

# Nitrate retention in a sand plains stream and the importance of groundwater discharge

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**Abstract** We measured net nitrate retention by mass balance in a 700-m upwelling reach of a third-order sand plains stream, Emmons Creek, from January 2007 to November 2008. Surface water and groundwater fluxes of nitrate were determined from continuous records of discharge and from nitrate concentrations based on weekly and biweekly sampling at three surface water stations and in 23 in-stream piezometers, respectively. Surface water nitrate concentration in Emmons Creek was relatively high (mean of  $2.25 \text{ mg NO}_3\text{-N l}^{-1}$ ) and exhibited strong seasonal variation. Net nitrate retention averaged

$429 \text{ mg NO}_3\text{-N m}^{-2} \text{ d}^{-1}$  and about 2% of nitrate inputs to the reach. Net nitrate retention was highest during the spring and autumn when groundwater discharge was elevated. Groundwater discharge explained 57–65% of the variation in areal net nitrate retention. Specific discharge and groundwater nitrate concentration varied spatially. Weighting groundwater solute concentrations by specific discharge improved the water balance and resulted in higher estimates of nitrate retention. Our results suggest that groundwater inputs of nitrate can drive nitrate retention in streams with high groundwater discharge.

**Keywords** Focused discharge · Groundwater · Mass-balance · Nitrogen · Sediments

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## Introduction

Humans have dramatically altered the nitrogen cycle during the past several decades, doubling the amount of fixed nitrogen worldwide (Galloway et al. 2008, Schlesinger 2009). Global increases in fertilizer production and application and increases in nitrogen oxide generated by burning fossil fuels are major causes for increases in the amount of available nitrogen in ecosystems. Expansion of cropland for biofuel production may lead to further increases in nitrogen loading to surface water and groundwater in agricultural regions (Donner and Kucharik 2008).

These changes have resulted in increases in the fluxes and concentrations of nitrate in many streams and rivers throughout the world (Howarth et al. 1996; Donner et al. 2002). Increased nitrate loading by rivers has been linked to hypoxia and other types of degradation in downstream ecosystems (Rabalais et al. 2002, Diaz and Rosenberg 2008).

There is increasing evidence of substantial nitrate uptake and transformation in streams and rivers (Alexander et al. 2000; Bernhardt et al. 2003; Mulholland 2004; Mulholland et al. 2008). Accurate empirical estimates of nitrate retention in streams are critical for parameterizing large-scale models of nitrate retention and transport (Seitzinger et al. 2002) and for balancing global nitrogen budgets (Schlesinger 2009). Processes contributing to nitrate retention in streams include assimilatory uptake by autotrophs and by heterotrophic microbes (e.g. Stelzer et al. 2003) and dissimilatory uptake by microbes (Burgin and Hamilton 2007). Nitrate retention in streams can occur in surficial compartments (biofilms, periphyton) and in sediments, often associated with surface water-groundwater interfaces (Duff et al. 2008). Nitrate retention in sediments can occur where nitrate in surface water penetrates the hyporheic zone (Triska et al. 1989a) and where groundwater discharges to surface water (Chestnut and McDowell 2000, Puckett et al. 2008).

There are two main ways to assess nitrate retention in streams: (1) whole-reach nutrient releases (bulk forms of nitrate or  $^{15}\text{NO}_3$ ) and (2) mass balance. The nutrient release approach typically involves a short-term (<24 h) injection of nitrate into a stream. Nitrate added during a short-term release to the surface water may not come into contact with all zones of nitrate processing in the sediments, especially at depth in upwelling locations, which could underestimate or overestimate in-stream nitrate retention at the reach-scale (Triska et al. 1989b; Poole et al. 2008). The time for an injected solute to reach equilibrium with nitrate retention processes in stream sediments will depend on physical properties such as hydraulic conductivity of sediments and the direction of vertical hydraulic gradient (Valett et al. 1996). Advantages of adding labeled forms of nitrate to stream reaches include the ability to measure gross uptake rates and to determine mechanisms of nitrogen retention (e.g. denitrification). The mass balance approach typically estimates net nitrate retention as

the difference between input and output fluxes of nitrate in a stream and usually is based on a longer time period than estimates from solute injections, allowing for the assessment of temporal variability in nutrient retention. An advantage of the mass balance approach is the ability to incorporate nitrate processing at depth into models of nitrate retention at the reach scale. Most estimates of nitrate retention in streams are based on selected months or seasons during the year (e.g. Hill 1983; Burns 1998; Mulholland et al. 2008). Annual empirical estimates of nitrate retention in streams are uncommon (but see House et al. 2001; Hoellein et al. 2007; Roberts and Mulholland 2007).

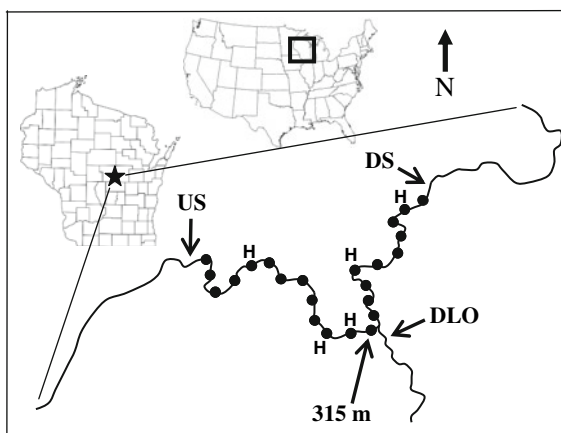
Nitrate retention in deeper sediments may be particularly important in groundwater-fed streams (Triska et al. 1984; Duff et al. 2008; Puckett et al. 2008). Streams draining watersheds with high permeability soils and sediments (e.g. sand plains) tend to receive considerable quantities of groundwater because of the high infiltration rates through the relatively coarse materials (Kraft and Stites 2003). Groundwater inputs to bedrock streams and those draining watersheds with low-porosity sediments tend to be lower. The concentration of nitrate in groundwater discharging to streams is often considerably different than the concentration in surface water (Valett et al. 1996; Kemp and Dodds 2001; Lewis et al. 2007). Thus, not accounting for groundwater inputs to a reach, or inaccurately representing them, particularly in streams where they are substantial, could have large effects on the rates of nutrient retention determined by mass balance studies. However, many mass balance studies of nitrate retention in streams do not consider groundwater inputs (e.g. Cooper and Cooke 1984; House et al. 2001; Brooks and Lemon 2007) or determine groundwater inputs of nitrate based on a small number of samples (Burns 1998) or by indirect measurements (Roberts and Mulholland 2007). Because of variability in the grain-size distribution of soils and sediments and its influence on hydraulic conductivity, discharge of groundwater within and among stream reaches is often not uniform (i.e. focused discharge, Lowry et al. 2007). Focused discharge may need to be considered when estimating nitrate retention, especially when there is variability in the nitrate concentration among groundwater flow paths that discharge to a stream.

We measured net nitrate retention by mass balance for almost 2 years in a primarily groundwater-fed sand plains stream in Central Wisconsin in the United States. Our main objectives were: (1) to quantify net nitrate retention at the reach-scale while incorporating extensive measurements of groundwater inputs through space and time and (2) to consider the influence of groundwater discharge on seasonal variation in net nitrate retention. Our study is the first, to our knowledge, to explicitly relate nitrate retention to temporal variation in groundwater discharge in a stream.

