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Evaluation of denitrification in an urban stream receiving wastewater effluent

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Abstract Urban streams often contain elevated concentrations of nitrogen (N) which can be amplified in systems receiving effluent from wastewater treatment plants (WWTP). In this study, we evaluated the importance of denitrification in a stream draining urban Greensboro, NC, USA, using two approaches: (1) natural abundance of ^{15}N–NO_3^- in conjunction with background NO_3^-–N concentrations along a 7 km transect downstream of a WWTP; and (2) C_2H_2 block experiments at three sites and at three habitat types within each site. Overall lack of a longitudinal pattern of $\delta^{15}N$–NO_3^- and NO_3^-–N, combined with high concentrations of NO_3^-–N suggested that other factors were controlling NO_3^-–N flux in the study transect. However, denitrification did appear to be significant along one portion of the transect. C_2H_2 block experiments showed that denitrification rates were much higher downstream of the WWTP compared to upstream, and showed that denitrification rates were highest in erosional and depositional areas downstream of the WWTP and in erosional areas upstream of the plant. Thus, the combination of the two methods for evaluating denitrification provided more insight into the spatial dynamics of denitrification activity than either approach alone. Denitrification appeared to be a significant sink for NO_3^-–N upstream of the WWTP, but not downstream. Approximately 46% of the total NO_3^-–N load was removed via denitrification in the upstream, urban section of the stream, while only 2.3% of NO_3^-–N was lost downstream of the plant. This result suggests that controlling NO_3^-–N loading from the plant could result in considerable improvement of downstream water quality.

Keywords Denitrification · Nitrogen · $\delta^{15}N$–NO_3^- · Urban stream · Wastewater

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Introduction

Urban streams are often heavily impacted by excessive nutrient loading due to inputs from nonpoint sources such as lawn fertilizers, septic tanks and sewage overflows (Wahl et al. 1997), pet waste (NCDENR 2004) and loss of pervious surfaces which reduce buffering capacity and facilitate runoff to adjacent streams (Paul and Meyer 2001). Point source pollution from industrial or wastewater treatment effluent also contributes markedly to nutrient enrichment in streams (Carpenter et al. 1998; Dyer and Wang 2002; Haggard et al. 2005; Marti et al. 2004).

Excessive N loading degrades water quality by altering the structure and function of biological communities (Inwood et al. 2005; Kemp and Dodds 2002; Ulseth and Hershey 2005). Streams heavily impacted by nitrogen often show lower nutrient removal capacity resulting in massive downstream export (Inwood et al. 2005; Paul and Meyer 2001), and eutrophication of receiving waters (Carpenter et al. 1998; Richardson et al. 2004; Wahl et al. 1997).

Previous studies have found that headwater streams play a major role in reducing N loads to downstream areas because of their capacity to retain N through various biogeochemical mechanisms (Inwood et al. 2005; Mulholland et al. 2004; Peterson et al. 2001; Wolheim et al. 2001). Mineralization of organic matter results in previously bound organic N being released as inorganic forms (Heaton 1986). In addition to biological assimilation and sorption to sediments, ammonium (NH_4^+) quickly undergoes nitrification as it spirals downstream (Mulholland et al. 2004; Peterson et al. 2001). Due to energetic constraints, microbes assimilate NH_4^+ more readily than nitrate (NO_3^-–N). Furthermore, inputs of NO_3^-–N are often much higher than NH_4^+ inputs into stream ecosystems. As a result of these processes, NO_3^-–N uptake lengths are often ten times greater than NH_4^+ uptake lengths (Peterson et al. 2001). Therefore, mechanisms controlling the fate of NO_3^-–N are of major significance to stream health.

One of the most important biogeochemical mechanisms governing N transformation is denitrification, which is carried out by heterotrophic bacteria under certain environmental conditions (e.g., Seitzinger 1988). Denitrification is considered to be an important ecological sink because it removes nitrogen from the system largely as an inert gas whereas other mechanisms, such as microbial assimilation, leave various forms of biologically available N in the system for later processing and transformation (Bohlke et al. 2004; Martin et al. 2001; Royer et al. 2004). The primary factors controlling denitrification rates include concentration of NO_3^-, a labile organic carbon (OC) source, and anoxic or near anoxic conditions (Knowles 1982; Trevors and Starodub 1987). Temperature, pH, and hydrological and sediment characteristics are also important factors controlling denitrification (Kellman and Hillaire-Marcel 1998; Knowles 1982; Richardson et al. 2004). In the absence of oxygen, denitrifying microbes reduce nitrogen oxides (NO_3^- and/or NO_2^-) and use them as terminal electron acceptors in the oxidation of organic matter, ultimately resulting in the production of nitrous oxide (N_2O) followed by dinitrogen gas (N_2) (Duff and Triska 2000; Knowles 1982; Martin et al. 2001; Seitzinger 1988; Tiedje et al. 1989).

Several methods exist for quantifying denitrification rates, but one of the most widely used methods involves the acetylene block technique (Duff and Triska 1990; Martin et al. 2001; Tiedje et al. 1989), which inhibits the reduction of N_2O to N_2 (Seitzinger et al. 1993; Sorensen 1978; Yoshinari et al. 1977). The stable isotope ratio of NO_3^-–N relative to a standard has also recently been used to assess denitrification over longitudinal transects (Kellman and Hillaire-Marcel 1998). Changes in the ratio of the heavier isotope to the lighter isotope (^{15}N:^{14}N), or fractionation, provide information regarding biogeochemical processes (Peterson and Fry 1987). In NO_3^-–N rich streams, denitrifying microbes will utilize the lighter isotope leading to enrichment of ^{15}N–NO_3^-. Thus, increasing $\delta^{15}N$–NO_3^- values with decreasing NO_3^-–N concentrations along a stream transect suggests denitrification is the major process controlling NO_3^-–N concentration (Chang et al. 2002; Kellman and Hillaire-Marcel 1998; Sebilo et al. 2003). However, if the relationship between $\delta^{15}N$–NO_3^- and NO_3^-–N is not inversely proportional, denitrification is not likely a dominant process in the system (Kellman and Hillaire-Marcel 1998).

