Denitrification in sediments of headwater streams in the southern Appalachian Mountains, USA.

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(ABSTRACT)

We investigated variations in resource availability (nitrate and labile organic carbon, LOC) as determinants of denitrification in sediments of streams in the southern Appalachian Mountains, USA. Stream water and sediments were sampled seasonally in two streams of contrasting nitrate availability, Noland Creek (high NO_3-N) and Walker Branch (low NO_3-N). Eight additional streams with varying nitrate levels were sampled once during summer. Stream sediments were incubated at ambient stream temperatures, and nitrous oxide accumulation was quantified following acetylene inhibition of nitrous oxide reduction. Denitrification potential was greater in Noland Creek than Walker Branch and was generally greater in sediments from the higher-nitrate streams. In autumn and spring, nitrate and LOC amendments indicated that denitrification potential in Walker Branch sediments was nitrate limited, with temperature having no effect on rates. Denitrification potential in Noland Creek sediments was not limited by nitrate, but temperature had a significant effect. When Noland Creek seasonal data were corrected to a common temperature, no seasonal differences in denitrification potential were detected. Nitrate-N in the 10 surveyed streams ranged from 10 to 549 μg/L, with the highest NO_3-N levels and denitrification rates generally occurring in the higher elevation streams in the GSMNP. We found that nitrate availability, more than LOC availability, controls potential denitrification in these streams.

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Introduction

Several mass balance studies have investigated nitrogen (N) dynamics at the catchment scale (Vitousek and Reiners 1975, Bormann and Likens 1979, Hedin et al. 1994, Swank and Vose 1997), yet few studies of this nature have been conducted in streams (Triska et al. 1984, Webster and Swank 1985, Burns 1998). Stream studies have led us to the conclusion that stream exports of N are often less than catchment inputs, suggesting significant retention processes for N in catchments. Sinks for N in catchments are considered to be primarily terrestrial (e.g. vegetation uptake, incorporation into soil organic matter, etc.), while in-stream sinks have been largely ignored. Several recent studies have suggested that in-stream processes may be important N sinks, exerting significant controls on N outputs from catchments (Holmes et al. 1996, Mulholland and Hill 1997).

Denitrification may be a significant mechanism for nitrogen loss in streams (Swank and Caskey 1982, Cooke and White 1987, Triska et al. 1993, Bradley et al. 1993). Denitrification is a microbially-mediated process in which facultative bacteria oxidize organic carbon while using nitrate as an alternative electron acceptor (Rosswall 1981). The end product is nitrogen gas – nitrous oxide (N_2O) and dinitrogen gas (N_2). In contrast to the assimilatory pathway of nitrate reduction, which produces ammonium for biosynthesis under aerobic conditions, denitrification ultimately removes available nitrogen from an ecosystem.

In streams characterized by high concentrations of nitrate, denitrification may improve surface-water quality and thereby offset the negative effects of nitrogen enrichment (Ventullo and Rowe 1982, Holmes et al. 1996, Jordan and Weller 1996). In streams with low concentrations of nitrate and where nitrogen is limiting to algae and microbes, additional loss of nitrate through denitrification can negatively affect ecosystem production (Neilsen et al. 1990, Rysgaard et al. 1994). Yet, our understanding of nitrogen loss from streams via denitrification is limited (Sinsabaugh 1994) because denitrification is potentially regulated by complex hydrological, biological, chemical, and geological factors.

We investigated the patterns of seasonal variation and role of resource availability on denitrification potential in the sediments of headwater streams in the southern Appalachian Mountains. Two streams of contrasting nitrate availability (West Fork of Walker Branch and Noland Creek) were selected as primary sites for the investigation. Denitrification potential was

also surveyed in eight additional streams with different nitrate concentrations located near the two primary study streams. The specific objectives of this study were: (*i*) to characterize the denitrification activity in the sediments of two headwater streams of contrasting nitrate; (*ii*) to identify temporal variation in denitrification; and (*iii*) to determine whether nitrate or labile organic carbon limit denitrification potential.

Study Sites

The West Fork of Walker Branch is located within the Ridge and Valley province in eastern Tennessee (35°58'N, 84°17'W) and drains a 38.4 ha catchment at an elevation of 265 m located on the U.S. Department of Energy's Oak Ridge National Laboratory Reserve (Figure 1). It arises from 4 perennial springs, resulting in base flows of generally 5-10 L/s (Mulholland et al. 1997). The width and depth of the West Fork of Walker Branch during base flow are approximately 1.5-3.0 m and 5.0-10.0 cm, respectively. The streambed consists of heterogeneous cobble, gravel, and fine-grained organic-rich sediments, with sections of exposed dolomitic bedrock (Mulholland et al. 1997). Baseflow alkalinity and pH are moderately high, with values of 2-3 meq/L and 7.8-8.2, respectively (Mulholland 1992). Riparian vegetation is primarily oaks, red maple, yellow poplar, and other mesophytic hardwoods.

Noland Creek drains the 17.4-ha Noland Divide Watershed (NDW) located in the Great Smoky Mountains National Park (GSMNP) (35°34'N, 83°28'W), situated at 1692 m elevation along the Tennessee-North Carolina border (Shubzda et al. 1995). Noland Creek is a poorly buffered high elevation stream with high nitrate concentrations due to high rates of atmospheric deposition and low rates of nitrogen uptake by vegetation in the catchment (Nodvin et al. 1995). The poor nitrate retention observed in mature forests of Noland Divide Watershed is likely exacerbated by the high levels of atmospheric nitrate deposition and increased acidity. Consequently, N exports from Noland Creek often exceed catchment inputs, resulting in a measurable N budget imbalance (Flum and Nodvin 1995). The soils of the Noland Creek watershed are poorly buffered inceptisols formed from the underlying Thunderhead Sandstone (King et al. 1968, Johnson et al. 1991). The overstory vegetation of the catchment is dominated by mature stands of red spruce, yellow birch, beech, and hemlock. The riparian understory

consists of dense rhododendron, contributing to stream shading (Johnson et al. 1991). The streambed of Noland Creek consists of large boulders and woody debris dams along riffle/run reaches.

In addition to the West Fork of Walker Branch and Noland Creek, 4 additional streams on the Oak Ridge reservation (East Fork of Walker Branch, Upper White Oak Creek, Gum Hollow, and Pinhook Branch) and 4 additional streams in the GSMNP (Rattlebox Creek, Mossy Creek, Hickory King Branch, and Beech Flats Prong) were studied. Stream elevations ranged from 244 to 1646 m. The streams on the Oak Ridge reservation were expected to have relatively low nitrate concentrations, whereas the streams in the GSMNP were expected to vary greatly in nitrate concentration depending on elevation.

Methods

Sampling and analyses

Seasonal sampling in Walker Branch and Noland Creek included measurements of stream temperature, conductivity, nitrate-N (NO_3-N), and dissolved organic carbon (DOC), and collection of sediments. Sampling was conducted in mid-autumn 1998 and in winter, spring, and summer 1999. The survey of the additional 8 streams was conducted in summer 1999, at which time stream water pH was also measured.

Following measurement of stream temperature and conductivity, water for chemical analyses was filtered (0.4 μm membrane filters), placed on ice, and then frozen on return to the laboratory. Nitrite and nitrate-N concentrations were determined by the cadmium-reduction method using a Bran Luebbe TRAACS 800 auto-analyzer (reported as NO_3-N because nitrite is probably minimal in these streams with dissolved oxygen concentrations near saturation). DOC was determined using a Shimadzu TOC-5000 total organic carbon analyzer. Stream water pH was measured on unfiltered samples in the laboratory using an Orion pH meter. An additional liter of unfiltered water was collected for use in the denitrification assays.

