CONTROLS ON NITROGEN INPUTS, LOADS, AND IN-STREAM CONCENTRATIONS IN THE ALTAMAHA RIVER, GEORGIA, AND BEYOND

by

SYLVIA CATHERINE SCHAEFER

(Under the Direction of Merryl Alber)

ABSTRACT

Human activity has increased the availability of reactive nitrogen (N), an element of critical importance to life. The broad goal of this dissertation was to understand which sources of N to a watershed reach rivers.

Nitrogen input budgets were calculated for 18 watersheds on the United States west coast. Fertilizer was the most important source of new N, with atmospheric deposition second. N export was best correlated with streamflow variations, which explained 66% of the variance. Including inputs explained an additional 16% of the variance. Riverine N export averaged 12% of inputs. Percentage export was also best related to streamflow. These results were likely due to the large range of streamflows across the study region, which may have overwhelmed other factors contributing to N export.

An in-depth examination of the Altamaha River watershed (Georgia, USA) found that riverine N concentrations and cumulative loads were best related to population density, rather than input factors. Concentrations were highest in the upper watershed and lowest in blackwater streams and a sampling station downstream of a dam. Isotopic analysis of nitrate suggested that most in-stream nitrate is derived from sewage or manure. Where

the concentration was particularly low, an atmospheric signal was observed, suggesting that background nitrate is of atmospheric origin. N in this system appeared to be lost primarily on the watershed surface, as only a small proportion of inputs reached the stream, N was transported downstream conservatively, and an isotopic mixing model fit the data well.

The Altamaha is comparable to other watersheds worldwide in its nitrate concentrations and population density. A metadata analysis of medium-sized (2,000-50,000 km^2) watersheds, which are under-studied relative to large watersheds, revealed that nitrate concentrations are generally low (<25 μM). We found a relationship between population density and riverine NO_3^- concentration, which varied among continents and latitudinal zones. A combination of population density, population density without access to improved sanitation, temperature, precipitation, slope, and fertilizer use, explained 45% of the variation in nitrate concentrations. The relationship had less explanatory power than in large watersheds, potentially due to increased variation in NO_3^- concentrations with decreased watershed size.

INDEX WORDS: nitrogen, nitrate, concentration, nitrate isotopes, watersheds, Net Anthropogenic Nitrogen Input (NANI), Altamaha River, population density

CONTROLS ON NITROGEN INPUTS, LOADS, AND IN-STREAM CONCENTRATIONS IN THE ALTAMAHA RIVER, GEORGIA, AND BEYOND

by

SYLVIA CATHERINE SCHAEFER

B.A., University of Virginia, 2001

M.S., University of Georgia, 2006

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2014

CONTROLS ON NITROGEN INPUTS, LOADS, AND IN-STREAM CONCENTRATIONS IN THE ALTAMAHA RIVER, GEORGIA, AND BEYOND

by

SYLVIA CATHERINE SCHAEFER

Major Professor: Merryl Alber

Committee: Jay Brandes
James T. Hollibaugh
Charles Hopkinson
C. Rhett Jackson
Samantha Joye

Electronic Version Approved:

Julie Coffield
Interim Dean of the Graduate School
The University of Georgia
August 2014

DEDICATION

To Adrian, whose love and support has made this possible.

ACKNOWLEDGEMENTS

I must first and foremost thank my advisor, Merryl Alber, for her guidance on this project. Thanks also to the rest of my committee—Jay Brandes, Tim Hollibaugh, Chuck Hopkinson, Rhett Jackson, and Mandy Joye, for their advice and assistance. In particular, this project would not have been possible without the help of Jay Brandes, who was exceedingly generous with his time and equipment (and who keeps me updated on Florida Man!). Kim Hunter in the Joye lab and Emily Davenport in the Hopkinson lab helped me run samples and allowed me to use their equipment to do so. Joan Sheldon and Natalie McLenaghan let themselves be dragged sampling in sketchy rural south Georgia with me. Funding for this research was provided by NSF and the GCE-LTER. Moral support for this research was provided by members of the Alber lab past and present, including Caroline McFarlin, Joan Sheldon, Natalie McLenaghan, and Christine Hladik. Thanks also to my other friends, especially Heather "Epic Adventures" Reader and the members of the Classic City Band, for helping me keep my sanity. And last but certainly not least, thanks to my family and especially to my husband Adrian for their love and support through the years.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Nitrogen (N) pollution is now a crucial issue facing coastal ecosystems worldwide (Howarth et al. 2002). Inputs of reactive N to the global cycle have increased nine-fold since the invention of the Haber-Bosch process (Galloway and Cowling 2002), and have doubled since 1970 alone (Galloway et al. 2008). Aquatic systems are particularly vulnerable to N enrichment, as they are subject to eutrophication, which results in numerous deleterious effects including but not limited to fish kills, toxic algal blooms, submerged aquatic vegetation loss, and loss of water clarity (Bricker et al. 2007). The problem of eutrophication must be managed on a watershed scale; therefore a comprehensive understanding of nitrogen dynamics in the watershed is essential.

The broad goal of my research is to understand which sources of N to a watershed reach the water, and what transformations occur as it is transported downstream. Below, I first review the N cycle and the behavior of stable isotopes in nitrogen transformations, and then discuss the transport of N through watersheds and methods for investigating these processes, both in the field and through modeling.

1.1 THE NITROGEN CYCLE

Nitrogen is a nutrient needed by all organisms. It most commonly occurs in the triple-bonded form of gaseous N_2, which makes up 70% of Earth's atmosphere but is not easily available to organisms. In natural systems, nitrogen can be fixed abiotically, via

lightning, or more commonly, through biological processes (Galloway et al. 2004). The organic nitrogen in organisms is released as ammonium (NH_4^+) upon the organisms' demise. This form of nitrogen is labile and can either be taken back up by primary producers or converted to nitrate (NO_3^-) by nitrifiers. Nitrate can be converted back to ammonium via dissimilatory nitrate reduction to ammonium (DNRA), or be converted back to N_2 via denitrification or anaerobic ammonium oxidation (anammox), closing the nitrogen cycle (Figure 1). In the early 1900s, the Haber-Bosch process was invented, which allowed the conversion of atmospheric N_2 into reactive ammonia for fertilizer and explosives, and the nitrogen cycle has been drastically altered ever since (Galloway and Cowling 2002).

Natural biological N fixation is of importance in both marine and freshwater systems (Howarth et al. 1988b). The enzymes involved in N fixation require trace elements such as iron and molybdenum, and these elements are more readily available in freshwater than in marine systems. This contributes to the observation that freshwater systems tend not to be as limited by N (Howarth et al. 1988a), although there is some disagreement about this (Smith 1984, Howarth and Paerl 2008, Schindler and Hecky 2009). Molybdenum, in particular, is less available to N-fixing organisms in the presence of sulfate, which is common in ocean water (Howarth et al. 1988a). (Conversely, phosphorus is less available in freshwater systems, since sulfate prevents dissolved inorganic phosphorus scavenging by iron oxides (Canfield et al. 2005).)

