The dark side of the hyporheic zone: depth profiles of nitrogen and its processing in stream sediments

ROBERT S. STELZER*, LYNN A. BARTSCH[†], WILLIAM B. RICHARDSON[†]
AND ERIC A. STRAUSS[‡]

Department of Biology and Microbiology, University of Wisconsin Oshkosh, Oshkosh, WI, U.S.A.
[†]*Upper Midwest Environmental Sciences Center, United States Geological Survey, La Crosse, WI, U.S.A.*
[‡]*Department of Biology, University of Wisconsin La Crosse, La Crosse, WI, U.S.A.*

SUMMARY

1. Although it is well known that sediments can be hot spots for nitrogen transformation in streams, many previous studies have confined measurements of denitrification and nitrate retention to shallow sediments (<5 cm deep). We determined the extent of nitrate processing in deeper sediments of a sand plains stream (Emmons Creek) by measuring denitrification in core sections to a depth of 25 cm and by assessing vertical nitrate profiles, with peepers and piezometers, to a depth of 70 cm.

2. Denitrification rates of sediment slurries based on acetylene block were higher in shallower core sections. However, core sections deeper than 5 cm accounted for 68% of the mean depth-integrated denitrification rate.

3. Vertical hydraulic gradient and vertical profiles of pore water chloride concentration suggested that deep ground water upwelled through shallow sediments before discharging to the stream channel. The results of a two-source mixing model based on chloride concentrations suggested that the hyporheic zone was very shallow (<5 cm) in Emmons Creek.

4. Vertical profiles showed that nitrate concentration in shallow ground water was about 10–60% of the nitrate concentration of deep ground water. The mean nitrate concentrations of deep and shallow ground water were 2.17 and 0.73 mg NO_3-N L^{-1}, respectively.

5. Deep ground water tended to be oxic (6.9 mg O_2 L^{-1}) but approached anoxia (0.8 mg O_2 L^{-1}) after passing through shallow, organic carbon-rich sediments, which suggests that the decline in the nitrate concentrations of upwelling ground water was because of denitrification.

6. Collectively, our results suggest that there is substantial nitrate removal occurring in deep sediments, below the hyporheic zone, in Emmons Creek. Our findings suggest that not accounting for nitrate removal in deep sediments could lead to underestimates of nitrogen processing in streams and catchments.

Keywords: biogeochemistry, denitrification, ground water, nitrate, peepers

Introduction

The supplies of available nitrogen in many aquatic ecosystems have been increasing and are projected to continue to rise (Tesoreiro *et al.*, 2007; Dubrovsky *et al.*, 2010) to the likely detriment of ecosystems (Diaz & Rosenberg, 2008). Because of this, there is growing interest in processes that can retain or remove

Correspondence: Robert S. Stelzer, Department of Biology and Microbiology, University of Wisconsin Oshkosh, 800 Algoma Blvd.,
Oshkosh, WI 54901, U.S.A.. E-mail: stelzer@uwosh.edu

available nitrogen in streams and rivers (Mulholland *et al.*, 2008). Processes contributing to nitrate removal in streams include assimilatory uptake by autotrophs and heterotrophic microbes (e.g. Stelzer, Heffernan & Likens, 2003) and dissimilatory reduction, including denitrification, by microbes (Burgin & Hamilton, 2007). Several investigators have shown that denitrification rates are governed by nitrate supply, redox state and organic carbon availability (Arango *et al.*, 2007; Groffman *et al.*, 2009). Where nitrate and organic carbon supplies are high and redox conditions are favourable (anoxia), spatially discrete areas with high rates of denitrification, or ''hotspots'', can occur. These conditions often occur in riparian zones, stream channels and hyporheic zones, and several investigators have measured high rates of denitrification and nitrate decline in these locations (e.g. Hill *et al.*, 2000; Richardson *et al.*, 2004; Mulholland *et al.*, 2008). Many empirical studies of nitrogen processing in streams have focussed on surficial sediments. For example, the vast majority of denitrification measurements of stream sediments are based on shallow (<5 cm) sediment cores (Fig. 1). Nitrate processing in deeper sediments below the hyporheic zone has received less attention by lotic ecologists and biogeochemists (Fischer *et al.*, 2005; Inwood, Tank & Bernot, 2007). If rates of denitrification and other types of nitrogen processing are high in deep sediments, not accounting for this could lead to underestimates of nitrate removal in ecosystems.
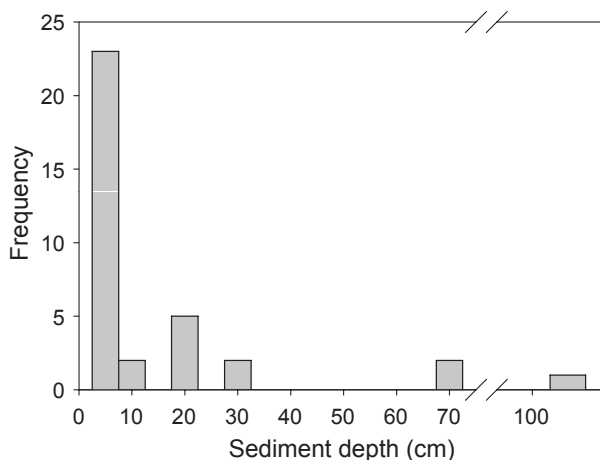
The hyporheic zone is typically defined as the portion of the alluvial aquifer in which surface water and ground water mix (Gooseff, 2010). Triska *et al.* (1989) defined the hyporheic zone as the depth at which pore water is made up of more than 10% but less than 98% of channel water. Based on Triska's definition, sediments below the hyporheic zone would be expected to be minimally influenced by surface water from the stream channel. However, particulate organic carbon (POC) from the stream channel may affect nitrogen processing in deep sediments where it frequently becomes buried (Herbst, 1980; Metzler & Smock, 1990; Laterell & Naiman, 2007). Particulate organic carbon often accumulates at the margins of the wetted channel and in pools and can become buried by bed movement and redistribution of fine sediments during spates. Burial of organic carbon that originated or was processed in stream channels may promote denitrification in deep sediments by supplying carbon and by establishing favourable redox conditions (i.e. oxygen depletion by heterotrophic microbes that utilise the organic carbon).

In the Central Sand Ridge Ecoregion in Central Wisconsin, streams tend to receive high inputs of nitrate from discharging ground water. We previously showed that in Emmons Creek, a third-order stream in this ecoregion, groundwater discharge explained 57–65% of whole-stream nitrate retention during a two-year mass balance study (Stelzer *et al.*, 2011). This suggested that processes in deep-stream sediments, through which the ground water upwelled before reaching the surface water, contributed to whole-stream nitrate retention. Other investigators have also suggested that available nitrogen is retained or removed along upwelling flow paths in deep sediments (Duff *et al.*, 2008; Puckett *et al.*, 2008). The main objective of the current study was to determine how denitrification and groundwater nitrate concentration changed with sediment depth in a nitrate-rich sand plains stream. We measured denitrification rates on sectioned sediment cores and constructed fine-scale vertical profiles of groundwater nitrate and chloride concentrations using pore water samplers (peepers) positioned within piezometer nests. Our study is one of the first to show high rates of denitrification coupled with evidence of in situ nitrate loss in deep-stream sediments.