## Methods

### Site description

Emmons Creek is a third order stream located in the Central Sand Ridges Ecoregion in Central Wisconsin (Fig. 1). The terrain is flat to gently rolling and soils are sandy and well drained (Kraft and Stites 2003). The land cover in the Emmons Creek watershed is dominated by a mix of hardwood forests, oak savanna, and irrigated row-crop agriculture. The study reach was about 700 m in length with a mean wetted channel width of 4.9 m and wetted channel area of 3607 m<sup>2</sup>. The land bordering the reach for



**Fig. 1** A map of the 700-m study reach in Emmons Creek, Wisconsin bound by the Upstream Station (US, 44°17.780 N, 089°14.601 W) and the Downstream Station (DS, 44°17.827 N, 089°14.366 W). Deans Lake Outflow (DLO), the Emmons Creek surface water sampling location at 315 m, the locations of the piezometers in the longitudinal transect (filled circles) and horizontal transects (H) are indicated

several hundred meters on each side is mostly hardwood forest and grassland that was formerly in agriculture and is now part of the Emmons Creek Fishery Area. Emmons Creek is a mesic groundwater-fed stream (Poff and Ward 1989), which, like many sand plain streams, receives abundant supplies of groundwater from precipitation and irrigation water that recharges in upland areas, flows through the aquifer and then discharges to the stream (Browne and Guldan 2005). The study reach also receives surface water from a small tributary, Deans Lake Outflow (Fig. 1). Based on a water balance (see below) surface water discharge increases along the 700 m reach by 7% due to groundwater inputs and by 6% due to the tributary, on average. Based on a habitat survey we conducted in 2007, the substrate in the wetted channel of Emmons Creek is primarily sand (42%) although silt (30%) and gravel (10%) are also abundant. Areas of silt and plant beds, consisting primarily of *Veronica*, a submergent macrophyte, are common near the margins of the wetted channel. Thirteen percent of the stream bottom was covered in macrophytes during June 2007. Epilithic chlorophyll-*a* ranged from 60 to 160 mg m<sup>-2</sup> during 2006 and 2007 (Eggert, unpublished data). Mean annual temperature of the surface water is about 10°C (annual range is 3–14°C), typical of northern temperate streams. The surface water and groundwater at the sampling locations was oxalic, with moderate conductivity, and slightly alkaline pH (Table 1). Soluble reactive phosphorus concentration is very low (<4 μg PO<sub>4</sub>-P l<sup>-1</sup>). The total dissolved nitrogen (TDN) in Emmons Creek surface water and associated groundwater is dominated by nitrate (over 98% of TDN based on a limited number of TDN samples during the summer), and surface water nitrate concentration is relatively high (up to 2.6 mg NO<sub>3</sub>-N l<sup>-1</sup>, Table 1), which is typical of streams draining agricultural watersheds, including those in Wisconsin (Kraft and Stites 2003; Saad 2008; Stanley and Maxted 2008).

### Surface water stations and piezometers

Surface water monitoring stations were established at the upstream and downstream ends of the study reach (Upstream and Downstream Stations), and at Deans Lake Outflow (DLO), about 3 m upstream from the confluence with Emmons Creek, for hydrologic and

**Table 1** Chemical and physical characteristics of Emmons Creek at the Upstream and Downstream Stations, of Deans Lake Outflow, and of ground water sampled from the piezometers

Location	Discharge ( $l\ s^{-1}$ )	WT (°C)	DO ( $mg\ l^{-1}$ )	Cond ( $mS\ cm^{-1}$ )	pH	SRP (mg $PO_4^{3-}$ $P\ l^{-1}$ )	$Cl^{-}$ ( $mg\ l^{-1}$ )	$NO_3^{-}$ -N ( $mg\ l^{-1}$ )	$NH_4^{+}$ -N ( $mg\ l^{-1}$ )	DON ( $mg\ l^{-1}$ )	DOC ( $mg\ l^{-1}$ )
Upstream Station	403 (33)	9.1 (3.7)	10.5 (1.3)	373 (13)	7.8	0.003 (0.001)	3.14 (0.22)	2.29 (0.23)	0.010 (0.006)	0.011	2.26
Downstream Station	456 (40)	9.1 (3.9)	10.4 (2.1)	371 (13)	7.9	0.003 (0.001)	3.07 (0.21)	2.22 (0.24)	0.016 (0.012)	0.011	2.49
Deans Lake Outflow	25 (4)	13.0 (7.3)	9.2 (1.5)	325 (18)	8.3	0.002 (0.001)	2.94 (0.30)	1.48 (0.52)	0.088 (0.051)	0.030	3.42
Ground water	27 <sup>a</sup> (17)	13.0 (1.2)	7.3 (1.7)	356 (31)	7.9 (0.1)	–	1.87 (0.15)	2.57 <sup>b</sup> (0.08)	ND	0.016 (0.041)	0.92 (0.49)

WT water temperature, DO dissolved oxygen, Cond specific conductivity, SRP soluble reactive phosphorus, DON dissolved organic nitrogen, DOC dissolved organic carbon. Means and (SD) in most cases are for 2007–2008. SRP data are from 2006 to 2007. Ground water data for some parameters were limited to the following dates: WT, DO, Cond are from June and August 2007; pH,  $NH_4^{+}$ -N are from August 2007. DON and DOC data are from August 2007. ND not detectable ( $<0.005\ mg\ NH_4^{+}\ l^{-1}$ )

<sup>a</sup> Measured by difference

<sup>b</sup> Unweighted for specific discharge

chemical monitoring (Fig. 1). In addition, surface water samples were collected in Emmons Creek 315 m upstream of the Downstream Station which is near the midpoint of the reach and 5 m upstream of the DLO confluence. Twenty-three piezometers were installed in a longitudinal transect in the middle of the thalweg of Emmons Creek at about 30-m intervals at a mean sediment depth (to midscreen) of 48 cm. Each piezometer was constructed of CPVC (1.2 cm inner diameter). The terminal 4.5 cm was the screened interval which consisted of 3 mm holes covered with 100  $\mu m$  Nitex mesh. The piezometers were installed in the stream sediments in order to characterize the hydrologic and chemical properties of groundwater discharging to Emmons Creek through the stream bed, in keeping with the conceptual model by Browne and Guldan (2005) about the dynamics of groundwater discharge to streams in this region. In about half of the piezometers, frozen water (at the static head level above the streamwater surface) made sampling from these units difficult or impossible in the winter. A piezometer was installed adjacent to the primary piezometer in these cases, cut off at the stream water surface. Primary piezometers and the cut-off piezometers were used to collect groundwater samples during the winter. At five locations (60, 210, 345, 375, and 555 m upstream of the Downstream Station, Fig. 1) lateral transects of piezometers ( $n = 47$ , mean sediment depth 48 cm) were installed that spanned the wetted channel at 0.5 m intervals. The piezometer at or near the center of each lateral transect was also part of the longitudinal transect. The piezometers in the lateral transects were used to determine if nitrate concentration in groundwater sampled from piezometers in the longitudinal transect was representative of groundwater at similar depth throughout the wetted channel.

#### Nutrient sampling—surface water

Surface water was collected from the thalweg for nutrients about every week at the Upstream and Downstream Stations from September 2005 to December 2008 (172 sampling dates). Stream water was collected with 60 ml plastic syringes and filtered in the field (odd weeks) or collected in 2 l HDPE amber containers and returned to the lab for filtration (even weeks). Water was collected about weekly from Deans Lake Outflow from May 2006 to

December 2008 and filtered in the field. From November 2007 to December 2008 surface water samples from Emmons Creek were collected weekly at the 315 m sampling location and filtered in the field. All surface water samples were collected within a 1 h interval on odd weeks and within a 3 h interval on even weeks and were syringe filtered through Whatman GF/F filters (25 mm) into HDPE containers.

