates cannot be accurately estimated without considering both the competing metabolic pathway provided by oxygen and the physical–chemical environment that defines the availability of metabolically important solutes to benthic and hyporheic microorganisms.

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