Approximately 1 kg (wet weight) of sediment from each of 12 depositional areas (~4-10 cm deep) was collected from each stream on each sampling date using a 385-cm^3 aluminum corer, then transferred to 1.6-L whirlpaks. Sediment samples were stored at ~4^oC until each denitrification assay was initiated, approximately 4 to 24 h following sample collection.

Denitrification assays

Sediment denitrification rates were estimated based on the accumulation of nitrous oxide (N_2O) after addition of acetylene to inhibit N_2O reduction (Tiedje et al. 1989, Holmes et al. 1996). Approximately 150 g (wet weight) of each sediment sample was transferred to an ashed 250-mL media bottle and unfiltered stream water was added to bring the total volume to 125 mL. The microcosms were capped and sealed with septa-fitted screw-top lids.

Anoxia was induced by purging the microcosms with helium for 15 min. Acetylene (C_2H_2) was generated through the dissolution of calcium carbide in water and was collected in a gas sampling bag for each experiment. Acetylene was added to each microcosm to yield 10% C_2H_2 by total volume. Following vigorous mixing, microcosms were left to equilibrate for a few minutes and excess pressure was released, bringing the internal pressure to ~1 atm. Microcosms were incubated at approximate ambient stream temperature for 12 h.

Gas headspace samples (8 mL) were collected using gas tight syringes after 1, 6, and 12 h. Prior to headspace sampling, microcosms were shaken vigorously. The headspace samples were injected into pre-evacuated vacutainers, sealed with silicone, and stored at room temperature until they could be analyzed for N_2O (within 8 weeks). Following the last N_2O sampling, the microcosms were opened and the sediments dried at 60^{o}C for ~96 h. Sediment was weighed, combusted at 500^{o}C for 5 h, and reweighed to obtain percent organic matter (% OM) content and ash free dry mass (AFDM).

Nitrous oxide concentrations were determined using a Perkin Elmer gas chromatograph fitted with a ^{63}Ni electron capture detector. The P10 argon/methane carrier gas flow averaged 30 mL/min. Column and detector temperatures were 50^{o}C and 300^{o}C, respectively. Autumn, winter, and spring headspace samples were analyzed using a backflush-to-vent system (Tiedje et al. 1989) requiring a 3-mL sample injection. The gas chromatograph was subsequently reconfigured to eliminate the need for back-flushing and headspace samples from the summer assays were analyzed using a direct injection method (J. Duff, USGS, Menlo Park, CA, personal communication) requiring a sample injection of only 100 µL. Nitrous oxide standards ranging from 0.015-0.36 µg/L were made from 99.8% pure N_2O and 100% helium stock gases using a standard dilution series.

Denitrification rates were calculated based on differences in nitrous oxide concentrations measured at 6 and 12 h. Total mass of N_2O in the headspace was calculated using the headspace N_2O concentrations and total microcosm volumes following correction for reduced N_2O solubility in the aqueous phase with an appropriate temperature-dependent Bunsen coefficient (Knowles 1979). Denitrification rates were calculated per gram AFDM (gAFDM) and per gram dry mass (gDM). Denitrification rates determined in these assays represent maximum potential denitrification rates in these streams under anoxic conditions. *In situ* stream denitrification rates likely would be lower because sediments are naturally hypoxic.

Differences in denitrification rates between streams were compared using a t-test across each sampling date. Seasonal variation in denitrification rates for each stream was investigated using of non-parametric ANOVA's on ranked rates (Kruskal-Wallis) due to the skewed distribution and lack of normality among rates for each season. These analyses were conducted on rates normalized to gAFDM as well as gDM when appropriate.

Nutrient limitations

To identify how nitrate or labile organic carbon (LOC) availability may limit rates of denitrification, microcosms identical to those described above were set up with nutrient amended stream water during autumn 1998 and spring 1999 sampling periods. Unfiltered stream water was amended with 200 mg NO_3-N/L (as KNO_3), 1 mg $C_{12}H_6O_{12}$-C/L (as dextrose), or both (Holmes et al. 1996). Six microcosms were set up for each of the three amendments and all were run concurrently with the 12 unamended microcosms described above. Gas headspace samples for N_2O were collected, stored, and analyzed as above.

Treatment effects were investigated using a one-way ANOVA for each stream and each sampling date. The Dunnett's post hoc MCT was used to identify significant differences between samples of a respective treatment as compared to the unamended samples. Scheffe's post hoc MCT was also conducted to identify significant differences among samples of the 3 treatments.

Temperature effects

Relationships between denitrification rate and temperature were determined for sediments collected in winter and summer 1999 in Walker Branch and Noland Creek. For this experiment,

12 sediment samples collected from each stream were pooled and homogenized. For each stream, 5 microcosms were set up with unamended stream water and incubated at each of 4 temperatures (4, 9, 12, and 18^{o}C ± 1^{o}C). Gas headspace samples were collected, stored, and analyzed as described above. Q_{10} coefficients were calculated for each stream for winter and summer samplings as e^{10k}, where k is the slope of the regression between the ln-transformed denitrification rates against temperature (Tank 1992).

Stream survey

The stream survey was conducted over a 3-wk period during the summer 1999 sampling season. The Oak Ridge streams were sampled on 17 June 1999 and the GSMNP streams were sampled on 28 June 1999. Eight 1-kg sediment samples were collected from sites of fine sediment accumulation in each stream. Unfiltered stream water was collected and used in the acetylene inhibition experiments for each stream, as described above. Samples were incubated at the average ambient stream temperatures of each site group (16^{o}C for all Oak Ridge streams and 14^{o}C for all Smoky Mts. streams). The Walker Branch and Noland Creek samples obtained in this survey were also used as summer samples in the seasonal investigation of denitrification potential in sediments from these two streams.

Results

Stream characteristics

Characteristics of the study streams and their sediments are summarized in Table 1. The average NO_3-N concentration observed in Noland Creek was about 25 times greater than that observed in Walker Branch (paired t-test, p=0.0004). Minimum and maximum NO_3-N concentrations in Walker Branch were observed during autumn (7.4 µg/L) and summer (50.0 µg/L) sampling, respectively. The minimum (620 µg/L) and maximum (682 µg/L) NO_3-N concentrations in Noland Creek were observed during autumn and winter sampling, respectively. Average DOC concentrations in Walker Branch and Noland Creek were 0.32 mg/L and 0.78 mg/L, respectively. DOC in Noland Creek was significantly greater than that of Walker Branch (paired t-test, p=0.0427). DOC in Walker Branch ranged from 0.11-0.54 mg/L, while DOC in Noland Creek ranged from 0.55-1.25 mg/L. Maximum DOC concentrations were observed in

Table 1: Temporal variation in biogeochemical data measured quarterly from October 1998 through June 1999 in Walker Branch and Noland Creek. Annual means and standard error, SE, were calculated for each site's parameters using seasonal data. *(m.d.=missing data; * denotes significant differences between the means of each site in t-test, $p<0.05$; %OM data were arcsin-square root transformed for analysis)*

Stream	Season	NO3-N (μg/L) *	DOC (mg/L) *	Stream Temperature (^{o}C)	% Organic Matter in Sediments *
Walker Branch	Fall	7.4	0.54	11.3	2.11%
	Winter	20.0	m.d.	11.8	2.28%
	Spring	19.4	0.32	13.0	1.74%
	Summer	50.0	0.11	15.3	2.29%
	Mean	24.2	0.32	12.9	2.11%
	SE	9.1	0.11	0.9	0.0013
Noland Creek	Fall	620	1.25	12.3	2.30%
	Winter	682	0.55	4.7	3.34%
	Spring	634	0.63	8.4	2.08%
	Summer	549	0.71	11.8	3.14%
	Mean	621	0.78	9.3	2.72%
	SE	27.5	0.16	1.8	0.0031

autumn in both streams.