#### Nutrient sampling—groundwater

Groundwater was sampled from the longitudinal transect of piezometers at about 2 week intervals (even weeks) from January 2007 to December 2008 with a 60 ml syringe attached to polypropylene tubing (0.6 mm inner diameter). Groundwater from the lateral piezometer transects was sampled on October 10, 2007 using a Global Water peristaltic pump. A water volume equivalent to the volume of the piezometer was removed from each piezometer prior to sample collection. All groundwater samples were filtered through Whatman GF/F filters in the field into HDPE containers. All surface water and groundwater samples were transported on ice from the field to the lab within 10 h of collection and stored at  $-20^{\circ}\text{C}$ .

#### Hydrology—surface water

Discharge at the Upstream and Downstream Stations was determined using the dilution-gauging method (Gordon et al. 2004). Sodium bromide (typically  $0.6\text{ kg Br l}^{-1}$ ) was injected at a constant rate (typically about  $1\text{ ml s}^{-1}$ ) with a Watson-Marlow 504S peristaltic pump for several hours into a riffle at a constricted portion of the channel about 50 m upstream of the Upstream Station. After plateau concentration of bromide had been reached at the Downstream Station (determined by collecting samples in a time series during previous releases at varying discharge) water samples for bromide were collected at five points along lateral transects at the Upstream and Downstream Stations and syringe-filtered through Whatman GF/F (25 mm) in the field. Bromide releases were conducted eight times during 2007 and 2008 at varying discharge in Emmons Creek. Typically, multiple data points could be collected during a single bromide release, especially

during periods of rapidly changing discharge. Discharge at Deans Lake Outflow was determined using the velocity-area method. Water velocity was measured with a Price-type Pygmy current meter. An estimation of discharge using the dilution-gauging method in Deans Lake Outflow compared favorably (12% lower) to the estimate using the velocity-area method.

Stream stage at the Upstream and Downstream Stations was measured and logged every ten minutes during the mass-balance study using Solinst 3001 Leveloggers (A linear relationship between discharge at the Upstream and Downstream Stations during 2008 was used to estimate discharge at the Downstream Station for a period during Jan 8, 2007 to March 12, 2007 when the Downstream Levelogger was not functioning). Pressure measured underwater with the Leveloggers was corrected for changes in atmospheric pressure using a Solinst 3001 Barologger placed in the riparian zone. Rating curves were produced that related discharge to stage based on linear regression (mean  $r^2 = 0.90$ ). Stage at the Upstream and Downstream Stations was used to predict discharge at the Upstream and Downstream Stations respectively. Stage at the Upstream Station was used to predict discharge at Deans Lake Outflow. Separate rating curves were prepared for discharge estimates in 2007 ( $n = 13$  for Emmons Creek,  $n = 7$  for Deans Lake Outflow) and 2008 ( $n = 6$  for Emmons Creek and  $n = 4$  for Deans Lake Outflow). Groundwater discharge ( $G$ ) to Emmons Creek was determined by subtracting the combined discharge at the Upstream Station and at Deans Lake Outflow from discharge at the Downstream Station. This resulted in continuous estimates of discharge for Emmons Creek surface water (Upstream Station, Downstream Station), for Emmons Creek groundwater, and for Deans Lake Outflow.

#### Hydrology—groundwater

The position of the water level in the longitudinal piezometers relative to the level of the stream surface water was measured with a Solinst water level meter biweekly during 2007 and 2008. Vertical hydraulic gradient (VHG) was calculated at each piezometer location using the following equation (Dahm et al. 2006):

$$VHG = \frac{\Delta h}{\Delta L} \quad (1)$$

where  $\Delta h$  is the difference in head between the water level in the piezometer and at the stream surface,  $\Delta L$  is the distance between the top of the stream bed and the midpoint of the screened interval of the piezometer.

Vertical hydraulic gradient was not measured during winter when frozen water in the piezometers prevented determination of static head level. Horizontal hydraulic conductivity ( $K_h$ ;  $\text{cm s}^{-1}$ ) of the stream sediments was determined by using slug tests in the longitudinal piezometers on three occasions during summer of 2007. A fixed volume of groundwater was added to each piezometer, the return time to the static head level was recorded, and  $T_0$  was determined (Hvorslev 1951). We used the following equation (Dahm et al. 2006):

$$K_h = \frac{(r^2) \times \ln(L_p/R)}{2 \times L_p \times T_0} \quad (2)$$

where  $r$  is the radius of the piezometer,  $L_p$  length of screened area,  $R$  radius of screened area,  $T_0$  time lag for water to return to 37% of initial change in water level.

We assumed that the sediments were homogenous and isotropic within a piezometer sampling location and therefore set  $K_h$  equal to vertical hydraulic conductivity ( $K_v$ ). Specific discharge ( $q$ ;  $\text{cm}^3 \text{cm}^{-2} \text{s}^{-1}$ ) was calculated for each piezometer location based on the vertical hydraulic conductivity and VHG using the following equation:

$$q = K_v \left( \frac{\Delta h}{\Delta L} \right) \quad (3)$$

We estimated the contribution of surface water and groundwater to shallow porewater using a two-source mixing model with chloride concentrations (Hill and Lymburner 1998). We sampled sediment porewater (5–10 cm sediment depth), water from the piezometers (assumed to represent groundwater), and surface water at each piezometer location on May 28, 2009 for chloride concentration. Porewater was collected with a modified mini-point sampler (after Duff et al. 1998). Piezometers and surface water were sampled as described previously. All samples were syringe-filtered through Whatman GF/F (25 mm). Only those porewater samples with chloride concentration constrained by the surface water and groundwater end members (17 of 20 cases) were considered.

## Nutrient analysis

Nitrate, chloride, and bromide were measured using a Dionex ICS-1000 ion chromatograph equipped with an IonPac AS14A column. Ammonium and soluble reactive phosphorus were measured colorimetrically after APHA (1992) and Solarzano (1969) respectively, with a Thermo Spectronic Aquamate spectrophotometer. Total dissolved nitrogen (TDN) and dissolved organic carbon were measured on a Shimadzu (Kyoto, Japan) TOC-V CPH+TNM with an ASI-V autosampler using the nonpurgable organic carbon (NPOC)/total nitrogen analysis. Dissolved organic nitrogen (DON) was determined by subtracting nitrate and ammonium concentrations from TDN. Quality control procedures during ion chromatography included injections of NIST (U.S. National Institute of Standards and Technology) traceable standards (recovery was typically  $100 \pm 2\%$ ) and routine duplicate analysis of samples (CV was consistently less than 0.5%).

During even sampling weeks throughout the year the specific conductivity, dissolved oxygen, and water temperature at the Upstream and Downstream Stations and at Deans Lake Outflow were measured with a YSI 85 field meter. These parameters were measured in groundwater collected from the longitudinal piezometers in June and August in 2007 (Table 1).

