

Project Completion Report (R/UW-GSI-002)

**A Basin-Scale Denitrification Budget for a Nitrate Contaminated Wisconsin Aquifer:
A Study at the Groundwater/Surface Water Interface**

Period of contract: July 1, 2000 – June 30, 2002

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TABLE OF CONTENTS

	Page
PROJECT SUMMARY.....	3
INTRODUCTION.....	5
PROCEDURES AND METHODS.....	7
RESULTS AND DISCUSSION.....	9
CONCLUSIONS/IMPLICATIONS/RECOMMENDATIONS.....	13
REFERENCES.....	14

LIST OF FIGURES

Figure 1. Land use within the Little Plover River study area.....	5
Figure 2. Nested groundwater sub-basins of the LPR study area.....	6
Figure 3. Cumulative discharge along the stream corridor.....	7
Figure 4. Distribution of total nitrate between excess N ₂ and nitrate in groundwater immediately before discharge to the stream.....	9
Figure 5. The load of excess N ₂ carried into the LPR as groundwater discharges.....	10
Figure 6. Relationship between the cumulative loads of total nitrate, nitrate and excess N ₂ along the stream corridor.....	11

LIST OF TABLES

Table 1. Characteristics of groundwater discharge to the LPR from the four sub-basins.....	10
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PROJECT SUMMARY

Title: A Basin-Scale Denitrification Budget for a Nitrate Contaminated Wisconsin Aquifer: A Study at the Groundwater/Surface Water Interface

Project ID: R/UW-GSI-002

Investigators:

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Period of Contract: July 1, 2000 to June 30, 2002

Background/Need:

Nitrate is a pervasive and increasing groundwater contaminant in Wisconsin. Many studies have found that nitrate is relatively conservative in groundwater. However, others have shown that nitrate can be transformed to nitrogen gas (denitrified) when reducing conditions are encountered along a groundwater flowpath (e.g., within riparian soil). Inadequate knowledge of groundwater denitrification hinders the development and application of accurate mass balance models for management of nitrate pollution.

Objectives:

The goal of this study was to achieve a better understanding of groundwater denitrification as a basin-scale control of nitrate concentrations and export from Wisconsin basins.

Methods:

The quantity and quality of groundwater discharging to the Little Plover River in Central Wisconsin was measured using a network of miniature wells at the groundwater/surface water interface (0.6 m beneath the streambed, distributed at 60-meter intervals over 10 km of headwater stream channel). Sampling surveys of all sites (n=160) were conducted in the summers of 2000 and 2001. A subset of sites (n=30) comprising a downwelling/upwelling sequence was sampled periodically. The concentration and load of denitrified-N carried into each 60-m stream segment via groundwater were quantified from the concentration of dissolved nitrogen gas (N₂) in excess of atmospheric equilibrium. Total groundwater nitrate was estimated from the sum of dissolved nitrate-N and excess N₂-N gas.

Results and Discussion:

For the average stream segment, 22 percent of groundwater nitrate-N (nitrate-N + denitrified-N) was discharged to surface water as excess N₂ gas (denitrified N). Higher denitrified N percentages were associated with low dissolved oxygen and high dissolved organic carbon of shallow (e.g., riparian soil) groundwater flowpaths. Lower denitrified N percentages were associated with indicators of deeper groundwater flowpaths (e.g., low DOC). Summed across all stream segments, the cumulative loads of denitrified N and nitrate-N were 45 kg/day and 157 kg/day, respectively, representing a basin-wide denitrification rate of 22.1 percent. Extrapolated

to an annual basis and expressed in terms of basin yield, these data indicate that approximately 57 kg/ha/yr were leached to groundwater as nitrate-N, of which 44 kg/ha/yr were released to surface water as nitrate-N. The remaining 13 kg/ha/yr were released to surface water as excess N₂-N (10 kg/ha/yr) or were transformed to excess N₂-N as surface water recharged groundwater in downwelling stream segments (3 kg/ha/yr).

Conclusions/Implications/Recommendations:

This study provides an aquifer-wide estimate of denitrification (13 kg/ha/yr) for a moderately thick (50-200 ft) surficial aquifer typical of many glacial/alluvial aquifers in agricultural settings in Wisconsin. Our results show that the transformation of NO₃⁻ to N₂ gas is quantitatively significant for the nitrate budget of agriculturally impacted aquifers. Approximately 22.1 percent of the total nitrate recharge to the aquifer (57 kg/ha/yr) was transformed to N₂ gas in groundwater. Groundwater denitrification appeared to be electron donor-limited in partially oxygenated intermediate and regional groundwater flow from remote upland recharge areas. Nitrate concentrations introduced by agricultural activity probably exceed the electron donating capacity of dissolved species in these flow systems. This suggests that increased loadings of nitrate to groundwater in the upland recharge areas will not be further offset or mitigated by biological nitrate removal via denitrification. In contrast, groundwater denitrification appeared to be nitrate-limited in a DOC-rich, local groundwater flow system associated with the near-stream environment. The near-stream, local groundwater flow system appeared to have additional capacity to buffer the stream's nitrate load against further increases in nitrogen loadings within the LPR landscape.

Because the annual recharge of the LPR aquifer is mostly derived during the rapid infiltration of coarse texture upland soils, intermediate and regional groundwater flow systems, which dominate the discharge to the LPR, carry low amounts of DOC to fuel the respiratory consumption NO₃⁻ by denitrifiers and have O₂ levels that potentially inhibit the activity of denitrifiers. Thus, our findings for the LPR basin may represent a lower-bound index of aquifer-wide denitrification. Similar studies should be performed in other basins to determine how basin characteristics (e.g., soils and geomorphology) affect aquifer-wide denitrification and further work should be done to clarify in what environments most denitrified N is generated (e.g., near-stream environment vs. intermediate and regional flowpaths).