Fig. 1 Frequency of sediment depths used in studies of denitrification based on sediment cores or grabs. Data are based on a Web of Science search of papers published from 2000 through 2009.

Methods

Study site description

Emmons Creek is a third-order, pre-dominantly groundwater-fed stream located in the Central Sand Ridges Ecoregion in Central Wisconsin (Fig. 2). Ground water in this ecoregion tends to have high nitrate concentrations, particularly in ground water with more recent recharge dates (Browne & Guldan, 2005; Tesoreiro *et al.*, 2007). Surface water in Emmons Creek and ground water discharging to the stream average about 2.3 and 2.6 mg NO$_3$-N L^{-1}, respectively (Stelzer *et al.*, 2011). The terrain in the Emmons Creek catchment is flat to gently rolling, and soils are sandy and well drained (Kraft & Stites, 2003). The land cover is a mix of hardwood forests, oak savanna, grasslands and irrigated row-crop agriculture. The dominant substratum in the wetted channel of Emmons Creek is sand (42%), followed by silt (30%) and gravel (10%) (Stelzer *et al.*, 2011). Silty areas are common near the margins of the wetted channel, as are beds of submergent macrophytes, especially *Veronica*. The study was conducted in a 700-m upwelling reach (Fig. 2), demarcated by upstream and downstream monitoring stations. More details about the study site and physical and chemical characteristics of Emmons Creek are described in Stelzer *et al.* (2011).

Piezometer nest and peepers

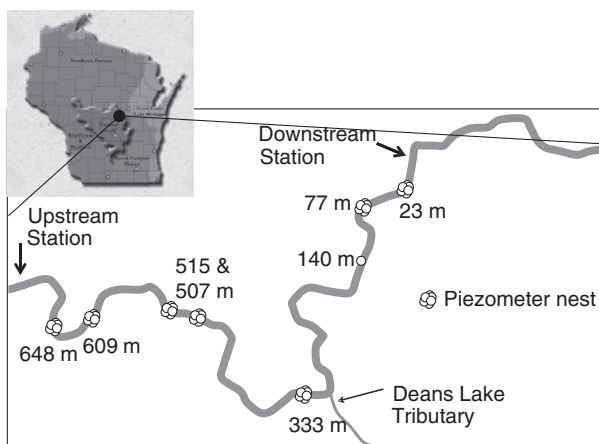Six piezometer nests, each consisting of six piezometers positioned at different sediment depths (grand

Fig. 2 Map of the study reach of Emmons Creek. The positions of the piezometer nests and of a single piezometer at 140 m are indicated in metres from the downstream station.

mean 50 cm to midscreen, range 23–71 cm) within a 1-m^2 area, were placed in upwelling locations of Emmons Creek (Fig. 2).

Upwelling was confirmed at these locations by the measurement of vertical hydraulic gradient (VHG). Piezometers consisted of chlorinated polyvinyl chloride pipe (1.2 cm inner diameter). The terminal 4.5 cm was the screened interval, which consisted of 3-mm holes covered with 100-μm Nitex mesh. A couple of months after a piezometer nest was installed at position 515 m (distance is relative to downstream station), an additional nest was installed at position 507 m (Fig. 2, see pore water sampler description). A single piezometer was also installed at position 140 m. Piezometers were placed in sediments that consisted pre-dominantly of a silt and/or sand layer that was at least 35 cm thick. This minimum sediment thickness was used so that peeper samplers could be deployed and that sediment cores (for denitrification) could be collected. In most cases, piezometer nests were placed in stream sediments near the edge of the wetted channel, where fine sediments tended to accumulate. Modified Pore Water Hesslein Samplers (peepers; 47 cm long × 10.5 cm wide, Rickly Hydrological, Columbus, OH, U.S.A.), with twenty-eight 10-mL cells at *c.* 1-cm vertical resolution, were deployed in the centre of each piezometer nest in June and September of 2009 and April of 2010 to determine nitrate and chloride concentration profiles to 20- to 30-cm sediment depths. The uppermost cells in the peepers extended into the water column and were used to sample surface water. After the June 2009 deployment, a peeper was deployed in the 507-m piezometer nest, instead of the 515 m nest, because sediments in the latter nest allowed for deeper placement of the peeper in the sediments. Before deployment, peepers were allowed to equilibrate in degassed deionised water for at least 24 h in the laboratory. After initial equilibration, the peepers were fitted with nylon 0.2-μm membranes (Biodyne A), transported to the field and deployed. Water samples from the peeper cells were collected after 4 weeks using sterile 10-mL syringes equipped with 18-gauge needles, placed in 15-mL low-density polyethylene containers and stored at −20 °C in the laboratory.

Ground water was collected from each piezometer nest that contained a peeper near the mid-point of the peeper incubation period (for the June 2009 deployment, this occurred 9 days after peepers were

sampled), syringe-filtered in the field through Whatman GF/Fs and stored at −20 °C in the laboratory. Ground water collected from the piezometers was considered deep ground water, while water collected from the peepers (between 5 and 20–30 cm depth) was considered shallow ground water. During the piezometer sampling events, the depth of the piezometers and the distances from the top of the piezometers to the surface water and to the static head were measured (the latter with a level tape; Solinst Canada Ltd., Georgetown, Ontario, Canada). These data were used to calculate the VHG for each piezometer (Dahm *et al.*, 2006). A two end-member mixing model using chloride concentrations was used to determine the contribution of surface water and deep ground water to shallow pore water (5-cm sediment depth) after Hill & Lymburner (1998). The surface water end member and shallow pore water component was based on chloride concentrations of peeper samples, while the deep groundwater end member was based on the mean chloride concentration of the piezometer samples, per peeper location and sampling date. Nitrate and chloride concentrations of the peeper and piezometer water samples were used to produce solute profiles to a depth of about 60–70 cm.

Denitrification measurements

Denitrification rates of stream sediments were measured in August and November of 2009 and May 2010, during or in close proximity to the peeper incubation periods. Eight sediment cores (up to 25 cm in length) were collected with a 7.6-cm-diameter polycarbonate corer and extracted with a plunger constructed from PVC and an expandable stop plug. During extraction, cores were cut into 5-cm sections and placed in individual plastic bags. Cores were collected within or in close proximity (0.5 m) of each piezometer nest. Cores were also collected in the vicinity of the piezometer at position 140 m. Ground water was pumped and collected from the piezometer in each nest at the deepest sediment depth for use in the denitrification incubations. Within several hours of collection, the core sections and ground water were returned to the laboratory in coolers and stored in a Fisher Isotemp Model 307C incubator (ThermoFisher Scientific, Waltham, MA, U.S.A.) at ambient sediment temperature.