## Mass balance model

The ecosystem boundaries of the study reach were defined longitudinally by the Upstream and Downstream Stations, laterally by the edges of the wetted channel, and vertically as the mean midscreen depth of the piezometers (ca. 48 cm). Net nitrate retention ( $R_f$ ) in the reach was determined by mass balance using the following model:

$$R_f = U_f + G_f + T_f - D_f \quad (4)$$

where  $U_f$  is the nitrate flux at the Upstream Station,  $G_f$  is the nitrate flux in groundwater,  $T_f$  is the nitrate flux in the tributary (Deans Lake Outflow),  $D_f$  is the nitrate flux at the Downstream Station.

Surface water fluxes ( $U_f$ ,  $D_f$ , and  $T_f$ ) and groundwater flux ( $G_f$ ) were determined daily by the time integration method (Stelzer and Likens 2006) between January 8, 2007 and November 10, 2008. For days on which nutrient concentrations were

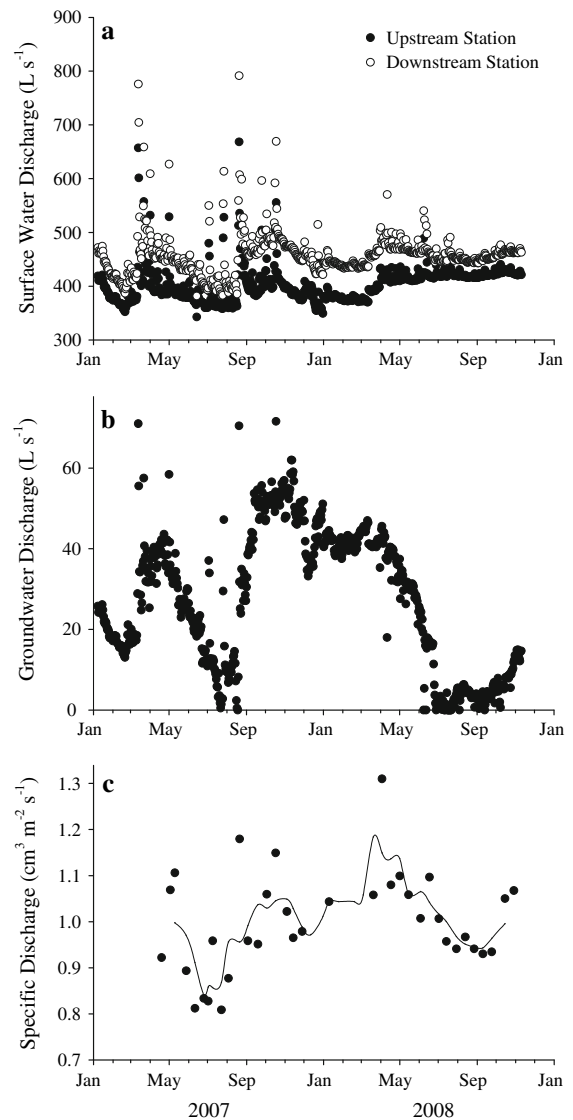
measured, daily fluxes were calculated by multiplying nutrient concentration by mean daily discharge. For days without nutrient concentration data, the mean of the two adjacent measured concentrations in the time series was multiplied by the mean daily discharge to determine fluxes. Groundwater nutrient concentrations were weighted by specific discharge ( $q$ ), an approach that was used in a study of nutrient retention in a tropical stream (Chestnut and McDowell 2000). Only groundwater nutrient concentrations from the longitudinal transect of piezometers were used in the mass balance model. We expressed nitrate retention per area of stream bottom in the wetted channel (areal net retention rate) and as a percentage of the sum of all inputs ( $U_f$ ,  $G_f$ ,  $T_f$ ) to the reach. Net retention of chloride was determined using this model to assess water balance. If chloride behaved conservatively in the reach, we would predict chloride retention to be zero.

We made several assumptions when determining net nitrate retention with the mass-balance model: (1) Groundwater discharged to surface water throughout the entire reach, and (2) there was no loss of surface water from the stream channel laterally or to deep groundwater. Nitrate wet deposition directly on the wetted stream channel was not included as an input in the mass balance model. The solute sampling regime did not target storm events. Because surface water nitrate concentration tends to decrease as surface water discharge increases in Emmons Creek (Stelzer, unpublished data), nitrate flux was probably overestimated during storm events. This probably resulted in increased uncertainty in estimates of nitrate retention during high flow periods. However, Emmons Creek exhibits very low hydrological flashiness ( $F = 0.025$ , based on the Richards-Baker flashiness index (Baker et al. 2004)) and baseflow accounts for most of the annual discharge. Thus, a sampling regime that included targeting stormflow events probably would not change our overall conclusions about nitrate retention in Emmons Creek.

## Results

### Hydrology

Emmons Creek has a hydrograph typical of a mesic groundwater stream (Fig. 2a). Most discharge peaks



**Fig. 2** **a** Mean daily surface water discharge at the Upstream and Downstream Stations in Emmons Creek. **b** Mean daily groundwater discharge to Emmons Creek measured as the difference between Emmons Downstream and the sum of Emmons Upstream and Deans Lake Outflow. **c** Specific discharge ( $q$ ) of groundwater to Emmons Creek. The curve fit represents a four-time unit (about 2 months) running average

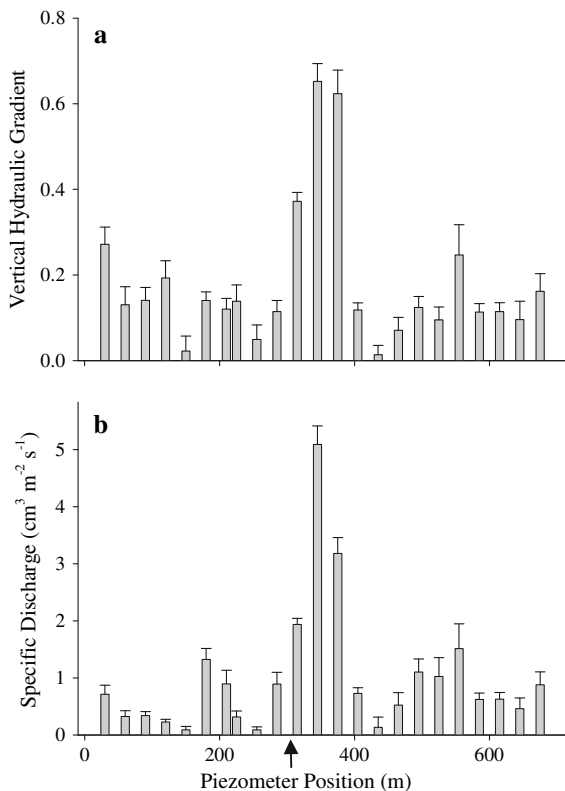
in March and April were associated with snowmelt. Large storm events, such as those in August and September of 2007, tended to be followed by higher baseflow discharge, presumably due to increased groundwater recharge. Surface water discharge tended to decrease throughout the growing season, especially in 2007. Groundwater discharge ( $G$ ) to the

reach, measured by difference, tended to be high in spring and autumn and low in the summer (Fig. 2b). In 2007 and 2008 steady, sharp decreases in groundwater discharge occurred from May through August. The specific discharge ( $q$ ) time series was generally consistent with the indirect measurements of groundwater discharge (Fig. 2b, c). Vertical hydraulic gradient (VHG) was consistently positive throughout the reach and averaged 0.18 (Fig. 3a). These results are consistent with the gain in surface water discharge along the reach (Fig. 2a). Specific discharge ( $q$ ) showed a similar pattern to that of VHG (Fig. 3b). Differences in the spatial pattern of VHG and  $q$  were due to variation in hydraulic conductivity among piezometer locations. The relatively high values of  $q$  in the middle portion of the reach (315–375 m, Fig. 3b) suggested that focused discharge of groundwater occurred at that location. The chloride mixing model showed that groundwater contributed 90%, on