Labile forms of organic N are mineralized into ammonium (NH_4^+) by heterotrophic organisms. NH_4^+ is the most accessible form of inorganic nitrogen for autotrophs because it is most similar to the redox state of cells (Canfield et al. 2005). This sets up a

competition for NH_4^+ between primary producers and organisms involved in the next step of the nitrogen cycle, nitrification.

Nitrification is a two-step microbially mediated process that converts NH_4^+ to nitrate (NO_3^-) via the intermediate NO_2^-. The two steps are performed by different species of bacteria, and the first (NH_4^+ to NO_2^-) is generally considered to be the rate-limiting step, such that NO_2^- rarely accumulates in the environment (Canfield et al. 2005). In addition to substrate availability, nitrification can be limited by other factors such as the presence of organic carbon (Strauss and Lamberti 2000), high or low pH (Strauss et al. 2002), or low temperature (Strauss et al. 2004).

Denitrification is the major sink for reactive nitrogen. This pathway converts NO_3^- to N_2 via the intermediates of NO_2^-, NO, and N_2O, and takes place under anoxic conditions. It is common in virtually all aquatic systems (Seitzinger 1988). Rates of denitrification are affected primarily by the substrate (NO_3^-) supply (e.g. Richardson et al. 2004), but other factors such as organic carbon content (Arango and Tank 2008) or organism activity (Cornwell et al. 1999) can also play a role. More labile forms of organic carbon are able to support higher rates of denitrification (Pfennig and McMahon 1996). Denitrification also takes place in soils and groundwater. In these areas, in addition to NO_3^- supply, organic matter content and saturation state can affect denitrification rates (Luo et al. 1999, Pabich et al. 2001). Anaerobic ammonium oxidation (anammox) is another potential sink for reactive nitrogen. First discovered in wastewater treatment systems, anammox converts one atom each of NO_3^- and NH_4^+ to N_2 (Mulder et al. 1995). The process has since been found to be important in the natural environment as well (Francis et al. 2007), and has been observed in many oxygen minimum zones in the

ocean (e.g. Dalsgaard et al. 2003, Thamdrup et al. 2006, Hamersley et al. 2007), where it could account for up to 29% of total N losses from the system (Dalsgaard et al. 2003). While anammox bacteria are also common in freshwater and brackish systems (Penton et al. 2006), the importance of the process in these environments is thought to be small (Koop-Jakobsen and Giblin 2009).

One other important link in the nitrogen cycle is dissimilatory nitrate reduction to ammonium (DNRA). In DNRA, NO_3^- is reduced to NO_2^- and then NH_4^+ under anaerobic conditions. This process has been found to be a more important sink for nitrate than denitrification in both terrestrial (Rutting et al. 2008) and aquatic ecosystems (An and Gardner 2002, Rutting et al. 2008). DNRA may increase in importance over denitrification when conditions are highly reducing, since denitrification is inhibited when free sulfide concentrations are high. In addition, ammonifiers may be better competitors at low or variable NO_3^- concentrations (Jorgensen 1989). Like most pathways in the nitrogen cycle, DNRA is controlled primarily by substrate availability (Silver et al. 2001).

1.2 N STABLE ISOTOPES AND THE N CYCLE

The natural abundance of stable isotopes can be used to investigate the transformations of N compounds in the N cycle. Microbial processes such as nitrification and denitrification prefer the lighter ^{14}N isotope to ^{15}N. Thus, over time, the substrate becomes enriched in the heavier isotope while the product becomes depleted if the substrate is not limiting. By comparing the isotopic signature of the substrate and the product it is possible to tell if the reaction has taken place (Mariotti et al. 1981). Because of the wide variety of transformations that N can undergo, it is useful to look at both

$^{14}N/^{15}N$ and $^{16}O/^{18}O$ isotopes in NO_3^-. In fact, $\delta^{18}O$ may be a more sensitive isotope to use in tracing NO_3^- transformations (Wankel et al. 2006), although O in NO_3^- equilibrates relatively rapidly with the O in H_2O.

Nitrification results in a $\delta^{15}N$ enrichment in ammonium and a depletion in nitrate due to preferential oxidation of $^{14}NH_4^+$. The isotopic fractionation factors associated with nitrification range from 13 to 36‰, resulting in $\delta^{15}N$ depletion of the resulting NO_3^- (Canfield et al. 2005). In the Delaware estuary, nitrification has been observed to result in residual $\delta^{15}N$-NH_4^+ values of up to +40‰ (Cifuentes et al. 1989). A difference of 24.5‰ between NH_4^+ and NO_3^-, similar to that observed in laboratory studies of nitrification, was found in Ise Bay, Japan (Sugimoto et al. 2008). Dähnke et al. (2008) analyzed both $\delta^{15}N$ and $\delta^{18}O$ in nitrate in the Elbe estuary and found NO_3^- in the turbidity maximum of the estuary to be depleted slightly in ^{15}N and greatly in ^{18}O, suggesting that nitrification was the source of NO_3^-.

Denitrification enriches the residual NO_3^- in both ^{15}N and ^{18}O. The increase in $\delta^{15}N$-NO_3^- is generally about twice that of $\delta^{18}O$- NO_3^- (Mayer 2005). The isotopic fractionation factors observed during denitrification range widely, from approximately 5 to 40‰ (Lehman et al. 2003). The magnitude of fractionation can vary depending on whether or not denitrification is limited by the availability of substrate. In most cases, sedimentary denitrification has only a small effect on the isotopic fractionation of NO_3^- because this process is generally limited by NO_3^- diffusion from the overlying water (Brandes and Devol 1997, Sebilo et al. 2003). In contrast, denitrification in the water column results in much greater fractionation (Brandes and Devol 1997). Riparian denitrification similarly has a larger fractionation factor, and the effect of this process can be distinguished within

large river systems (Sebilo et al. 2003). Cey et al. (1999) found denitrification to occur in groundwater, based on concurrent increases in $\delta^{15}N$ and $\delta^{18}O$ values in nitrate. In groundwater, ^{15}N and ^{18}O fractionate in a linear relationship, with a ^{15}N:^{18}O fractionation ratio of between 1.7 and 2.1 (Böttcher et al. 1990, Cey et al. 1999).

Other nitrogen transformations also affect isotope ratios. Assimilation of N by phytoplankton results in a positive enrichment in $\delta^{15}N$- NO_3^- (Waser et al. 1998, Granger et al. 2004, Needoba et al. 2004). Oxygen isotope fractionation during NO3- assimilation is similar to that of N, leading to a 1:1 increase in the two ratios (Granger et al. 2004). Dissimilatory nitrate reduction to ammonium (DNRA) has not been as well-studied, but produces ^{15}N-depleted ammonium (Mccready et al. 1983), and so would presumably also leave the remaining pool of nitrate enriched in ^{15}N (Lehmann et al. 2004).