The day after cores and ground water were collected, the sediment core sections were subjected to the acetylene block denitrification assay (Richardson *et al.*, 2004; Groffman *et al.*, 2006). After each core section was homogenised, 25 mL of sediment, 20 mL of ground water and 5 mL of 1 mg L^{-1} chloramphenicol solution (to arrest enzyme synthesis) were added to glass incubation vessels (246-mL canning jars) fitted with grey butyl septa. The ground water added to the sediments came from a piezometer within 1 m of where each sediment core was collected. Anoxic conditions in the vessels were established with a series of helium flushes. Immediately after the addition of 20 mL of acetylene, vessels were placed on a shaker (Innova Model 2000) at 175 rpm in the incubator at ambient sediment temperature (13 °C for August 2009 and May 2010 incubations, 9 °C for November 2009 incubations). Head space gas was sampled with a 5-mL syringe at *c.* 30-min intervals during the *c.* 90-min incubations (i.e. three times per incubation) and was immediately transferred to evacuated 2-mL serum vials. Within 3 weeks of the incubations, nitrous oxide (N_2O) concentration in the vials was measured on a Hewlett-Packard Model 5890 gas chromatograph fitted with a 3.18-mm column packed with Porapak R media and a 63Ni electron capture detector at the US Geological Survey Upper Midwest Environmental Sciences Center (UMESC) in La Crosse, WI, U.S.A. Nitrous oxide concentration in the head space was first converted from volumetric to mass units and then to total N_2O (water plus gas phase) using the equations in Groffman *et al.* (1999). Denitrification rates were calculated as the rate of nitrous oxide (N_2O) production during the *c.* 90-min incubations. Nitrous oxide concentration was not routinely measured at time zero (acetylene addition). However, nitrous oxide concentration was assumed to be zero at time zero because samples collected at time zero showed N_2O concentration to be below or at the limit of detection. In cases where the relationship between N_2O concentration and time was non-linear, only the linear portion was used to calculate N_2O production. Samples from the ground water used in the denitrification incubations were collected for the measurements of nitrate, dissolved organic carbon, sulphate and chloride concentrations, filtered through Whatman GF/Fs and stored at −20 °C.

Dissolved oxygen and temperature measurements

Dissolved oxygen of pore water at 5-cm sediment depth was measured on three occasions (August of

2009 and April and August of 2010) within the areas enclosed by each piezometer nest using an oxygen microelectrode (Microelectrodes, Inc., Bedford, NH, U.S.A.). Pore water temperature was measured by inserting a handheld thermometer into the sediment. Dissolved oxygen of deep ground water was measured with an YSI 85 field meter. Ground water was pumped from two piezometers per nest using a peristaltic pump and sent to a flow cell in which the dissolved oxygen probe was inserted, to minimise oxygen exchange between the water and atmosphere.

Nutrient analysis

Nitrate, chloride and sulphate concentrations were measured using a Dionex ICS-1000 ion chromatograph (Dionex Corporation, Sunnyvale, CA, U.S.A.) equipped with an IonPac AS14A column. Dissolved organic carbon (DOC) concentration was measured using an OI Analytical Model 1030 W TOC analyzer (OI Analytical, College Station, TX, U.S.A.).

Sediment characterisation

A 15-mL subsample was collected from each sediment core section the day after denitrification incubations and stored at −20 °C. Samples were dried at 60 °C, weighed, combusted at 500 °C for 4 h and reweighed. These measurements were used to calculate ash-free dry mass, % organic matter and bulk density. The remaining sediment in each core section was dried at 105 °C for several days. After removal of all particulate organic matter greater than 1 cm in length, the sediments were ground with a mortar and pestle to break apart aggregates. This material was then passed through a set of standard brass sieves placed in a sieve shaker (Endecotts Octagon 200). All materials that passed through or were retained on a 63-μm sieve were considered silt and sand, respectively, based on the Wentworth scale. Material retained on a 2-mm sieve was considered larger than sand. Size fractions were determined gravimetrically.

Statistical analysis

Nested two-way ANOVA was used to determine the effects of core location and core section on denitrifi-

cation rate and sediment characteristics (% organic matter, % silt, bulk density). Core section was nested within core location in the ANOVA model, and samples collected on different dates were used as replicates. One-way ANOVA was used to compare denitrification rates and sediment characteristics among seasons. Only the top four core sections (0–5, 5–10, 10–15 and 15–20 cm) were included in the ANOVA models because of a low number of replicates for the 20- to 25-cm core section. One-way ANOVA was used to compare the nitrate and DOC concentrations of ground water used in the denitrification incubations among seasons. Pearson correlations were used to assess the strength of relationships between denitrification rates and sediment characteristics. Systat v. 13 was used for all statistical analyses.

Results

Denitrification

Denitrification rates were highest in the top 5 cm of sediments and decreased with sediment depth (Table 1, Fig. 3, ANOVA $F_{24,61} = 2.46$, $P < 0.01$). However, denitrification at sediment depths below 5 cm accounted for 68% of the total (core integrated) denitrification rate, on average. Denitrification rates also varied among core locations (ANOVA $F_{7,24} = 3.61$, $P < 0.05$). Denitrification rates were highest on average in August (6.46 μg N$_2$O-N cm^{-2} h^{-1} when integrated throughout the 25-cm core), decreased in November (5.27 μg N$_2$O-N cm^{-2} h^{-1}) and decreased further in May (3.60 μg N$_2$O-N cm^{-2} h^{-1}) (ANOVA $F_{2,90} = 5.407$, $P < 0.01$) (Fig. 3). The nitrate concentrations of ambient ground water used in the denitrification incubations were relatively high and similar among seasons with means (+SD) of 2.20 (0.81), 2.41

Table 1 Grand means (and SD) of denitrification rates from Emmons Creek sediments based on measurements in August, November and May

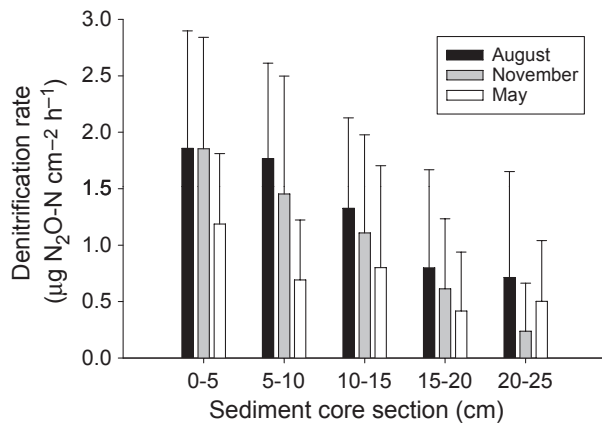| Core section (cm)	Denitrification rate (μg N$_2$O-N cm^{-2} h^{-1})		
	Mean	SD	N
0–5	1.63	0.92	24
5–10	1.30	0.92	24
10–15	1.08	0.85	24
15–20	0.59	0.65	21
20–25	0.46	0.63	13

Fig. 3 Mean (+SD) denitrification rates of Emmons Creek sediments by core section for August and November of 2009 and May of 2010.

