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DENITRIFICATION AND SEDIMENT-WATER NUTRIENT EXCHANGE IN THE UPPER NEUSE RIVER

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ABSTRACT

We conducted quarterly (spring, summer, fall and winter) determinations of denitrification at representative sites in the mainstem Neuse River and tributaries. Sites within the Piedmont included Crabtree Creek above and below the Cary Wastewater Treatment Plant and the mainstem Neuse River in Smithfield. Sites within the Coastal Plain included Nahunta Swamp and the mainstem Neuse River at Fort Barnwell. Denitrification measurements were made on intact sediment cores using the N_2/Ar and isotope pairing techniques. We measured nitrate (NO_3^--N) and ammonium (NH_4^+-N) concentrations in the water column at the time of core collection and evaluated rates of sediment-water exchange of NO_3^--N, NH_4^+-N and dissolved oxygen (O_2) in conjunction with denitrification rate determinations. Nitrate concentrations in the stream or river water varied over an order or magnitude, from about 10 to 110 μmol L^{-1}, while NH_4^+-N concentrations were lower, varying from 0.6 to 10.8 μmol L^{-1}. Sediments consistently consumed dissolved O_2, with rates varying from 256 to 1418 μmol m^{-2} h^{-1}. Sediment-water exchange of NO_3^--N and NH_4^+-N was not always observed. However, non-zero values always showed release of NH_4^+-N from sediments and consumption of NO_3^--N by sediments at rates ranging to 104 and 102 μmol m^{-2} h^{-1}, respectively. Most cores actively denitrified with rates ranging to 222 μmol N_2-N m^{-2} h^{-1}. On average, coupled nitrification-denitrification accounted for 66% of total denitrification. Lowest rates of denitrification were generally observed during December, when the water temperature was lowest at 4°C. No other relationship was identified between rates of denitrification and measured physicochemical variables within or among sites or dates as sampling frequency and replication were low. The limited data from this survey study suggests that denitrification represents a sink for ≤5% for the NO_3^--N load to the mainstem Neuse River and tributary streams.

(denitrification, nitrate, rivers, coupled nitrification-denitrification, N loss, isotope pairing, N_2/Ar)

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SUMMARY AND CONCLUSIONS

This study was aimed at providing broad coverage of spatial and temporal variability in denitrification and sediment-water exchange of inorganic-N and dissolved O_2 at representative sites in the mainstem Neuse River and tributary streams in both the Piedmont and Coastal Plain provinces of North Carolina. At least some intact core samples collected at each of five study sites during each season actively denitrified when rates were assessed using the highly sensitive, but labor-intensive N_2/Ar and isotope pairing techniques. Although all sediments consumed dissolved O_2, non-zero fluxes of nitrate (NO_3^--N) and ammonium (NH_4^+-N) were not consistently observed. The pattern of nitrogenous nutrient exchange at the sediment-water interface for non-zero fluxes was consistent, however, as sediments consumed NO_3^--N and released NH_4^+-N. Values for all biological and chemical variables showed high spatial and temporal variability as expected in a survey study characterized by limited replication. Although this study was not designed to identify relationships among variables, rates of denitrification were lowest when the water temperature was lowest in December. Although denitrification is actively occurring in lotic freshwaters of the Neuse watershed, rates (0 to 222 μmol N_2-N m^{-2} d^{-1}) are generally low when compared to core- or chamber-based estimates for other systems with similar water column concentrations of NO_3^--N. Extrapolation of these limited data to larger spatiotemporal scales indicates that denitrification represents a small in-river sink (≤5%) for NO_3^--N in the Neuse watershed. If this estimate proves accurate, denitrification plays a lesser role in the N budget of the mainstem Neuse River and tributary streams than for many other lotic systems for which estimates are available.

RECOMMENDATIONS

Our study was designed to determine whether denitrification was an active component of the N cycle in the Neuse River system. We found relatively low rates of denitrification in all seasons at all stations. We extrapolated this limited to get a qualitative estimate of the sink strength of this process in the in-stream N cycle of the Neuse River system and found that denitrification removed a mass of N equivalent to ≤5% of the nitrate (NO_3^-N) in the overlying water. However, this estimate is not firmly based from the perspective that the methodology used here (core-determined rates) does not embrace the spatial heterogeneity in the physical structure of the bottom substrate that affects rates of material exchange at the sediment-water interface. Further, our methodology does not simulate local variations in upwelling and downwelling that characterizes the hyporheic zone and also influences rates of denitrification and associated biological activity. Other techniques such as mass balance assessments or N_2/Ar open channel methods are better suited to provide firm estimates of total in-river N loss or N loss to denitrification, respectively, as these give information on longer spatiotemporal scales. We recommend further study utilizing appropriate methodology if a firmly based estimate of denitrification or total in-river N loss is deemed necessary to guide policy and management decisions in the Neuse River watershed.

1. INTRODUCTION

1.1 Background

Nitrogen is considered to be the nutrient most frequently limiting primary production in coastal marine and estuarine environments (Ryther and Dunstan 1971; Nixon 1995). Prior to significant human influence, the production of biologically available N from previously inaccessible atmospheric N in terrestrial ecosystems was restricted largely to N_2 fixation by specialized procaryotes characterized by limited distribution. However, N_2 fixation through fossil fuel combustion, legume cultivation and fertilizer production have increased dramatically over the last two decades to that extent that the magnitude of anthropogenic and natural N_2 fixation terms are roughly equal in contemporary global N budgets (Schlesinger 1997; Galloway et al. 2003; Galloway 2005). Increases in anthropogenic N_2 fixation have been linked to enhanced N inputs to aquatic ecosystems worldwide. The negative consequences to coastal waters of accelerated anthropogenic N_2 fixation include increased rates of primary production (Nixon 1995; Paerl 1997; Jackson et al. 2001) the global expansion of harmful algal blooms, (Hallegraef 1993; Richardson 1996; Paerl et al. 2003), bottom water hypoxia and anoxia (Justic et al. 1993; Rabalais et al. 1996), habitat degradation (Winn and Knott 1992; Lenihan and Petersen 1997; Diaz and Solow 1999), loss of biodiversity (Cloern 2001; Rabalais 2002) and alterations of food web structure and function (Jackson et al. 2001). Nitrogen overenrichment has been identified as the single most important threat to coastal marine environments (Howarth et al. 2002; NRC 2000; Rabalias 2002).