Different sources of NO_3^- that reach a stream can have different isotopic compositions of both N and O and can therefore be identified by their isotopic signature. Chang et al. (2002) found that $\delta^{18}O$- NO_3^- in streams whose watersheds were dominated by urban areas reflected the isotopic signature of atmospheric deposition, whereas $\delta^{15}N$- NO_3^- in streams whose watersheds contained land used for livestock agriculture reflected the signature of manure. Increased cropland in watersheds has been shown to result in increased $\delta^{15}N$- NO_3^-, although the concentration of NO_3^- and the variability in $\delta^{15}N$ decrease with increasing stream order due to the mixing of waters from different streams (Lefebvre et al. 2007). The proportion of a watershed consisting of residential land has also been found to affect $\delta^{15}N$ in both sediments and consumers in small streams (Lake et al. 2001) as well as in suspension-feeding mussels in a salt marsh (McKinney et al.

2001). McClelland et al. (1997) found $\delta^{15}N$ in estuarine primary producers to be positively related to the contribution of wastewater, which tends to be enriched in ^{15}N.

The isotopic composition of oxygen atoms can also provide insight into N sources. A dual-isotope approach to the analysis of NO_3^- has been used to distinguish between atmospheric, fertilizer, soil nitrification, and manure and sewage (Mayer 2005). Mayer et al. (2002) analyzed NO_3^- in water samples taken at the most downstream USGS water quality gauging stations of 16 northeastern U.S. rivers for ^{15}N and ^{18}O. They found $\delta^{15}N$-NO_3^- to be higher in watersheds with higher levels of agricultural and urban land use, and that nitrogen stemming from wastewater and manure were the dominant sources of in-stream nitrate in these watersheds, whereas the in-stream nitrogen in less developed watersheds was primarily from soil nitrification. $\delta^{18}O$- NO_3^- values, on the other hand, showed a slight positive correlation with the importance of atmospheric deposition as an input to watersheds. The $\delta^{18}O$ did not correlate with fertilizer inputs, which suggested that nitrate-containing fertilizers did not contribute directly to in-stream NO_3^- concentrations, although other N-containing fertilizers or microbially processed nitrate-containing fertilizer could not be ruled out. While in-stream denitrification was known to be significant, the isotopic values did not reflect this process (Mayer et al. 2002). Spoelstra et al. (2001) found that microbially nitrified NO_3^-, rather than atmospheric deposition, was a source of stream NO_3^- based on both N and O isotopes.

The isotopic composition of NO_3^- can also be used to elucidate seasonal and spatial patterns within a watershed. While it is difficult to draw definitive conclusions from isotopes due to the variety of potential N sources and transformations along a river reach, data from sub-watersheds of a large system can provide information on the relative

importance of different N sources and on the relative contribution of different sub-watersheds to downstream flow, as well as information on differences between seasons. Battaglin et al. (2001) used $\delta^{15}N$ and $\delta^{18}O$ in nitrate to investigate the sources of N in the watershed of the Mississippi River. They collected water samples for analysis on the Mississippi River itself and on its tributaries over the course of a year. They found differences among sites, as well as evidence for seasonal shifts in isotope abundance. $\delta^{15}N$ and $\delta^{18}O$ values on the main stem of the Mississippi increased from spring to fall while flow decreased. These observations suggested that the proportions of water and nitrate deriving from sources such as groundwater and agricultural tile drainage were not the same over the course of the year.

In streams, since conservative mixing with a marine end-member is not a consideration, declines in N concentrations along the reach of a stream suggest some mechanism of loss (Kellman and Hillaire-Marcel 1998). NO_X concentrations in the Altamaha River are significantly higher upstream than closer to the coast (Weston et al. 2009), suggesting that there may be an in-stream loss in this system. Corresponding changes in $\delta^{15}N$ and $\delta^{18}O$ can provide insight on the mechanisms behind such a loss (Kellman and Hillaire-Marcel 1998). In their study of the Mississippi River watershed, Battaglin et al. (2001) collected two Lagrangian sets of samples, allowing them to track nitrate transformations in a given parcel of water over time. They concluded that NO_3^- losses were a result of assimilation rather than denitrification, since the shift in $\delta^{15}N$-NO_3^- along the river reach was negative, which is not consistent with what would be expected from denitrification (Battaglin et al. 2001). It is not clear why they reached this

conclusion, however, since N isotope fractionation of the substrate during assimilation is generally also positive (Waser et al. 1998, Granger et al. 2004, Needoba et al. 2004).

1.3 NITROGEN IN THE WATERSHED

Once reactive nitrogen has been applied to the watershed surface it begins its journey through the watershed. Nitrogen applied in the form of ammonium is rapidly converted to nitrate by nitrifiers within the soil (Starr et al. 1974), whereas organic nitrogen must be mineralized to ammonium first. Aber et al. (1998) found that chronic nitrogen additions to forests resulted in an initial increase in nitrogen mineralization. On a longer time scale, however, they found nitrogen mineralization rates to decrease. Nitrification rates increased as well, but since the increases in cycling rates were small compared to the N additions, nitrogen was still efficiently retained in the forest system.

Both NO_3^- and DON can leach from soils, although DON is usually a smaller component of N leaching than NO3-. A study of agricultural soils in Germany found DON to contribute between 6 and 21% of total N losses from soil (Siemens and Kaupenjohann 2002). In forest soils, increased atmospheric deposition has been found to increase leaching of both NO_3^- and DON, with DON contributing up to about half of leached N (Pregitzer et al. 2004).

Nitrate leaching from soils varies with land use and soil characteristics. In forests, the C:N ratio of soils can affect nitrate leaching. A study of European forest soil databases found increased nitrate leaching at low C:N ratios (Gundersen et al. 1998). Similar results were obtained in North America, where the C:N ratio of soil was affected by the tree species present (Lovett et al. 2002). In contrast, leaching of NO3- from agricultural soils, which receive higher applications of nitrogen than forests, is affected by management

strategies, soil characteristics, and climate (Dinnes et al. 2002). Management strategies such as incorporation of straw can reduce nitrate leaching from these types of lands (Beaudoin et al. 2005). Organic farming practices have been found to reduce nitrate loss from agricultural soils, although plowing in of nitrogen fixing-cover crops resulted in substantial NO3- leaching (Knappe et al. 2002). Other studies, however, have shown cover crops to reduce soil nitrate leaching (Staver and Brinsfield 1998). Soil type is also important, as more porous agricultural soils have been found to leach more NO_3^- (Beaudoin et al. 2005). Climate can also play a factor, with greater precipitation resulting in greater nitrate leaching from soils (Hatfield and Prueger 2004).