This study contributes to a body of evidence that groundwater denitrification can substantially affect the load of nitrate delivered to aquifer-fed streams and that groundwater denitrification is an important factor controlling the collective release of N to large river systems from small agricultural basins.

Related Publications: none yet submitted

Key Words: nitrate, aquifer denitrification, groundwater/surface water interface, excess nitrogen gas.

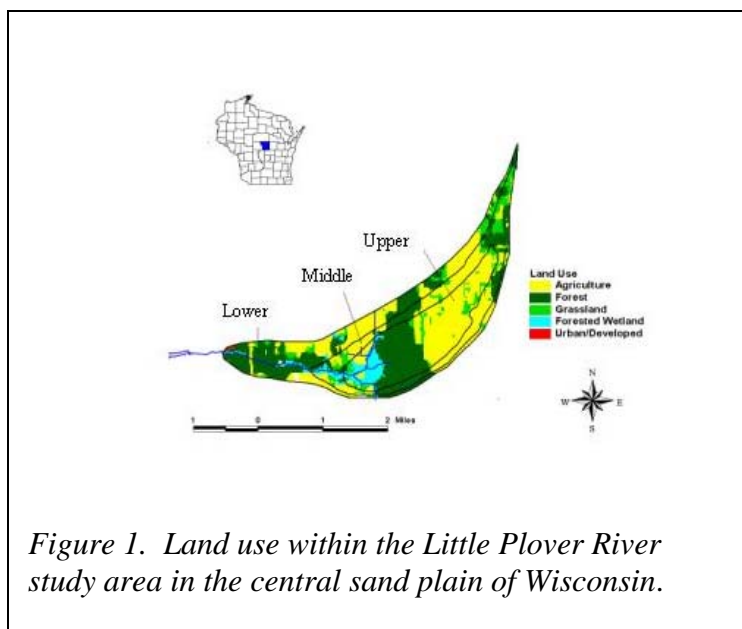
Funding: UWS and USGS

INTRODUCTION

Nitrate (NO_3^-) contamination of shallow groundwater is pervasive in Wisconsin and has affected the quality of small aquifer-fed tributaries to large river systems. These developments are partly explained by the steep rise in agricultural chemical fertilizer use since about 1960. Statewide, more than 10 percent of Wisconsin's wells currently exceed the nitrate drinking water standard, but across predominantly agricultural districts the exceedence rate averages about 20 percent (LeMasters and Baldock, 1995). Parallels have emerged between surface water quality of aquifer-fed streams and historical use of N fertilizer within agricultural basins. For example, Mechenich and Kraft (1997) reported that nitrate concentrations (as nitrogen) increased from 2.4 mg/l in 1967 to 8 mg/l in 1996 in the baseflow of the Little Plover River in the central sand plain of Wisconsin (Figure 1). Mass balance calculations for nitrate loadings in the central sand plain suggested that nitrate concentrations in groundwater and surface water could nearly double before leveling off under likely agricultural land use scenarios.

The relationship of nitrate contamination in groundwater and surface water in small streams is important to understand because the discharge of nitrate to surface water potentially 1) provides a regionalized indication of the quality of an aquifer's drinking water supply, and 2) indicates possible impacts to freshwater fauna (e.g., Kincheloe et al., 1979; Hecnar et al., 1995). Moreover, the release of nitrate from aquifers into small streams that collectively drain to large river systems is a threat to the health of coastal estuarine ecosystems (e.g., Gulf of Mexico at the mouth of the Mississippi River, e.g., Rabelais et al., 1996).

However, the interpretation of surface water trends in nitrate is difficult. Residence times of groundwater within surficial unconsolidated aquifers, on the order of decades, are similar to the timescale of major historical agricultural land use changes. In addition, chemical/biological reactions in the aquifer may efficiently remove nitrate before groundwater discharges to a stream. Thus, for example, currently low nitrate loads within a small stream could reflect groundwater recharged during earlier periods of lower fertilizer applications, biological removals by denitrification (microbial reduction of NO_3^- to N_2) or assimilation within the riparian soils, or a combination thereof. While relationships between aquifer residence time, historical and spatial variations in land use practices and nitrate are increasingly accessible by modeling approaches, too little is currently known of subsurface biological nitrate removals to account for their effects.



Objectives:

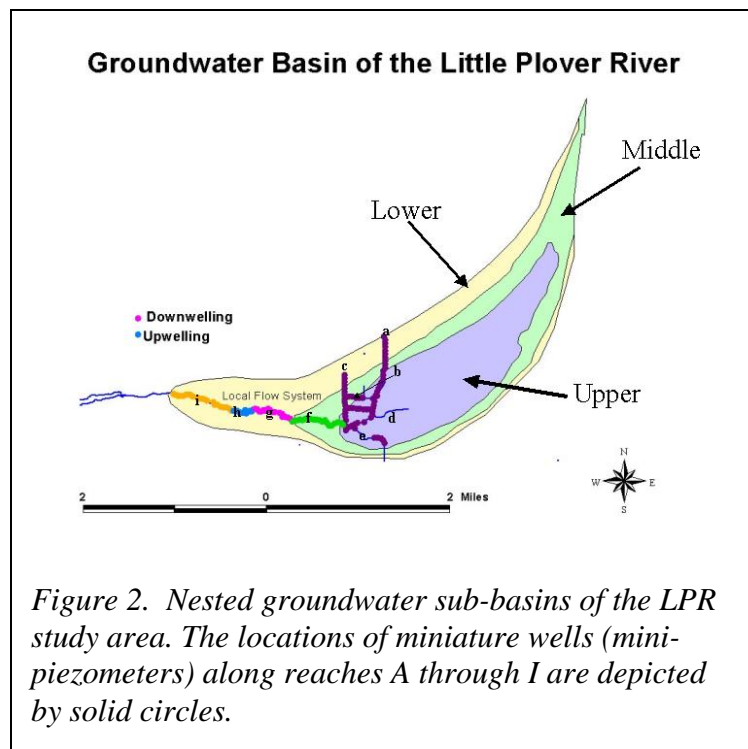
In this study we attempted to achieve a better understanding of groundwater denitrification as a basin-scale control of nitrate in aquifer-fed streams in Wisconsin. The specific objectives were to 1) quantify aquifer-wide denitrification by measuring the excess N_2 gas (relative to atmospheric equilibrium) carried in groundwater immediately before it discharges to the Little Plover River (Figure 1), and 2) evaluate possible flowpath controls (such as interception of reducing substrates in the near-stream environment) on aquifer denitrification.

Study Area:

The Little Plover River drains approximately 1300 ha of the Wisconsin central sand plain (Figure 1). The stream originates in perennial wetland seeps and agricultural ditches immediately below the Arnott moraine, flows west to the Wisconsin River, and includes cumulatively 10-km of first and second order channel at the westernmost point of the study area. On an average annual basis, most streamflow (90 percent) is derived when groundwater discharges from surficial deposits of glacial drift and gravelly outwash to the stream (Weeks et al., 1965).

The LPR basin consists of a set of nested groundwater sub-basins, which contribute baseflow (sustained flow from groundwater discharge) to different reaches of the stream (Figure 2). The Upper sub-basin discharges to the headwater agricultural ditches (reaches A-C) and wetland meanders (reaches D and E). The Middle and Lower sub-basins contribute flow to reach F and reach I. The recharge area of the Local sub-basin fluctuates dynamically with daily and seasonal moisture conditions, is too small to delineate in Figure 2, and feeds a local groundwater flow system connected to reaches G and H. The stream channel contributes recharge to the local groundwater flow system in losing reach G and receives discharge from the local groundwater flowsystem in gaining reach H.

The average daily baseflow load of nitrate (as nitrogen) in the LPR was approximately 156 kg/day in 1996 at the downstream end of the study area (Albertson, 1998). Extrapolated to annual basis this represents a yield of approximately 44 kg/ha/yr of nitrate-N from the basin. It is uncertain to what extent the load and yield of nitrate are mitigated by the conversion of nitrate to nitrogen gas by denitrification.



PROCEDURES AND METHODS

Network of Wells at the Groundwater/Surface Water Interface:

Miniature wells (minipiezometers) were installed at 160 locations (Figure 2) along 10-km of LPR stream channels, including three headwater agricultural ditches (reaches A, B, and C), two meandering headwater channels (reaches D, E), which originated in wetland, and one meandering second order channel (reaches F, G, H and I). The installations were uniformly spaced at 60-m intervals along the centerline of stream channel. The well screens were positioned 60-cm below the streambed to allow sampling of groundwater immediately before it discharged to the stream (reaches A-F, H, I) or immediately after it was recharged by surface water (reach G).

The minipiezometers installed for the study were made of 0.5 cm i.d., polyethylene tubing with 2.5 cm screens. The well screens had 8 to 10 lines of perforations created in four to five passes through a sewing machine. A stainless steel rod was inserted into the minipiezometer so the screen could be pushed into the sediment to the desired depth. The rod was then removed and the tubes were left in place throughout the study.

Groundwater Discharge/Recharge Measurements:

The rate of groundwater entry or exit for each stream segment was obtained using:

$$Q_i = q_i l_i w_i$$

where w_i and l_i are the i^{th} segment's length and average width, respectively, and q_i represents specific discharge calculated using Darcy's Law (Lee and Cherry, 1978)

$$q_i = -K_i (dh_i/dz_i)$$

Here K_i represents the hydraulic conductivity of the sediment and dh_i/dz_i , the hydraulic gradient. Hydraulic conductivity, K_i , was measured using a falling head test (Hvorslev, 1951). The hydraulic gradient was determined from the height (dh_i) of the static head relative to the streamwater surface and the depth of the well screen (dz_i) below the streambed.

An independent check of the accuracy of Q_i measurements was made by comparing the cumulative segment discharge (ΣQ_i) along the stream corridor to measured streamflow (Figure 3). The values of Q_i were summed from the upstream end to downstream end of each reach and the reaches were summed in sequence from A to I.