Microbial denitrification is a respiratory process where electron transport phosphorylation is coupled to the sequential reduction of nitrogenous oxides ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$) in the absence of O_2 by facultative anaerobes. Proximal controls on denitrification include O_2 concentration, as well as the availability of NO_3^- and metabolizable organic-C (Tiedje 1994) . Nitrate supply is frequently determined by rates of aerobic processes, namely N mineralization (organic-N $\rightarrow NH_4^+$) and nitrification ($NH_4^+ \rightarrow NO_3^-$). The latter is an energy-yielding activity for specialized chemoautotrophs (nitrifying bacteria) and microbial nitrification and denitrification are frequently coupled at oxic-anoxic interfaces (e.g. Jenkins and Kemp 1984; An and Joye 2001)

Denitrification closes a major loop in the global nitrogen cycle. Biologically active NO_3^- is transformed to inert N_2 gas by denitrification, removing from the ecosystem N that would otherwise be available to fuel primary production. Barring physical export, denitrification provides the only mechanism for permanent N removal from the active pool in aquatic ecosystems, as other fates and transformations such as microbial assimilation or sediment burial leave biologically available N in the system for additional processing (Royer et al. 2004). The percent removal of N inputs to aquatic ecosystems by denitrification can be substantial. Seitzinger et al. (2002) estimated that of the biologically active N entering the stream/river systems draining 16 watersheds in the eastern US, 30 to 70% is removed primarily through denitrification. Estuarine systems show 10 to 80% removal of biologically active N inputs, depending on morphometry and water residence time (Seitzinger 1988; Nixon et al. 1996), while the figure is >80% for continental shelf regions of the North Atlantic (Seitzinger and Giblin

1996). Globally, denitrification is thought be increasing in response to accelerated anthropogenic N_2 fixation (Galloway 2005).

Although denitrification is a clearly a key process in N cycling dynamics in aquatic and terrestrial ecosystems, point determination of rates of denitrification is problematic. Direct measurement of N_2 production is difficult due to high background concentrations in both air and water. Attempts to purge N_2 from overlying water in sediment cores and subsequently measure rates of N_2 accumulation (Seitzinger et al. 1980) require long preincubation times and suffer from limited availability of instrumentation with sufficient precision to detect small changes in N_2. Alternate techniques have been developed, but all have their shortcomings as well as advantages. Acetylene has been used to inhibit the reduction of N_2O to N_2 (Martin et al. 2001), followed by the precise determination of N_2O by electron capture gas chromatography. The acetylene reduction assay allows a large number of samples to be processed, providing an indication of spatiotemporal variability in denitrification rates (Groffman et al. 2006). However, co-inhibition of NO_3^- production by nitrification (Seitzinger et al. 1993), physical disturbance of the sediment-water system (Koike and Sorensen 1988) and incomplete inhibition of N_2O reduction (Christensen et al. 1990) can lead to serious underestimation of denitrification rates. An isotope pairing technique (Nielsen 1992) involves the addition of $^{15}NO_3^-$ to overlying water in a sediment core and mass spectrometric determination of the accumulation of $^{29}N_2$ and $^{30}N_2$ gas. This method can differentiate between denitrification supported by water column-derived NO_3^- and coupled nitrification-denitrification (Steingruber 2001), but can give an erroneous estimate of denitrification if $^{15}NO_3^-$ enrichment increases substrate availability in N-limited systems (Lohse 1996). Denitrification rates in aquatic systems have also been estimated from the change in N_2/Ar ratios in water overlying sediment cores (Kana 1998). Argon serves as a stable conservative tracer and a membrane inlet mass spectrometer is used to precisely assess the time course for change in the ratio of these two gases to estimate the rate of denitrification. This method provides rapid, real-time data over short (hours) incubation periods and requires small (7 ml) samples. However, N_2 scavanging by O^- during the formation of NO^- inside the ion source of the mass spectrometer can lead to a decrease in the apparent N_2/Ar ratio with increasing O_2 concentration, leading to an erroneous estimate for denitrification (An et al. 2001). The isotope pairing and N_2/Ar methods are more labor intensive and costly than the acetylene block technique, but are generally considered to give more reliable estimates of rates of denitrification in aquatic systems (Groffman et al. 2006).

The Neuse River Estuary in North Carolina has experienced the increasingly frequent development of nuisance algal blooms, bottom water hypoxia and recurring massive summer fish kills (Burkholder et al. 1997; Paerl et al. 1998; Mallin 2000), all symptoms of nutrient overload attributed to recent urbanization and agricultural intensification in the watershed (McMahon & Woodside 1997). Under Section 303(c) of the Clean Water Act, states are charged with the responsibility of developing and adopting scientifically defensible water quality criteria that protect the designated uses of ambient waters (USEPA 2001). It is generally agreed that N is the nutrient in excess in the Neuse River Estuary (Paerl et al. 2004). Consequently, in 1997 the North Carolina Division of Water Quality implemented regulations (15A NCAC 2B.0232, 1997) aimed at achieving a 30% reduction in the N load to the Neuse River relative to 1991 to 1995 levels.

In further response to the declining water quality of the Neuse River Estuary, the Neuse River Estuarine Modeling and Monitoring Project (ModMon; www.unc.edu/ims/neuse/modmon) was undertaken with the overall objective of developing an understanding of ecosystem function. In particular, two of the many goals were to: (a) monitor estuarine nutrient trends and nitrogen cycling dynamics; and (b) calibrate, verify and validate models being used to predict estuarine response to the mandated 30% reduction in the N load.

In addition to the ongoing ModMon effort for the Neuse Estuary, the North Carolina Department of Environment and Natural Resources (DENR) has developed the framework for a Nutrient Transport Model for the upper Neuse River. The model was designed to predict the average annual nutrient load arriving at a downstream location given upstream nutrient inputs assuming nutrient removal via a first order decay process. Further it was intended to encompass the geographical extent of the catchment and the range of annual flow and nutrient loading conditions. Consequently, the model was split into high flow and low flow categories designed to represent summer conditions of low flow when nonpoint loading is minimal and winter-spring conditions of faster transport and higher nutrient loading. Flow conditions were subdivided into four location categories to distinguish the Piedmont from the Coastal Plain and tributaries from the mainstream Neuse River.

A study of water quality trends in the Neuse River basin (Stow et al. 2001) provides indirect evidence that denitrification may be a significant sink for nitrogen. A 10 year increase in basin-wide N sources due to anthropogenic activity was not reflected in increased nitrogen loading to the river. The analysis of Stow et al. (2001) showed that <10% of the N input to the watershed was exported to the river, while downstream damping of NO_3^- suggested in-stream loss to denitrification. Clearly, an important consideration to both modeling efforts is an understanding of internal factors that control N concentrations in the Neuse River, including not only denitrification, but also sediment-water N exchange. Improved predictive capability regarding upstream N processing will enhance our ability to predict the magnitude and seasonal pattern of N loading to the Neuse River Estuary.

1.2 Objectives

The overall objective of this research was to provide broad coverage of spatial and temporal variability in denitrification and sediment-water N exchange in the upper Neuse River. To this end we:

1. Measured rates of denitrification and sediment-water N exchange in sediment cores under controlled laboratory conditions.
2. Sampled quarterly (spring, summer, winter and fall) to encompass various combinations of water temperature, flow and nutrient loading;
3. Sampled permanent sites that are geographically representative of the watershed, i.e. the Neuse River mainstream and tributaries in the Piedmont and Coastal Plain.

4. Determined the relative importance of NO_3^- from the overlying water and coupled nitrification-denitrification in suppling substrate for denitrification.
5. Assessed the relationship between rates of denitrification and environmental controls such as water temperature and NO_3^- concentration of the ovelying water.

We utilized both the isotope pairing and N_2/Ar techniques to assess rates of denitrification on each sampling date. The effort and expense allowed the processing of relatively few cores, but was consistent with our primary focus, namely obtaining the most reliable estimates of the rate of denitrification. Accurate estimates for the denitrification rate taken seasonally at representative sites provide the information necessary to determine: (a) whether in-stream denitrification is an important sink term for N in the Neuse River watershed; (b) identify spatial and temporal variations in denitrification to guide in the design of future studies; (c) enhance the capability of the Nutrient Transport Model for the upper Neuse River to predict downstream N decay; and (d) benefit the ModMon effort directed toward simulating processes that determine water quality in the Neuse River and Neuse River Estuary for various nutrient loading and hydrologic scenarios.