The percent organic matter in sediments from Walker Branch and Noland Creek did not vary significantly among seasons. However, the mean %OM content in Noland Creek sediments was significantly greater than that of Walker Branch (ANOVA, p=0.0261). Noland Creek water temperatures (4.7-12.3 ^{o}C) were more variable than those in Walker Branch (11.3-15.3 ^{o}C) throughout the sampling year. Noland Creek water temperatures were also typically lower than those of Walker Branch, except in autumn.

Effects of temperature

Temperature had a significant effect on denitrification rates in Noland Creek sediments during both winter (r^2=0.46, p=0.0011) and summer (r^2=0.70, p<0.0001) (Figure 2). Q_{10} coefficients of 1.29 and 2.49 were determined for winter and summer, respectively. No significant effect of temperature on denitrification was detected for WB sediments – no denitrification activity was observed in any Walker Branch samples during experiments testing for temperature effects.

Denitrification in streams of contrasting nitrate availability

Denitrification rates in Noland Creek were quite high, averaging ~891 ng N_2O $gAFDM^{-1}$ h^{-1}, whereas denitrification rates in Walker Branch were much lower, averaging ~61 ng N_2O $gAFDM^{-1}$ h^{-1}, and more variable (Table 2). Eighty percent of the denitrification rates observed in Walker Branch microcosms were zero. Denitrification rates were significantly greater in Noland Creek sediments than in sediments from Walker Branch in all sampling seasons (t-tests, p<0.05; Table 2). No significant seasonal variation in Walker Branch denitrification rates was observed, regardless of data normalization (p>0.05, Figure 3). Nonparametric analysis of variance of denitrification rates normalized to gAFDM and gDM in Noland Creek sediments among seasons detected significant seasonal variation (p= 0.02 and 0.01, respectively) (Figure 4, A and B). However, seasonal variation was no longer significant upon correction of these denitrification rates to a common temperature (8.5^{o}C) using the mean Q_{10} value (1.89). Seasonal denitrification rates in each stream normalized to either gAFDM or gDM were not significantly correlated with any of the individual stream characteristics presented in Table 1.

Table 2: Denitrification rates (ng N_2O $gAFDM^{-1}$ h^{-1}) in sediments from streams of contrasting NO_3-N availability. Standard error of the means (SE) are indicated in parentheses. Rates were significantly different between sites for all seasons ($p < 0.05$).

Season	Walker Branch	Noland Creek
Fall	12.12 (3.50)	1004.27 (289.91)
Winter	50.09 (14.46)	299.50 (86.46)
Spring	152.09 (43.90)	1231.21 (355.42)
Summer	30.88 (8.91)	1041.49 (300.65)
Annual Mean (SE)	61.29 (22.90)	890.98 (132.88)

Nutrient limitations

Additions of nutrients to the sediment microcosms yielded consistent results for both the autumn 1998 and spring 1999 experiments for each site (Figure 5). In Walker Branch, additions of nitrate and nitrate plus labile organic carbon (LOC) significantly increased the denitrification rates in sediments, with increases of 5- to 50-fold for spring and autumn respectively (ANOVA followed by Dunnett's MCT, $p<0.0001$). Additions of LOC alone did not significantly change denitrification rates from those in the unamended controls. Results of subsequent Scheffe's multiple comparisons tests confirmed that rates of denitrification in Walker Branch were statistically similar in microcosms amended with nitrate and nitrate plus LOC during both seasons. In contrast to Walker Branch, none of the nutrient additions significantly enhanced denitrification rates in sediments from Noland Creek during either season.

Survey of denitrification rates in streams along a nitrate gradient

Nitrate-N in the 10 streams sampled in the nitrate gradient study ranged from 10 to 549 μg/L, with the highest NO_3-N levels generally occurring in the higher elevation streams in the GSMNP (Table 3). DOC concentrations in these streams ranged from 0.11 to 1.38 mg/L and sediment organic matter content varied from 1.52 to 4.11%. Conductivity and pH values ranged from 12.6 to 282 μS and 5.76 to 8.37, respectively, and were consistently lower in the streams of the GSMNP compared to streams on the Oak Ridge reservation.

Sediment denitrification rates were most strongly related to stream water NO_3-N concentrations ($r^2=0.74$, $p=0.0014$) (Figure 6). Denitrification was generally higher in sediments from the higher elevation streams ($r^2 = 0.45$), and this relationship was stronger when data were normalized to grams dry sediment ($r^2 = 0.77$). Denitrification rates were negatively related to stream water pH ($r^2=0.69$, $p=0.0031$), conductivity ($r^2=0.53$, $p=0.0173$), and temperature ($r^2=0.43$, $p=0.0391$), but were not significantly related to percent sediment organic matter and DOC concentration.

Table 3:
located in Oak Ridge, TN, and the second group of sites is located in the Great Smoky Mountains National Park, NC/TN. Elevations were estimated across the sampling reach.

Site	Elevation (m)	[NO_3-N] (μg/L)	[DOC] (mg/L)	% Organic Matter	Conductivity (μS)	Stream Water pH	Stream Temp. (^oC)
East Fork Walker Branch	270	43.0	0.27	2.47%	242.0	8.37	14.4
West Fork Walker Branch	265	50.0	0.11	3.79%	282.0	7.76	15.3
Upper White Oak	271	77.4	0.28	1.83%	246.0	7.67	15.0
Gum Hollow	250	85.7	0.63	3.05%	276.0	7.93	17.0
Pinhook Branch	244	97.6	0.75	4.11%	253.0	7.89	18.6
Rattlebox Creek	610	10.2	1.38	2.14%	12.6	6.30	18.2
Mossy Rock Creek	668	63.0	1.08	3.06%	25.6	6.84	18.8
Hickory King Branch	521	376	0.46	3.42%	20.9	6.24	13.9
Beech Flats Prong	1250	532	0.31	2.84%	41.0	5.99	11.6
Noland Creek	1646	549	0.71	1.52%	14.0	5.76	11.8

A stepwise multiple regression analysis (Table 4) identified nitrate concentration as the primary predictor of denitrification rates normalized by gAFDM (model R^2=0.74, p=0.0014). The stepwise incorporation of conductivity (model R^2=0.83) into the model, followed by elevation (R^2=0.93) explained an additional 19% of variation in denitrification rates (p=0.0008). Interestingly, elevation was identified as the sole significant predictor (R^2=0.77, p=0.0009) of denitrification rates normalized to gDM.

Table 4: Summary of stepwise selection of predictor variables in multiple regression with denitrification rates normalized by either gAFDM or gram dry weight. Default '*significance level for entry*' = 0.15. * Overall model was significant at p =0.0008.

Dependent Variable	Predictor Variable	Model Coefficient	Partial R^2	Model R^2	F	*p*
Denitrification Rate * (ng N_2O $gAFDM^{-1}$ h^{-1})	NO_3-N	25.76	0.74	0.74	22.96	0.001
	Conductivity	17.37	0.08	0.83	3.4	0.11
	Elevation	6.76	0.1	0.93	8.63	0.03
Denitrification Rate (ng N_2O gDM^{-1} h^{-1})	Elevation	-1.89	0.77	0.77	26.6	0.0009

Discussion

Factors influencing denitrification rates

In our two-stream comparison, Noland Creek denitrification rates were an order of magnitude greater than those of Walker Branch. The range of denitrification rates observed in sediments from both streams are similar to those previously summarized by Korom (1982) for sand and gravel sediments (Smith and Duff 1988, Smith et al. 1991). While denitrification rates in sediments from Walker Branch were generally lower than those observed in most areas of Sycamore Creek (Holmes et al. 1996), they were similar to denitrification rates observed in sediments from the upwelling hyporheic zones and areas 1-5 m into the parafluvial flowpaths of this nitrogen-limited Sonoran Desert stream. In contrast, the average annual denitrification rate in sediments from Noland Creek, on the other hand, was more than 2 times the maximum denitrification rates observed by Holmes et al. (1996).