In this study, we focused on the role of denitrification as a sink for N in NBC above versus below the WWTP. We used two different techniques to evaluate denitrification across varying spatial scales. We know of no other study that has combined these two approaches to examine both site specific and longitudinal patterns in denitrification. An earlier study conducted in North Buffalo Creek, Greensboro, NC, USA found NH_4^+–N concentrations up to 2.5 mg/l and NO_3^-–N concentrations as high as 8.0 mg/l at a site 6 km below the North Buffalo Creek Wastewater Treatment Plant (NBC WWTP) (Hershey et al. 2004). Although there was an observed decline in NH_4^+–N with distance downstream, there was not an overall decline in DIN indicating that nitrification was significant, such that overall DIN concentrations were in excess of microbial demand, and nitrogen retention efficiency was low. Based on these results from NBC and other studies of nitrogen dynamics in

urban streams, we hypothesized that longitudinal patterns in $\delta^{15}N$–NO_3^- vs. NO_3^-–N, which are indicative of denitrifying activity, could be used to evaluate whether denitrification is a significant sink for N in a stream with high point-source loading of NO_3–N. We also hypothesized that denitrification rates in an urban stream would be higher downstream of a WWTP, where NO_3^-–N concentration is higher, compared to upstream. Finally, we hypothesized that at uniform NO_3^-–N concentrations, denitrification rates vary among different habitat types in urban streams. We predicted that erosional habitats would yield higher denitrification rates than the depositional habitats and mid-channel laminar flow areas because erosional substrates were dominated by clay, which is likely to support anaerobic microsites and, therefore, higher denitrification. To test theses hypotheses, we measured $\delta^{15}N$–NO_3^- and NO_3^-–N along a transect in North Buffalo Creek below the North Buffalo Creek Wastewater Treatment Plant (NBC WWTP). We also directly measured denitrification in erosional, depositional, and laminar flow habitats within each of three sites, two sites downstream of the WWTP and one site upstream. Finally, we evaluated whether the observed longitudinal patterns in $\delta^{15}N$–NO_3^- vs. NO_3^-–N were consistent with observed direct measurements of denitrification.

Materials and methods

Study sites

This research was conducted in North Buffalo Creek (NBC) which is located in Greensboro, NC, USA, and originates in the headwaters of the Cape Fear River Basin. Greensboro is a moderate-sized city with a population size of 238,000 (City of Greensboro, Planning Department, 2005). Two wastewater treatment plants serve the City of Greensboro. The T.Z. Osborne plant serves the southern portion of the city and is located on South Buffalo Creek. The northern portion of the city is served by the NBC WWTP with the effluent draining into NBC just before it leaves the city limits (City of Greensboro, Water Resources 2005). The NBC WWTP contributes approximately 50% of the baseflow discharge to NBC (Hershey et al. 2004), but the plant effluent originates primarily in the Greensboro water supply reservoirs, which occur in the Reedy Fork watershed. NBC WWTP contributes approximately 45,000 to 60,000 m^3/l of treated effluent to NBC (Ulseth and Hershey 2005). The plant employs a final treatment stage involving sand filtration tanks and addition of sodium hypochlorite for removal of pathogenic bacteria, but no nutrient removal (City of Greensboro, Water Resources 2005). The NBC WWTP is permitted to discharge 8 mg/l of ammonium (NH_4^+) in winter and 4 mg/l in summer. Currently, no regulations exist for NO_3^-–N concentrations in the effluent (Ed Osborne, NBC WWTP, personal communication). Phosphorous (P) concentrations also were elevated below the WWTP compared to upstream sites. Ulseth and Hershey (2005) found that average P concentrations were less than 0.01 mg/l upstream while concentrations ranged from 0.08 to 0.38 mg/l downstream of the WWTP.

Land use above the NBC WWTP is primarily urban while the reach below the WWTP is rural and largely forested. NBC has been designated as impaired and listed on the Environmental Protection Agency's 303(d) list due to poor quality fish and benthic invertebrate communities in addition to multiple violations of fecal coliform bacteria standards which prohibit rating for recreational use (NCDENR 2004). The stream reach chosen for this research began approximately 7 stream km above the NBC WWTP and extended approximately 14 stream km below the WWTP (Fig. 1).

Three sites were selected for studies of denitrification rates using the acetylene block technique. Fairview was the most urban site lying in the northeast section of Greensboro. Riparian habitat at Fairview extended approximately 10 m from the bank with a grass buffer extending beyond riparian cover. Minorwood was located approximately 1.72 stream km below NBC WWTP. Minorwood was primarily forested and in an area with low housing density. Rankin was located outside of the city limits and approximately 8.13 stream km from NBC WWTP with densely forested riparian habitat on both sides. The stream transect chosen for isotope sampling began at Rankin and extended downstream.

Field procedures

Samples for $\delta^{15}N$–NO_3^- isotope analysis were taken from a canoe along a transect that began at Rankin