2. METHODS

2.1 Study site

The study sites are located within the 16,582 km^2 Neuse River watershed. The Neuse River originates northwest of Raleigh, NC, at the confluence of the Flat and Eno rivers and flows about 320 km east through the Piedmont and Coastal Plain provinces toward Pamlico Sound. The river becomes a tidal estuary below Kinston. Land uses within the watershed are agricultural (29.3%), urban (13.5%), forest (38.5%) and wetlands (14.3%), while a small percentage is barren. The Piedmont is characterized by rolling hills and narrow, forested floodplains. Soils are a highly erodable clay underlain by fractured rock. In contrast, the Coastal Plain is flat and the river is lined with swamp and marsh or bottomline hardwood forests on sandy soil. A detailed description of the Neuse River and watershed are given in Lunetta *et al.* (2003).

Five sampling locations within the Neuse River watershed were selected for quarterly (spring, summer, winter and fall) denitrification rate determinations. Selection of sampling locations (Fig. 1) was guided by the NC Division of Water Quality to include tributary and mainstream Neuse River sites in both the Piedmont and Coastal Plain provinces. Sampling was conducted above and below the wastewater treatment facility at the Crabtree Creek sampling location to assess the influence of a point nutrient source on denitrification and sediment -water nutrient exchange. All sites were situated near USGS gauging stations to gain an accurate assessment of river discharge (Table 1).

Table 1. Location and description of sampling sites.

Site	Location		USGS Station No.	Description
Crabtree Creek - A	35.84N	78.78W	02087275	Piedmont tributary stream Above Cary WWTP[δ]
Crabtree Creek - B	35.84N	78.78W	02087275	Piedmont tributary stream Below Cary WWTP
Smithfield	35.56N	78.33W	02087570*	Piedmont region Mainstem Neuse River
Nahunta Swamp	35.49N	77.81W	02091000	Coastal Plain region Tributary stream
Fort Barnwell	35.31N	77.30W	02091814	Coastal Plain region Mainstem Neuse River

[δ]WWTP = Wastewater Treatment Plant
*Gauge height data only available at this site.

2.2 Sample collection

Sediment cores were collected by hand into acrylic tubes of 6.4 cm i.d. x 30 cm length or 10 cm i.d. x 35 cm length for cores to be used for denitrification rate determinations by the N_2/Ar and isotope pairing (IPT) methods, respectively. Acrylic tubes containing roughly 50% undisturbed sediment and 50% overlying water were sealed on both ends with rubber stoppers. In conjunction with core collection at each site, water temperature determinations were made with a

hand-held thermistor probe and bulk river water was collected into 25-L opaque carboys. Sediment and water samples were transported to UNC-Chapel Hill and stored at 4°C.

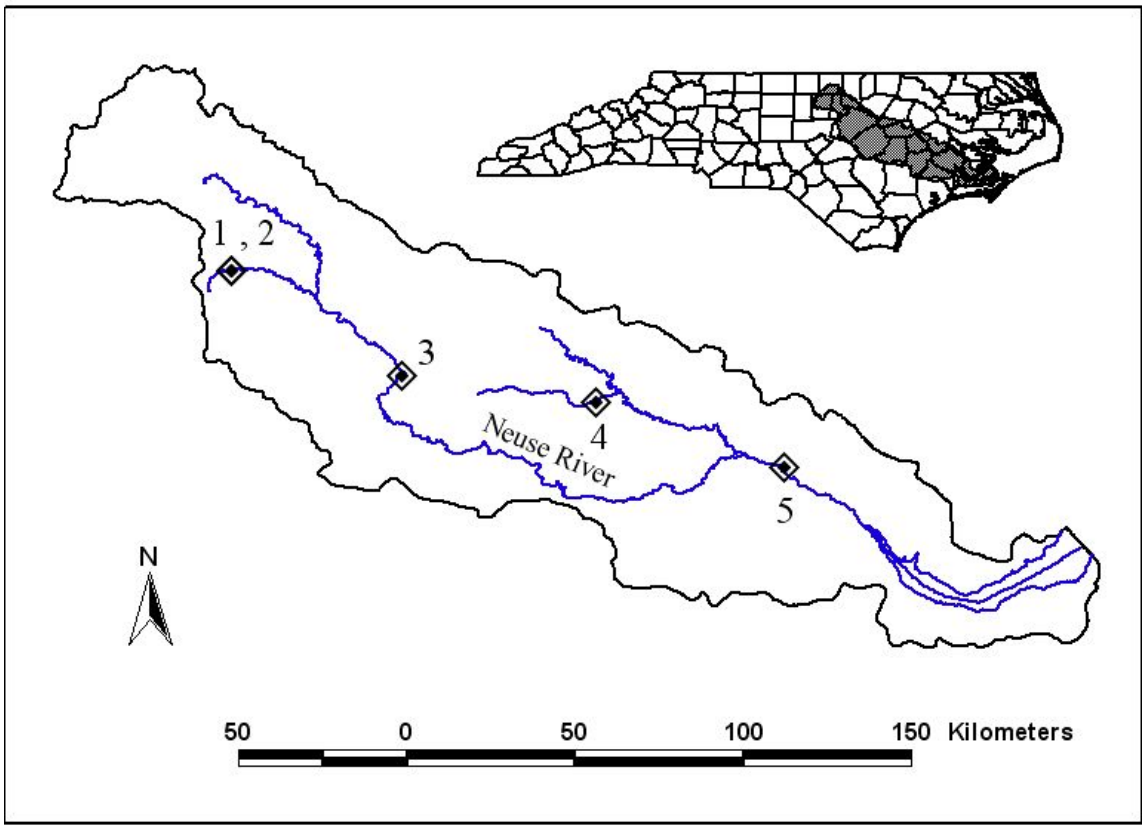

Figure 1. Location of sampling sites on the mainstem Neuse River and representative tributary streams. Sampling sites included Crabtree Creek above (1) and below (2) the Cary Wastewater Treatment facility, the mainstem Neuse River at Smithfield (3), the tributary stream Nahunta Swamp (4) and the mainstem Neuse River at Fort Barnwell (5).

2.3 Experimental

2.3.1 N_2/Ar-determined rates of denitrification

The morning following collection, sediment and water samples to be used for denitrification rate measurements by the N_2/Ar method were transported on ice (cores) or at ambient temperature (water) to Horn Point Environmental Lab (Cambridge, MD) where cores and water were equilibrated in site-specific incubation tanks at the the temperature of sample collection for ~ 8 h. Experiments were initiated by capping duplicate cores from each site with gas-tight tops fitted with two sample ports and an attached stir bar suspended 6 cm from top. Core tubes were sited around a central carousel housing 4 equidistant magnets that rotated the stir bars at 40 rpm to simulate river flow in the overlying water without disruption to the sediment. At zero time and three ~evenly spaced intervals thereafter, overlying water was withdrawn from the outlet port

into 7 mL glass stoppered tubes for N_2/Ar analysis. An additional 50 ml sample was withdrawn for NO_3^--N and NH_4^+-N analysis. A gravity-feed system replenished each core with site water via the inlet port during sample removal. A sediment-free blank from each site was similarly treated throughout each experiment. Experiments had a duration of 6 to 24 h depending on water temperature and were conducted in the dark; Lower water temperatures required a longer observational period. However, the length of all experiments was set to restrict dissolved oxygen consumption to <50% of the measured zero time concentration.