Noland Creek had considerably higher levels of NO_3-N and organic carbon availability (as DOC and percent organic matter in sediments) than the West Fork of Walker Branch. Until now, the degree to which these factors affect denitrification in stream sediments in the southern Appalachian mountains has not been investigated. Our results clearly show that nitrate availability, rather than labile organic carbon availability, controls the potential for denitrification in Walker Branch. Enrichment of the Walker Branch sediments with NO_3-N, or with NO_3-N and LOC, resulted in significant increases in denitrification rates. Similar results were reported for Sycamore Creek where nitrate additions generated a 2-10 fold increase in denitrification rates in hyporheic and parafluvial sediments and approximately a 30-fold increase in denitrification in the bank sediments (Holmes et al. 1996). In contrast, neither nitrate nor LOC appeared to limit denitrification in sediments from Noland Creek.

Contrary to results of our stream survey, other studies have shown that LOC availability is highly correlated with denitrification (Knowles 1982, Tiedje et al. 1982). The insignificant increase in denitrification rates in sediments from Noland Creek and Walker Branch when DOC availability was maximum (e.g. during autumn months) relative to other seasons, and the lack of response in denitrification rates when the LOC concentration was increased during the nutrient amendment experiments strongly suggests that carbon availability is not the driving factor for denitrification in sediments from these streams. It is possible that the lack of response in

denitrification following LOC additions was due to a metabolic shift toward dissimilatory reduction of nitrate to ammonium as observed by Knowles (1982), although the relatively low ambient LOC availability year-round in these streams likely precludes this metabolic phenomenon.

The results of our stream survey further emphasized that nitrate rather than carbon availability is the primary determinant of denitrification in sediments from streams in the southern Appalachian Mountains. Where nitrate availability was limited, as in the lower-elevation Oak Ridge streams, denitrification potential was low regardless of carbon availability or ambient stream temperatures. Where nitrate concentrations were high and neither nitrate nor LOC limitations were evident, as in Noland Creek, denitrification rates were high and were primarily influenced by temperature.

Interestingly, denitrification rates in sediments from Rattlebox Creek were comparable to those of other GSMNP stream sediments at similar elevations, despite the fact that Rattlebox Creek exhibited the lowest nitrate concentrations in the surveyed gradient. There are several possible seasons why the denitrification rates in Rattlebox Creek were much higher than would be predicted based on its background NO_3-N concentration. First, NO_3-N concentrations during the survey may not be typical of NO_3-N available during much of the year, and the observed activity of the denitrifier community may reflect a higher longer-term NO_3-N concentrations. Second, the highest DOC concentration observed in this survey was measured in Rattlebox Creek. Although we have demonstrated that DOC does not significantly affect denitrification in Noland Creek and Walker Branch, it certainly could have contributed to the denitrification activity observed during this survey.

Our stream survey also suggested that additional stream characteristics may have influenced the rates of denitrification in stream sediments from the southern Appalachian Mountains. Not surprisingly, elevation was a secondary predictor of denitrification rates ($gAFDM^{-1}$) in these streams (and the primary predictor for rates normalized to gDM), as identified in the stepwise regression model. The higher elevation streams in the southern Appalachian Mountains generally receive among the highest atmospheric inputs of nitrate in North America (Nodvin et al. 1995). Shubzda et al. (1995) also demonstrated that N-deposition increases with increasing elevation in this region, so NO_3-N concentrations are higher in the higher elevation streams (Flum and Nodvin 1995).

The appearance of conductivity as a significant predictor in the model was initially confusing. The significant correlation between denitrification and conductivity is probably the result of the large contrast in bedrock weathering between the Oak Ridge and Smoky Mountains stream catchments. The high conductivity of the Oak Ridge streams are likely attributed to relatively high rates of weathering of the underlying dolomite formations, while the highly resistant sandstone formations of the GSMNP produce streams with lower pH and total ionic strength.

Stream pH has been shown to indirectly influence denitrification in stream sediments. Several studies have shown that acidic conditions may reduce or even completely inhibit denitrification in stream sediments (Klemedtsson et al. 1977, Knowles 1982, Davidson and Swank 1987), although Palumbo et al. (1987) found that bacterial numbers and activity in sediment were not necessarily correlated with the pH of overlying water. Our study suggests a significant negative relationship between pH and denitrification, a pattern opposite to most studies. This is probably because nitrate concentrations and pH were inversely related in our study, with the high elevation, high NO_3-N GSMNP streams (e.g. Noland Creek, Beech Flats, and Hickory King Branch) exhibiting significantly greater denitrification rates than the lower elevation, higher pH streams in the Oak Ridge area. We suspect that the high inputs nitrate from atmospheric deposition in high elevation streams in the southern Appalachian Mountains simply sustain endogenous assemblages of denitrifying organisms in otherwise suboptimal, mildly acidic conditions.

Seasonal variation in denitrification rates

We hypothesized that denitrification in stream sediments would vary seasonally, in response to seasonal variation in those factors which potentially regulate denitrification (e.g. nitrate and carbon availability). Significant seasonal variation in denitrification activity was not observed in this study despite reported seasonal variation in such factors as nitrate and carbon availability in Noland Creek (Flum and Nodvin 1995, Nodvin et al. 1995, National Trends Network, NADP Office, IL, unpubl. data) and Walker Branch (Elwood and Turner 1989, Mulholland 1992, Mulholland and Hill 1997). Instead, seasonal variation in stream temperature contributed to seasonal variation in denitrification rates in Noland Creek sediments.

Within-site variability among denitrification rates

The variability observed in denitrification rates among sediment samples in each stream was relatively high, especially those from Walker Branch. Such variability in denitrification rates confirms the substantial patchiness of denitrification hotspots observed in studies in other streams (Parkin 1987, Steinhart et al. in press). Several studies have shown that denitrification activity is higher in fine-textured sediments because the probability of anaerobic conditions is greater (Groffman and Tiedje 1989, Garcia-Ruiz et al. 1998, Steinhart et al. in press). In contrast, depositional areas characterized by more coarsely textured substrates are likely to be more oxic and support less active denitrifier assemblages. Therefore, these areas have a reduced potential for denitrification (Rysgaard et al. 1994).

We hypothesized that normalization of N_2O data to gAFDM would reduce some of the variability associated with heterogeneous organic content of sediments and differential nutrient retention among samples. However, statistical analyses of denitrification rates in these streams were consistent regardless of normalization to organic matter in the sediments.

The preparation time allotted for experimental setup through the helium-flushing step was presumed to be sufficient for sample warming and re-acclimatization, yet a lag in denitrification activity was nonetheless observed early in the incubations. Sudden shifts to anoxia in laboratory experiments through such treatments as helium flushing do not permit denitrifiers to acclimatize to optimum denitrifying conditions as they would if oxygen were depleted gradually (i.e. through a natural cascade of metabolic processes, Knowles 1982). Additionally, the time required for re-acclimatization to ambient stream temperature following short-term sample storage at 4^oC is uncertain. Denitrification activity observed in the early stages of our denitrification assays may, therefore, reflect conditions and activity of denitrifying enzymes which existed at the time of sample collection (Limmer and Steele 1982). However, we feel that our denitrification rates calculated using nitrous oxide accumulation between the 6- and 12-h sampling approximate the potential denitrification one might expect to find in completely anoxic microsites in sediments from these streams.