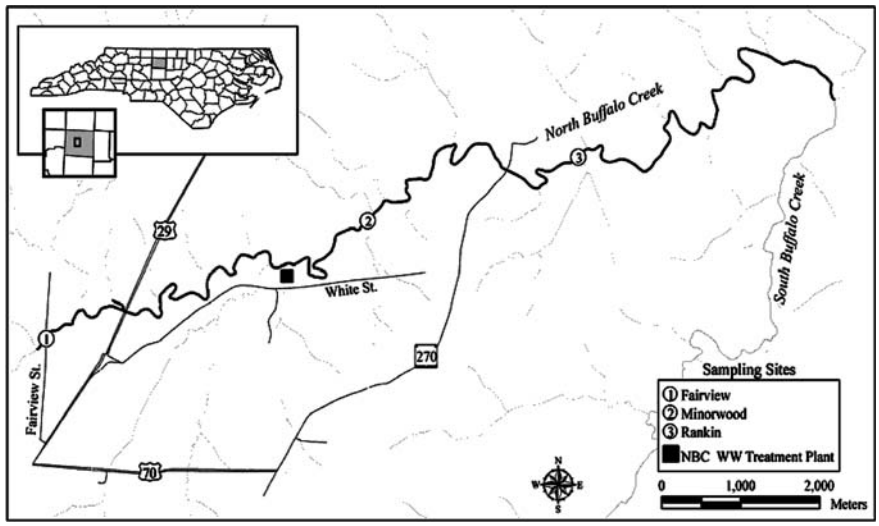

Fig. 1 Map of study sites on North Buffalo Creek in Greensboro, NC

Mill Road on October 9, 2004 and July 26, 2005 (Fig. 1). Samples were taken approximately every 500 m proceeding downstream, and distances were determined by GPS. The stream transect sampled on October 9, 2004 extended 3.5 stream km with a total of 8 stations sampled. A second sampling date was added the following summer to assess any differences between the fall and summer patterns. Mean stream temperatures were 18°C and 25°C for October 9, 2004 and July 26, 2005, respectively. Additionally, we extended the length of the study transect sampled on July 26, 2005 to approximately 7 stream km with 15 sampled stations in an effort to evaluate longer longitudinal patterns. All streamwater samples for isotope analysis were taken from mid-channel laminar flow areas using 60 ml syringes. Syringes were flushed three times prior to use. Syringe water was filtered through Whatman glass fiber filters with a nominal pore size of 0.7 μm into acid washed 250 ml HDPE bottles. New filters were used for each sample filtration to prevent isotopic contamination between samples. Samples for NH_4^+–N and NO_3^-–N analyses were also taken at each station and were filtered in the same manner. All samples were kept on ice until they were returned to the laboratory. NH_4^+ concentrations were determined manually by the phenol hypochlorite method while NO_3^-–N analysis was determined by copper cadmium reduction (Parsons et al. 1984). Discharge was not measured directly as part of this study transect. However, a USGS gage station (02095500) located at Rankin Mill Road provided discharge at one point, and there are no major tributaries along the transect. Mean discharge at Rankin Mill Road on October 9, 2004 and July 26, 2005 were 29 ft^3/s and 17 ft^3/s, respectively. Average width in this study transect is approximately 16 m.

In order to determine relative importance of stream habitat types and longitudinal effects on denitrifying activity, a stratified random sampling approach was implemented to incorporate three different stream habitat types: erosional areas, mid-channel laminar flow areas and depositional zones. A total of 36 cores were randomly collected from each of the three habitat types at Fairview, Minorwood and Rankin (Fig. 1). Dissolved oxygen (DO), pH and temperature were measured at each site using a multiparameter probe (YSI 6920). Stream flow characteristics for sites where denitrification was measured using C_2H_2 block technique are presented in Table 1. These sites were chosen largely because of their accessibility to the stream and proximity to the WWTP. Substrate composition was not quantified. However, we made some general observations. Substrate composition above the WWTP was composed of more sand and silt than the downstream sites. Minorwood appeared to have considerably more clay in the erosional areas than either Fairview or Rankin with less sandy substrate except in the mid-channel laminar flow area. Rankin had mixed substrates in each habitat type and much greater prevalence of boulders and cobble in the streambed.

Table 1 Proportion of water column NO_3–N load removed through benthic denitrification

Site	Mean habitat weighted denitrification rate (mg N_2O–N m^{-2} d^{-1})	Discharge (m^3 d^{-1})	Velocity (m d^{-1})	Width (m)	NO_3–N load (mg m^{-1} d^{-1})	Proportion NO_3–N removed through denitrification % d^{-1}
Fairview (upstream)	0.33	5211.21	30285.93	12	21713.36	46%
Rankin (downstream)	19.39	39145.21	13957.40	16	11,743,563.70	2.3%

We could not collect and process samples from all sites and habitats on a single day. We chose to block our sampling by habitat type in order to minimize the possibility of precipitation compromising longitudinal comparisons. One habitat type was sampled from each of the three sites on one sampling day (n = 4 cores from each site), followed by sampling of the other two habitat types on two additional days. Note however, that all sampling events occurred within a 6-day period and there was no precipitation during that time, thus between habitat comparisons should not be compromised by the sampling design. Cores were extracted randomly from mid-channel laminar flow areas on 6 September 2005. Depositional and erosional habitat sampling occurred on September 11, 2005, and September 12, 2005, respectively. Polycarbonate cylinders 20 cm in length and 5 cm in diameter were used to collect intact sediment cores from each habitat type at each site. Cores were sealed with acrylic tops that were equipped with polypropylene male adapters fitted with septa and #10 rubber stoppers were used to seal the bottom. Cores were transported on ice to the laboratory.

Laboratory procedures

Samples from both transect sampling dates were removed from ice upon return to the laboratory and immediately processed following Mulholland et al. (2004). The samples were boiled with 5 g NaCl and 3 g of MgO to drive off existing NH_4^+ followed by an acid diffusion procedure to reduce NO_3^- to NH_3, which was adsorbed onto an acidified filter. Samples were analyzed for $\delta^{15}N$ on a DELTA Plus Advantage configured for continuous flow analysis through a Carlo Erba NC2100 Elemental Analyzer at the Colorado Plateau Stable Isotope Lab at Northern Arizona University. The laboratory reported standard deviations for sample standards range from 0.13–0.17‰ ($\delta^{15}N$). The reference standard is atmospheric N_2 and the sample $\delta^{15}N$ is determined from the following equation (Peterson and Fry 1987):

$$\delta^{15}N(‰) = \left[\left({}^{15}N/{}^{14}N_{sample}\right)/\left({}^{15}N/{}^{14}N_{standard}\right) - 1\right] * 1000$$

Intact sediment cores used for quantification of denitrification rates were treated with C_2H_2 within 18 h of sampling. Welding grade C_2H_2 was purified to remove contaminants following Hyman and Arp (1987). Nonfiltered streamwater was collected from each site and used to treat to the cores corresponding with that site. Overlying water was replaced with streamwater containing 10% C_2H_2 and each core was sampled immediately after addition of C_2H_2 for the ambient concentration of N_2O, followed by sampling at 12, 24 and 36 h. Following sample extraction, 10% C_2H_2 streamwater replaced the sample volume extracted from the core headspace to continue the incubation period and dilution was considered in rate calculation. The vials chosen for gas sampling had been previously tested to ensure that no N_2O leakage would occur within the incubation period. Sample vials were purged with ultra high purity N_2 and returned to atmospheric pressure prior to core sampling.