Nitrogen and dissolved O_2 gas fluxes were determined by high precision (±0.05%) analysis of aqueous phase ratios of N_2 and O_2 to Ar in a Balsars Prisma QMS 200 quadrapole mass spectrometer. The system was modified by addition of a sample loop that allowed gas extraction from aqueous samples in a low pressure (10^{-6} mbar) environment. Extracted gases were passed through a liquid N_2 cryotrap to remove residual CO_2 prior to entering the mass spectrometer. The instrument was optimized to measure only atomic mass units (amu) of O_2 (32 amu), N_2 (28 amu) and Ar (40 amu) in the separating field. Argon served as a stable conservative tracer against which changes in N_2 and O_2 were assessed. The instrument was standardized with air-equilibrated deionized water maintained at constant temperature and pressure in conjunction with gas solubility values given in Colt (1984). A detailed description of the membrane inlet mass spectrometric system is given by Kana (1998).

Overlying core water collected for nutrient analysis at each sampling time was immediately filtered (25 mm GF/F glass membranes) and frozen. Samples were subsequently analyzed manually (NH_4^+-N) or by automated flow injection colorimetry (NO_3^--N) by the phenol-hypochlorite and Cu-Cd reduction methods, respectively (Parsons et al. 1984).

Net fluxes of NH_4^+-N, NO_3^--N, O_2 and N_2 between the sediment and overlying water were calculated from the time-linear rate of change in concentration over the observational period accounting for the volume of overlying water and and sediment surface area of each core. Fluxes were corrected for changes observed in water column blanks.

2.3.2 Isotope Pairing-determined rates of denitrification

The isotope pairing technique was used to determine rates of denitrification at the Curriculum for Marine Science at UNC-CH within 7 d of sample collection. Sediment cores stored at 4^{o}C were subcored with the barrel of a 60 mL plastic syringe (incubator) that had been modified by removal of the tapered tip. The incubator was adjusted to include 3 cm of sediment and the bottom was sealed. A teflon plunger was used to seal the incubator top leaving about 15 mL space between the surface of sediment and the plunger.

Artificial bottom water with $^{15}NO_3^-$ tracer was made by dissolving $Na^{15}NO_3$ (Cambridge Isotope Laboratories Inc. USA) in deionized water to give an 18 to 81 μmol L^{-1} $^{15}NO_3^-$ solution. The artificial bottom water was stored in 4 liter dark bottle. One liter of "control" bottom water was prepared similar as artificial bottom water without adding $^{15}NO_3^-$ tracer. Then the artificial

bottom water and "control" bottom water were bubbled with an artificial air mixture of He and O_2.

A 3 h preincubation process was initiated. Teflon tubes (1/8" o.d.) carried artificial bottom water from the dark bottle to the incubators through a peristaltic pump and a similar tube delivered the "control" bottom water to the control incubator, all at a flow rate of about 4 mL min^{-1}. This preincubation replaced the original bottom water with artificial bottom water and allowed the $^{15}NO_3^-$ tracer to diffuse into the sediment and reach a steady state. During preincubation, the artificial bottom water and "control" bottom water were continuously bubbled with artificial air to minimize background N_2 gas in the waters, and all incubators are kept in water bath at the *in situ* temperature in darkness.

Following pre-incubation, the incubators were sealed with gas-tight septum and a portion of the bottom water was replaced with artificial air (10 cm^3, 1 atm) to give a sediment-water-air incubation system. The incubators were placed in a SR2 magnetic stirrer under darkness and maintained at the temperature of sample collection. The overlying water was gently stirred by a small (8mm L×1.5 mm D) teflon coated magnet stir bar positioned ~1.5 cm above the sediment-water interface.

An incubation was terminated by vigorously shaking the incubator for about 15 s, equilibrating dissolved N_2 between the gas and aqueous phase. The gas phase was sampled by displacing N_2-free deionized water in a sealed glass vial with headspace gas from the incubator through activation of the plunger that sealed the top of the incubator. The incubator without a tracer served as control. Incubators were sacrificed at hourly intervals to 3 h following initiation of each experiment.

The gas samples were analyzed on a GC-MS mass spectrometer (Finnigan MAT 252). Subsamples of gas (100 μL) were injected into a carrier gas flow of He and possible interfering gases were separated from N_2 in the gas chromatographer. All three N_2 species ($^{28}N_2$, $^{29}N_2$, and $^{30}N_2$) were measured and excess $^{29}N_2$ and $^{30}N_2$ were calculated using the background $^{29}N_2/^{28}N_2$ and $^{30}N_2/^{28}N_2$ ratios determined from the control.

Denitrification rate were calculated from the production rates of $^{29}N_2$ and $^{30}N_2$. Denitrification rates fueled by $^{15}NO_3^-$ tracer ($D15$) and $^{14}NO_3^-$ ($D14$) were estimated by (Nielsen 1992):

$$D15 = J^{29}N_2 + (2 \times J^{30}N_2), \quad (1)$$

and

$$D14 = J^{29}N_2 + (2 \times J^{28}N_2); \quad (2)$$

where $J^{28}N_2$, $J^{29}N_2$, and $J^{30}N_2$ represent production rates of these nitrogen species. Because the production rate of $^{28}N_2$ ($J^{28}N_2$) is not readily measured, an estimate of $D14$ (D^14) is obtained from:

$$D\text{^}14 = D15 \times \frac{J^{29}N_2}{2 \times J^{30}N_2} \quad (3)$$

It was assumed that the only nitrate in overlying water was $^{15}NO_3^-$ tracer. $^{14}NO_3^-$ in sediment is produced by nitrification from NH_4^+. Hence, the rate of denitrification consuming $^{15}NO_3^-$ ($D15$) is supported by nitrate from the overlying water (i.e., external nitrate); while the rate of

denitrification consuming $^{14}NO_3^-$ (*D*14) is called coupled nitrification-denitrification and is supported by nitrate produced on site through nitrification. This incubation experiment was developed to simulate the field condition and determine the *in situ* denitrification rate.

2.3.4 Statistical analysis and calculations

Relationships between variables were assessed by correlation analysis (α=0.05). The percent of NO_3^--N removed by denitrification was calculated based on NO_3^--N loading and measured denitrification rates. Loading rates were calculated by multiplying discharge times the NO_3^--N concentration and dividing by river width (Kemp and Dodds 2002a), with estimated daily loss calculated as the denitrification rate divided by the NO_3^--N load times the water velocity (Inwood et al. 2005).

3. RESULTS

Water temperatures varied from 4 to 29°C, reflecting the season of collection (Table 2). Water discharge reflected catchment size. Tributary streams (Crabtree Creek and Nahunta Swamp) showed discharges of generally <3 m^3s^{-1}, while recorded discharges in mainstem Neuse stations were an order of magnitude or more higher (Table 3). Discharge at the time of sample collection was 10 to 85% lower than the historic mean for the month in which samples were collected except during the 14 August sampling session when discharge exceeded the historic means by as much as 415%.