Catchment significance of denitrification in southern Appalachian Mountain streams

Denitrification in stream sediments may contribute to measurable loss of NO_3-N from catchments in the southern Appalachian Mountains, particularly when NO_3- concentrations are relatively high (>300 g/L). The 97.5 -ha Walker Branch catchment receives ~10 kg N ha^{-1} y^{-1} (or 975 kg N y^{-1}) from atmospheric deposition, while ~0.2 kg N ha^{-1} y^{-1} (19.5 kg N y^{-1}) is lost from the catchment as stream export, mostly as nitrate (P. J. Mulholland, personal communication). Nodvin et al. (1995) reported that the 17.4-ha Noland Divide Watershed receives ~20 kg N ha^{-1} y^{-1}(or 348 kg N y^{-1}), ~17 kg N ha^{-1} y^{-1} (296 kg N y^{-1}) of which is lost from the catchment via stream export. Our study suggests that approximately 50, 39, and 568 ng N_2O-N $gAFDM^{-1}$ h^{-1} could be lost via denitrification in the East and West Forks of Walker Branch and Noland Creek sediments, respectively. Assuming that 505 $gAFDM/m^2$ of benthic particulate organic matter, as reported for Walker Branch (Mulholland 1997), is typical of streams in the southern Appalachian Mountains (Webster and Meyer 1997), denitrification in the East and West Forks of Walker Branch and Noland Creek sediments produces 0.17, 0.22, and 2.52 g N_2O-N m^{-2} y^{-1}, respectively. Finally, the East and West Fork of Walker Branch constitute 540 m^2 and 1260 m^2 of the entire 975,000-m^2 catchment, respectively (P. J. Mulholland, personal communication). The streambed area of Noland Creek is unknown, but based on estimates of streambed area throughout the region (Webster and Meyer 1997), Noland Creek constitutes 1%, or 1740 m^2, of the entire 174,000-m^2 catchment. Using these values, we calculate that 0.34 kg N/y and 4.38 kg N/y may be lost from these catchments via denitrification in Walker Branch (East and West Forks combined) and Noland Creek sediments, respectively. Thus, denitrification in stream sediments is ~0.03% of total N inputs in the Walker Branch catchment and 1.3% of total N inputs in the Noland Divide catchment. Nitrogen loss via stream N export is ~57-fold higher than N loss via denitrification in Walker Branch sediments and ~68-fold higher than N loss via denitrification in Noland Creek sediments.

The proportion of N loss from the catchment via denitrification in these stream sediments appears to be small, especially considering that these calculations are based on potential denitrification rates and that perhaps as little as 2.5% of these rates may be expected *in situ* (Tiedje et al. 1982). However, Holmes et al. (1996) proposed that even modest rates of denitrification have significant impact on the fate of stream water nitrate, particularly in nitrogen-

limited streams. Future investigations should focus on the magnitude to which *in situ* denitrification serves as a mechanism of nitrate removal in streams draining areas of high atmospheric deposition like those found in the southern Appalachian Mountains.

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Appendix – Figure Captions

Figure 1: Location of Oak Ridge sites sampled in this study, ordered by numbers 1-5 for West Fork Walker Branch, East Fork Walker Branch, Upper White Oak Creek, Pinhook Branch, and Gum Hollow; and location of Great Smoky Mountains National Park sites sampled in this study, ordered by numbers 6-10 for Rattlebox Creek, Mossy Creek, Hickory King Branch, Noland Creek, and Beech Flats Prong.

Figure 2: The effect of temperature on denitrification rates in Noland Creek sediments in winter and summer 1999.

Figure 3: Seasonal variation in denitrification rates (±1 SE) in sediments from Walker Branch normalized to (A) gAFDM and (B) gDM. No significant differences in denitrification rates were detected among seasons ($p<0.05$).

Figure 4: Seasonal variation in denitrification rates (±1 SE) in sediments from Noland Creek normalized to (A) gAFDM and (B) gDM, both before and after correction to common temperature (8.5^{o}C) using the mean Q_{10} value (1.89). Significant seasonal variation was detected in rates ($gAFDM^{-1}$ and gDM^{-1}) among seasons before temperature correction.

Figure 5: Nutrient limitations on denitrification rates (±1 SE) in Walker Branch and Noland Creek sediments. Amendments that yielded denitrification rates significantly different from those of the unamended controls are indicated by a letter different from that of the unamended sample set (ANOVA followed by Dunnett's MCT, $p<0.0001$). Statistical codes for fall 1998 experiment are in lower case letters and those for spring 1999 are in upper case letters.

Figure 6: Linear regressions between denitrification rates and stream characteristics from stream sites surveyed in summer 1999. Open circles represent Oak Ridge streams and closed circles represent GSMNP streams.