All vials were analyzed for N_2O concentration using electron capture gas chromatography within 48 h of completing the incubation period. Nitrous oxide partitioning between the aqueous and gas phase was temperature-corrected using the Bunsen coefficient (0.544) corresponding to N_2O at room temperature (25°C) (Tiedje 1982). N_2O concentration for a given sample was determined by the time-linear

change in concentration between sampling points, normalized to a square meter surface area and reported as mg N_2O–N/m^2/day.

Once the incubation period was complete, approximately 3 cm of the top sediment layer was placed into a drying oven at 50°C to obtain dry mass (DM). Samples were then combusted at 550°C to obtain ash free dry mass and (AFDM) in order to calculate percent organic matter (% OM). Percent OM of the sediment was determined for two of the four cores randomly chosen from each habitat type in each site. The percentages were then averaged together to obtain one value for % OM for each habitat type in each site.

Statistical analyses

Statistical results were evaluated at the $\alpha = 0.05$ significance level. The relationship between $\delta^{15}N$–NO_3^- and NO_3^-–N was determined by simple linear regression using separate analyses for the two different transects. Longitudinal changes in NO_3^-–N and NH_4^+–N concentrations in the reach used for stable isotope evaluation were also determined by regressing the respective nutrient concentrations on the distance downstream from the WWTP.

Stepwise multiple regression analysis was used to determine potential predictors of denitrification rates. Forward stepwise selection analysis revealed significance of habitat and sites only, therefore, % OM, NO_3^-–N, pH and DO were dropped from the model and the significant covariates were analyzed individually by one-way ANOVA to determine significance between sites. Two-way ANOVA was used to determine significance of site, habitat and the interaction between site and habitat on denitrification rate. Denitrification rates were log transformed to correct for unequal variance. Site means were compared using Tukey's HSD procedure. Because there was a significant interaction between habitat and site (see Results), additional one-way ANOVA models were constructed for each site to determine differences in denitrification rates between habitat types and means were compared using Tukey's HSD. Mean rates in the depositional areas at Fairview are the result of two cores as opposed to the original four because two cores were eliminated from analysis due to nonlinear production of N_2O through time.

We estimated the proportion of NO_3^-–N removed through benthic denitrification upstream and downstream of the WWTP based on NO_3^-–N load to those areas and the mean habitat weighted denitrification rates. We calculated the upstream and downstream NO_3^-–N loading per meter stream width by multiplying discharge by NO_3^-–N concentration and dividing by stream width (Kemp and Dodds 2002). Our estimate for daily loss of NO_3^-–N to denitrification upstream and downstream of the WWTP was obtained using the following equation (Inwood et al. 2005):

$$\frac{\text{Mean habitat weighted denitrification rate}}{NO_3 - \text{N Load}} * \text{Stream water velocity}$$

Discharge and stream water velocity data for Rankin Mill Road were obtained from the USGS gage station (02095500) located at that site. However, USGS data were not available for Fairview or Minorwood. Therefore, we used data from the Church Street USGS gage (02095271) which is located approximately 1.8 stream km from Fairview. Streamflow characteristics for the core sampling period are presented in Table 1. A transect perpendicular to stream flow was established in order to determine the proportion of relative habitat types at each site. The width of each habitat type in one linear meter of stream distance within the transect was estimated. Mean denitrification rates for Fairview and Rankin Mill Road were adjusted for the mean rates observed in each habitat type to the approximate proportion of each habitat type at those sites.

Results

Natural abundance of $\delta^{15}N$–NO_3^-

Nutrient samples taken along a 3.5 km transect beginning at Rankin Mill Road on October 9, 2004, revealed high DIN concentrations throughout the transect (Fig. 2a, b). Nitrate and NH_4^+ decreased significantly along the transect. Based on the slope of the linear regression, NH_4^+ decreased at a rate of 0.02 mg/l/km and NO_3^-–N decreased at a rate of 0.6 mg/l/km. $\delta^{15}N$–NO_3^- increased significantly with distance downstream (Fig. 2c). Simple linear regression of $\delta^{15}N$–NO_3^- on NO_3^-–N was not significant (Fig. 2d).

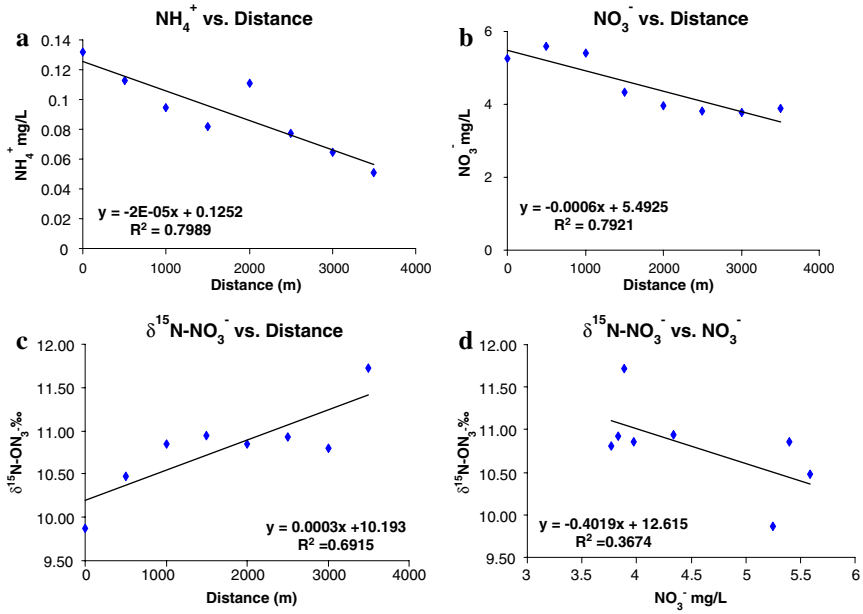

Fig. 2 Dissolved inorganic nitrogen concentrations and natural abundance of $\delta^{15}N–NO_3^-$ along a 3.5 km stream transect taken on October 9, 2004. Results from natural abundance of $\delta^{15}N–NO_3^-$ to NO_3^- concentrations exhibit nonlinear patterns which were not significant