As nitrogen moves towards stream channels, it enters riparian zones flanking the stream. The riparian zones of streams are generally thought to be zones of nitrate removal from groundwater, but the extent to which they are able to do so is dependent on landscape characteristics (Vidon and Hill 2004). In areas where riparian flow is shallow, allowing water to interact with organic matter and vegetation, NO_3^- removal is effective, particularly where NO3- flux is low (Hill 1996). Jacinthe et al. (1998) found pockets of organic matter within the groundwater flow path to be extremely important for NO_3^- removal. In riparian zones that are in contact with a large aquifer, groundwater may not come in contact with vegetation and therefore these types of riparian zones will not be as effective in removing NO_3^- (Hill 1996). The soil texture and slope of the groundwater table may also have an effect on NO_3^- removal efficiency (Vidon and Hill 2004). Urban riparian zones have been shown to be less efficient at removing NO_3^- due to the fact that lower water tables limit contact between groundwater and the upper soil layers that are most efficient at denitrifying (Groffman et al. 2002).

Nutrient cycling in riverine wetlands and riparian zones is impacted by the geomorphology of these areas. Well-aerated areas, such as levees, tend to accumulate nitrification-derived NO_3^-, while lower-lying and therefore more anoxic regions are dominated by denitrification (Johnston et al. 2001). A study of French wetlands found topography to be the most important factor controlling denitrification rates (Clement et al. 2002). Groundwater age also plays a role. Younger groundwater in the Pamlico watershed in North Carolina was found to contain excess N_2, indicative of denitrification (Tesoriero et al. 2005). NO_3^- in groundwater is often thought to be of anthropogenic origin, but recent studies suggest that subterranean anthropogenic N can also take the forms of NH_4^+ or DON (Bowen et al. 2007, Kroeger et al. 2007).

Further transformations occur within streams and their streambeds. A large body of evidence suggests that the majority of in-stream nitrogen transformations take place in small headwater streams because these small streams make up the majority of the total length of a drainage network (Peterson et al. 2001). Therefore, changes in nitrogen processing in these small streams would have a disproportionate effect on the N delivered to larger streams (Alexander et al. 2007). In addition, channel depth is negatively correlated with nitrogen removal since it reduces the supply of particulate N to the sediments, where most removal occurs, and therefore larger streams are less efficient removers of N (Alexander et al. 2000). However, this does not mean that large rivers are not important players in watershed-scale nitrogen processing. A model by Seitzinger et al. (2002) found that while the percentage of inputs removed in a stream reach was greater in headwater streams, the mass of nitrogen removed in higher-order streams was greater due to the overall greater inputs to larger rivers. An application of the SPARROW

model (discussed in greater detail below) similarly found that in large rivers, which tend to have a longer total reach length and therefore a longer total residence time, the increased residence time outweighs reduced channel depth (Alexander et al. 2000). Debris dams in streams that increase residence time have been shown to increase ammonium uptake rates, particularly in summer months, due to increased organic matter trapping (Claessens et al. 2010).

Once water reaches a stream, it does not travel exclusively within the channel; shallow subsurface (known as "hyporheic") flows along the streambed also occur (Figure 2). Poole et al. (2008) proposed the concept of "hydrologic spirals." A molecule in the stream that enters a hyporheic flow path, re-emerges into the stream, and then enters another hyporheic flow, is considered to have completed one hydrologic spiral. Spiral length is defined as the distance that a molecule travels downstream over the course of completing a given spiral. These spirals have an important influence on ecosystem dynamics in streams, and can greatly complicate studies of nutrient uptake in a reach, since spirals originating outside the reach under study could emerge in that reach or could start within the reach and emerge further downstream. Only hyporheic flows that both begin and end within a reach and that are shorter than the temporal duration of the experiment will not bias studies of biotic nutrient uptake (Poole et al. 2008). Since nutrients often enter hyporheic flow paths along with the water, biochemical transformations can occur in these zones as well, although the primary effect of hyporheic flows is to increase the residence time of solutes (Bencala 2005). Both NO_3^- uptake and nitrification has been shown to occur along hyporheic flow paths, thus both of these processes can be significant sinks of N from river water (Hinkle et al. 2001).

The nitrogen in both rivers and groundwater eventually reach the coastal zone, where it can contribute to eutrophication. Although studies of estuarine eutrophication tend to focus on inorganic forms of N (e.g. Rabalais et al. 2002, Camargo and Alonso 2006), organic nitrogen can account for up to 90% of the N that reaches estuaries via rivers, and can therefore be a substantial contributor to eutrophication in the coastal zone (Seitzinger and Sanders 1997). Groundwater may also discharge directly to receiving estuaries. The concentrations of solutes in groundwater are often higher than the concentrations in estuarine water (Bowen et al. 2007), suggesting that groundwater can be an important source of nutrients to coastal systems. Paerl (1997) found both groundwater and N from direct atmospheric deposition to estuarine surface waters to be of importance in a wide range of coastal systems, suggesting that a comprehensive approach to managing all inputs of N is necessary.

1.4 MEASURING WATERSHED N CYCLING

Adding a known quantity of nutrients to streams is an effective way to trace their movement through a system. For example, Gooseff et al. (2004) added NO_3^- to streams in the McMurdo Dry Valleys in Antarctica in order to calculate NO_3^- uptake and denitrification potentials in benthic microbial mats and hyporheic zones in the stream. Both benthic mats and hyporheic flows were found to be important in NO_3^- removal, with dissimilatory reduction to NO_2^- accounting for about 20% of removal. Richey et al. (1985) added ammonium, urea, and nitrate to a small stream in Hubbard Brook Experimental Forest and examined rates of transformation, finding nitrification to be the dominant in-stream transformation. When performing these types of studies, it is

important to repeat experiments over time since nutrient uptake patterns can change seasonally (Claessens et al. 2010).

The addition of nutrients can also be used to examine nutrient limitation. Grimm and Fisher (1986) used this approach in the Sonoran Desert by adding both N and P to streams and observing the effect on chlorophyll a, production, and respiration, finding that nitrogen was limiting. However, the technique requires that N be added in quantities that are often well above natural in-stream concentrations (e.g. Gooseff et al. 2004). Adding nutrient concentrations at several levels and regressing these against measured uptake lengths can help circumvent this problem by allowing extrapolation to ambient nutrient conditions (Payn et al. 2005).

Another approach, one which does not require large additions of N that may be well beyond normal environmental concentrations, is through the use of trace amounts of the stable isotope ^{15}N. This approach was used with great success in the LINX (Lotic Intersite Nitrogen eXperiment) project. LINX experiments added continuous drips of small amounts of ^{15}N-labeled NO_3^- to streams. Samples for ^{15}N- NO_3^-, ^{15}N-N_2O, and ^{15}N-N_2 were then taken downstream, allowing researchers to calculate NO3- uptake and denitrification rates and distances (Hall et al. 2009, Mulholland et al. 2009). The studies found that NO_3^- uptake lengths increased with increasing discharge and NO_3^- concentrations, whereas increased primary production decreased uptake lengths. These findings imply that uptake by primary producers was the primary nitrate sink, but that their efficiency declined with increased nitrate concentrations (Hall et al. 2009). Other experiments relied on the addition of ^{15}N-labeled NH_4^+ to examine stream nitrogen cycling (Tank et al. 2000) and, in particular, nitrification (Mulholland et al. 2000). Tank

et al. (2000) found high ammonium demand in the Appalachian streams they studied. Other projects have used ^{15}N tracer studies as a basis for a compartmental stream nitrogen cycle model (Wollheim et al. 1999).