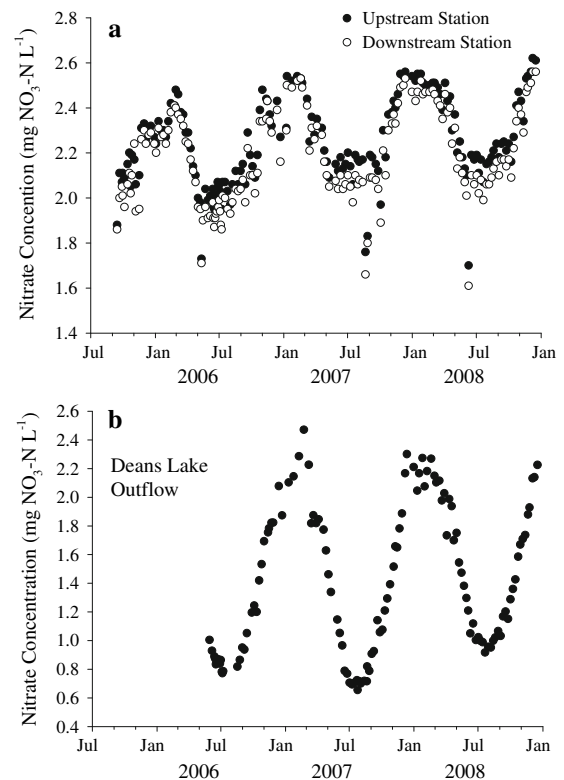
average ( $\pm 7\%$  SD,  $n = 17$ ), to pore water at 5 to 10 cm depth throughout the reach.

### Nutrient concentrations

Surface water nitrate concentrations showed a strong and repeatable pattern of seasonal variation at both Emmons Upstream and Downstream Stations (Fig. 4a). The highest concentrations occurred during winter and the lowest concentrations occurred during the summer. Nitrate concentration in Deans Lake Outflow showed strong seasonal variation and its time series had greater amplitudes than the Emmons Creek surface water time series (Fig. 4b). There was less seasonal variation in groundwater nitrate concentration than in Emmons Creek surface water (Fig. 4a, 5a). Mean groundwater nitrate concentration ( $2.57 \text{ mg NO}_3\text{-N l}^{-1}$ ) was higher than mean surface water nitrate concentration ( $2.25 \text{ mg NO}_3\text{-N l}^{-1}$ )

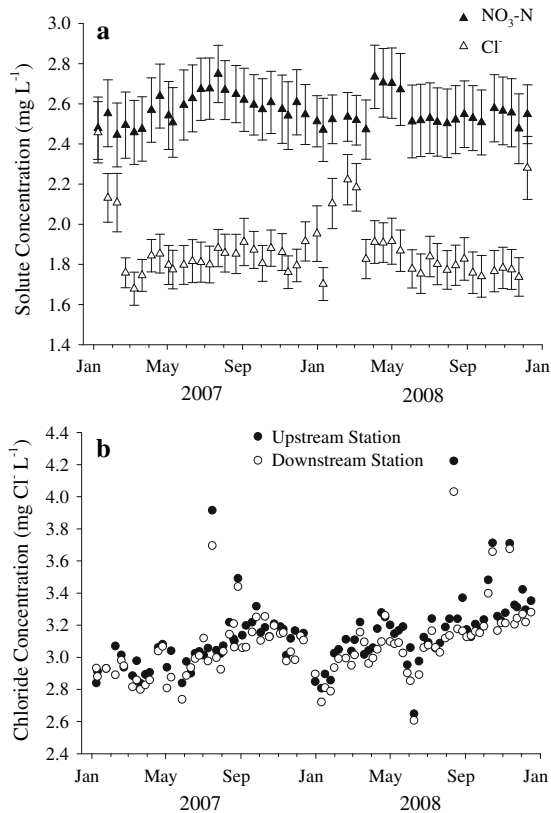


**Fig. 3** **a** Mean (+SD) vertical hydraulic gradient (VHG) and **b** Mean (+SD) specific discharge ( $q$ ) by piezometer position as distance from the Downstream Station. The time period is 8 January 2007 to 24 November 2008 for VHG and  $q$ . The position of Deans Lake Outflow is indicated with an *arrow*



**Fig. 4** **a** Surface water nitrate concentration in Emmons Creek at the Upstream and Downstream Stations. Outliers from 20 August 2007 for the Upstream and Downstream Stations ( $1.10$  and  $1.05 \text{ mg NO}_3\text{-N l}^{-1}$ ) are not shown. **b** Surface water nitrate concentration in Deans Lake Outflow





**Fig. 5** **a** Mean ( $\pm$ SE) groundwater nitrate and chloride concentrations from piezometers. **b** Surface water chloride concentration in Emmons Creek at the Upstream and Downstream Stations

during the 2-year mass balance study (Fig. 4a, 5a; Table 1; Paired *t*-test  $P < 0.001$ ).

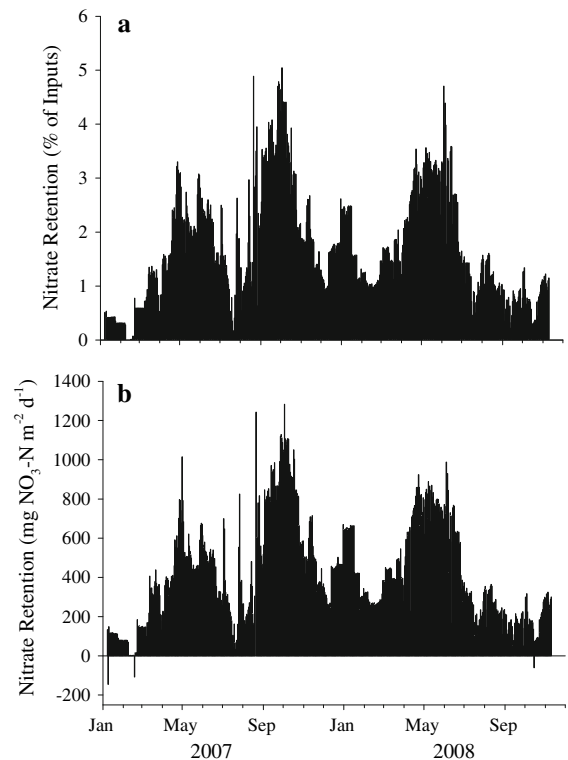
There was moderate variation in groundwater nitrate concentration among piezometer locations in the longitudinal transect with unweighted means per location ranging from 0.37 to 3.34 mg NO<sub>3</sub>-N l<sup>-1</sup>. Groundwater from piezometers with the highest *q* (at 315–345 m, Fig. 3b) had relatively high nitrate concentrations. This largely contributed to the higher overall mean nitrate concentration in groundwater when concentrations were weighted by *q* (2.69 mg NO<sub>3</sub>-N l<sup>-1</sup>) than when they were unweighted (2.57 mg NO<sub>3</sub>-N l<sup>-1</sup>) (Paired *t*-test  $P < 0.001$ ). Mean nitrate concentration of groundwater collected from the lateral piezometer transects was similar to the mean nitrate concentration from those piezometers in the lateral transects that were also part of the longitudinal transect (2.50 vs. 2.61 mg NO<sub>3</sub>-N l<sup>-1</sup>; Mann–Whitney  $P > 0.05$ ). This result suggests that

the nitrate concentration of the groundwater collected from the longitudinal piezometers was representative of groundwater at similar depth throughout the wetted channel.