Nutrient analysis of samples taken from the 7 km transect on July 26, 2005 again showed high DIN concentrations (Fig. 3a, b). Ammonium decreased significantly along the 7 km transect at rate of 0.009 mg/l/km. Nitrate was variable and did not decrease significantly. $\delta^{15}N–NO_3^-$ appeared to increase slightly along the study transect but was not significant (Fig. 3c). The relationship between $\delta^{15}N–NO_3^-$ and NO_3^-–N was not significant (Fig. 3d). However, an isolated portion of this study transect suggested evidence of denitrification. An inversely proportional relationship between $\delta^{15}N–NO_3^-$ on NO_3^-–N from 1500 to 2500 m of the study transect was significant (Fig. 3b, c). In that part of the transect, NO_3^-–N decreased sharply. Thus, the increase in $\delta^{15}N–NO_3$ combined with the decreasing

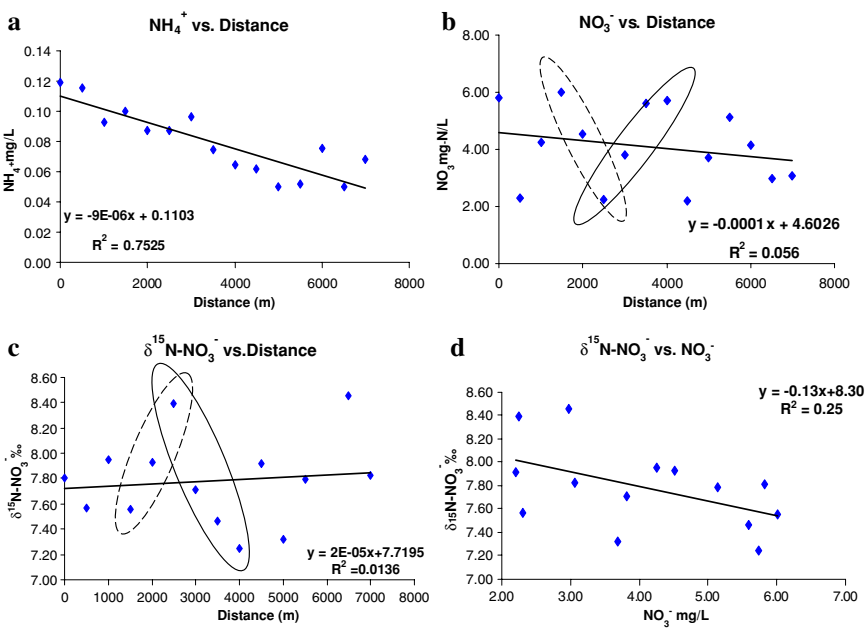

Fig. 3 Dissolved inorganic nitrogen concentrations and natural abundance of $\delta^{15}N–NO_3^-$ along a 7 km stream transect taken on July 26, 2005. Relationships between $\delta^{15}N–NO_3^-$ and NO_3^- were not significant. Dashed ellipses show significant relationship between decreasing NO_3^- and increasing $\delta^{15}N–NO_3^-$ indicating denitrification. Solid ellipses show increasing NO_3^- with decreasing $\delta^{15}N–NO_3^-$ not indicative of denitrification

NO_3^-–N suggests that denitrification was an important process in that portion of the transect. The 2500–4000 m portion also revealed an inverse relationship between $\delta^{15}N$–NO_3^- vs. NO_3^-–N (Fig. 3b, c). However, in that section of the transect, NO_3^-–N increased while $\delta^{15}N$–NO_3^- decreased.

Physical and chemical parameters from sediment core sampling sites

Values of physical and chemical variables at each of the core sampling sites taken in September 2005 are presented in Table 2. pH did not differ significantly between sites. Dissolved oxygen was significantly higher in Fairview than Minorwood and Rankin although concentrations between Minorwood and Rankin did not differ significantly. Mean % OM did not differ significantly among sites or among habitat types, except that at Minorwood, % OM was significantly higher in the erosional area than in the depositional and mid-channel area.

Denitrification rates between sites

Minorwood and Rankin both had significantly higher mean denitrification rates than Fairview, although rates between the two downstream sites were not significantly different from each other. Mean denitrification rates for Fairview, Minorwood and Rankin were 0.58, 35.49, and 22.64 mg N_2O–$N/m^2/d$, respectively. Mean NO_3^-–N concentrations between Minorwood and Rankin were not significantly different although both sites had significantly higher NO_3^-–N concentrations than Fairview (Table 2).

Denitrification rates between habitat types

Mean rate for the depositional, erosional and mid-channel laminar flow areas at Fairview were 0.28, 1.26 and 0.05 mg N_2O–$N/m^2/d$, respectively (Fig. 4a). Mean denitrification rates were not significantly different between depositional areas and mid-channel laminar flow areas at Fairview, whereas erosional areas had significantly higher rates of denitrification than both depositional and laminar flow areas.

Mean denitrification rate for the depositional, erosional, and mid-channel laminar flow areas at Minorwood were 50.27, 47.96, and 8.23 mg N_2O–$N/m^2/day$, respectively (Fig. 4b). Both depositional and erosional areas at Minorwood were significantly higher than in mid-channel laminar flows areas. Depositional and erosional areas did not differ significantly.