1.5 MODELS OF WATERSHED NITROGEN DYNAMICS

While field measurements are useful in gaining an understanding of nitrogen dynamics within particular reaches, it can be difficult to scale these studies up to a larger watershed. Models are the best way of understanding nitrogen dynamics at a large spatial scale, and a great variety has been developed.

Some of the simplest models use a straightforward mass balance. This approach compares nitrogen inputs to exports without attempting to understand transformations of N as it moves through the watershed. Nitrogen sources to the watershed surface are summed and compared to downstream riverine exports (Howarth et al. 1996). This approach was used by the SCOPE Nitrogen Project. Boyer et al. (2002) constructed N budgets for 16 watersheds in the northeastern United States that took into account inputs from fertilizer, atmospheric deposition, biological N fixation, and net food and feed import. They found 25% of N inputs to watersheds to be exported to coastal systems, although the proportion exported differs in other regions (Schaefer and Alber 2007). Other authors (Van Breemen et al. 2002) calculated that half of the N not exported by the stream was lost in gaseous form, mostly from denitrification, with the rest accounted for by food and wood export and increases in N storage in soils and biomass. Other researchers have constructed similar models that compare N inputs to the land to outputs at the mouth of a river. Green et al. (2004) used a global grid-based model to compare inputs and exports. This study found an average export of 18% of nitrogen inputs,

although exports ranged from 0 to 100% of inputs, with the export of an individual basin highly dependent on both watershed temperature and hydrology.

Mass balances using an import/export approach are usually done on an annual to multi-year time scale, which can miss important processes that take place on shorter time scales. A nitrogen mass balance of the Androscoggin River that combined in-stream and snowpack N concentration measurements with USGS flow data found that approximately half of the river's annual load of inorganic nitrogen was delivered during the spring snowmelt period (Oczkowski et al. 2006).

More complex models can incorporate spatial information, often in the form of GIS layers describing watershed characteristics such as soil textures, land use, and climatic patterns. Many of these models also include more explicit descriptions of the actual processes taking place.

The SWAT (Soil and Watershed Assessment Tool) model calculates flow, sediment, and chemical loads in a watershed using inputs that include hydrological, climatic, and soil characteristics, as well as information on management of agricultural activities in the basin. The approach subdivides basins into subwatersheds or grid cells, and does not require calibration, allowing it to be used on basins without monitoring stations (Arnold et al. 1998). This makes SWAT a useful tool for exploring different management scenarios (Jayakrishnan et al. 2005). The model has been extended to include algorithms from biogeochemical models to improve its description of N dynamics, particularly in soils, to make it more useful in scenario analysis (Pohlert et al. 2007).

SPARROW (SPAtially Referenced Regression On Watershed attributes) is a USGS model described as a "hybrid-mechanistic statistical catchment model" (Elliot et al.

2005). It develops a regression between land surface loading and downstream solute concentration (Smith et al. 1997). A delivery term is used to estimate the proportion of on-land sources that reach a stream, and an in-stream decay coefficient is used to describe their behavior in the stream (Smith et al. 1997). This model has been applied to a wide variety of watersheds throughout the United States (e.g. Alexander et al. 2000) as well as other regions (e.g. Alexander et al. 2002).

Other models have also been developed. Seitzinger et al. (2002) developed a model to calculate N removal within a river network based on the ratio of depth to travel time in rivers and lakes, since this property affects the contact time of water with the bottom sediments in which nitrogen removal typically occurs. This model showed that denitrification was one of the largest sinks for nitrogen within the watershed studied.

1.6 DISSERTATION OVERVIEW

There are many ways to approach a study of the nitrogen cycle, all of which provide useful insights into the many ways in which environmental conditions combine to affect N processing. My dissertation uses several approaches. Chapter 2 examines the controls on the percentage of new anthropogenic nitrogen inputs to the watershed surface that are exported in streamflow from watersheds on the west coast of the United States using a simple mass balance watershed nitrogen budget similar to that of the SCOPE studies (Boyer et al. 2002). Chapter 3 examines the Altamaha River in Georgia, USA, in depth by combining an N input budget with a year-long series of measurements of in-stream N concentration and the stable isotope composition of NO_3^-, particulate N, and H_2O in order to elucidate controls on N processes. Chapter 4 presents a global perspective, focusing on small- and medium-sized watersheds throughout the world based on published

measurements of NO_3^- concentrations in comparison with watershed characteristics derived through GIS.

REFERENCES

Aber, J., W. McDowell, K. Nadelhoffer, A. Magill, G. Berntson, M. Kamakea, S. McNulty, W. Currie, L. Rustad, and I. Fernandez. 1998. Nitrogen saturation in temperate forest ecosystems - Hypotheses revisited. *Bioscience* 48:921-934.

Alexander, R. B., E. W. Boyer, R. A. Smith, G. E. Schwarz, and R. B. Moore. 2007. The role of headwater streams in downstream water quality. *Journal of the American Water Resources Association* 43:41-59.

Alexander, R. B., A. H. Elliott, U. Shankar, and G. B. Mcbride. 2002. Estimating the sources and transport of nutrients in the Waikato River Basin, New Zealand. *Water Resources Research* 38.

Alexander, R. B., R. A. Smith, and G. E. Schwarz. 2000. Effect of stream channel size on the delivery of nitrogen to the Gulf of Mexico. *Nature* 403:758-761.

An, S. M. and W. S. Gardner. 2002. Dissimilatory nitrate reduction to ammonium (DNRA) as a nitrogen link, versus denitrification as a sink in a shallow estuary (Laguna Madre/Baffin Bay, Texas). *Marine Ecology-Progress Series* 237:41-50.

Arango, C. P. and J. L. Tank. 2008. Land use influences the spatiotemporal controls on nitrification and denitrification in headwater streams. *Journal of the North American Benthological Society* 27:90-107.

Arnold, J. G., R. Srinivasan, R. S. Muttiah, and J. R. Williams. 1998. Large area hydrologic modeling and assessment - Part 1: Model development. *Journal of the American Water Resources Association* 34:73-89.

Battaglin, W. A., C. Kendall, C. C. Y. Chang, S. R. Silva, and D. H. Campbell. 2001. Chemical and isotopic evidence of nitrogen transformation in the Mississippi River, 1997-98. *Hydrological Processes* 15:1285-1300.