Figure 1

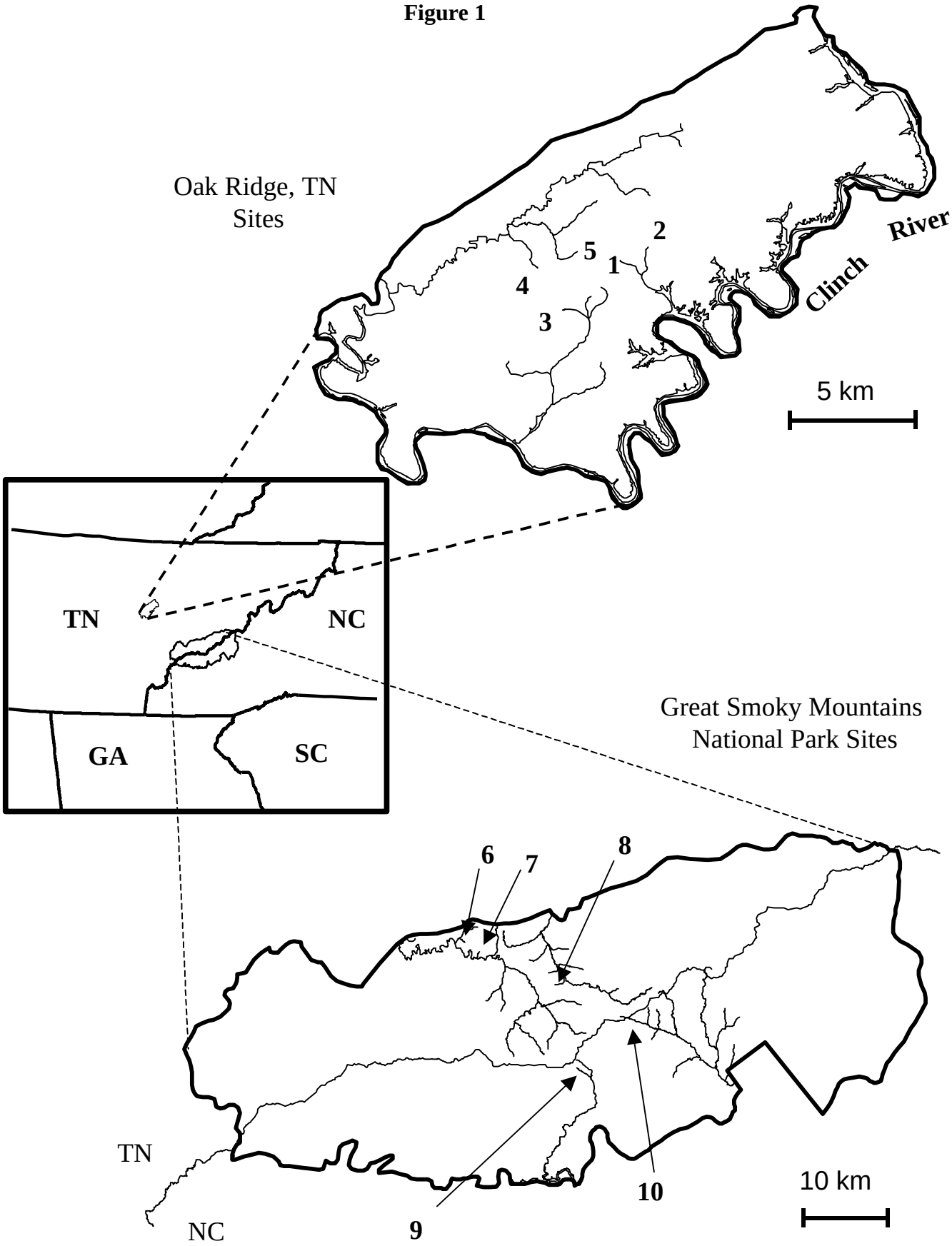

Oak Ridge, TN
Sites
1
2
3
4
5
Clinch River
5 km
TN
NC
GA
SC
Great Smoky Mountains
National Park Sites
6
7
8
9
10
TN
NC
10 km

Figure 2

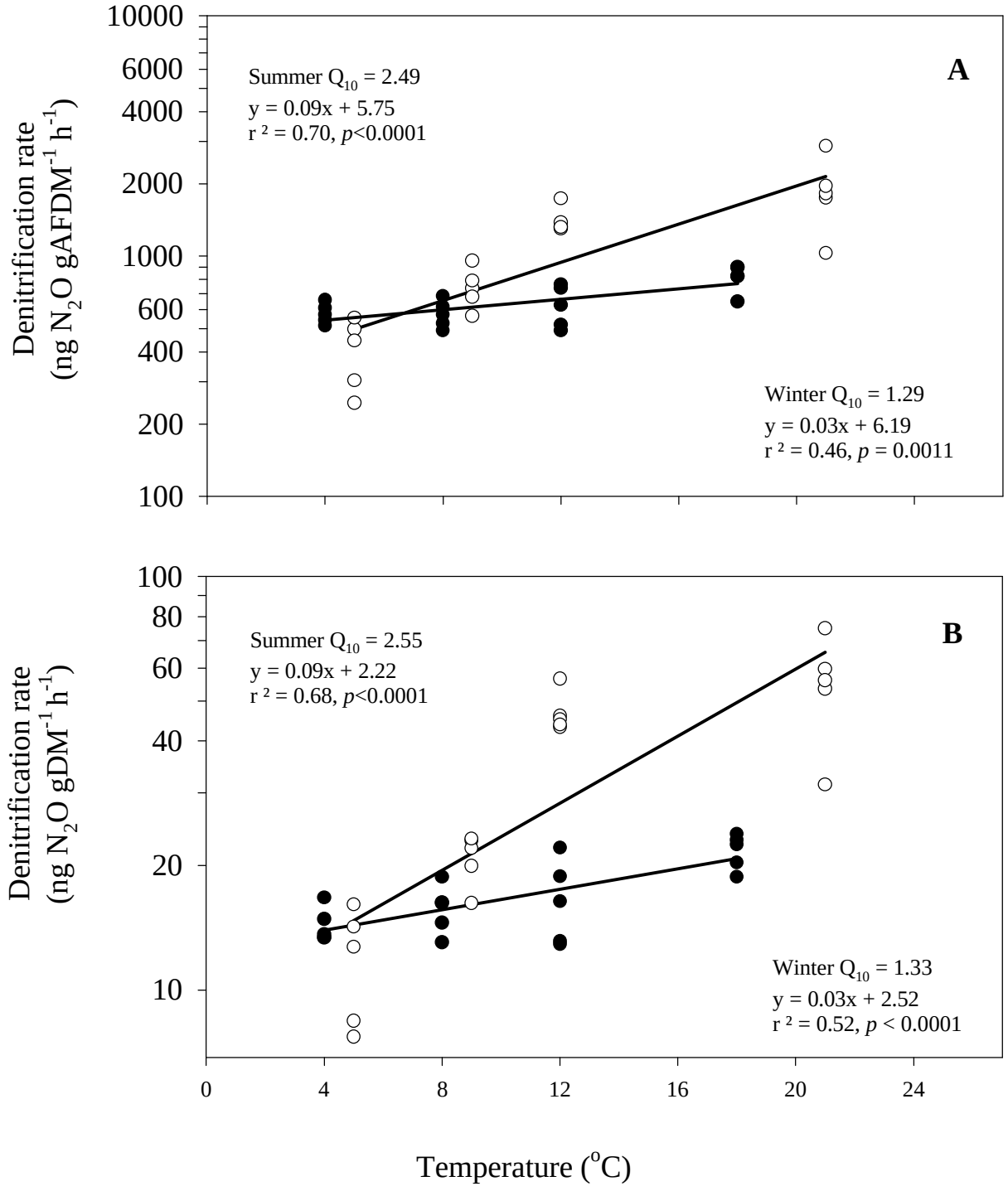

A
Summer Q_{10} = 2.49
y = 0.09x + 5.75
r ² = 0.70, *p*<0.0001
Winter Q_{10} = 1.29
y = 0.03x + 6.19
r ² = 0.46, *p* = 0.0011
Denitrification rate (ng N_2O gAFDM^{-1} h^{-1})
10000
6000
4000
2000
1000
600
400
200
100
B
Summer Q_{10} = 2.55
y = 0.09x + 2.22
r ² = 0.68, *p*<0.0001
Winter Q_{10} = 1.33
y = 0.03x + 2.52
r ² = 0.52, *p* < 0.0001
Denitrification rate (ng N_2O gDM^{-1} h^{-1})
100
80
60
40
20
10
0
4
8
12
16
20
24
Temperature (^{o}C)