Denitrification patterns observed at Rankin were very similar to those at Minorwood. Mean estimates of denitrification rates for the depositional, erosional, and mid-channel laminar flow areas at Rankin were 35.25, 27.08 and 5.58 mg N_2O–$N/m^2/d$, respectively (Fig. 4c). Rates between the erosional and depositional areas at Rankin did not differ significantly and both of these areas had significantly higher rates than the mid-channel laminar flow areas.

Table 2 Chemical and physical characteristics at each site

Site	NO_3^-–N (mg/l)	NH_4^+–N (mg/l)	pH	Dissolved oxygen (mg/l)	Temperature (°C)	Mean percent organic matter
Fairview	0.05	0.06	6.6	9.4	23.2	
Depositional						2.63
Erosional						5.83
Mid-channel						3.1
Minorwood	4.74	0.33	6.5	8.1	24.9	
Depositional						0.92
Erosional						2.54
Mid-channel						1.15
Rankin	4.82	0.40	6.6	7.6	23.1	
Depositional						1.44
Erosional						2.4
Mid-channel						1.44

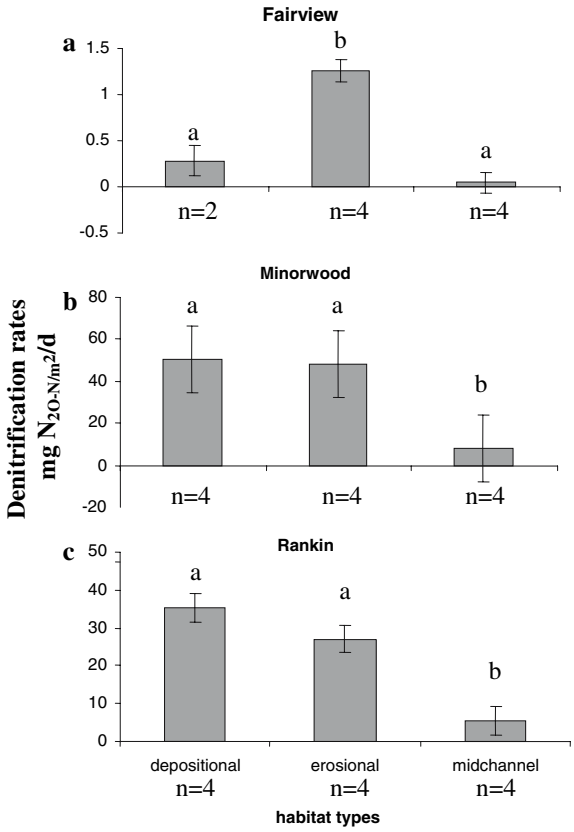

Fig. 4 Differences in denitrification rates between habitat types at each site. Bars with the same letters are not significant

Discussion

Natural abundance of δ^{15}N–NO_3^-

Dissolved inorganic nitrogen concentrations were high throughout the study reach, which is similar to many other streams draining urban land use (Grimm et al. 2005; Wahl et al. 1997). Changes in nutrient concentrations typically reflect point source input during baseflow conditions (Carpenter et al. 1998; Marti et al. 2004) and all samples were taken from NBC at low flow. The significant declines in NH_4^+–N in both transects (Figs. 2a, 3a) were likely due to biological assimilation and nitrification (Bradley et al. 1995; Hershey et al. 2004; Peterson et al. 2001). Peterson et al. (2001) reported that benthic uptake of NH_4^+–N removed more than half the NH_4^+–N as did nitrification from various headwater streams across North America. However, they also found higher nitrification rates in streams with higher NO_3^-–N concentrations (Peterson et al. 2001). Biomass of nitrifying bacterial communities have been reported to increase downstream of WWTP's (Montuelle et al. 2003). Some of the NO_3^-–N present in the water column in NBC is likely the result nitrification (Hershey et al. 2004). However, NO_3^-–N concentrations were too high to be a result of this process alone and probably reflect direct NO_3^-–N input from WWTP and possibly other sources as well. This conclusion is supported by the observation that NO_3^-–N above the WWTP was two orders of magnitude lower than the downstream sites.

Nitrate decreased significantly along the transect in October 2004 but not in July 2005 (Figs. 2b, 3b). The significant decline in NO_3^-–N concentrations in October 2004 suggested that a sink for NO_3^-–N is present, although persistent elevated concentrations throughout the transect indicate supply remained in excess of demand. The absence of a significant downstream NO_3^-–N decline in July 2005 also indicate that N input from the WWTP exceeded the stream's retention capacity resulting in excessive downstream export of NO_3^-–N. Marti et al. (2004) found considerably lower nutrient retention efficiency in headwater streams receiving effluent from wastewater treatment plants compared to reference streams. They measured dissolved inorganic nitrogen (DIN) retention efficiency in 15 streams in Spain and discovered uptake lengths ranging from 0.14 to 29 km (Marti et al. 2004). Similarly, Haggard et al. (2005) found greater nitrogen transformation than retention in a 3rd order rural stream in Arkansas receiving wastewater effluent. The results of their study indicated high nitrification rates, but estimates of NO_3^- uptake lengths ranged from 3 to 12 km demonstrating low retention efficiency and large scale effects on stream quality due to the point source discharge. Although NBC is a 4th order stream, the results of this study suggest that it is exhibiting low N retention and exporting large quantities of N to downstream areas.