Beaudoin, N., J. K. Saad, C. Van Laethem, J. M. Machet, J. Maucorps, and B. Mary. 2005. Nitrate leaching in intensive agriculture in Northern France: Effect of farming practices, soils and crop rotations. *Agriculture Ecosystems & Environment* 111:292-310.

Bencala, K. E. 2005. Hyporheic Exchange Flows.in M. G. Anderson, editor. *Encyclopedia of Hydrologic Sciences*. U.S. Government.

Böttcher, J., O. Strebel, S. Voerkelius, and H. L. Schmidt. 1990. Using Isotope Fractionation of Nitrate Nitrogen and Nitrate Oxygen for Evaluation of Microbial Denitrification in a Sandy Aquifer. *Journal of Hydrology* 114:413-424.

Bowen, J. L., K. D. Kroeger, G. Tomasky, W. J. Pabich, M. L. Cole, R. H. Carmichael, and I. Valiela. 2007. A review of land-sea coupling by groundwater discharge of nitrogen to New England estuaries: Mechanisms and effects. *Applied Geochemistry* 22:175-191.

Boyer, E. W., C. L. Goodale, N. A. Jaworsk, and R. W. Howarth. 2002. Anthropogenic nitrogen sources and relationships to riverine nitrogen export in the northeastern USA. *Biogeochemistry* 57:137-169.

Brandes, J. A. and A. H. Devol. 1997. Isotopic fractionation of oxygen and nitrogen in coastal marine sediments. *Geochimica Et Cosmochimica Acta* 61:1793-1801.

Bricker, S., B. Longstaff, W. Dennison, A. Jones, K. Boicourt, C. Wicks, and J. Woerner. 2007. Effects of Nutrient Enrichment in the Nation's Estuaries: A Decade of Change. National Centers for Coastal Ocean Science, Silver Spring, MD.

Camargo, J. A. and A. Alonso. 2006. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. *Environment International* 32:831-849.

Canfield, D. E., B. Thamdrup, and E. Kristensen. 2005. *Aquatic Geomicrobiology*. Elsevier Academic Press, San Diego. 640 pp.

Cey, E. E., D. L. Rudolph, R. Aravena, and G. Parkin. 1999. Role of the riparian zone in controlling the distribution and fate of agricultural nitrogen near a small stream in southern Ontario. *Journal of Contaminant Hydrology* 37:45-67.

Chang, C. C. Y., C. Kendall, S. R. Silva, W. A. Battaglin, and D. H. Campbell. 2002. Nitrate stable isotopes: tools for determining nitrate sources among different land uses in the Mississippi River Basin. *Canadian Journal of Fisheries and Aquatic Sciences* 59:1874-1885.

Claessens, L., C. L. Tague, P. M. Groffman, and J. M. Melack. 2010. Longitudinal and seasonal variation of stream N uptake in an urbanizing watershed: effect of organic matter, stream size, transient storage and debris dams. *Biogeochemistry* 98:45-62.

Clement, J. C., G. Pinay, and P. Marmonier. 2002. Seasonal dynamics of denitrification along topohydrosequences in three different riparian wetlands. *Journal of Environmental Quality* 31:1025-1037.

Cornwell, J. C., W. M. Kemp, and T. M. Kana. 1999. Denitrification in coastal ecosystems: methods, environmental controls, and ecosystem level controls, a review. *Aquatic Ecology* 33:41-54.

Dähnke, K., E. Bahlmann, and K. Emeis. 2008. A nitrate sink in estuaries? An assessment by means of stable nitrate isotopes in the Elbe estuary. *Limnology and Oceanography* 53:1504-1511.

Dalsgaard, T., D. E. Canfield, J. Petersen, B. Thamdrup, and J. Acuna-Gonzalez. 2003. N-2 production by the anammox reaction in the anoxic water column of Golfo Dulce, Costa Rica. *Nature* 422:606-608.

Dinnes, D. L., D. L. Karlen, D. B. Jaynes, T. C. Kaspar, J. L. Hatfield, T. S. Colvin, and C. A. Cambardella. 2002. Nitrogen management strategies to reduce nitrate leaching in tile-drained midwestern soils. *Agronomy Journal* 94:153-171.

Elliot, A. H., R. B. Alexander, G. E. Schwarz, U. Shankar, J. P. S. Sukias, and G. B. McBride. 2005. Estimation of nutrient sources and transport for New Zealand using the hybrid mechanistic-statistical model SPARROW. *Journal of Hydrology* (NZ) 44:1-27.

Francis, C. A., J. M. Beman, and M. M. M. Kuypers. 2007. New processes and players in the nitrogen cycle: the microbial ecology of anaerobic and archaeal ammonia oxidation. *Isme Journal* 1:19-27.

Galloway, J. N. and E. B. Cowling. 2002. Reactive nitrogen and the world: 200 years of change. *Ambio* 31:64-71.

Galloway, J. N., F. J. Dentener, D. G. Capone, E. W. Boyer, R. W. Howarth, S. P. Seitzinger, G. P. Asner, C. C. Cleveland, P. A. Green, E. A. Holland, D. M. Karl, A. F. Michaels, J. H. Porter, A. R. Townsend, and C. J. Vörösmarty. 2004. Nitrogen cycles: past, present, and future. *Biogeochemistry* 70:153-226.

Galloway, J. N., A. R. Townsend, J. W. Erisman, M. Bekunda, Z. C. Cai, J. R. Freney, L. A. Martinelli, S. P. Seitzinger, and M. A. Sutton. 2008. Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. *Science* 320:889-892.

Gooseff, M. N., D. M. McKnight, R. L. Runkel, and J. H. Duff. 2004. Denitrification and hydrologic transient storage in a glacial meltwater stream, McMurdo Dry Valleys, Antarctica. *Limnology and Oceanography* 49:1884-1895.

Granger, J., D. M. Sigman, J. A. Needoba, and P. J. Harrison. 2004. Coupled nitrogen and oxygen isotope fractionation of nitrate during assimilation by cultures of marine phytoplankton. *Limnology and Oceanography* 49:1763-1773.

Green, P. A., C. J. Vorosmarty, M. Meybeck, J. N. Galloway, B. J. Peterson, and E. W. Boyer. 2004. Pre-industrial and contemporary fluxes of nitrogen through rivers: a global assessment based on typology. *Biogeochemistry* 68:71-105.

Grimm, N. B. and S. G. Fisher. 1986. Nitrogen limitation in a Sonoran Desert Stream. *Journal of the North American Benthological Society* 5:2-15.

Groffman, P. M., N. J. Boulware, W. C. Zipperer, R. V. Pouyat, L. E. Band, and M. F. Colosimo. 2002. Soil nitrogen cycle processes in urban Riparian zones. *Environmental Science & Technology* 36:4547-4552.

Gundersen, P., I. Callesen, and W. de Vries. 1998. Nitrate leaching in forest ecosystems is related to forest floor C/N ratios. *Environmental Pollution* 102:403-407.