There was less seasonal variation in chloride concentration in Emmons Creek surface water than in nitrate concentration (Fig. 5b). Chloride concentration in groundwater tended to be relatively high during a few dates in the winter but stayed within 1.6 and 1.9 mg l<sup>-1</sup> during most of the year (Fig. 5a).

#### Nitrate retention and flux

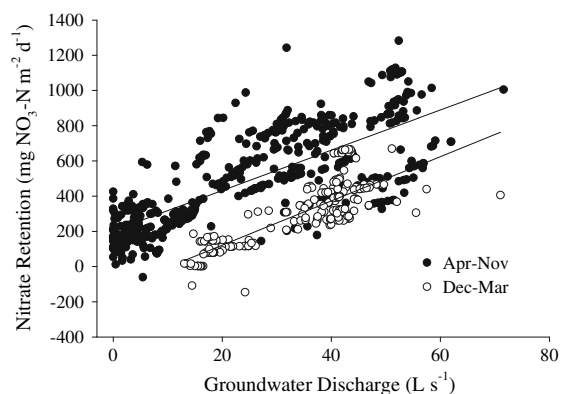
Net nitrate retention as a percentage of all inputs to the reach was 2–4% during the late spring of both years and in autumn of 2007 and typically 1% or less during the summer and winter (Fig. 6a). Mean net nitrate retention was 1.7% during the 2-year study. Mean net nitrate retention was 1.4% when groundwater nitrate concentration was not weighted by



**Fig. 6** Mean daily net nitrate retention in Emmons Creek as a percentage of nitrate inputs to the study reach (**a**) and as an areal rate (**b**). Outliers from 20 August 2007 (13.5% in **a**, 3136 mg NO<sub>3</sub>-N m<sup>-2</sup> d<sup>-1</sup> in **b**) are not shown

specific discharge. Chloride retention as a percentage of inputs was variable throughout the study but averaged  $-0.5$  and  $-0.8\%$  when weighted and unweighted by specific discharge, respectively. Mean net nitrate retention was  $18\%$  when it was calculated as a percentage of inputs that increase discharge (groundwater and Deans Lake Outflow) from upstream to downstream in the study reach. Areal net nitrate retention (mean of  $429 \text{ mg NO}_3\text{-N m}^{-2} \text{ d}^{-1}$ ) tended to be high during spring and autumn and low during winter and summer (Fig. 6b). Areal net nitrate retention showed a peak during April, May, and early June during both 2007 and 2008. A large peak in net nitrate retention occurred during autumn 2007 but retention was much more muted during autumn 2008.

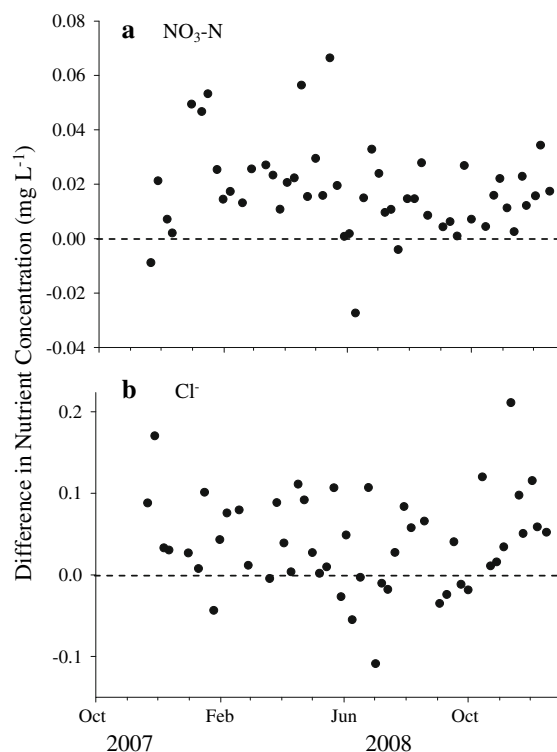
Areal net nitrate retention was positively correlated with the magnitude of groundwater discharge (Fig. 7). Most data points for the warmer months (April through November) clustered separately from data points for the colder months (December through March). Groundwater discharge explained  $57\%$  of the variation in net nitrate retention during April through November and  $65\%$  during December through March. The slopes of the linear regressions of net nitrate retention on groundwater discharge were similar for Apr–Nov ( $11.4$ ) and Dec–Mar ( $12.6$ ) and each regression was highly significant ( $P < 0.001$ ). At a given



**Fig. 7** The relationship between mean daily net nitrate retention and groundwater discharge in Emmons Creek for warmer months (April through November, slope =  $11.4$ ,  $y$ -intercept =  $203$ ,  $r^2 = 0.57$ ) and colder months (December through March, slope =  $12.6$ ,  $y$ -intercept =  $-130$ ,  $r^2 = 0.65$ ) from 8 January 2007 to 10 November 2008. An outlier from 20 August 2007 ( $3136 \text{ mg NO}_3\text{-N m}^{-2} \text{ d}^{-1}$ ,  $70 \text{ l s}^{-1}$ ) was not included in the regression

groundwater discharge, net nitrate retention tended to be higher during the warmer months. For example at about  $20 \text{ l s}^{-1}$  net nitrate retention was three to six-fold higher during the warmer months. At about  $40 \text{ l s}^{-1}$ , net retention was two to three-fold higher during the warmer months.

A comparison of solute concentrations in surface water between the Upstream Station and at  $315 \text{ m}$  (the upper portion of the reach unaffected by Deans Lake Outflow, Fig. 1) revealed that nitrate and chloride concentrations were mostly higher at the Upstream Station than at  $315 \text{ m}$  (i.e. a positive difference in Fig. 8a, b). Given that mean groundwater nitrate concentration in the portion of the reach upstream of the  $315 \text{ m}$  location (between  $315$  and  $675 \text{ m}$ ) was *higher* than that of surface water at the Upstream Station ( $2.42$  vs.  $2.29 \text{ mg NO}_3\text{-N l}^{-1}$ ), the decrease in surface water nitrate concentration from the Upstream Station to  $315 \text{ m}$  suggests that net nitrate retention occurred. The decline in chloride



**Fig. 8** The difference in nutrient concentrations between the Emmons Upstream Station and the  $315 \text{ m}$  surface water location, which is immediately upstream of Deans Lake Outflow (a positive value indicates that concentration is higher at the Upstream Station) for nitrate (a) and chloride (b)

concentration between the Upstream Station and 315 m (Fig. 8b) is consistent with conservative mixing of chloride between upwelling groundwater (mean  $1.83 \text{ mg Cl}^{-1} \text{ l}^{-1}$  between 315 and 675 m) and surface water (mean  $3.14 \text{ mg Cl}^{-1} \text{ l}^{-1}$  at Upstream Station).

## Discussion

### Hydrology

The consistently positive vertical hydraulic gradient throughout the study reach and the results of the chloride mixing model (90% contribution of groundwater to porewater at 5–10 cm sediment depth) suggest that the water sampled from the piezometers represented groundwater that moved upward through the sediments before discharging to the surface water. The results of the mixing model suggest that the hyporheic zone (where surface water and groundwater mix) is very shallow in Emmons Creek.