Table 2 Solute concentrations of ground water used in the denitrification incubations

Piezometer nest	Month	NO_3-N (mg L^{-1})	Cl$^-$ (mg L^{-1})	SO_4-S (mg L^{-1})	DOC (mg L^{-1})
23	Aug	2.05	2.41	3.91	0.14
	Nov	2.75	2.60	3.29	0.14
	May	2.07	2.64	3.95	0.31
77	Aug	2.34	1.26	1.65	0.14
	Nov	2.37	1.39	1.73	0.38
	May	2.20	1.41	2.46	0.30
140	Aug	3.42	1.73	2.46	0.14
	Nov	3.51	1.84	2.68	0.25
	May	3.46	1.97	2.43	0.25
333	Aug	2.99	1.87	2.43	0.31
	Nov	3.01	1.88	2.51	0.18
	May	2.97	1.97	2.48	0.22
507	Aug	1.85	2.03	2.77	0.21
	Nov	2.13	2.30	3.54	0.18
	May	1.02	2.02	3.92	0.39
515	Aug	1.04	2.07	4.45	0.27
	Nov	1.25	2.22	3.54	0.28
	May	1.06	1.66	2.28	0.47
609	Aug	2.62	1.53	1.84	0.15
	Nov	2.80	1.56	1.94	0.29
	May	2.82	1.64	1.97	0.14
648	Aug	1.32	1.43	2.02	0.21
	Nov	1.43	1.82	2.48	0.23
	May	1.32	2.21	2.63	1.00

Piezometer nest location is given as distance from the downstream station at Emmons Creek in metres.

(0.77) and 2.12 (0.93) mg NO_3-N L^{-1} for August, November and May, respectively (Table 2, ANOVA $F_{2,21} = 0.25$, $P = 0.779$). Dissolved organic carbon concentrations were very low with a mean (+SD) of 0.27

(0.018) mg L^{-1} and also did not differ among seasons (Table 2, ANOVA $F_{2,21} = 2.84$, $P = 0.081$).

Sediment composition

The organic matter ($F_{24,60} = 5.54$, $P < 0.001$) and silt content ($F_{24,61} = 4.15$, $P < 0.001$) of the sediment used in the denitrification incubations were higher in shallower core sections than in deeper sections, while bulk density increased with sediment depth ($F_{24,60} = 2.79$, $P < 0.01$) (Table 3). In many cases, organic-rich, silty sediments in the upper core sections transitioned to sediments with lower organic matter and silt content in the deeper sections. However, in some cores, % organic matter was as high as 10–20% in the 20- to 25-cm core section. Per cent organic matter and % silt were highly correlated ($r = 0.948, P < 0.001$). Mean per cent organic matter ($F_{2,89} = 0.288$, $P = 0.750$), % silt ($F_{2,90} = 0.533$, $P = 0.589$) and bulk density ($F_{2,89} = 1.239$, $P = 0.295$) of the sediment used in the denitrification incubations did not differ among seasons. There was a weak positive relationship between denitrification rate and % organic matter ($r = 0.261$, $P = 0.007$) and a weak negative relationship between denitrification rate and bulk density ($r = -0.310$, $P = 0.001$). There was a positive, non-significant correlation between denitrification rate and % silt ($r = 0.176$, $P = 0.072$).

Solute profiles

The VHG was consistently positive for all piezometers throughout the study period with a range of 0 (one case) to 0.250 and a grand mean of 0.095 (Table 4). The chloride profiles were consistent with the VHG results. Chloride concentrations in the shallow ground water sampled with the peepers were usually very

Table 3 Mean (and SD) % organic matter, % silt and bulk density of sediments from Emmons Creek core sections used in the denitrification incubations

Core section (cm)	% Organic	% Silt	Bulk density (g cm^{-3})	N
0–5	24.0 (15.2)	22.5 (13.3)	0.34 (0.31)	23–24
5–10	18.2 (14.9)	18.1 (13.4)	0.52 (0.37)	24
10–15	12.4 (10.8)	10.8 (8.6)	0.68 (0.44)	24
15–20	8.4 (8.3)	8.4 (7.6)	0.85 (0.46)	21
20–25	6.5 (7.3)	8.0 (6.1)	0.90 (0.42)	13

All units are based on dry mass.

Table 4 Mean sediment depth to midscreen and vertical hydraulic gradient (VHG) for the six piezometers from each piezometer nest in Emmons Creek

Piezometer nest	Piezometer	1	2	3	4	5	6
23	Sediment depth (cm)	61	68	55	47	53	49
	VHG	0.186	0.227	0.061	0.056	0.095	0.069
77	Sediment depth	48	44	61	37	50	35
	VHG	0.085	0.095	0.091	0.131	0.091	0.109
333	Sediment depth	48	30	42	59	36	41
	VHG	0.224	0.104	0.124	0.211	0.146	0.171
507/515	Sediment depth	67	32	54	40	55	37
	VHG	0.038	0.016	0.066	0.019	0.060	0.018
609	Sediment depth	58	64	48	38	46	37
	VHG	0.111	0.109	0.123	0.124	0.131	0.151
648	Sediment depth	63	39	51	35	52	66
	VHG	0.026	0.022	0.033	0.025	0.032	0.041

Piezometer nest location is given as the distance from the downstream station at Emmons Creek in metres.
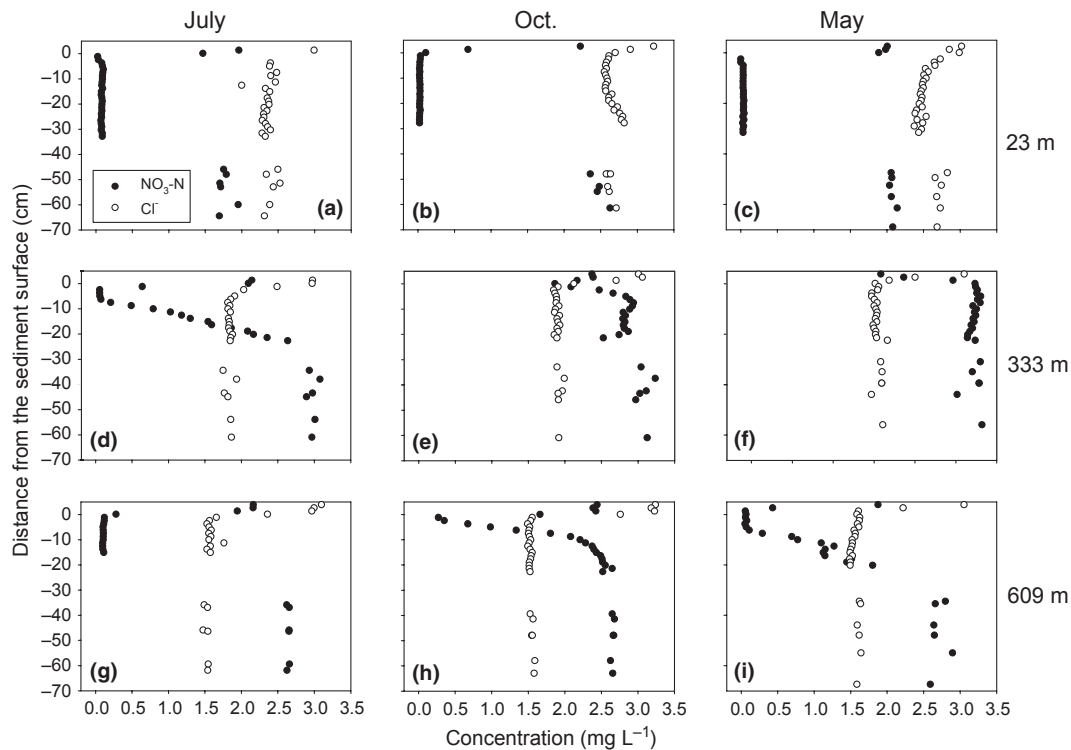

Fig. 4 Representative nitrate and chloride concentration profiles from Emmons Creek at 23, 333 and 609 m during July and October of 2009 and May of 2010 (months indicate when peepers were sampled). Data from shallow sediments (typically less than 30 cm depth) and deep sediments are from peepers and piezometers, respectively. Surface water solute concentrations are indicated at positive distances from the sediment surface.

similar to the concentrations in deeper ground water sampled with the piezometers (Figs 4 & 5). Concentrations of chloride in the surface water were consistently higher than those in both deep and shallow ground water (Fig. 4). The results of the two end-member mixing model using chloride indicated that on average, surface water contributed only 5% to the pore water at 5-cm sediment depth, with 95%

coming from ground water. Collectively, these results suggest that deep ground water upwelled through the sediments before discharging to the surface water and that the hyporheic zone, as defined by Triska *et al.* (1989), was very shallow in Emmons Creek.