Hall, R. O., J. L. Tank, D. J. Sobota, P. J. Mulholland, J. M. O'Brien, W. K. Dodds, J. R. Webster, H. M. Valett, G. C. Poole, B. J. Peterson, J. L. Meyer, W. H. McDowell, S. L. Johnson, S. K. Hamilton, N. B. Grimm, S. V. Gregory, C. N. Dahm, L. W. Cooper, L. R. Ashkenas, S. M. Thomas, R. W. Sheibley, J. D. Potter, B. R. Niederlehner, L. T. Johnson, A. M. Helton, C. M. Crenshaw, A. J. Burgin, M. J. Bernot, J. J. Beaulieu, and C. P. Arango. 2009. Nitrate removal in stream ecosystems measured by N-15 addition experiments: Total uptake. *Limnology and Oceanography* 54:653-665.

Hamersley, M. R., G. Lavik, D. Woebken, J. E. Rattray, P. Lam, E. C. Hopmans, J. S. S. Damste, S. Kruger, M. Graco, D. Gutierrez, and M. M. M. Kuypers. 2007. Anaerobic ammonium oxidation in the Peruvian oxygen minimum zone. *Limnology and Oceanography* 52:923-933.

Hatfield, J. L. and J. H. Prueger. 2004. Impacts of changing precipitation patterns on water quality. *Journal of Soil and Water Conservation* 59:51-58.

Hill, A. R. 1996. Nitrate removal in stream riparian zones. *Journal of Environmental Quality* 25:743-755.

Hinkle, S. R., J. H. Duff, F. J. Triska, A. Laenen, E. B. Gates, K. E. Bencala, D. A. Wentz, and S. R. Silva. 2001. Linking hyporheic flow and nitrogen cycling near

the Willamette River - a large river in Oregon, USA. *Journal of Hydrology* 244:157-180.

Howarth, R. and H. W. Paerl. 2008. Coastal marine eutrophication: Control of both nitrogen and phosphorus is necessary. *Proceedings of the National Academy of Sciences of the United States of America* 105:E103-E103.

Howarth, R. W., G. Billen, D. Swaney, A. Townsend, N. Jaworski, K. Lajtha, J. A. Downing, R. Elmgren, N. Caraco, T. Jordan, F. Berendse, J. Freney, V. Kudeyarov, P. Murdoch, and Z. L. Zhu. 1996. Regional nitrogen budgets and riverine N&P fluxes for the drainages to the North Atlantic Ocean: Natural and human influences. *Biogeochemistry* 35:75-139.

Howarth, R. W., R. Marino, and J. Cole. 1988a. Nitrogen fixation in freshwater, estuarine, and marine ecosystems. 2. Biogeochemical controls. *Limnology and Oceanography* 33:688-701.

Howarth, R. W., R. Marino, J. Lane, and J. Cole. 1988b. Nitrogen fixation in freshwater, estuarine, and marine ecosystems. 1. Rates and importance. *Limnology and Oceanography* 33:669-687.

Howarth, R. W., A. Sharpley, and D. Walker. 2002. Sources of nutrient pollution to coastal waters in the United States: Implications for achieving coastal water quality goals. Estuaries 25:656-676.

Jacinthe, P. A., P. M. Groffman, A. J. Gold, and A. Mosier. 1998. Patchiness in microbial nitrogen transformations in groundwater in a riparian forest. *Journal of Environmental Quality* 27:156-164.

Jayakrishnan, R., R. Srinivasan, C. Santhi, and J. G. Arnold. 2005. Advances in the application of the SWAT model for water resources management. *Hydrological Processes* 19:749-762.

Johnston, C. A., S. D. Bridgham, and J. P. Schubauer-Berigan. 2001. Nutrient dynamics in relation to geomorphology of riverine wetlands. *Soil Science Society of America Journal* 65:557-577.

Jorgensen, K. S. 1989. Annual Pattern of Denitrification and Nitrate Ammonification in Estuarine Sediment. *Applied and Environmental Microbiology* 55:1841-1847.

Kellman, L. and C. Hillaire-Marcel. 1998. Nitrate cycling in streams: using natural abundances of NO_3^--delta N-15 to measure in-situ denitrification. *Biogeochemistry* 43:273-292.

Knappe, S., U. Haferkorn, and R. Meissner. 2002. Influence of different agricultural management systems on nitrogen leaching: results of lysimeter studies. *Journal of Plant Nutrition and Soil Science* 165:73-77.

Koop-Jakobsen, K. and A. E. Giblin. 2009. Anammox in Tidal Marsh Sediments: The Role of Salinity, Nitrogen Loading, and Marsh Vegetation. *Estuaries and Coasts* 32:238-245.

Kroeger, K. D., P. W. Swarzenski, W. J. Greenwood, and C. Reich. 2007. Submarine groundwater discharge to Tampa Bay: Nutrient fluxes and biogeochemistry of the coastal aquifer. *Marine Chemistry* 104:85-97.

Lake, J. L., R. A. McKinney, F. A. Osterman, R. J. Pruell, J. Kiddon, S. A. Ryba, and A. D. Libby. 2001. Stable nitrogen isotopes as indicators of anthropogenic activities

in small freshwater systems. *Canadian Journal of Fisheries and Aquatic Sciences* 58:870-878.

Lefebvre, S., J. C. Clement, G. Pinay, C. Thenail, P. Durand, and P. Marmonier. 2007. N-15-nitrate signature in low-order streams: Effects of land cover and agricultural practices. *Ecological Applications* 17:2333-2346.

Lehman, M. F., P. Reichert, S. M. Bernasconi, A. Barbieri, and J. A. McKenzie. 2003. Modelling nitrogen and oxygen isotope fractionation during denitrification in a lacustrine redox-transition zone. *Geochimica Et Cosmochimica Acta* 67:2529-2542.

Lehmann, M. F., D. M. Sigman, and W. M. Berelson. 2004. Coupling the 15N/14N and 18O/16O of nitrate as a constraint on benthic nitrogen cycling. *Marine Chemistry* 88:1-20.

Lovett, G. M., K. C. Weathers, and M. A. Arthur. 2002. Control of nitrogen loss from forested watersheds by soil carbon: Nitrogen ratio and tree species composition. *Ecosystems* 5:712-718.

Luo, J., R. W. Tillman, and P. R. Ball. 1999. Factors regulating denitrification in a soil under pasture. *Soil Biology & Biochemistry* 31:913-927.

Mariotti, A., J. C. Germon, P. Hubert, P. Kaiser, R. Letolle, A. Tardieux, and P. Tardieux. 1981. Experimental-Determination of Nitrogen Kinetic Isotope Fractionation - Some Principles - Illustration for the Denitrification and Nitrification Processes. *Plant and Soil* 62:413-430.