Sample Collection and Analysis:

Water samples were field filtered (0.45 μm nitrocellulose membrane filter), chemically preserved (as appropriate), and stored at 4 °C for chemical analysis of dissolved solutes (nutrients, major ions, DOC, DIC,

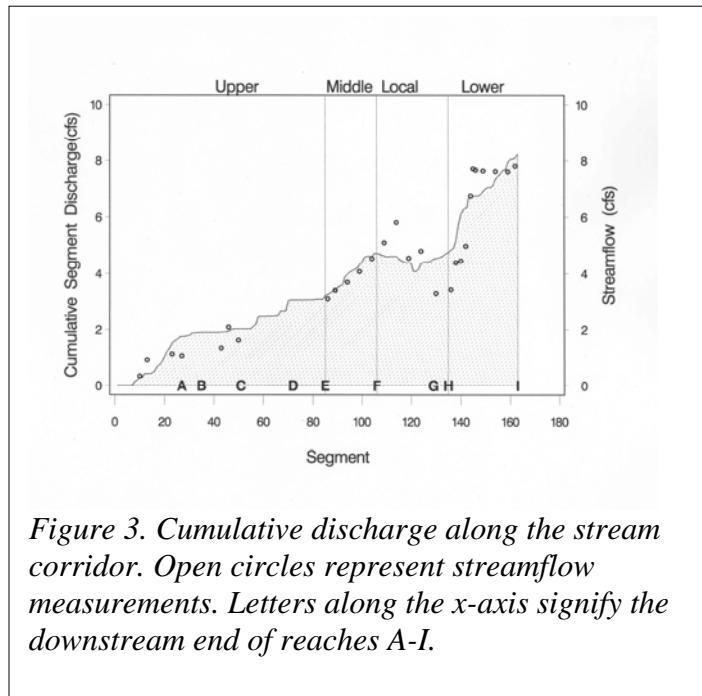


Figure 3. Cumulative discharge along the stream corridor. Open circles represent streamflow measurements. Letters along the x-axis signify the downstream end of reaches A-I.

silica and alkalinity) in the lab. Water temperature, pH, specific conductance, and dissolved oxygen, and oxidation reduction potential were measured before sample collection using a YSI sonde. Surface water samples were collected as grab samples. Groundwater samples were collected under suction by attaching a peristaltic pump to the minipiezometer. A sealed flow-through cell was connected to the outlet of the peristaltic pump to obtain sonde readings.

Dissolved gases (Ar, N₂, O₂, CO₂, CH₄, N₂O, chlorofluorocarbons) were collected from groundwater and surface water using an in-field gas stripping approach (Browne, 2002). At the time of collection, barometric pressure was recorded using the YSI sonde. Total dissolved gas pressure was determined using a dissolved gas saturation monitor calibrated in percent saturation mode relative to ambient barometric pressure. The stripped gas was maintained in gas-tight 10-ml syringes until the mole fraction (X_i) of each analyte could be determined in the lab by gas chromatography. Analyses were completed within 2 to 5 days of sample collection. The partial pressure of each gas analyte (P_i) was obtained by multiplying its mole fraction by the total dissolved gas pressure. The concentration of dissolved gas was calculated from Henry's Law relationships.

Measurement of Excess N₂ (Denitrified N):

The measured partial pressure of Ar, a nonbiogenic reference gas, was used to determine the amount of dissolved N₂ gas derived from atmospheric equilibration prior to aquifer recharge (Vogel et al., 1981; Martin et al., 1995). Dissolved concentrations of N₂ gas in excess of atmospheric equilibrium were assumed to result from the microbial transformation of NO₃⁻ to N₂ within groundwater. An estimate of the original total concentration of nitrate before denitrification was obtained as the sum of measured NO₃⁻ and excess N₂ concentrations:

$$[\text{Total_NO}_3^-] = [\text{NO}_3^-] + 2[\text{excess_N}_2]$$

The ratio, ξ , of excess N₂ gas to total NO₃⁻ was used as an index of the progress of the denitrification reaction within groundwater immediately before it discharged to the stream (gaining segments) or immediately after it was recharged by surface water (losing segments):

$$\xi = \frac{2[\text{excess_N}_2]}{[\text{Total_NO}_3^-]}$$

Estimation of Loads (kg/day) and Annual Yield (kg/ha/yr) of Excess N₂:

The loads of nitrogen in kg-N/day entering or exiting each stream segment as NO₃⁻ and excess N₂ were estimated using:

$$\text{Load}_i = Q_i C_i$$

where C_i is the concentration of NO₃⁻ nitrogen or excess N₂ nitrogen. Segment loads were summed (ΣLoad_i) from the upstream end to downstream end of each reach and reaches were summed in sequence from lowest to highest letter (Figure 2) to obtain cumulative loads. The cumulative load values for NO₃⁻ were compared to the actual NO₃⁻ loads measured within the stream itself at several positions along the stream corridor as an independent check of the accuracy of segment loads (See Figure 6). Actual nitrate loads within the stream ($Q_{\text{strm}} C_{\text{strm}}$ in kg/day) were obtained from the measured streamflow (Q_{strm}) and concentration (C_{strm}).

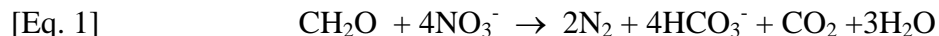
Estimates of the annual basin yield of nitrate and excess N₂ in kg/ha/yr (as nitrogen) were extrapolated from the cumulative segment loads from reaches A-I. The cumulative load in kg/day was multiplied by 365 and divided by total basin area (1294 ha) to obtain basin yield.

RESULTS AND DISCUSSION

Figure 4 illustrates the concentration excess N₂ detected in groundwater 0.6 m below the stream/sediment interface of each 60-m stream segment. These data reflect the composition of groundwater shortly before it discharged to the stream (in gaining stream segments) or shortly after surface water

recharged groundwater (in losing stream segments). The data reveal that some of the nitrate recharged to groundwater in the upland landscape was converted to benign N₂ gas before being discharged to the stream. In addition they show that some nitrate discharged to the stream was converted to N₂ gas when it reentered groundwater through the losing stream segments of reach G. Denitrification reaction progress (ξ) approached 100 percent in reaches G and H of the Local sub-basin and was notably high in headwaters segments within reaches D and E of the Upper sub-basin. What these locations

appeared to have in common, and what distinguishes them from other segments, were an available supply of reactive electron donor in the form of DOC (Figure 4) to fuel denitrification (eq. 1) as well as low dissolved oxygen (a condition conducive to denitrification).