Rapid temporal fluctuations in discharge are common in urban stream systems due to changes such as loss of pervious surfaces and channel modifications to upland urban habitats (Paul and Mayer 2001). Furthermore, nonpoint source inputs are typically reflected in streams with elevated discharge during heavy rain events (Carpenter et al. 1998). A previous study in this stream transect closely examined the temporal variation in nutrient concentrations associated with

fluctuations in discharge during rain events. They found that nutrient concentrations increased upstream of the WWTP plant during storm events, but changes in nutrient concentrations downstream of the WWTP during different flow conditions were not significant (Ulseth and Hershey 2005). Sites upstream of the WWTP appeared to be mitigating nutrient inputs while downstream sites were receiving inputs beyond demand. Increased discharge, channel width and water residence time affect the ability of a stream system to mitigate N inputs (Alexander et al. 2000). Greater discharge below the WWTP, in addition to increased point and nonpoint sources of N are influencing the capacity of NBC to retain N as evidenced by consistently elevated NO_3^-–N concentrations.

Multiple processes can affect $\delta^{15}N$–NO_3^- leading to progressive enrichment in the water column downstream of a NO_3^-–N point source. Nitrifying bacteria preferentially use $^{14}NH_4^+$–N leading to enrichment of $^{15}NO_3^-$–N (DeBruyn and Rasmussen 2002). Autotrophs and denitrifiers also preferentially utilize the lighter isotope (Kellman and Hillaire-Marcel 1998; Peterson and Fry 1987). Thus, evaluation of denitrification using natural abundance of $\delta^{15}N$–NO_3^- and NO_3^-–N integrates these distinct processes to measure a net effect. Consequently, the technique of regressing $\delta^{15}N$–NO_3^- on NO_3^-–N is sensitive to variation in the relative importance of fractionation processes and variations in loading as well as the $\delta^{15}N$ signal of that load.

Kellman and Hillaire-Marcel (1998) used natural abundance of $\delta^{15}N$–NO_3^- to evaluate denitrifying activity in a NO_3^-–N rich stream adjacent to an agricultural field. Their study, which evaluated patterns of $\delta^{15}N$–NO_3^- and NO_3^-–N over a 700 m transect, found evidence of denitrification. Mayer et al. (2002) measured natural abundance of $\delta^{15}N$–NO_3^- and NO_3^-–N at the outlet of sixteen watersheds in the northeastern US. Although complementary studies in this region found significant denitrifying activity in all rivers in their study, they concluded that sampling outlets alone was not an effective approach with this technique and that longitudinal evaluation of $\delta^{15}N$–NO_3^- in the rivers would likely be more conclusive (Mayer et al. 2002). Our study attempted to use ratios of $\delta^{15}N$–NO_3^- and NO_3^-–N to evaluate denitrification activity over a relatively long stream transect ($\sim$7 km). Effluent from wastewater treatment plants provide a sustained input of N (McClelland et al. 1997) that is isotopically distinct from other anthropogenic sources (Wayland and Hobson 2001). NO_3^-–N produced from microbially processed human and animal waste has a $\delta^{15}N$ value ranging from +7 to + 20‰ (Mayer et al. 2002; McClelland and Valiela 1997) compared to $\delta^{15}N$–NO_3^- of less than +5‰ found in predominantly forested headwaters streams in the northeastern U.S. with low NO_3^-–N (Mayer et al. 2002). $\delta^{15}N$–NO_3^- values measured in NBC ranged from 7.07 to 12.40‰, which is indicative of a strong influence of wastewater. Fractionation should be large at high NO_3^-–N, leading to an inverse relationship between $\delta^{15}N$–NO_3^- and NO_3^-–N if denitrification is important (Kellman and Hillare-Marcel 1998). The patterns we observed of $\delta^{15}N$–NO_3^- and NO_3^-–N were not significant on either date (Figs. 2d, 3d). The absence of a clear spatial pattern between these variables suggested that denitrification was not the dominant process influencing NO_3^-–N flux despite the high denitrification rates we observed using the C_2H_2 block technique. This could reflect some combination of high background NO_3^-–N, and inputs from other sources such as groundwater, small tributaries, runoff and/or nitrification.

Denitrification did appear to dominate a 1 km section of the 7 km transect studied on 26 July 2005. Isolation of the portion 1.5–2.5 km below WWTP resulted in a significant inverse relationship between $\delta^{15}N$–NO_3^- and NO_3^-–N (Fig. 3d), suggesting denitrification. Although the isolated portion 2.5–4.0 km below WWTP also resulted in inversely proportional relationships between $\delta^{15}N$–NO_3^- and NO_3^-–N, the pattern could not have been derived from denitrification because NO_3^-–N increased while $\delta^{15}N$–NO_3^- decreased, rather than the reverse pattern observed in the 1.5–2.5 km section. Kellman and Hillaire-Marcel (1998) found similar spatial variation resulting from this technique. However, results of their study are closely correlated with seasonal effects of fertilizer applications associated with agricultural practices in addition to assessment of a shorter transect than our study. It is likely that dilution from non-wastewater sources along other parts of the transect could have influenced the observed pattern in $\delta^{15}N$–NO_3^- and NO_3^-–N more strongly than did denitrification such that this method was not sensitive enough to evaluate the importance of denitrification as a N sink over a 7 km distance. There are several homes located

adjacent to the stream along the study transect. Thus, septic tank seepage or lawn fertilizer could also have affected DIN (Mayer et al. 2002). A narrower sampling scheme and shorter longitudinal transects may improve detection of lateral inputs that may be influencing the patterns. Although the results of the C_2H_2 reduction technique (discussed below) showed relatively high denitrification rates at specific sites below the WWTP, the longitudinal patterns between $\delta^{15}N$–NO_3^- and NO_3^-–N and consistently elevated NO_3^-–N concentrations indicate that no overall reduction in N is occurring in NBC.

Spatial variability in denitrification rates (C_2H_2 reduction)

Denitrification is primarily limited by three major factors: availability of labile organic carbon, presence of NO_3^-–N substrate and anaerobiosis (Seitzinger 1988). Clearly, elevated NO_3^-–N are present below the plant. DOC (which we did not measure) is also typically very high in wastewater effluent (Bradley et al. 1995; DeBruyn and Rasmussen 2002; Haggard et al. 2005). Thus, higher rates of denitrification observed below the WWTP are most likely driven by NO_3^-–N and DOC.