Table 2. Inorganic nitrogen concentrations (± 1 standard deviation) in water overlying of duplicate cores and river water temperature at the time of sample collection during this study.

Site	Date	Temperature (°C)	NH_4^+-N (μmol L^{-1})	NO_3^--N (μmol L^{-1})
Crabtree Creek - A	19 December 00	4.0	4.3 (0.6)	30.0 (1.2)
Crabtree Creek - B		4.0	4.8 (1.2)	10.6 (0.4)
Smithfield		5.0	5.1 (0.2)	61.5 (3.1)
Nahunta Swamp		5.0	5.7 (0.6)	60.6 (0.8)
Fort Barnwell		4.5	2.6 (0.4)	47.4 (1.8)
Crabtree Creek - A	8 May 01	19.0	4.5 (1.3)	29.8 (8.6)
Crabtree Creek - B		20.0	4.0 (0.7)	68.1 (0.1)
Smithfield		20.0	4.9 (0.2)	55.9 (1.4)
Nahunta Swamp		22.0	5.5 (0.6)	57.7 (22.5)
Fort Barnwell		18.5	2.1 (0.4)	47.7 (0.4)
Crabtree Creek - A	14 August 01	29.0	9.4 (0.1)	11.2 (0)
Crabtree Creek - B		29.0	10.8 (1.4)	17.3 (0.4)
Smithfield		26.0	8.9 (1.6)	24.2 (3.3)
Nahunta Swamp		29.0	3.7 (0.3)	38.1 (0.1)
Fort Barnwell		24.5	1.8 (0.2)	32.3 (0.1)
Crabtree Creek - A	18 November 01	13.5	5.4 (0.7)	110.5 (0.7)
Crabtree Creek - B		13.5	2.7 (0.0)	98.9 (17.1)
Smithfield		11.5	0.8 (0.1)	39.2 (0.4)
Nahunta Swamp		16.0	0.6 (0.3)	31.7 (0.5)
Fort Barnwell		11.0	2.4 (0.5)	46.7 (1.3)

Ammonium concentrations at the time of sample collection varied from 0.6 to 10.8 μmol L^{-1}, ranging over a factor about 18 (Table 2). Nitrate concentrations were higher, varying from 10.6 to 110.5 μmol L^{-1}. However, NO_3^--N concentrations showed a smaller range compared with NH_4^+-N, about an order of magnitude. Nitrate comprised 54 to 98% of the DIN pool and averaged 88%. No site-to-site differences were apparent in concentrations of either nitrogenous

nutrient on any date. Likewise, no trends were obvious within a sampling site when concentrations of either nutrient were compared across dates.

Table 3. Discharge data at USGS gauging stations on the date of sample collection and the historic mean discharge (parentheses) for the month in which samples were collected. Historic records cover at least ten years.

Site	Date	Station #	$m^3 s^{-1}$ Discharge
Crabtree Creek - A,B	19 December 00	02087275	2.7 (3.0)
Smithfield		02087570	1.92 (2.03)*
Nahunta Swamp		02091000	1.1 (2.0)
Fort Barnwell		02091814	48 (113)
Crabtree Creek - A,B	8 May 01	02087275	0.4 (1.4)
Smithfield		02087570	1.57 (2.08)
Nahunta Swamp		02091000	0.3 (1.7)
Fort Barnwell		02091814	36 (100)
Crabtree Creek - A,B	14 August 01	02087275	9.2 (3.3)
Smithfield		02087570	3.08 (1.98)
Nahunta Swamp		02091000	7.9 (1.9)
Fort Barnwell		02091814	59 (19)
Crabtree Creek - A,B	18 November 01	02087275	0.3 (2.1)
Smithfield		02087570	7 (19)
Nahunta Swamp		02091000	1.31 (1.86)
Fort Barnwell		02091814	18 (19)

*Flow data unavailable for Smithfield. Data given are gauge heights (m).

Ammonium fluxes were highly variable across both sampling sites, with means for duplicate cores varying from 0 to 104 μmol m^{-2} h^{-1} (Table 4). The high standard deviation for duplicate cores points to high variability on a scale of meters in factors affecting NH_4^+-N flux. Nonetheless, non-zero NH_4^+-N fluxes were always positive, indicating release of nutrient from the sediment to the water column. Nitrate fluxes showed variability markedly similar to those for NH_4^+-N, with values varying from 0 to -102 μmol m^{-2} h^{-1} (Table 4). However, fewer non-zero values were observed, and fluxes were uniformly negative, indicating loss of NO_3^--N from the water to the sediment. Again, sitewise meter-scale variability in factors affecting NO_3^--N flux is apparent in the large standard deviations associated with values for duplicate cores on any date. No site-to-site differences were apparent in fluxes for either nutrient on any date and no seasonal trends could be identified for either nutrient when fluxes were compared for each site across dates.

Unlike fluxes for nitrogenous nutrients, non-zero dissolved O_2 fluxes were consistently observed in both cores at every site on every sampling date (Table 5). Moreover, variability between duplicate cores was considerably less than for NO_3^--N or NH_4^+-N. Fluxes were always negative,

indicating O_2 consumption by sediments. Overall, values ranged over a factor of <5, from -256 to -1206 μmol m^{-2} h^{-1}. Site-wise, no differences in flux were found when values for a given date were compared. Date-wise, however, the lowest fluxes for each site except Crabtree Creek - B were associated with the coldest sampling session, 19 December 2000.

Table 4. Ammonium and NO_3^--N fluxes in duplicate cores at each site. Dissolved O_2 fluxes and denitrification rate determinations were also made for these cores. Data in parentheses represent ± 1 standard deviation. Positive and negative fluxes represent sediment release and consumption of nutrient, respectively.

Site	Date	NH_4^+-N flux (μmol m^{-2} h^{-1})	NO_3^--N flux (μmol m^{-2} h^{-1})
Crabtree Creek - A	19 December 00	12.3 (17.4)	0
Crabtree Creek - B		0	0
Smithfield		28.8 (8.1)	0
Nahunta Swamp		0	0
Fort Barnwell		10.7 (15.1)	-6.9 (9.7)
Crabtree Creek - A	8 May 01	0	--63.4 (89.6)
Crabtree Creek - B		0	0
Smithfield		11.3 (16.0)	-57.9 (81.8)
Nahunta Swamp		36.3 (51.3)	0
Fort Barnwell		0	-61.8 (20.4)
Crabtree Creek - A	14 August 01	0	0
Crabtree Creek - B		104.2 (23.5)	0
Smithfield		0	0
Nahunta Swamp		5.5 (7.7)	0
Fort Barnwell		8.3 (11.7)	0
Crabtree Creek - A	18 November 01	19.7 (2.5)	0
Crabtree Creek - B		9.6 (13.5)	0
Smithfield		54.6 (37.1)	-70.7 (16.2)
Nahunta Swamp		5.3 (7.5)	-22.1 (18.6)
Fort Barnwell		15.0 (7.8)	-102.1 (45.9)

Denitrification was observed in at least one of duplicate cores on most occasions (14 of 20 pairs of cores) when rates were assessed by the N_2/Ar technique (Table 5). Average rates for duplicate cores varied from 0 to 222 μmol N_2-N m^{-2} h^{-1}, while the overall average rate of denitrification was 41 μmol N_2-N m^{-2} h^{-1}. Denitrification rates showed high meter-scale variability when duplicates cores for each site and date were compared, in agreement with data for nutrient fluxes. In accord with the data for O_2 flux, lowest non-zero rates of denitrification were generally recorded during December. Rates of denitrification showed no correlation with fluxes of NH_4^+-N or NO_3^--N that were simultaneously measured on the same cores.