Most nitrate profiles (Fig. 4) indicated substantial nitrate removal at sediment depths below the hyporheic zone, while a minority of profiles suggested less nitrate removal. In most cases, nitrate concentration decreased in the zone between the deep and shallow ground water (Fig. 4). In some cases, the decrease in nitrate concentration in the shallow sediments was captured by the peeper samplers (Fig. 4d,e,h,i). In other cases, there was an abrupt difference between the nitrate concentrations in the deep ground water and the shallow ground water (Fig. 4a,b,c,g). In most cases, there was a gap in the solute profile because of difficulties in inserting the full length of the peeper into the sediments (gravel beneath finer-grained sediment often limited peeper insertion depth). The mean nitrate concentrations of deep and shallow ground water were 2.17 and 0.73 mg NO_3-N L^{-1}, respectively. When concentrations of solutes from the peepers were expressed as a percentage of deep groundwater concentrations, nitrate in the shallower ground water was reduced to about 10–60% of that in the deep ground water (Fig. 5). These profiles suggested that nitrate removal was highest in July, declined in October and declined further in May

(Fig. 5). This decrease in inferred nitrate removal across seasons qualitatively agreed with the decline in denitrification rates (Fig. 3). In July, nitrate concentrations of the shallow ground water, when expressed as a percentage of deep groundwater concentrations, were more consistently low than in the other months, as reflected in the low variation about the means (Fig. 5a). Deep ground water associated with Emmons Creek was oxic (means were 6.9 mg O_2 L^{-1} and 65% O_2 saturation, Table 5). Dissolved oxygen was much lower in shallow pore water at 5-cm sediment depth (means were 0.8 mg O_2 L^{-1} and 8% saturation, Table 5).

Discussion

The denitrification results and nitrate profiles suggest that substantial nitrate processing occurs below the interface where ground water and surface water mix in Emmons Creek. Although denitrification rates were higher in shallow sediments, sediments deeper than 5 cm accounted for 68% of the total denitrification rates per core, on average. The substantial denitrification rates measured below 5-cm sediment depth, and the evidence of in situ nitrate removal in deeper sediments, suggest that denitrifying bacteria, organic carbon and anoxic conditions were present in these sediments. Although oxygen was detected in the shallow pore water of Emmons Creek (Table 5), it is
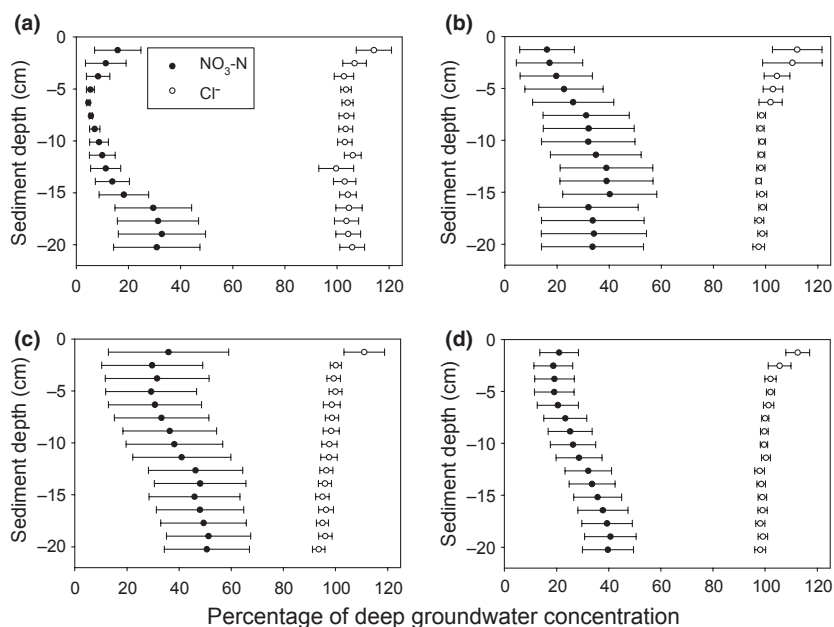

Fig. 5 Mean (+SE) nitrate and chloride concentrations in the shallow ground water of Emmons Creek (peepers) expressed as a percentage of associated deep groundwater solute concentrations (piezometers). A. July 2009, B. October 2009, C. May 2010, D. Grand means based on all three peeper deployment dates. Months indicate when peepers were sampled.

Table 5 Mean (and SD) dissolved oxygen for deep ground water from piezometers and for shallow pore water at 5-cm sediment depth in Emmons Creek

Piezometer nest	Deep ground water		Shallow pore water	
	(mg O$_2$ L^{-1})	(% saturation)	(mg O$_2$ L^{-1})	(% saturation)
23	6.43 (0.98)	61.75 (10.25)	0.21 (0.21)	2.12 (2.13)
77	8.54 (1.07)	81.90 (12.59)	0.26 (0.19)	2.58 (1.89)
333	8.71 (0.08)	81.10 (0.14)	2.74 (2.92)	26.68 (28.58)
507/515	5.13 (2.18)	50.00 (21.35)	0.23 (0.21)	2.26 (2.08)
609	8.98 (0.08)	82.05 (1.34)	1.12 (0.54)	11.00 (5.46)
648	3.40 (0.02)	33.15 (0.21)	0.47 (0.32)	4.64 (3.14)

Piezometer nest location is given as the distance from the downstream station at Emmons Creek in metres.

likely that anoxic microsites were present (Fenchel, King & Blackburn, 1998). Few previous studies have assessed variation in denitrification rates with sediment depths in lotic ecosystems. Inwood *et al.* (2007) found that over 88% of the denitrification activity came from the top 5 cm of sediment in 15-cm sediment cores from an agricultural stream. Fischer *et al.* (2005) showed that potential denitrification rate decreased substantially with sediment depth in a large river. However, they found that denitrification in deep sediments, when summed across depths, accounted for 45% of the total denitrification rate at one location. Lefebvre, Marmonier & Pinay (2004) found no clear relationship between denitrification rates and stream sediment depths. Other investigators have shown denitrification can occur up to several metres deep in soils (Starr & Gillham, 1993; Hill *et al.*, 2000). Together, these results suggest that not accounting for denitrification rates in deep sediments or soils may lead to underestimates of areal denitrification rates. Our mean depth-integrated areal denitrification rate of 5.06 μg N$_2$O-N cm^{-2} h^{-1} was higher than most published denitrification rates in stream sediments from unamended (e.g. Richardson *et al.*, 2004; Schaller, Royer & David, 2004; Arango *et al.*, 2007; Herrman, Bouchard & Moore, 2008) and from nutrient-amended incubations (e.g. Sheibley *et al.*, 2003; Bartkow & Udy, 2004). The depth-integrated aspect of the Emmons Creek denitrification rate and the high nitrate concentrations in the ground water used in the incubations probably contributed to the higher denitrification rate relative to most previous studies of denitrification in stream sediments.