Because high DOC concentrations probably reflect shallow groundwater flowpaths (riparian/hyporheic flowpaths), we concluded that the denitrification progress of these segments probably occurred in the near-stream environment. The smaller indices of denitrification progress typical of the low DOC stream segments likely reflect denitrification reactions remote from the stream along intermediate and regional groundwater flowpaths.

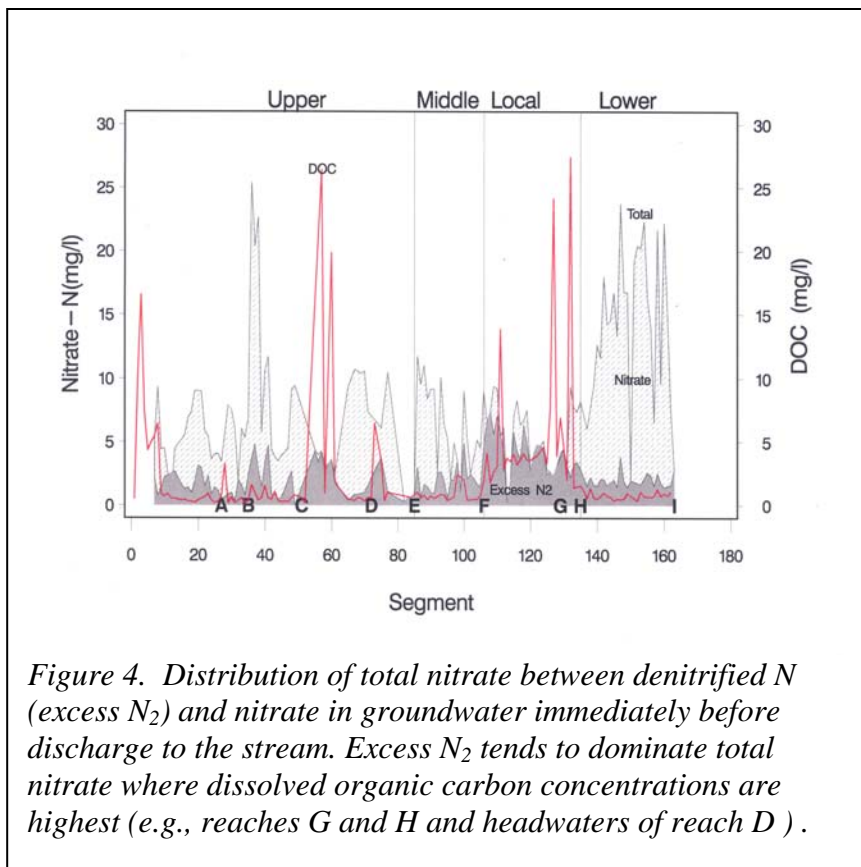


Figure 4. Distribution of total nitrate between denitrified N (excess N₂) and nitrate in groundwater immediately before discharge to the stream. Excess N₂ tends to dominate total nitrate where dissolved organic carbon concentrations are highest (e.g., reaches G and H and headwaters of reach D).

Excess N₂ in Intermediate and Regional Groundwater Flowpaths:

The concentrations and loads of excess N₂ from the Upper, Middle and Lower sub-basins (Table 1) provide an indication of the importance of denitrification along intermediate to regional groundwater flowpaths in the LPR. Groundwater discharged from these sub-basins mostly originates in upland recharge areas remote from the stream (Figure 2). Estimates of the average

Table 1. Characteristics of groundwater discharge to the LPR from the four sub-basins (Figure 2). Values represent mean and standard deviations of 60-m stream segments sampled for each sub-basin. Flags (* or **) identified weighted means: * weighting by discharge and ** weighting by total-NO₃ load.

Sub-Basin	Stream Segments (n)	mg/l								%				Apparent Age (yr)*	Load (kg/day)						
		Nitrate*		Excess N ₂ *		Total NO ₃ *		DOC*		ξ **		O ₂ Sat*			Discharge (cfs)		Nitrate		Excess N ₂		
Upper	42	4.90	0.87	1.73	0.26	6.63	0.78	2.59	1.96	21.3	24.8	72.7	8.3	18.8	3.0	7.00E-02	8.14E-02	0.840	1.545	0.297	0.428
Middle	20	4.72	0.86	1.99	0.29	6.72	0.90	0.80	0.13	29.6	23.9	85.8	7.3	27.6	3.9	7.49E-02	6.45E-02	0.878	1.149	0.371	0.429
Local	29	1.34	0.41	4.34	0.26	5.68	0.39	5.03	0.98	68.2	15.8	9.3	1.5	3.8	1.5	9.26E-04	7.75E-02	-0.097	0.673	0.375	0.480
Lower	25	9.84	1.76	1.78	0.13	11.62	1.72	0.73	0.10	13.2	23.6	61.0	5.7	13.7	4.1	1.32E-01	1.34E-01	3.793	4.310	0.574	0.543
Total	116	6.08	1.33	2.18	0.35	8.26	1.22	1.89	1.35	22.0	27.0	64.1	8.7	16.3	3.7	6.63E-02	1.01E-01	1.227	2.617	0.387	0.472

time of travel to the stream based on dissolved chlorofluorocarbons measurements (Busenberg and Plummer, 1992) are provided in Table 1 as “Apparent Age.” Mean aquifer residence-times before discharge to the stream ranged from approximately 14 years for the Lower sub-basin to 28 years in the Middle sub-basin (Table 1).