Table 5. Dissolved O_2 flux and rate of denitrification measured in duplicate cores at each site using the N_2/Ar technique. Data in parentheses represent ± 1 standard deviation. A negative flux represents net O_2 consumption by the sediment.

Site	Date	Dissolve O_2 flux (μmol m^{-2} h^{-1})	Denitrification rate (μmol N_2-N m^{-2} h^{-1})
Crabtree Creek - A	19 December 00	-357 (154)	10.0 (14.1)
Crabtree Creek - B		-278 (11)	19.9 (28.1)
Smithfield		-361 (147)	24.4 (12.2)
Nahunta Swamp		-365 (25)	30.3 (8.1)
Fort Barnwell		-439 (118)	60.4 (32.2)
Crabtree Creek - A	8 May 01	-1418 (874)	46.2 (65.3)
Crabtree Creek - B		-763	40.2 (18.6)
Smithfield		-1024 (250)	45.0 (2.6)
Nahunta Swamp		-694 (150)	23.0 (22.9)
Fort Barnwell		-846 (622)	0
Crabtree Creek - A	14 August 01	-681 (88)	0
Crabtree Creek - B		-256 (177)	0
Smithfield		-647 (411)	71.7 (13.2)
Nahunta Swamp		-1187 (411)	100.6 (35.2)
Fort Barnwell		-791 (136)	106.8 (56.9)
Crabtree Creek - A	18 November 01	-457 (41)	24.6 (24.3)
Crabtree Creek - B		-335 (83)	0
Smithfield		-1206 (173)	222.1 (9.5)
Nahunta Swamp		-502 (30)	0
Fort Barnwell		-468 (85)	0

Non-zero values of denitrification were observed on 13 of 15 cores when rates were measured using the isotope pairing technique (Table 6). The total data showed lower variability than rates measured by the N_2/Ar method, with rates varying from 0 to 143 μmol N_2-N m^{-2} h^{-1}. As with N_2/Ar-determined rates, no pattern was apparent with respect to denitrification rates measured by isotope pairing when data were compared within or across sites or dates. The percent contribution of coupled nitrification-denitrification to total denitrification varied from 11 to 95% and averaged 66%. The relative importance of coupled nitrification-denitrification showed no correlation with denitrification rates. The overall average rate of denitrification determined by the isotope pairing technique was 33 μmol N_2-N m^{-2} h^{-1}, about 25% lower than the average for the N_2/Ar method. Comparison of denitrification rate estimates for each site on the same date by the two independent techniques showed no relationship (Fig. 2).

Gauging station data necessary for calculation of percent N loss to denitrification (discharge and velocity) was available on the sample dates only for the Fort Barnwell site. Discharge data from the date of sample collection and the mean value for velocity on the two nearest available dates bracketing the sample date were used to calculate percent N loss to denitrification for the

Crabtree Creek and Nahunta swamp sites. Neither velocity nor discharge data were recorded for the Smithfield site, so denitrification losses are not estimated. Losses to denitrification varied from 0.2 to 3.1% at Fort Barnwell, 0.2 to 23% at Crabtree Creek-A, 1.0 to 8.7% at Crabtree-B and 1.4 to 18% at Nahunta Swamp. The overall mean loss to denitrification was 5.0%.

Table 6. Concentration of $^{15}NO_3^-$-N in artificial streamwater added to sediment cores, rate of denitrification measured using the isotope pairing technique and calculated percent of total denitrification due to coupled nitrification-denitrification. A single rate determination for each site was made from the time course of ^{15}N accumulation in dinitrogen gas in the headspace of sediment-water incubators that were sacrificed hourly during each experiment.

Site	Date	$^{15}NO_3^-$-N (μmol L^{-1})	Denitrification rate (μmol N_2-N m^{-2} h^{-1})	Percent coupled
Crabtree Creek - A	8 May 01	20.0	0	N.A.[δ]
Crabtree Creek - B		71.0	0	N.A.
Smithfield		55.0	3.8	11
Nahunta Swamp		73.0	81.7	86
Fort Barnwell		48.0	14.2	53
Crabtree Creek - A	14 August 01	2.3	8.3	95
Crabtree Creek - B		18.8	72.5	93
Smithfield		23.3	142.9	90
Nahunta Swamp		36.8	51.3	66
Fort Barnwell		32.1	18.8	51
Crabtree Creek - A	18 November 01	110.0	11.7	46
Crabtree Creek - B		81.0	30.8	81
Smithfield		38.0	10.4	52
Nahunta Swamp		31.0	41.7	91
Fort Barnwell		39.0	6.7	38

[δ]N.A. = Not applicable

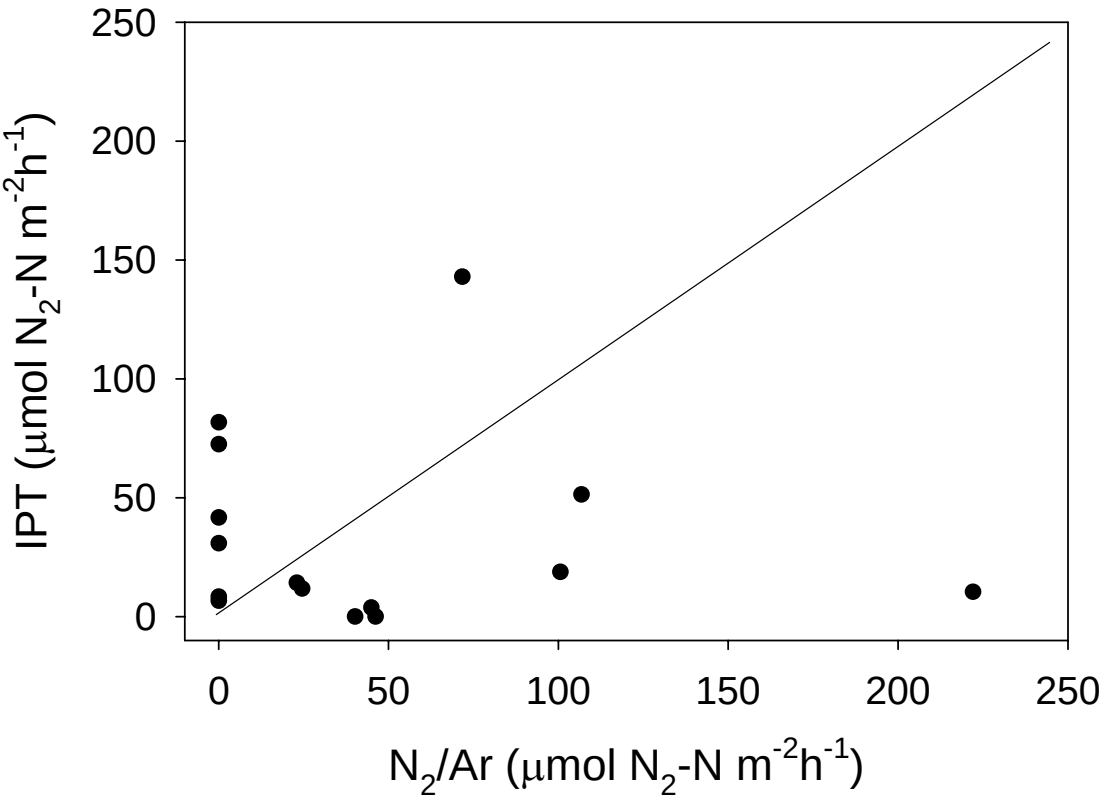

Figure 2. Comparison of denitrification rate estimates for each site on the same date using the isotope pairing (IPT) and N_2/Ar techniques. The line indicating a 1:1 relationship is shown.