We found a positive but weak correlation between denitrification rate and the % organic matter of the sediment. Many previous investigators have shown that denitrification rates in sediments (Arango *et al.*,

2007; Inwood *et al.*, 2007) and soils (Hill *et al.*, 2000; Groffman *et al.*, 2009) are positively related to organic carbon content. Solomon *et al.* (2009) showed a positive relationship between the percentage of fine particles and denitrification rates in sediments from a tropical stream. The relatively weak correlations between denitrification rate and our measured sediment characteristics indicate that there was considerable unexplained variation in denitrification rate. Variation in the abundance and activity of bacteria capable of denitrification and in the quality of organic carbon available to the bacteria (e.g. Baker & Vervier, 2004; Greenan *et al.*, 2006) are two potential sources for the unexplained variation in denitrification rates in Emmons Creek sediments.

Denitrification rates varied among seasons in Emmons Creek. The higher rates in August do not appear to be due to differences in the incubation temperatures among seasons. Incubation temperatures were 13 °C for the August and May denitrification incubations and 9 °C for the November incubations. The seasonal differences in denitrification rates were not likely due to bulk sediment characteristics (% organic matter, % silt, bulk density) or nitrate and dissolved organic carbon concentrations of ground water used in the incubations, as the magnitude of these variables did not vary among seasons. Other investigators have shown seasonal variation in denitrification rates in stream sediments, including higher rates during the summer when comparisons were made within substratum or habitat types (Richardson *et al.*, 2004; Schaller *et al.*, 2004; Arango *et al.*, 2007; Herrman *et al.*, 2008).

Several authors have pointed out limitations of using the acetylene block method in the laboratory for measuring denitrification rates (e.g. Groffman *et al.*, 2006). By creating ideal redox conditions for

denitrification in the incubation vessels (anoxia), this method could lead to higher denitrification rates than that occurring in situ. However, shallow pore water approached anoxia at most of the locations from which sediment cores for the denitrification incubations were obtained. This suggests that the induced anoxia during the denitrification incubations may have not had a large effect on the denitrification rates measured. Another limitation of the acetylene block method is that acetylene, in addition to blocking the conversion of nitrous oxide to dinitrogen (N_2), inhibits nitrification (Hynes & Knowles, 1978). In sediments where denitrification is tightly coupled to nitrification, this could lead to underestimates of denitrification. This tight coupling is probably more common in situations when nitrate availability is low. Because the source water for the incubations (deep ground water) had high nitrate concentrations, inhibition of nitrification probably did not cause denitrification to be underestimated in our study.

The profiles of nitrate concentration in ground water and shallow pore water (Figs 4 & 5) strongly suggest that nitrate was removed in sediments in situ and that a substantial amount of the nitrate loss occurred below the hyporheic zone. Nitrate concentration did not change as ground water moved upward through deep sediments (40–70 cm depth, Fig. 4), but changed abruptly or steadily in many cases as ground water passed through shallower sediments (10–40 cm). We think that the decline in nitrate concentration that occurred in the profiles may have been as a result of changes in redox conditions and POC content in sediments at moderate depths (about 20–40 cm). The shift from oxic conditions in deep ground water to those approaching anoxia in the shallow pore water (Table 5) suggests that ground water passed through a zone favourable for nitrate reduction before it discharged to the surface water. Burial of POC at depth probably contributed to the reducing conditions in the sediments by creating oxygen demand by aerobic microbes (Groffman, 1994). This POC would also provide sources of carbon and electron donors for denitrifying bacteria. The presence of POC in the deep sediments (Table 3) and the extreme hypoxic conditions suggest that denitrification contributed to the decline in nitrate concentration from deep to shallow ground water. The qualitative agreement between denitrification rates and changes in nitrate profiles across seasons (Figs 3 & 5) is consistent with denitrification contributing to the nitrate profile patterns observed. The proposed controls on nitrate concentrations in sediments near or below the groundwater–surface water interface described previously are similar to the redox- and carbon-driven controls described by Hedin *et al.* (1998) at the soil–stream interface and the general case of an advection-dominated system described by Seitzinger *et al.* (2006). Other investigators have shown similar decreases in nitrate concentration along upwelling flow paths in stream sediments (Pretty, Hildrew & Trimmer, 2006; Krauss *et al.*, 2009) and in soils (Hedin *et al.*, 1998).

The pattern of denitrification rate with sediment depth differed from the pattern of the nitrate concentration profiles. Denitrification rate was higher in the shallower core sections (Table 1), while most of the nitrate profiles suggested that there was greater in situ nitrate loss in relatively deep sediments (Fig. 4). The denitrification rates that we measured were probably closer to potential rates than the rates that occurred in situ. The deep ground water used in the denitrification incubations had relatively high nitrate concentrations. Because nitrate concentration tended to decrease as ground water moved upwards through the sediments (Fig. 4 & 5), there was probably less nitrate available for denitrification in the shallow sediments (<20 cm) in nature. By using deep ground water for the denitrification incubations, we may have overestimated denitrification rates, particularly in the shallower sediments. If denitrification rates were differentially overestimated in the shallower sediments, deeper sediments may have contributed even more to total denitrification in the sediments than we originally estimated.

Our results suggest that there is a zone in the deep sediments of Emmons Creek that is characterised by the presence of POC and very low oxygen concentrations. We think this zone may serve as important ecotone (*sensu* Gibert *et al.*, 1990) for nitrogen transformation. The VHG data (Table 4) and the results of the two end-member mixing model suggest that the hyporheic zone is very shallow, <5 cm on average, in Emmons Creek. This suggests that there is minimal contribution of surface water, and associated solutes, to moderately deep (10–40 cm) sediments. However, our observations of carbon-rich sediments in deep core sections and the presence of POC at depth (Table 3) suggest that POC that originates or is

processed in the stream channel becomes buried in the deep sediments of Emmons Creek. Without the contributions of POC from the surface channel, it is unlikely that nitrogen transformation in the deep sediments would occur to the same extent as we observed. Some investigators have proposed that buried POC makes significant contributions to carbon mineralisation in streams. Metzler & Smock (1990) suggested that burial of organic detritus in stream sediments could affect ecosystem metabolism and carbon cycling. Battin *et al.* (2003), using end-member mixing analysis, concluded that buried POC supported 61% of respiration in the hyporheic zone in White Clay Creek. Although these studies focused on carbon mineralisation, our results and the positive relationship between denitrification rates and carbon availability based on numerous studies suggest that buried POC could also produce denitrification hot spots in stream sediments. Investigators working in riparian zones have also suggested that organic carbon buried up to metres deep in soils is microbially available and may contribute to denitrification (Hill *et al.*, 2000; Gurwick *et al.*, 2008).