Consistent with the apparent age distribution, the Lower sub-basin had higher mean segment loadings of NO₃⁻ and total-NO₃ (reflecting more recent agricultural practices) than the Upper and Middle sub-basins. However, the comparatively low index of denitrification reaction progress (ξ=13.2%) observed for the Lower sub-basin suggests that increased loading of nitrate in upland recharge areas over the last several decades has not been matched by proportional increases in denitrification along intermediate and regional groundwater flowpaths to the LPR. Two factors that potentially limit denitrification progress along flowpaths to the LPR from upland recharge areas include 1) the availability of electron donors (notably organic carbon), and 2) inhibition by dissolved oxygen.

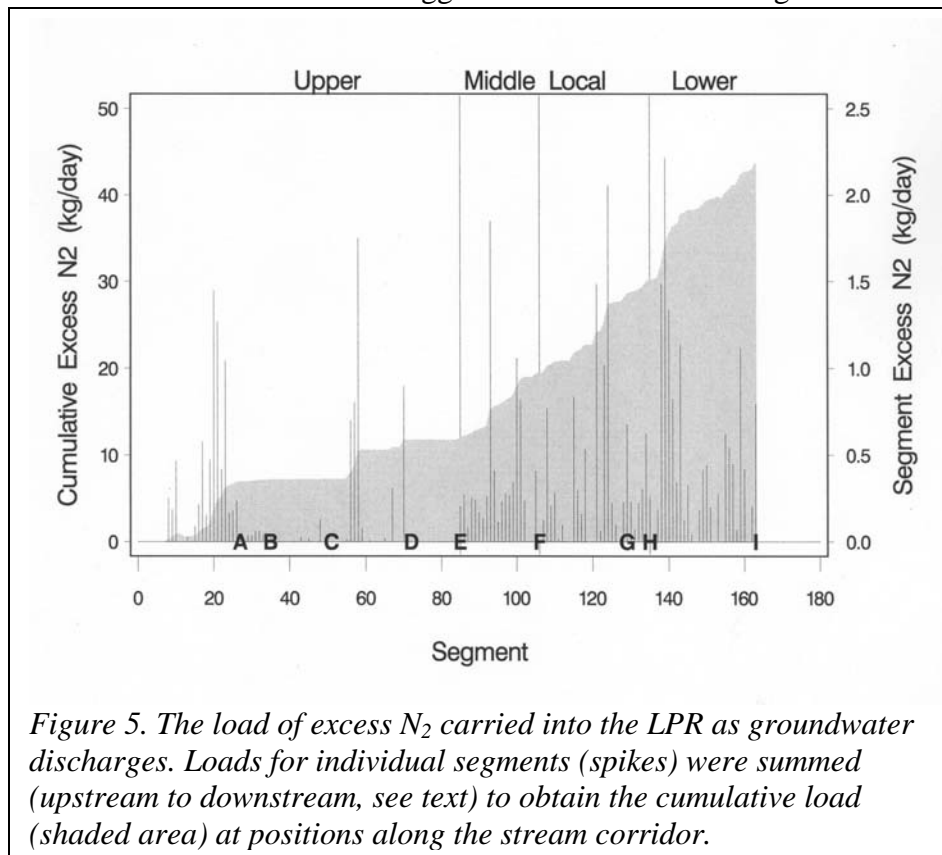


Figure 5. The load of excess N₂ carried into the LPR as groundwater discharges. Loads for individual segments (spikes) were summed (upstream to downstream, see text) to obtain the cumulative load (shaded area) at positions along the stream corridor.

Because organic carbon is a common electron donor for the heterotrophic denitrification process (Eq. 1), zones of high dissolved organic carbon (DOC) are important for denitrification along groundwater flowpaths (Komor, 1992). Potential zones of high DOC along intermediate and regional groundwater flowpaths of the LPR include the soil horizons and the near water table environment of upland recharge areas, patches of buried organic debris within glacial and alluvial aquifer substrate, and the riparian soils and hyporheic sediments of the near-stream environment. Bypass of these zones, or limited contact with them, will potentially limit excess N_2 production.

Two factors likely physically limit the contribution of excess- N_2 from the near-stream environment of the Upper, Middle and Lower sub-basins. First, most of the recharge area for the Upper, Middle and Lower discharge zones is remote from the LPR's riparian corridor. This suggests that as shallow groundwater flowlines from nearby recharge areas (riparian zones) and deeper flowlines from remote recharge areas converge in the stream subsurface, deeper flowlines will dominate the collective discharge thereby allowing large parcels of older DOC-poor groundwater to enter the stream from beneath the riparian soil. Consistent with this idea, groundwater discharged to stream segments of the Upper, Middle and Lower sub-basins 1) generally lacked high DOC concentrations (a condition contraindicative of flowpaths through organic matter-rich riparian soils) that could fuel denitrification in the near-stream environment (Figure 4), and 2) had apparent ages (Table 1) consistent with long travel-times from distant recharge areas. Second, due to riparian underflow, low-DOC upwelling waters continually wash the incoherent sandy sediments of the gaining stream segments, preventing an accumulation of organic matter-rich substrate to facilitate denitrification in the hyporheic zone.