4. DISCUSSION

Reduced discharge relative to historic means was observed on all sampling dates except 14 August 2001 (Table 3). This likely reflects the protracted drought that was recorded throughout North Carolina during 1998 through 2002 (Weaver 2005).

Ammonium and NO_3^--N (Table 2) concentrations generally fell within the 10^{th} to 90^{th} percentile for values recorded from 1996 to 2005 at Ambient Monitoring Sites that correspond to our sampling stations (NCDENR 2001, 2006). The observed concentrations of inorganic-N compare favorably with the range of values reported for other lotic systems in the state. Mallin et al. (2001, 2004) and Ensign and Mallin (2001) give mean NO_3^--N and NH_4^+-N concentrations varying from 0.5 to 37 μmol L^{-1} and 2.4 to 6.5 μmol L^{-1} for six rivers ranging from third to fifth order in the NC coastal plain.

Nitrate comprised 88% of DIN. Dominance of NO_3^--N in DIN of lotic systems has been attributed to anthropogenic activity regionally (Mallin et al. 1999) and worldwide (Turner et al. 2003). Thus, the high percent contribution of NO_3^--N to DIN in the Neuse River is consistent with the high level of agricultural activity and increasing development in the river basin.

Nutrient and dissolved O_2 fluxes have been rarely determined for lotic systems using core or chamber techniques, with most studies focusing on sediment-water NO_3^--N exchange. Our NO_3^--N fluxes of 0 to -102 μmol m^{-2} h^{-1} (Table 4) are lower than other reports for studies utilizing these methodologies. Duff et al. (1996) reported NO_3^--N fluxes of -65 to -470 μmol m^{-2} h^{-1} for a tropical forest stream. Hill et al. (1981) observed fluxes of -45 to -745 μmol m^{-2} h^{-1} for a temperate stream in a forested/agricultural area. Otherwise, Sain et al. (1977) and Robinson (1979) give NO_3^--N fluxes of -180 to -495 μmol m^{-2} h^{-1} for a spring-fed stream while van Kessel (1977) reported fluxes of -280 to -480 μmol m^{-2} h^{-1} for an irrigation ditch. The single study (Hill and Sanmugadas 1985) conducted on higher order systems (2 Ontario rivers) showed generally higher loss rates of NO_3^--N (-108 to -1225 μmol m^{-2} h^{-1}) than reported for streams.

The dissolved O_2 fluxes of -256 to -1418 μmol m^{-2} h^{-1} found in the mainstem Neuse River and tributary streams (Table 5) were lower than respiratory fluxes reported for other lotic systems using similar methodologies. Grimm and Fisher (1984) observed respiratory flux of -4000 to -5625 μmol m^{-2} h^{-1} for a Sonoran desert stream, while Fellows et al. (2001) reported values of -1280 to -1780 μmol m^{-2} h^{-1} for two New Mexican creeks. Higher rates of sediment O_2 consumption in these other studies relative to the Neuse system are probably due to increased labile substrates in surficial sediment and porewater, as water temperatures in these other studies were no higher than we found at our sites. Although we could find no studies assessing NH_4^+-N exchange at the sediment-water interface in lotic systems, our data showing either no exchange or efflux (Table 5) agrees well with an investigation in the intertidal region of the Great Ouse River (Trimmer et al. 1998), where sediments were a consistent source of NH_4^+-N and sink for O_2 and NO_3^--N.

Denitrification is generally considered to have the highest spatiotemporal variability of all microbial processes involved in N cycling dynamics (Tiedje et al. 1989), with coefficients of

variation exceeding 100% for multiple measurements at the same site (Parkin and Robinson 1989). Accordingly, replicate N_2/Ar-based rates of denitrification at the same site showed high variability (Table 5) and there was a lack of agreement between rates estimated by the N_2/Ar and isotope pairing techniques (Fig. 2) for the same date and site. Our denitrification rates of 0 to 222 μmol N_2-N m^{-2} h^{-1} generally fall toward the low end of rates reported across a wide range of lotic systems worldwide (Table 7), where maximum rates >500 μmol N_2-N m^{-2} h^{-1} are not uncommon. Most measurements have been conducted with the C_2H_2 inhibition technique. This method is widely acknowledged to underestimate denitrification rates (e.g. Seitzinger 1993), further emphasizing that rates of denitrification in the Neuse and its tributaries are relatively low.

Rates of denitrification are primarily governed by redox status and secondarily by the availability of NO_3^--N and labile C (Holmes et al. 1996; Duff et al. 1996; Kemp and Dodds 2002a), a trend that apparent in Table 7. It is unlikely that NO_3^--N limited denitrification here, as concentrations in the Neuse and tributary streams (Table 2) were generally well in excess of the value of <10 μmol L^{-1} considered to be the apparent K_m (half-saturation constant) for denitrification (Christensen et al. 1989). We have no data for organic-C, but consistently active consumption of O_2 by sediments (Table 5) and frequent efflux of NH_4^+-N (Table 4) suggests an adequate supply of high quality of organic matter to support microbial activity, including denitrification. Garcia-Ruiz et al. (1998b) reported an inverse correlation between sediment particle size and rates of denitrification in a survey of >30 British rivers, and suggested that fine particles limited O_2 diffusion and created a greater opportunity for anaerobiosis. Similarly, generally sandy sediments at all of our study sites may have promoted aeration, limiting the availability of anaerobic microsites that support denitrification.

Although coupled activity has been demonstrated to contribute significantly to total denitrification in freshwater sediments (Lorenzen et al. 1998) and in coastal marine sediments (Rysgaard et al. 1994; An and Joye 2001; Laursen and Seitzinger 2002b), little data are available for coupled nitrification-denitrification in lotic systems. Seitzinger (1988) estimated that coupled mineralization-nitrification-denitrification comprised >75% of total denitrification in the Potomac and Delaware rivers, while Laursen and Seitzinger (2002b) suggested that coupled nitrification-denitrification was important in a reach-scale study involving three North American rivers, based on diurnal differences in water column NO_3^--N concentrations and sediment denitrification rates. Strong correlations between nitrification and denitrification (Kemp and Dodds 2002a) or denitrifying enzyme activity (Richardson et al. 2004) have been interpreted to indicate a high contribution of coupled nitrification-denitrification to total denitrification in sediment of prarie streams and the upper Mississippi River, respectively. Our isotope pairing experiments showed that on average 66% of total denitrification was due to coupled nitrification-denitrification (Table 6). Although these data suggest that coupled activity is important in supplying NO_3^--N to denitrifiers in the Neuse system, the mixing regime in our cores may not have adequately simulated the physical properties that influence material exchange across the sediment-water interface, and hence, rates of biological activity. The lack of correlation between NO_3^--N consumption by sediments (cf. Tables 4, 5 and 6) and rates of total denitrification is consistent with a high contribution of coupled activity, but is also readily explained by spatial heterogeneity or assimilatory NO_3^--N reduction by benthic algae and heterotrophs.