We think the results of our study point to one of the problems with efforts to geographically compartmentalise ecosystem processes at ecotones. Based on many criteria, the processes that we identify in the deep sediments are part of the groundwater system and not the stream system *per se*. However, the POC in the deep sediments probably originated from or was processed in the stream channel. We choose to take a conservative approach about the role of the stream channel in these subhyporheic processes because: (i) we do not have direct evidence that the POC at depth originated or was processed in the stream and (ii) based on the current study, we cannot make a causal link between POC and nitrate processing in sediments. We are planning future experiments that would manipulate POC quantity and quality in deep sediments to determine how POC effects nitrogen processing. As this work progresses, we think we will be able to draw more definitive conclusions about the role of the stream channel in these subhyporheic processes.

Many models of hyporheic zone structure and function emphasise connections between surface water and the hyporheic zone (Jones & Holmes, 1996; Boulton *et al.*, 1998; Muholland & DeAngelis, 2000; Boulton *et al.*, 2010). An important paradigm in stream ecology is that surface water downwells into sediments and brings materials to the hyporheic zone where they are often biogeochemically transformed and returned to the surface water in some altered form (Jones, Fisher & Grimm, 1995; Valett *et al.*, 1996; Clilverd, Jones & Kielland, 2008; Boulton *et al.*, 2010). Less emphasis has been placed on the role of upwelling ground water on biogeochemical transformations at the surface water–groundwater interface (Triska *et al.*, 1989; Gibert *et al.*, 1990; Vervier, Gibert & Dole-Olivier, 1992; Krauss *et al.*, 2009). We suggest that more emphasis be placed on biogeochemical transformations that occur in deep sediments below the hyporheic zone, especially in streams with shallow hyporheic zones that receive substantial inputs of ground water. In particular, we think that consideration of the role of deep sediments in nitrogen processing will lead to a better understanding of nitrogen biogeochemistry in streams and catchments.

Acknowledgments

References

Arango C.P., Tank J.L., Schaller J.M., Royer T.V., Bernot M.J. & David M.B. (2007) Benthic organic carbon influences denitrification in streams with high nitrate concentration. *Freshwater Biology*, **52**, 1210–1222.

Baker M.A. & Vervier P. (2004) Hydrological variability, organic matter supply and denitrification in the Garonne River ecosystem. *Freshwater Biology*, **49**, 181–190.

Bartkow M.E. & Udy J.W. (2004) Quantifying potential nitrogen removal by denitrification in stream sediments at a regional scale. *Marine and Freshwater Research*, **55**, 309–315.

Battin T.J., Kaplan L.A., Newbold D. & Hendricks S.P. (2003) A mixing model analysis of stream solute

dynamics and the contribution of a hyporheic zone to ecosystem function. *Freshwater Biology*, **48**, 995–1104.

Boulton A.J., Findlay S., Marmonier P., Stanley E.H. & Valett H.M. (1998) The functional significance of the hyporheic zone in streams and rivers. *Annual Review of Ecology and Systematics*, **29**, 59–81.

Boulton A.J., Datry T., Kasahara T., Mutz M. & Stanford J.A. (2010) Ecology and management of the hyporheic zone: stream-groundwater interactions of running waters and their floodplains. *Journal of the North American Benthological Society*, **29**, 26–40.

Browne B.A. & Guldan N.M. (2005) Understanding long-term baseflow water quality trends using a synoptic survey of the groundwater-surface water interface, Central Wisconsin. *Journal of Environmental Quality*, **34**, 825–835.

Burgin A.J. & Hamilton S.K. (2007) Have we overestimated the role of denitrification in aquatic ecosystems? A review of nitrate removal pathways *Frontiers of Ecology and the Environment*, **5**, 89–96.

Clilverd H.M., Jones J.B. & Kielland K. (2008) Nitrogen retention in the hyporheic zone of a glacial river in interior Alaska. *Biogeochemistry*, **88**, 31–46.

Dahm C.N., Valett H.M., Baxter C.V. & Woessner W.W. (2006) Hyporheic zones. In: *Methods in Stream Ecology* (Eds F.R. Hauer & G.A. Lamberti), pp. 119–142. Elsevier, Boston.

Diaz R.J. & Rosenberg R. (2008) Spreading dead zones and consequences for marine ecosystems. *Science*, **321**, 926–929.

Dubrovsky N.M., Burow K.R., Clark G.M., Gronberg J.M., Hamilton P.A., Hitt K.J. *et al.* (2010) The quality of our Nation's waters – nutrients in the Nation's streams and groundwater, 1992–2004: U.S. geological survey circular 1350, 174 p.

Duff J.H., Tesoriero A.J., Richardson W.B., Strauss E.A. & Munn M.D. (2008) Whole-stream response to nitrate loading in three streams draining agricultural landscapes. *Journal of Environmental Quality*, **37**, 1133–1144. DOI: 10.2134/jeq2007.0187.

Fenchel T., King G.M. & Blackburn T.H. (1998) *Bacterial Biogeochemistry: The Ecophysiology of Mineral Cycling*. 2nd edn. Academic Press, London.

Fischer H., Kloep F., Wilzcek S. & Pusch M.T. (2005) A river's liver – microbial processes within a hyporheic zone of a large lowland river. *Biogeochemistry*, **76**, 349–371.

Gibert J., Dole-Olivier M.-J., Marmonier P. & Vervier P.. (1990) Surface water-groundwater ecotones. In: *The Ecology and Management of Aquatic-Terrestrial Ecotones* (Eds R.J. Naiman & H. Décamps), pp 199–206. United Nations Educational, Scientific, and Cultural Organization, Paris and Parthenon Publishers, Carnforth, UK.

Gooseff M.N. (2010) Defining hyporheic zones – advancing our conceptual and operational definitions of where stream water and groundwater meet. *Geography Compass* **4**, 945–955.

Greenan C.M., Moorman T.B., Kaspar T.C., Parkin T.B. & Jaynes D.B. (2006) Comparing carbon substrates for denitrification of subsurface drainage water. *Journal of Environment Quality* **35**, 824–829.

Groffman P.M. (1994) Denitrification in freshwater wetlands. *Current topics in wetland biogeochemistry*, **1**, 15–35.

Groffman P.M., Holland E.A., Myrold D.D., Robertson G.P. & Zou X. (1999) Denitrification. In: *Standard Methods for Long-Term Ecological Research* (Eds G.P. Robertson & D.C. Coleman & C.S. Bledsoe & P. Sollins), pp. 272–288. Oxford University Press, New York.

Groffman P.M., Altabet M.A., Böhlke J.K., Butterbach-Bahl K., David M.B., Firestone M.K. *et al.* (2006) Methods for measuring denitrification: diverse approaches to a difficult problem. *Ecological Applications* **16**, 2091–2122.

Groffman P.M., Butterbach-Bahl K., Fulweiler R.W., Gold A.J., Morse J.L., Stander E.K. *et al.* (2009) Challenges to incorporating spatially and temporally explicit phenomena (hotspots and hot moments) in denitrification models. *Biogeochemistry* **93**, 49–77.