### Nitrate retention

There was low net nitrate retention in Emmons Creek when expressed as a percentage of all inputs to the study reach. However, because nitrate fluxes were high, low nitrate retention expressed as a percentage of inputs translated to substantial nitrate retention on an areal basis. These rates were relatively high compared to those reported from other agriculturally influenced streams (e.g. Cooper 1990; Bernot et al. 2006; Mulholland et al. 2008). The relatively low mean chloride retention suggested that we quantified the important water fluxes in the reach. The decline in nitrate concentration from the Emmons Upstream Station to the 315 m location, despite the higher nitrate concentration in groundwater than in surface water, provided additional independent support that net nitrate retention occurred in Emmons Creek. Chestnut and McDowell (2000) used a similar method to infer net nutrient uptake based on longitudinal comparisons of surface water nutrients in a tropical stream.

Nitrate retention showed substantial seasonal variation. Peaks in net nitrate retention tended to occur in the spring and autumn. Our results suggest that the

high groundwater discharge and moderate temperatures during this period led to high net nitrate retention. Fifty-seven percent of the variation in areal net nitrate retention was explained by the magnitude of groundwater discharge in the warmer months, including most of spring and autumn (Fig. 7). We hypothesize that much of the nitrate retention in Emmons Creek occurs in sediments, particularly in organic rich sediments found abundantly at the wetted channel margins and associated with numerous plant beds. We think that nitrate retention processes (Triska et al. 1989a; Burgin and Hamilton 2007) in these sediments are limited by the flux of upwelling nitrate-rich groundwater to which microbes are exposed. If nitrate processing is limited by the flux of nitrate in groundwater, periods of high groundwater nitrate flux would tend to result in higher rates of net nitrate retention at the reach scale. The higher net nitrate retention in the warmer months than in the colder months at similar groundwater discharge, suggests that temperature-driven metabolic processes influenced nitrate processing rates in Emmons Creek.

Net nitrate retention was marginally higher and net chloride retention was closer to zero when groundwater nitrate and chloride concentration were weighted by specific discharge ( $q$ ). The difference in nitrate retention is attributable to relatively high nitrate concentration in groundwater sampled from piezometers with high  $q$ . In situations where there is larger spatial variation in specific discharge and/or nutrient concentrations among groundwater flow paths, weighting groundwater nutrient concentrations by  $q$  could have larger effects on estimates of nutrient retention. Information derived from detailed process studies of groundwater–surface water exchange (e.g. Poole et al. 2008) will also help determine when it is necessary to weight groundwater inputs by specific discharge.

Despite predictions from theory, many investigators have found a weak or no relationship between nutrient retention in streams and hydrologic properties such as transient storage (e.g. Martí et al. 1997; Hall et al. 2002; Roberts et al. 2007). However, other studies have linked nitrate retention in streams to hydrologic parameters including vertical hydraulic gradient (Valett et al. 1994), hydrologic retention (Valett et al. 1996) and hydrologic exchange (Puckett et al. 2008). Valett et al. (1994) showed that nitrate

concentration in surface water was related to spatial variation in the magnitude and direction of vertical hydraulic gradient in Sycamore Creek. Groundwater with relatively high nitrate concentration discharged to stream water with relatively low nitrate concentration. Although the magnitude of vertical hydraulic gradient varied spatially in the study reach of Emmons Creek, the direction of exchange between surface water and groundwater (i.e. upwelling) was more uniform than in Sycamore Creek. Based on a study of nitrate transport in the hyporheic zones of nitrate-rich agricultural streams, Puckett et al. (2008) suggest that physical properties of sediments affect nitrogen processing by influencing hydrological exchange between surface water and groundwater. Collectively, these results suggest that both the magnitude and direction of advective transport between surface water and groundwater need to be considered when using mass balance approaches to estimate nutrient retention in streams.

### Mechanisms

Potential mechanisms driving nitrate retention in Emmons Cr. include assimilatory and dissimilatory uptake by various processes (Hall and Tank 2003; Burgin and Hamilton 2007; Hoellein et al. 2007; Roberts and Mulholland 2007; Arango et al. 2008). As stated previously, the positive relationship between net nitrate retention and groundwater discharge suggest that processes occurring in the sediment played an important role in nitrate retention. Assimilatory uptake of nitrate by algae, by rooted aquatic plants (Cooper 1990; Birgand et al. 2007) and by sediment-dwelling bacteria all may have contributed to nitrate retention. Because the study reach exhibited consistent upwelling, plant beds and heterotrophic bacteria in the sediments probably received increased supply of nitrate when groundwater discharge was high.

Of the dissimilatory processes of nitrate uptake, denitrification is probably the most likely mechanism that could account for substantial nitrate retention (or removal) in Emmons Creek. Denitrification has been shown to be an important nitrate sink in streams at the reach scale (e.g. Laursen and Seitzinger 2004; Mulholland et al. 2008). In situ or lab-based incubations using cores also suggest that denitrification can be an important mechanism for

nitrate retention in shallow sediments under certain geochemical conditions (e.g. Richardson et al. 2004). Although the groundwater sampled from the piezometers in Emmons Creek was oxic (Table 1), several studies have suggested that denitrification can proceed at anoxic microsites in an otherwise fully oxic environment as long as other essential conditions are met (Fenchel et al. 1998; Mehnert et al. 2007). These conditions include a supply of nitrate, a carbon source, and a suitable electron donor, of which organic carbon and various metals (Puckett et al. 2008) can qualify. In addition, preliminary data from Emmons Creek on dissolved oxygen concentration of upwelling groundwater in the organic-rich sediments at the stream margins suggests that it is very low ( $< 1 \text{ mg O}_2 \text{ l}^{-1}$ ). We also have preliminary data (Stelzer, unpublished) that suggests denitrification rates are high in the fine (silty) sediments of the stream and that there are sharp gradients in porewater nitrate concentration (from high to low) along upwelling flowpaths. These data suggest that processes in the sediments, and denitrification in particular, may account for considerable nitrate retention in Emmons Creek. We compared published denitrification rates measured at reach scales to our estimates of areal net nitrate retention to explore whether denitrification could have been a major contributor to nitrate retention in Emmons Creek. Areal denitrification rates estimated in rivers using a variety of methods were considerably less than our net nitrate retention rate in some studies (Mulholland et al. 2008; Smith et al. 2008) and approached or exceeded our rate in other cases (Laursen and Seitzinger 2004; Pribyl et al. 2005). These results suggest that denitrification could account for all or a considerable amount of the nitrate retention in Emmons Creek.

### Stream ecosystem comparison

Net nitrate retention in Emmons Creek, expressed as a percentage of all inputs to the reach, was relatively *low* compared to several other mass balance studies (Hill 1983; Burns 1998; Mulholland 2004; Duff et al. 2008). In general, surface water nitrate concentrations were lower in these studies than in Emmons Creek, which may have contributed to the differences.