Distribution of sediment particle composition was not quantified, although some qualitative observations were made at each site. Highest denitrification rates at Fairview in the erosional habitat (Fig. 4a) may have been due to the higher clay content in erosional areas, compared to higher sand content in depositional and laminar flow areas. Stream habitats characterized by coarsely textured substrates likely contain oxic microsites (Martin et al. 2001). Therefore, it is probable that the pore water between the sand particles supported interstitial DO, which inhibited denitrification (Duff and Triska 1990; Martin et al. 2001), whereas higher denitrification rates in the erosional area were likely due to the greater abundance of clay particles observed in that habitat. Clay substrates typically result in higher denitrification rates because smaller microsites between particles in sediments encourage anaerobiosis (Garcia-Ruiz et al. 1998; Martin et al. 2001). Percent OM did not significantly explain differences in denitrification rates between habitat types in our models, therefore, variation in denitrification rates between habitat types are more likely due to substrate differences which control the presence or absence of oxygen. Percent OM in NBC was comparable to values reported in other studies from streams draining various land uses. Inwood et al. (2005) found that sediment organic matter content explained some of the variation in denitrification rates between urban, agricultural and forested streams. However, the primary predictor of rates in their study was NO_3^-–N. They also found higher rates in agricultural and urban streams than forested streams, although forested streams removed a greater proportion of NO_3^-–N relative to input than the other stream types. Martin et al. (2001) reported a range of % OM found in headwater streams of the southern Appalachian Mountains comparable to this study, but they also found % OM to be a nonsignificant predictor of denitrification.

At the two downstream sites, mid-channel laminar flow areas had significantly lower denitrification rates than the other two habitat types (Fig. 4b, c). Mid-channel laminar flow areas were sandy at both Minorwood and Rankin, although not as sandy as Fairview. Erosional areas at Minorwood had more clay than any other habitat at all sites. The disparity in sand composition between the sites is likely the result of Fairview being located in a more urbanized area, where sediment deposition is often high (Groffman and Boulware 2002; Paul and Meyer 2001). Furthermore, mean NO_3^-–N concentrations did not decrease from Minorwood to Rankin suggesting that the input from the WWTP is overloading microbial demand for NO_3^-–N.

The C_2H_2 reduction method used in this study coupled with measurement of natural abundance ratios of $\delta^{15}N$–NO_3 provides complementary information regarding denitrification in this NO_3^-–N rich stream. The results of both analyses show that denitrification is ineffective in reducing NO_3^-–N inputs to NBC below the WWTP. Evaluation of natural abundance of $\delta^{15}N$–NO_3^- in stream transects 1 km or less may be more appropriate where additional nonpoint or point sources of DIN are not suspected. While evaluation of the natural abundance of $\delta^{15}N$–NO_3 has some limitations, it is an informative method of evaluating longitudinal patterns of denitrification activity. The C_2H_2 block technique has been shown to be a very useful tool for quantifying denitrification in multiple stream ecosystems (Inwood et al. 2005; Kemp and Dodds 2002; Martin et al. 2001; Royer et al. 2004). We feel that when used in conjunction, these two methods can provide useful

information across varying spatial scales regarding denitrification in other stream systems.

Proportion of NO_3–N loss to denitrification

Based on the results of the C_2H_2 block technique, we estimated that benthic denitrification in the areas upstream of the WWTP removed approximately 46% of the total NO_3–N load daily. Although the downstream areas had significantly higher denitrification rates, the proportion of NO_3^-–N removed via denitrification was only 2.3% per day (Table 2). This difference is due to the greater discharge to downstream areas and greater NO_3–N concentration downstream versus upstream. Based on these estimates, denitrification appears to be an important sink for N upstream but of minor importance downstream of the WWTP.

The proportion of NO_3^-–N removed via denitrification in our study lies within the range of estimates reported in other studies. Inwood et al. (2005) found that estimates of NO_3^-–N loss to denitrification were significantly higher in forested headwater streams than in suburban and agriculturally influenced streams. Their estimates of NO_3^-–N removal for forested, agricultural and suburban headwater streams were 141, 31 and 18% d^{-1}, respectively. A wide range of removal estimates are reported in Royer et al. (2004) which focused primarily on agriculturally influenced streams (0.5–273% d^{-1}). Lower estimates of removal in their study coincided with higher agricultural drainage periods. Based on our estimates of NO_3^-–N loss in NBC, our data suggest that implementation of N-removal processes at the NBC WWTP could lead to significant reduction in N export from this stream.

Conclusions

Natural abundance of $\delta^{15}N$–NO_3^- has been used to evaluate denitrification activity with varying success (Kellman and Hillaire-Marcel 1998; Mayer et al. 2002; Sebilo et al. 2003). In our study this technique showed that denitrification was not the dominant process controlling nitrogen dynamics over a relatively long stream transect ($\sim$7 km) downstream of a WWTP. However, examination of isolated sections of the transect indicated that denitrification activity was significant in one portion, and not significant in other portions. Rates of denitrification determined by the C_2H_2 block technique in intact cores were comparable to those reported in other streams with high NO_3^-–N (Inwood et al. 2005; Royer et al. 2004), were much higher downstream of the WWTP compared to upstream, and showed that denitrification rates were high in erosional and depositional areas downstream of the plant and in erosional areas only at the upstream site. Thus, the combination of the two methods for evaluating denitrification provided more insight into the spatial dynamics of denitrication activity than either methods alone. Although denitrification did not result in an overall reduction of NO_3^-–N downstream of the city due to high N-loading from the WWTP, denitrification did appear to be a significant sink for NO_3^-–N in the urban section of NBC. This result suggests that reduction of point source N loading associated with the WWTP could significantly reduce export of N from the watershed. Furthermore, management of urban streams should consider the complexity of habitat types found in unaltered systems and implement strategies to maximize heterogeneity of streambeds. Understanding the conditions whereby denitrification is maximized as well as the spatial variability in N-loading is essential for managing downstream N-flux to large rivers.

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