Many studies have attempted to estimate total in-river N loss, or explicitly, N loss to denitrification (Table 8). Total in-river N losses include not only denitrification, but also assimilation into autotrophic biomass and in some cases sedimentation of particulate-N. All estimates are subject to criticism. Length and time scales can influence results. Seitzinger et al. (2002) found that N removal in drainage networks varied with stream order. Temporal changes in physical and chemical characteristics (discharge, depth, water residence time) can influence removal efficiency on time scales ranging to annual and distance scales from reach to the whole river system (Laursen and Seitzinger 2004). Chamber and core experiments may not reproduce *in situ* conditions affecting water exchange between at the sediment surface or between subsurface sediments and groundwater (Mulholland et al. 2004) and may not embrace the natural substrate heterogeneity (Sjoden et al. 1997), which alone can account for 30% of the variability in measured denitrification rates (Kemp and Dodds 2002b).

Our seasonal survey of denitrification at representative sites in the mainstem Neuse River and tributary streams was not intended to give a firmly based estimate of in-stream N removal via denitrification. Nonetheless, acknowledging the shortcomings of any estimate, comparison with other lotic waters provides at least a qualitative indication of the relative importance of this microbial process in the N biogeochemistry of the Neuse system. Our overall estimate of 5% N loss to denitrification falls toward the low end of data reported for rivers and streams worldwide (Table 8), which show values ranging from 1 to 141% for either total N loss or denitrification. Stream bottoms at all of our study sites except Nahunta Swamp were dominated by rocky substrates that likely show low or undetectable rates of denitrification. Thus, our value of 5% N loss to denitrification is an overestimate if the bottom substrate at the sampling sites is representative at larger scales of space. Our study using the most widely regarded (Groffman et al. 2006) enclosure-based methods indicates that denitrification actively removes N from the mainstem Neuse and tributary streams and gives rate estimates for suitable substrate that compare favorably with published rates using a variety of analytical techniques. Although our limited data suggest that denitrification provides a minor sink for N in the Neuse system, other techniques such as mass balance assessments or N_2/Ar open channel methods are better suited to provide firm estimates of total in-river N loss or N loss to denitrification, respectively, as these give information on longer spatiotemporal scales.

Table 7. Denitrification rate measurements in streams and rivers. $^{15}NO_3^-$-N tracer addition, N_2/Ar open channel and mass balance approaches determine rates on a reach scale from in *situ* measurements. All other rates are derived from core and chamber experiments in the laboratory.

Stream/river	Denitrification rate (μmol N_2-N m^{-2} h^{-1})	NO_3^--N (μmol L^{-1})	Method	Reference
Neuse River system	0 to 222	11 to 111	N_2/Ar, isotope pairing	this study
Sugar Creek, IN	120	64	$^{15}NO_3^-$-N tracer addition	Bohlke et al. (2004)
Walker Branch, TN	12	2	$^{15}NO_3^-$-N tracer addition	Mulholland et al. (2004)
Potomac River, MD	210 to 232	>1	direct N_2 flux	Seitzinger (1988)
Delaware River, NJ	166 to 344	-.	direct N_2 flux	Seitzinger (1988)
Gelbaek, Denmark	100 - 1400	285 to10,000	C_2H_2 inhibition	Christensen et al. (1990)
Rabis Baek, Denmark	40 to 460	-	C_2H_2 inhibition	Christensen and Sorensen (1988)
King's & Shane Cr., KS	0 to 9	0 to 55	C_2H_2 inhibition	Kemp and Dodds (2002)
Little Lost Man Cr., CA	0	3	C_2H_2 inhibition	Duff et al. (1984)
San Francisquito Cr., CA	27	57 to 71	C_2H_2 inhibition	Duff et al. (1984)
Sycamore Creek, AZ	3 to 13	2	C_2H_2 inhibition	Holmes et al. (1996)
Rivers, Ontario	110 to 1220	-	NO_3 loss	Hill and Sanmugadas (1985)
Salto River, Costa Rica	5 to 470	14	NO_3 loss & C_2H_2	Duff et al. (1996)
Stream sediments, UK	0 to 497	-	C_2H_2 inhibition	Garcia-Ruiz et al. (1998a)
River sediments, UK	20 to 660	35 to 750	C_2H_2 inhibition	Pattinson et al. (1998)
Agricultural streams, IL	<1 to 1070	8 to 715	C_2H_2 inhibition	Royer et al. (2004)
Streams, Denmark	420	-	C_2H_2 inhibition	Nielsen et al. (1990)
South Platte River, CO	0 to 15600	200 to 500	N_2/Ar open channel & mass balance	Pribyl et al. (2005)
Mississippi River, MO	14 to 285	13 to 186	C_2H_2 inhibition	Richardson et al. (2004)
Rivers, NJ, IL-IN	270 to 15800	-	N_2/Ar, open channel	Laursen and Seitzinger (2002a)

Table 8. Percent N removal in rivers.

River or stream	% N removed	Method	Reference
Neuse river and tributaries	5	N_2/Ar & isotope pairing in cores	This study
Kalamazoo River Watershed, MI	18 to 141	Denitrification in composite sediments	Inwood et al. (2005)
Dorn River, England	15	Denitrification in cores	Cooke and White (1987)
Potomac River	35	Denitrification in cores	Seitzinger (1988)
Delaware River	20	Denitrification in cores	Seitzinger (1988)
Duffin Creek, Ontario	6	Denitrification in cores, mass balance	Hill (1979, 1981, 1983)
Gelbaek, Denmark	1	Denitrification in cores	Christensen and Sorensen (1988); Christensen et al. (1990)
Neversink, NY	11 to 12	Mass balance	Burns (1998)
Purukohukohu, New Zealand	14 to 17	Mass balance	Cooper and Cooke (1984)
South Platte River, CO	34 to 45	N_2/Ar open channel & mass balance	Pribyl et al. (2005)
Upper Mississippi River, MO	6.9	Denitrification in cores	Richardson et al. (2004)
Agricultural stream, Quebec	≤50	isotopic signature of NO_3^-	Kellman et al. (1998)
Walker Branch, TN	16	$^{15}NO_3^-$ tracer addition	Molholland et al. (2004)
South Platte River, CO	50	Mass balance	Sjodin et al. (1997)
Drainages to North Atlantic Ocean	10 to 20	Mass balance; in-river N loss	Howarth et al. (1996)
16 watersheds, Northeastern U.S.	11	Mass balance; in-river N loss	Van Breemen et al. (2002)
16 drainage networks, Northeastern U.S.	37 to 76	Regression model	Seitzinger et al. (2002)

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