Thus, most of the excess N_2 from the Upper, Middle and Lower sub-basins was probably not produced in the near-stream environment. Remote production of excess N_2 could have occurred slowly and continuously over long periods of time as DOC-poor groundwater traveled to the stream discharge point (Vogel et al, 1981; Pucket and Cowdery,

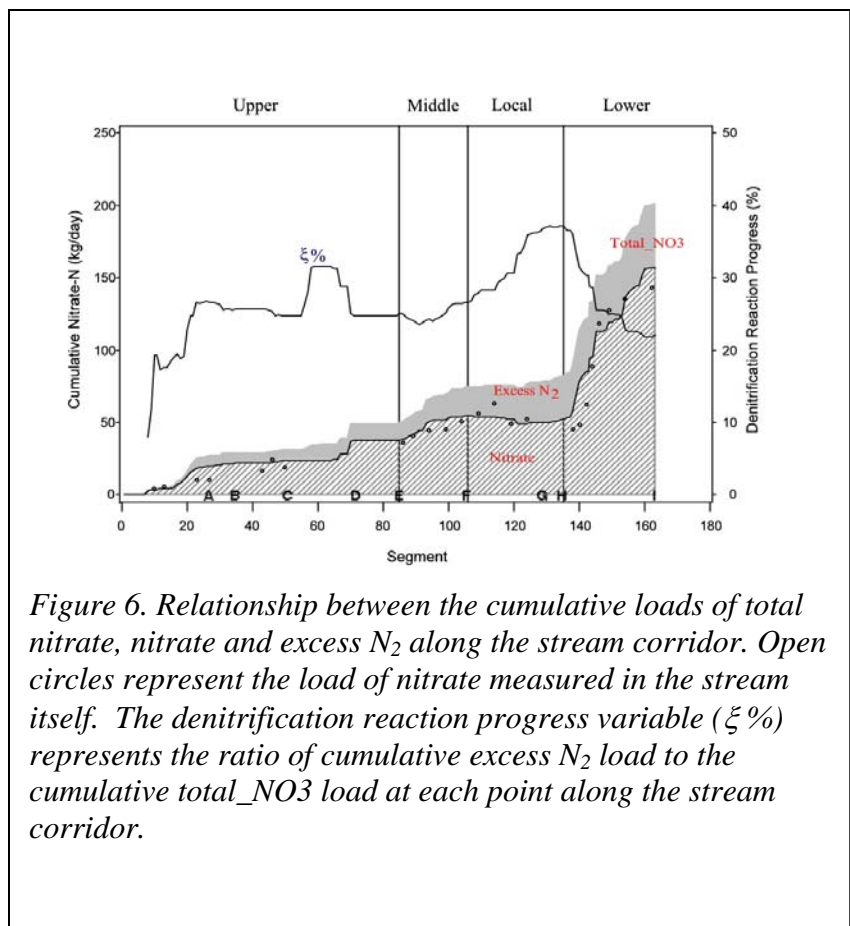


Figure 6. Relationship between the cumulative loads of total nitrate, nitrate and excess N_2 along the stream corridor. Open circles represent the load of nitrate measured in the stream itself. The denitrification reaction progress variable (ξ %) represents the ratio of cumulative excess N_2 load to the cumulative total NO_3 load at each point along the stream corridor.

2002). The average apparent age of groundwater discharge was 13.7 yr for the Lower sub-basin, 18.8 yr for the Upper sub-basin, and 27.6 yr for the Middle sub-basins. Based on these flowpath durations and the mean excess N₂ concentrations reported in Table 1, the effective denitrification rates for the Upper, Middle and Lower sub-basins were 0.0066, 0.0051 and 0.0092 mmol L⁻¹ yr⁻¹, respectively. However, denitrification may also have occurred in relatively rapid bursts during interception of organic carbon-rich subsurface environments (e.g., such as the near water table environment of an upland recharge wetland). Although a practical distinction between slow-sustained and rapid denitrification mechanisms cannot be drawn from the data, the similarity of excess-N₂ concentrations between the Upper, Middle and Lower sub-basins, despite wide variation in apparent age, weighs against the idea that denitrification is rate-limited along intermediate and regional flowpaths.

Dissolved oxygen is favored thermodynamically over nitrate as a terminal electron acceptor in the oxidation of organic carbon and may, therefore, inhibit microbial denitrification by favoring aerobic heterotrophs. Consistent with this idea, our results revealed a strong negative correlation between excess N₂ and O₂ (%sat) ($r^2=0.62$, $p < 0.0001$, $Y = 4.07 - 0.033 X$) in groundwater. Our results also revealed instances where excess N₂ and O₂ were coincident at relatively high dissolved O₂ levels (>50 percent O₂ saturation). These occurrences have a number of possible explanations. For example, denitrification may have occurred in anoxic hotspots along flowpaths to the stream (e.g., in patches of DOC-rich groundwater at the capillary fringe of upland recharge wetlands). It is also possible that facultative anaerobes used both O₂ and NO₃⁻ to support heterotrophic respiration (Komor, 1992; Robertson and Kuenen, 1984). An additional explanation is that sample collection (pumping from the minipiezometers) may have induced convergence of flowlines within the stream subsurface (e.g., mixing of deoxygenated, longer residence time and oxygenated, shorter residence time flowlines). However, the data did not provide a basis to distinguish which of these mechanisms (or others) may be responsible for the relationship between excess N₂ and dissolved oxygen.

Excess N₂ in Shallow Groundwater Flowpaths of the Local Sub-basin:

The production of excess-N₂ in Local sub-basin groundwater was not limited by the availability of electron donor (DOC) or inhibited by the presence of O₂. Reaches G and H had the highest mean concentration of DOC and the lowest mean concentrations of O₂ of all the sub-basins (Table 1). These reaches also had the youngest mean apparent age (3.8 ± 1.5 yr) of groundwater, suggesting a near-stream origin (riparian and hyporheic flowpaths) for the excess-N₂ burden. Cumulatively, reaches G and H accounted for 11 kg/day (25 percent) of the total excess-N₂ load (45 kg/day) to the LPR (Figure 5). Due to the denitrification of surface water nitrate recharged to groundwater within the losing segments of reach G, the overall mean segment load of nitrate was negative (-0.097 kg/day).