Figure 3

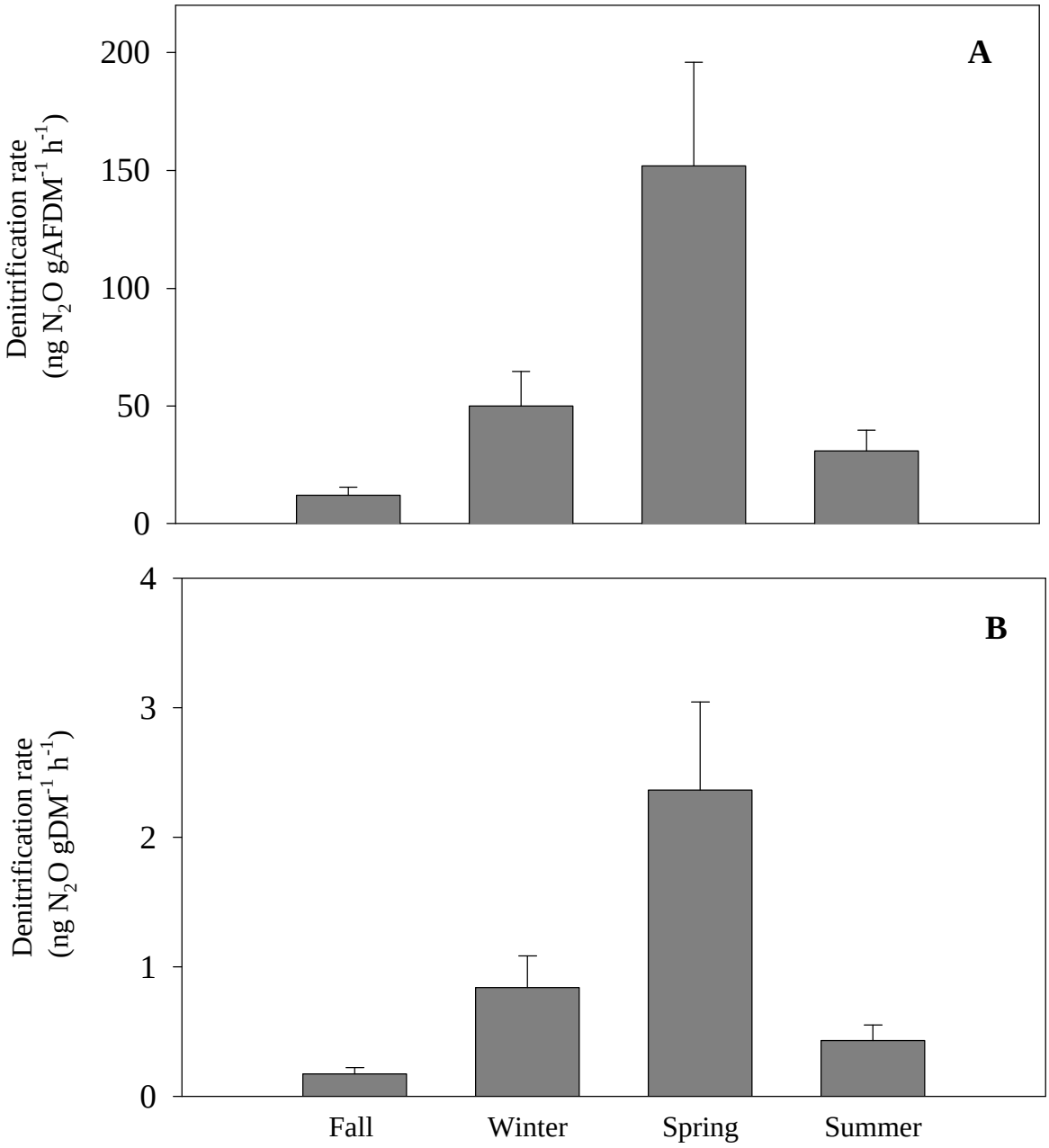

A
B
Denitrification rate
(ng N_2O gAFDM^{-1} h^{-1})
Denitrification rate
(ng N_2O gDM^{-1} h^{-1})
200
150
100
50
0
4
3
2
1
0
Fall
Winter
Spring
Summer

Figure 4

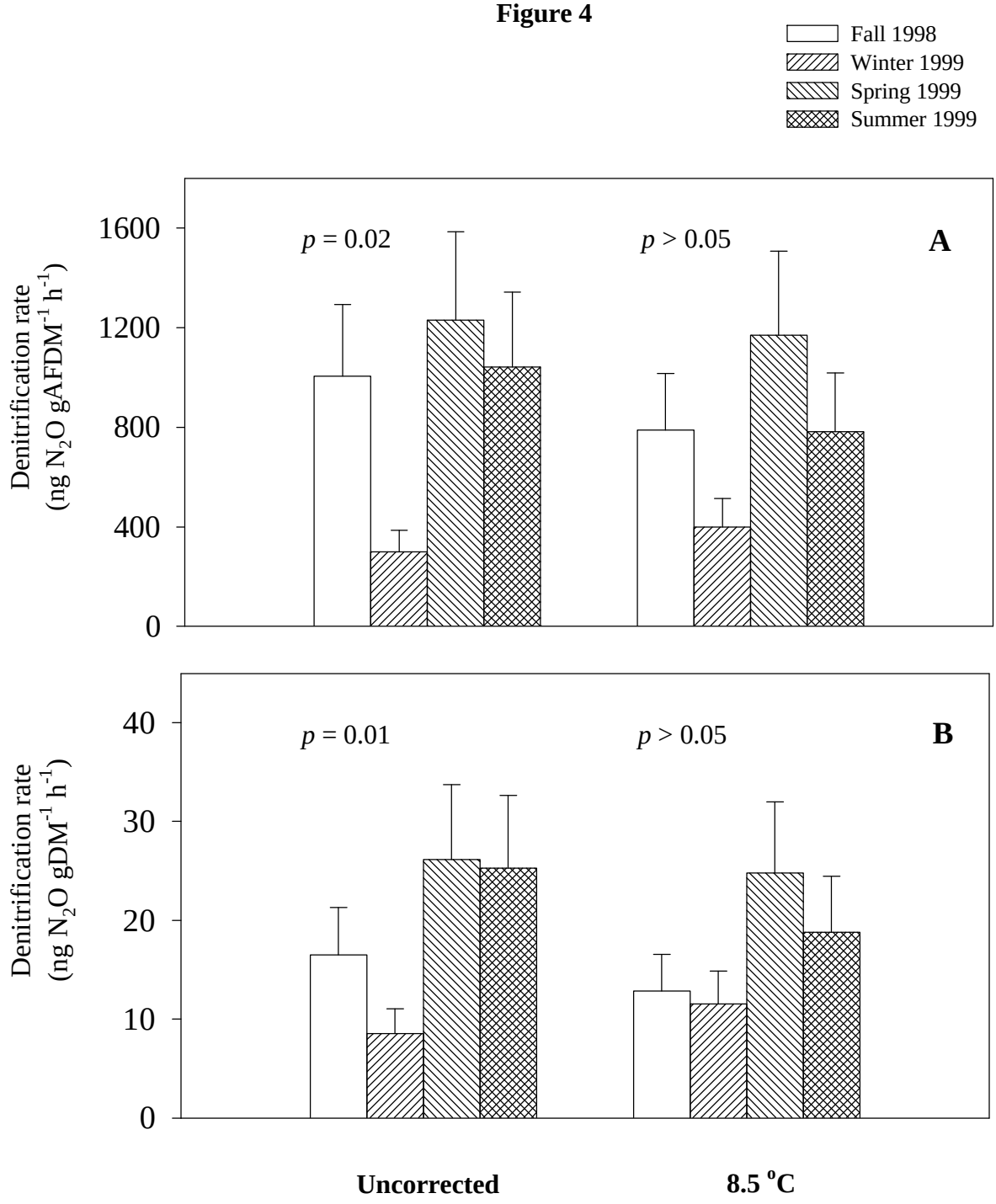

Fall 1998
Winter 1999
Spring 1999
Summer 1999
Denitrification rate
(ng N_2O gAFDM^{-1} h^{-1})
1600
1200
800
400
0
p = 0.02
p > 0.05
A
Denitrification rate
(ng N_2O gDM^{-1} h^{-1})
40
30
20
10
0
p = 0.01
p > 0.05
B
Uncorrected
8.5 °C