Gurwick N.P., Groffman P.M., Yavitt J.B., Gold A.J., Blazejewski G. & Stoldt M. (2008) Microbially available carbon in buried riparian soils in a glaciated landscape. *Soil Biology and Biochemistry*, **40**, 85–96.

Hedin L.O., von Fischer J.C., Ostrom N.E., Kennedy B.P., Brown M.G. & Robertson G.P. (1998) Thermodynamic constraints on nitrogen transformations and other biogeochemical processes at soil-stream interfaces. *Ecology* **79**, 684–703.

Herbst G.N. (1980) Effects of burial on food value and consumption of leaf detritus by aquatic invertebrates in lowland forest stream. *Oikos*, **35**, 411–424.

Herrman K.S., Bouchard V. & Moore R.H. (2008) An assessment of nitrogen removal from headwater streams in an agricultural watershed, northeast Ohio, U.S.A. *Limnology and Oceanography*, **53**, 2573–2582.

Hill A.R. & Lymburner D.J. (1998) Hyporheic zone chemistry and stream-subsurface exchange in two groundwater-fed streams. *Canadian Journal of Fisheries and Aquatic Sciences*, **55**, 495–506.

Hill A.R., Devito K.J., Campagnolo S. & Sanmugadas K. (2000) Subsurface denitrification in a forest riparian zone: the interactions between hydrology and supplies of nitrate and organic carbon. *Biogeochemistry*, **51**, 193–223.

Hynes R.K. & Knowles P. (1978) Inhibition by acetylene of ammonia oxidation in *Nitrosomonas europaea*. *FEMS Microbiology Letters* **4**, 319–321.

Inwood S.E., Tank J.L. & Bernot M.J. (2007) Factors controlling sediment denitrification in Midwestern streams of varying land use. *Microbial Ecology*, **53**, 247–258.

Jones J.B. & Holmes R.M. (1996) Surface-subsurface interactions in stream ecosystems. *Trends in Ecology and Evolution*, **11**, 239–242.

Jones J.B., Fisher S.G. & Grimm N.B. (1995) Nitrification in the hyporheic zone of a desert stream ecosystem. *Journal of the North American Benthological Society*, **14**, 249–258.

Kraft G.J. & Stites W. (2003) Nitrate impacts on groundwater from irrigated-vegetable systems in a humid north-central US sand plain. *Agriculture, Ecosystems and Environment*, **100**, 63–74.

Krauss S., Heathwaite L., Binley A. & Keenan P. (2009) Nitrate concentration changes at the groundwater-surface water interface of a small Cumbrian river. *Hydrological Processes*, **23**, 2195–2211.

Laterell J.J. & Naiman R.J. (2007) Sources and dynamics of large logs in a temperate floodplain river. *Ecological Applications*, **17**, 1127–1141.

Lefebvre S., Marmonier P. & Pinay G. (2004) Stream regulation and nitrogen dynamics in sediment interstices: comparison of natural and straightened sectors of a third-order stream. *River Research and Applications*, **20**, 499–512.

Metzler G.M. & Smock L.A. (1990) Storage and dynamics of subsurface detritus in a sand-bottomed stream. *Canadian Journal of Fisheries and Aquatic Sciences*, **47**, 588–594.

Muholland P.J. & DeAngelis D.L. (2000) Surface-subsurface exchange and nutrient spiraling. in: *Streams and Groundwaters* (Eds J.B. Jones & P.J. Mulholland), pp. 149–166. Academic Press, San Diego.

Mulholland P.J., Helton A.M., Poole G.C., Hall R.O. Jr, Hamilton S.K., Peterson B.J. *et al.* (2008) Stream denitrification across biomes and its response to anthropogenic nitrate loading. *Nature* **452**, 202–205. DOI: 10.1038/nature06686.

Pretty J.L., Hildrew A.G. & Trimmer M. (2006) Nutrient dynamics in relation to surface-subsurface hydrological exchange in a groundwater fed chalk stream. *Journal of Hydrology*, **330**, 84–100.

Puckett L.J., Zamora C., Essaid H., Wilson J.T., Johnson H.M., Brayton M.J. *et al.* (2008) Transport and fate of nitrate at the ground-water/surface-water interface. *Journal of Environmental Quality*, **37**, 1034–1050. DOI: 10.2134/jeq2006.0550.

Richardson W.B., Strauss E.A., Bartsch L.A., Monroe E.M., Cavanaugh J.C., Vingum L. *et al.* (2004) Denitrification in the upper Mississippi river: rates, controls, and contributions to nitrate flux. *Canadian Journal of Fisheries and Aquatic Sciences*, **61**, 1102–1112.

Schaller J.L., Royer T.V. & David M.B. (2004) Denitrification associated with plants and sediments in an agricultural stream. *Journal of the North American Benthological Society*, **23**, 667–676.

Seitzinger S., Harrison J.A., Böhlke J.K., Bouwman A.F., Lowrance R., Peterson B. *et al.* (2006) Denitrification across landscapes and waterscapes: a synthesis. *Ecological Applications*, **16**, 2064–2090.

Sheibley R.W., Duff J.H., Jackman A.P. & Triska F.J. (2003) Inorganic nitrogen transformations in the bed of the Shingobee River, Minnesota: integrating hydrological and biological processes using sediment perfusion cores. *Limnology & Oceanography*, **48**, 1129–1140.

Solomon C.T., Hotchkiss E.R., Moslemi J.M., Ulseth A.J., Stanley E.H., Hall R.O. *et al.* (2009) Sediment size and nutrients regulate denitrification in a tropical stream. *Journal of the North American Benthological Society*, **28**, 480–490.

Starr R.C. & Gillham R.W. (1993) Denitrification and organic carbon availability in two aquifers. *Ground Water*, **31**, 934–947.

Stelzer R.S., Heffernan J.B. & Likens G.E. (2003) The influence of dissolved nutrients and particulate organic matter quality on microbial respiration and biomass in a forest stream. *Freshwater Biology*, **48**, 1925–1937.

Stelzer R.S., Drover D.R., Eggert S.L. & Muldoon M.A. (2011) Nitrate retention in a sand plains stream and the importance of groundwater discharge. *Biogeochemistry*, **103**, 91–107. DOI: 10.1007/s10533-010-9449-y.

Tesoreiro A.J., Saad D.A., Burow K.R., Frick E.A., Puckett L.J. & Barbash J.E. (2007) Linking ground-water age and chemistry data along flow paths: implications for trends and transformations of nitrate and pesticides. *Journal of Contaminant Hydrology*, **94**, 139–155.

Triska F.J., Kennedy V.C., Avanzino R.J., Zellweger G.W. & Bencala K.E. (1989) Retention and transport of nutrients in a third-order stream in northwestern California: hyporheic processes. *Ecology*, **70**, 1893–1905.

Valett H.M., Morrice J.A., Dahm C.N. & Campana M.E. (1996) Parent lithology, surface-groundwater exchange, and nitrate retention in headwater streams. *Limnology and Oceanography* **41**, 333–345.

Vervier P., Gibert J. & Dole-Olivier M. (1992) A perspective on the permeability of the surface freshwater-groundwater ecotone. *Journal of the North American Benthological Society* **11**, 93–102.