Net nitrate retention in Emmons Creek, expressed on an areal basis, was relatively *high* compared to

many published studies of nitrate retention estimated by mass balance or by nutrient release (Table 2). Mean net areal nitrate retention in Emmons Creek was higher than the mean rates in several mass balance studies of similar duration (Cooper 1990; Bernhardt et al. 2003; Roberts and Mulholland 2007) but lower than the rates measured for two other studies (Cooper and Cooke 1984, House et al. 2001). Estimates of net nitrate uptake in Emmons Creek were also higher than most studies of nitrate uptake based on releases of bulk or  $^{15}\text{N}$ -enriched nitrate. A notable exception is the study by Hoellein et al. (2007) who found very high net nitrate uptake rates in forested streams in northern Michigan, USA (Table 2). The high nitrate concentrations in groundwater and surface water in Emmons Creek may have contributed to the relatively high areal nitrate retention rates. Comparisons across diverse stream ecosystems have shown positive relationships between nutrient uptake per unit area and nutrient concentration (Dodds et al. 2002; Mulholland et al. 2008). In addition, because the piezometers were placed at moderate sediment depth (ca. 48 cm) our estimates of net nitrate retention probably integrated processes occurring at a deeper sediment depth than in some

studies based on reach-scale nitrate releases, in which the duration of the solute injection and hydrologic properties can limit the penetration depth of added solutes (Hill and Lymburner 1998). It is likely that these factors contributed to the higher estimates of net areal nitrate uptake in Emmons Creek than in many previous studies. Our inclusion of deeper sediments within the stream ecosystem boundaries should be considered when comparing our estimates of nitrate retention with those from other studies, particularly studies based on solute injections.

#### Nitrate time series

Emmons Creek exhibits strong and repeatable seasonal variation in nitrate concentration but low variation at shorter temporal scales within a season, particularly during summer and winter. Many streams in nitrogen-rich watersheds show much greater variation at shorter (daily to weekly) and longer (e.g. seasonal) temporal scales (Kemp and Dodds 2001; Royer et al. 2004; Mehnert et al. 2007). Variation in precipitation, soil properties (e.g. porosity), degree of hydrologic alteration (e.g. tile drains), and the amount and timing of nitrogen availability

**Table 2** Published nitrate uptake rates in streams representing a variety of ecosystem types and stream sizes

Watershed type	Method	Study period	Nitrate uptake ( $\text{mg NO}_3\text{-N m}^{-2} \text{d}^{-1}$ )	Reference
Grassland	Mass balance	sp-aut	564	Cooper and Cooke (1984)
Agricultural	Mass balance	1 year	3	Cooper (1990)
Grassland	Mass balance	3 years	723 <sup>a</sup>	House et al. (2001)
Forested	Mass balance	2 years	104	Bernhardt et al. (2003)
Forested	Mass balance	2 years	-11 to 79 <sup>b</sup>	Roberts and Mulholland (2007)
Forested	Mass balance	2 years	429	Current study
Forested	$\text{NO}_3^-$	su-aut	75	Bernhardt et al. (2002)
Various	Various	Various	126 <sup>c</sup>	Ensign and Doyle (2006)
Agricultural	$^{15}\text{NO}_3^-$	2 years	1 <sup>a</sup>	Bernot et al. (2006)
Forested	$\text{NO}_3^-$	1 year	1360	Hoellein et al. (2007)
Various	$^{15}\text{NO}_3^-$	sp-su	19 <sup>d</sup>	Mulholland et al. (2008)
Agricultural	$^{15}\text{NO}_3^-$	sp-su	120 <sup>e</sup>	Mulholland et al. (2008)

Nitrate uptake is net for mass balance and bulk nitrate addition studies and gross for  $^{15}\text{NO}_3^-$  additions.  $\text{NO}_3^-$  and  $^{15}\text{NO}_3^-$  indicate form of nutrient added at the reach-scale. *sp* spring, *su* summer, *aut* autumn. Data are means unless otherwise indicated

<sup>a</sup> Measured during summer

<sup>b</sup> Range (mean not reported)

<sup>c</sup> Mean from third order streams

<sup>d</sup> Median of 22 streams in relatively undisturbed watersheds

<sup>e</sup> Median of 23 streams

contribute to these differences among stream ecosystems. Many streams in agricultural watersheds with extensive hydrologic alteration have high temporal variation in streamwater nitrate concentration. The Emmons Creek watershed has a history of agricultural activity. However, the sandy soils and relatively flat topography in this region limit surface runoff and promote infiltration of precipitation and irrigation water which results in relatively long time periods between recharge and discharge (Saad 2008). These factors probably buffer Emmons Creek against temporal variation in nitrogen supply (e.g. fertilizer application) seen in more runoff-dominated watersheds.

The seasonal variation in surface water nitrate concentration in Emmons Creek is probably driven by a combination of nitrate uptake in the stream and in the riparian zone. We suggest that in the winter Emmons Creek surface water is essentially groundwater that has been minimally modified by in-stream processes (the mean nitrate concentrations are strikingly similar between surface water, 2.46 mg NO<sub>3</sub>-N l<sup>-1</sup>, and groundwater, 2.49 mg NO<sub>3</sub>-N l<sup>-1</sup>, during the winter months, Paired *t*-test *P* = 0.20) due to slow rates of metabolism limited by cold temperatures. In the summer months, surface water nitrate concentration is lower than groundwater nitrate concentration (means are 2.06 vs. 2.60 mg NO<sub>3</sub>-N l<sup>-1</sup>, Paired *t*-test *P* < 0.001). It is well known that nitrate processing in riparian zones and in more upland areas can reduce the nitrate concentration along groundwater flow paths discharging to streams (e.g. Duff et al. 2007). However, the pattern of variation in nitrate concentration in the groundwater time series from Emmons Creek (Fig. 5a) does not suggest a strong riparian influence. Groundwater nitrate concentrations are not lower during the growing season than at other times of the year. The relatively high nitrate retention in Emmons Creek suggests that in-stream processes could contribute to the temporal variation in stream water nitrate concentration (Bernhardt et al. 2005). Seasonal variation in nitrate supply to Emmons Creek upstream of the study reach (e.g. high nitrate groundwater flow paths going dry in the summer) could also influence seasonal variation in surface water nitrate concentration. To further assess the roles of in-stream and upland processes on the seasonal nitrate decline in streams such as Emmons

Creek, assessments of nitrate processing at the watershed scale, coupled with in-stream nitrogen budgets, would probably be useful.

#### Implications for nitrogen removal from ecosystems

Our results agree with a growing number of studies that show many streams retain significant amounts of available nitrogen and need to be considered in whole-watershed nitrogen budgets (Alexander et al. 2000; Mulholland et al. 2008). It is likely that groundwater and surface water concentrations of nitrate associated with Emmons Creek and many other streams in the agricultural Midwestern United States are much higher than those before widespread human settlement and intensive agricultural activity (Smith et al. 2003). Projections of increasing human population size and increases in nitrogen fertilizer use at global scales (Donner and Kucharik 2008) suggest that amounts of fixed nitrogen added to ecosystems will continue to increase. Riparian and upland zones can remove substantial amounts of available nitrogen as groundwater and overland flow move from upland areas to streams (Hedin et al. 1998, Duff et al. 2007), and best management plans for ecosystems usually include maintenance of healthy riparian zones (Osborne and Kovacic 1993). Our work and that of others who have examined groundwater inputs of nitrate to streams suggest that a considerable amount of nitrogen can be retained or transformed along groundwater flow paths upwelling through stream sediments (Duff et al. 2008, Puckett et al. 2008). We suggest that more attention be given to stream sediments as potential hot spots for nitrate removal, especially in groundwater-fed streams. In particular, not accounting for nitrogen processing in deep sediments may lead to underestimates of nitrate retention in streams.

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