Figure 5

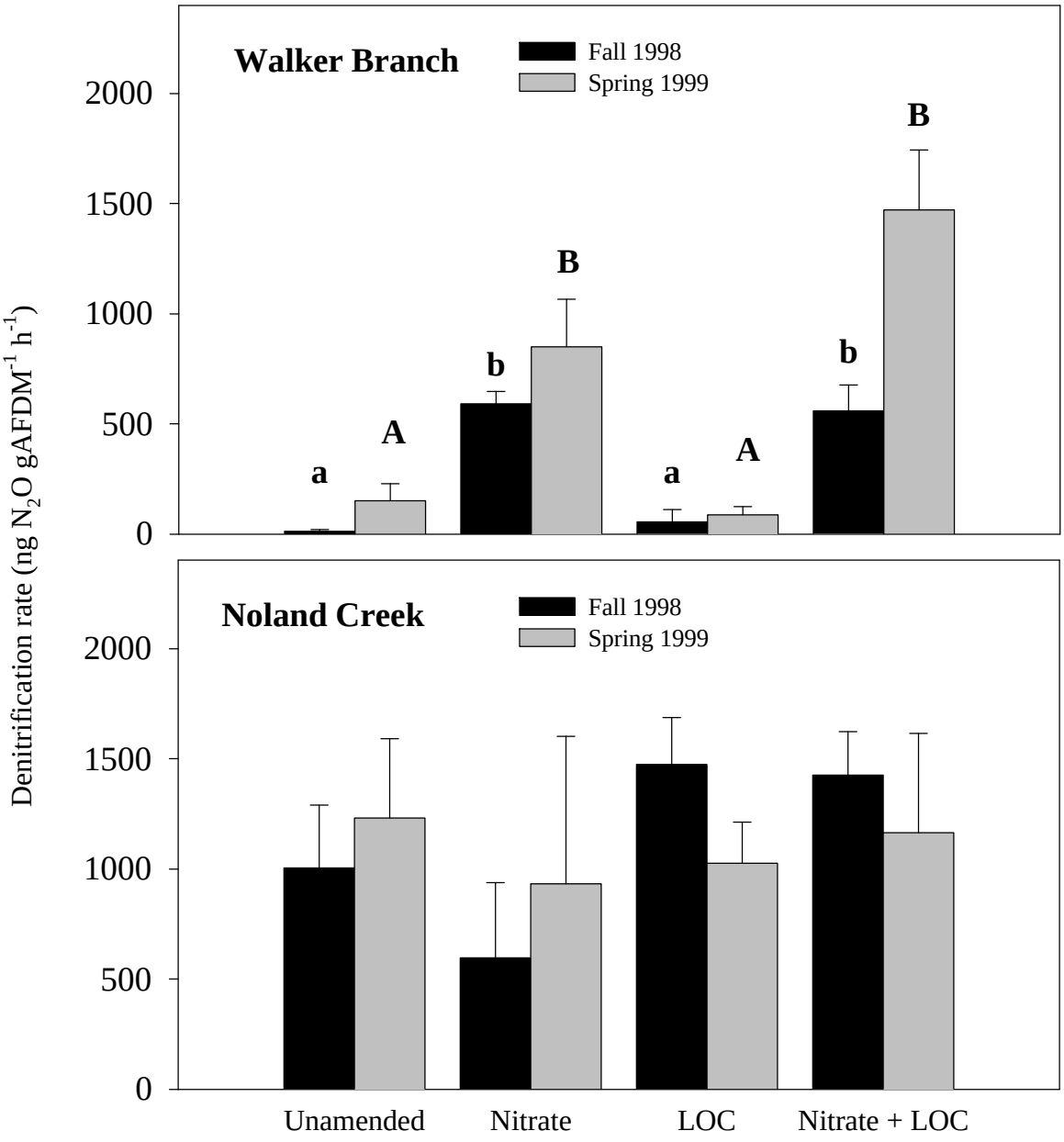
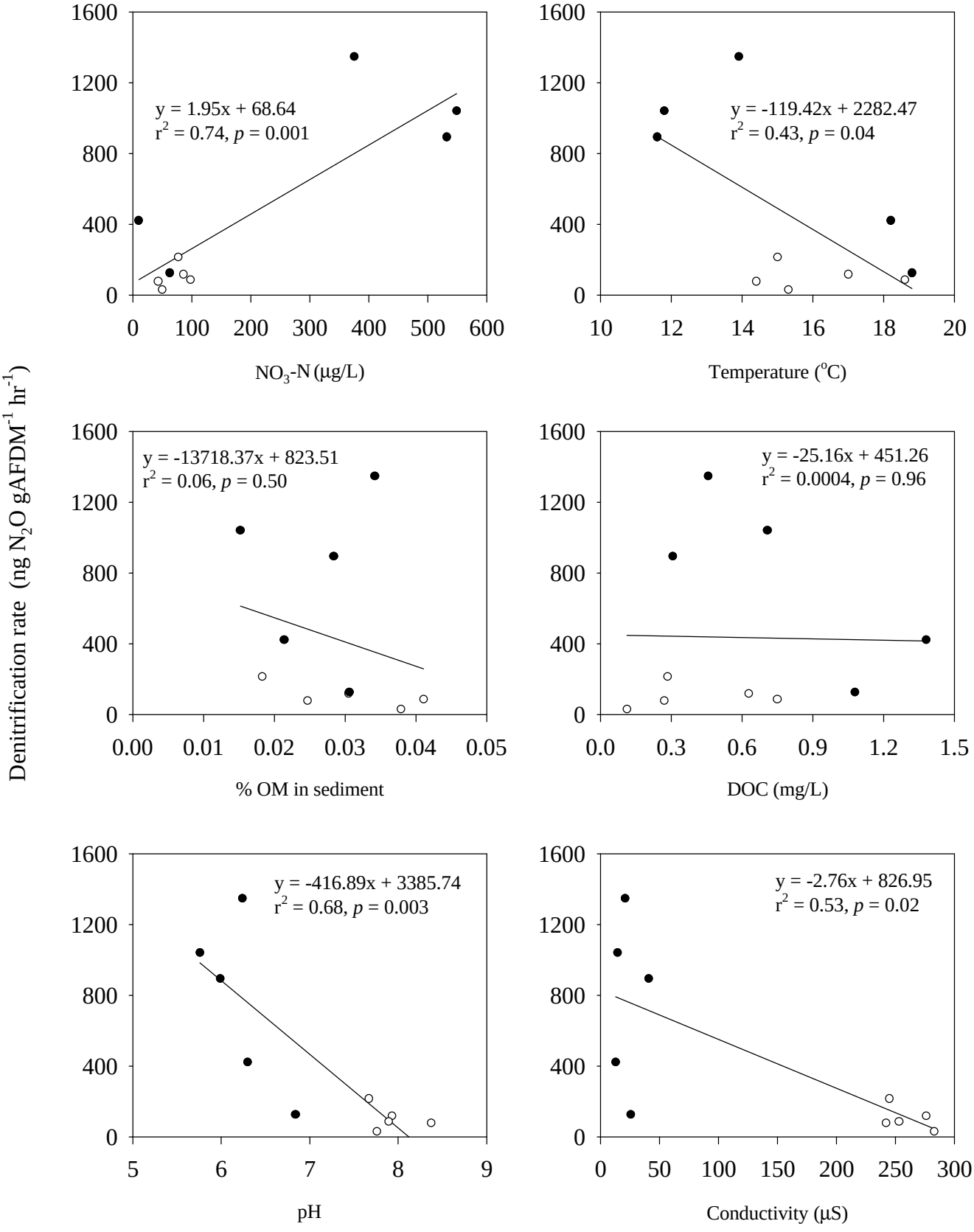

Walker Branch
Fall 1998
Spring 1999
Noland Creek
Fall 1998
Spring 1999
Denitrification rate (ng N_2O gAFDM^{-1} h^{-1})
0
500
1000
1500
2000
a
A
b
B
a
A
b
B
Unamended
Nitrate
LOC
Nitrate + LOC

Figure 6


Denitrification rate (ng N_2O gAFDM^{-1} hr^{-1})
y = 1.95x + 68.64
r^2 = 0.74, p = 0.001
NO_3-N (µg/L)
y = -119.42x + 2282.47
r^2 = 0.43, p = 0.04
Temperature (^{o}C)
y = -13718.37x + 823.51
r^2 = 0.06, p = 0.50
% OM in sediment
y = -25.16x + 451.26
r^2 = 0.0004, p = 0.96
DOC (mg/L)
y = -416.89x + 3385.74
r^2 = 0.68, p = 0.003
pH
y = -2.76x + 826.95
r^2 = 0.53, p = 0.02
Conductivity (µS)