The near-stream environment and hyporheic sediments of the Local sub-basin provide organic matter-rich environments for heterotrophic denitrification due to a continual influx of 1) particulate organic carbon (POC) and DOC from the stream in the losing segments, and 2) DOC from shallow riparian groundwater flowpaths in the gaining stream segments. This means that denitrification in groundwater of the Local sub-basin is potentially limited solely by NO₃⁻ availability and implies that reaches G and H have additional capacity to transform NO₃⁻ to excess N₂ in groundwater. Thus, unlike reaches fed by intermediate and regional groundwater

discharge, reaches G and H will likely continue to partially buffer the stream's nitrate load against further increases in nitrogen loadings within the landscape.

Basin-scale Production of Excess N₂ in the LPR Basin:

There was a close correspondence between the collective minipiezometer measurements at the 0.6 m depth and stream measurements. Figure 6 shows that nitrate loads measured within the stream itself agreed with the cumulative segment loadings of nitrate from groundwater. (The stream loads of excess N₂ did not correspond as closely to the cumulative segment loads of excess N₂. Oversaturation of the stream water relative to atmospheric N₂ induced a continual efflux of excess N₂ from the stream to the atmosphere along the stream corridor.) Good agreement was also obtained when cumulative segment chloride loads were compared to stream chloride loads and when cumulative segment discharge (Q_i) was compared to measured streamflow (Q_{strm}). These observations show that the sampling network performed well in providing a basin-scale or aquifer-wide viewpoint on groundwater quality currently discharging to the stream.

Figure 6 shows nitrogen discharged along the stream corridor as nitrate and excess N₂. Summed across all stream segments, the cumulative loads (as nitrogen) of excess N₂ and NO₃⁻ were 45 kg/day and 157 kg/day, respectively. Extrapolated to an annual basis and expressed in terms of basin yield, approximately 57 kg/ha/yr were leached to groundwater as nitrate-N, of which 44 kg/ha/yr were released to surface water as nitrate-N. The remaining 13 kg/ha/yr were released to surface water as excess N₂-N (10 kg/ha/yr) or were transformed to excess N₂-N (3 kg/ha/yr) as surface water recharged groundwater in downwelling stream segments. On a basin-scale the index of denitrification progress suggests that 22.1 percent of the nitrate leached to ground water was denitrified.

CONCLUSIONS/IMPLICATIONS/RECOMMENDATIONS

This study provides an aquifer-wide estimate of denitrification (13 kg/ha/yr) for a moderately thick (50-200 ft) surficial aquifer typical of many glacial/alluvial aquifers in agricultural settings in Wisconsin. Our results show that the transformation of NO₃⁻ to N₂ gas is quantitatively significant for the nitrate budget of agriculturally impacted aquifers. Approximately 22.1 percent of the total nitrate recharge to the aquifer (57 kg/ha/yr) was transformed to N₂ gas in groundwater. Ground water denitrification appeared to be electron donor-limited in partially oxygenated intermediate and regional groundwater flow from remote upland recharge areas. Nitrate concentrations introduced by agricultural activity probably exceed the electron donating capacity of dissolved species in these flow systems. This suggests that increased loadings of nitrate to groundwater in the upland recharge areas will not be further offset or mitigated by biological nitrate removal via denitrification. In contrast, groundwater denitrification appeared to be nitrate-limited in a DOC-rich, local groundwater flow system associated with the near stream environment. The near-stream, local groundwater flow system appeared to have additional capacity to buffer the stream's nitrate load against further increases in nitrogen loadings within the LPR landscape.

Average groundwater residence times in the LPR aquifer (13.4 yr to 27.6 yr) are apparently not sufficient to induce anoxic conditions typical of aquifer environments where denitrification progress approaches 100 percent (e.g., Postma et al., 1991). Because the annual recharge of the

LPR aquifer is mostly derived during the rapid infiltration of coarse texture upland soils, intermediate and regional groundwater flow systems, which dominate the discharge to the LPR, carry low amounts of DOC to fuel the respiratory consumption of NO_3^- by denitrifiers and have O_2 levels that potentially inhibit the activity of denitrifiers. Thus, our findings for the LPR basin may represent a lower-bound estimate of aquifer-wide denitrification.

The apparent rate of denitrification for the intermediate and regional flowpaths of the LPR ranged from 0.0051 to 0.0092 $\text{mmol L}^{-1} \text{yr}^{-1}$. These values are comparable to rates found in other field studies in sand and gravel aquifers (summarized in Puckett and Cowdery, 2002). Based on the assumption of slow, sustained reaction rates along intermediate and regional groundwater flowpaths, these calculations overlook the possibility of bursts of localized denitrification in electron donor-rich environments (e.g., near water table environments of upland recharge areas). Similar studies should be performed in other basins to determine how basin characteristics (e.g., soils and geomorphology) affect aquifer-wide denitrification and further work should be done to clarify in what environments most denitrified N is generated (e.g., near-stream environment vs. intermediate and regional flowpaths).

This study contributes to a body of evidence that groundwater denitrification can substantially affect the load of nitrate delivered to aquifer-fed streams and that groundwater denitrification is an important factor controlling the collective release of N to large rivers systems from small agricultural basins.

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