Mayer, B. 2005. Assessing sources and transformations of sulphate and nitrate in the hydrosphere using isotope techniques. Pages 67-89 in P. K. Aggarwal, J. R. Gat,

and K. F. O. Froehlich, editors. *Isotopes in the Water Cycle: Past, Present and Future of a Developing Science*. IEA, Netherlands.

Mayer, B., E. W. Boyer, C. Goodale, N. A. Jaworski, N. Van Breemen, R. W. Howarth, S. Seitzinger, G. Billen, L. J. Lajtha, M. Nosal, and K. Paustian. 2002. Sources of nitrate in rivers draining sixteen watersheds in the northeastern US: Isotopic constraints. *Biogeochemistry* 57:171-197.

McClelland, J. W., I. Valiela, and R. H. Michener. 1997. Nitrogen-stable isotope signatures in estuarine food webs: A record of increasing urbanization in coastal watersheds. *Limnology and Oceanography* 42:930-937.

Mccready, R. G. L., W. D. Gould, and R. W. Barendregt. 1983. Nitrogen Isotope Fractionation during the Reduction of NO_3^- to NH_4^+ by Desulfovibrio Sp. *Canadian Journal of Microbiology* 29:231-234.

McKinney, R. A., W. G. Nelson, M. A. Charpentier, and C. Wigand. 2001. Ribbed mussel nitrogen isotope signatures reflect nitrogen sources in coastal salt marshes. *Ecological Applications* 11:203-214.

Mulder, A., A. A. Vandegraaf, L. A. Robertson, and J. G. Kuenen. 1995. Anaerobic Ammonium Oxidation Discovered in a Denitrifying Fluidized-Bed Reactor. Fems *Microbiology Ecology* 16:177-183.

Mulholland, P. J., R. O. Hall, D. J. Sobota, W. K. Dodds, S. E. G. Findlay, N. B. Grimm, S. K. Hamilton, W. H. McDowell, J. M. O'Brien, J. L. Tank, L. R. Ashkenas, L. W. Cooper, C. N. Dahm, S. V. Gregory, S. L. Johnson, J. L. Meyer, B. J. Peterson, G. C. Poole, H. M. Valett, J. R. Webster, C. P. Arango, J. J. Beaulieu, M. J. Bernot, A. J. Burgin, C. L. Crenshaw, A. M. Helton, L. T. Johnson, B. R.

Niederlehner, J. D. Potter, R. W. Sheibley, and S. M. Thomas. 2009. Nitrate removal in stream ecosystems measured by N-15 addition experiments: Denitrification. *Limnology and Oceanography* 54:666-680.

Mulholland, P. J., J. L. Tank, D. M. Sanzone, W. M. Wollheim, B. J. Peterson, J. R. Webster, and J. L. Meyer. 2000. Nitrogen cycling in a forest stream determined by a N-15 tracer addition. *Ecological Monographs* 70:471-493.

Needoba, J. A., D. M. Sigman, and P. J. Harrison. 2004. The mechanism of isotope fractionation during algal nitrate assimilation as illuminated by the 15N/14N of intracellular nitrate. *Journal of Phycology* 40:517-522.

Oczkowski, A. J., B. A. Pellerin, C. W. Hunt, W. M. Wollheim, C. J. Vorosmarty, and T. C. Loder. 2006. The role of snowmelt and spring rainfall in inorganic nutrient fluxes from a large temperate watershed, the Androscoggin River basin (Maine and New Hampshire). *Biogeochemistry* 80:191-203.

Pabich, W. J., I. Valiela, and H. F. Hemond. 2001. Relationship between DOC concentration and vadose zone thickness and depth below water table in groundwater of Cape Cod, USA. *Biogeochemistry* 55:247-268.

Paerl, H. W. 1997. Coastal eutrophication and harmful algal blooms: Importance of atmospheric deposition and groundwater as "new" nitrogen and other nutrient sources. *Limnology and Oceanography* 42:1154-1165.

Payn, R. A., J. R. Webster, P. J. Mulholland, H. M. Valett, and W. K. Dodds. 2005. Estimation of stream nutrient uptake from nutrient addition experiments. *Limnology and Oceanography-Methods* 3:174-182.

Penton, C. R., A. H. Devol, and J. M. Tiedje. 2006. Molecular evidence for the broad distribution of anaerobic ammonium-oxidizing bacteria in freshwater and marine sediments. *Applied and Environmental Microbiology* 72:6829-6832.

Peterson, B. J., W. M. Wollheim, P. J. Mulholland, J. R. Webster, J. L. Meyer, J. L. Tank, E. Marti, W. B. Bowden, H. M. Valett, A. E. Hershey, W. H. McDowell, W. K. Dodds, S. K. Hamilton, S. Gregory, and D. D. Morrall. 2001. Control of nitrogen export from watersheds by headwater streams. *Science* 292:86-90.

Pfennig, K. S. and P. B. McMahon. 1996. Effect of nitrate, organic carbon, and temperature on potential denitrification rates in nitrate-rich riverbed sediments. *Journal of Hydrology* 187:283-295.

Pohlert, T., J. A. Huisman, L. Breuer, and H. G. Frede. 2007. Integration of a detailed biogeochemical model into SWAT for improved nitrogen predictions - Model development, sensitivity, and GLUE analysis. *Ecological Modelling* 203:215-228.

Poole, G. C., S. J. O'Daniel, K. L. Jones, W. W. Woessner, E. S. Bernhardt, A. M. Helton, J. A. Stanford, B. R. Boer, and T. J. Beechie. 2008. Hydrologic spiralling: The role of multiple interactive flow paths in stream ecosystems. *River Research and Applications* 24:1018-1031.

Pregitzer, K. S., D. R. Zak, A. J. Burton, J. A. Ashby, and N. W. MacDonald. 2004. Chronic nitrate additions dramatically increase the export of carbon and nitrogen from northern hardwood ecosystems. *Biogeochemistry* 68:179-197.

Rabalais, N. N., R. E. Turner, Q. Dortch, D. Justic, V. J. Bierman, and W. J. Wiseman. 2002. Nutrient-enhanced productivity in the northern Gulf of Mexico: past, present and future. *Hydrobiologia* 475:39-63.

Richardson, W. B., E. A. Strauss, L. A. Bartsch, E. M. Monroe, J. C. Cavanaugh, L. Vingum, and D. M. Soballe. 2004. Denitrification in the Upper Mississippi River: rates, controls, and contribution to nitrate flux. *Canadian Journal of Fisheries and Aquatic Sciences* 61:1102-1112.

Richey, J. S., W. H. Mcdowell, and G. E. Likens. 1985. Nitrogen Transformations in a Small Mountain Stream. *Hydrobiologia* 124:129-139.

Rutting, T., D. Huygens, C. Muller, O. Cleemput, R. Godoy, and P. Boeckx. 2008. Functional role of DNRA and nitrite reduction in a pristine south Chilean Nothofagus forest. *Biogeochemistry* 90:243-258.

Schaefer, S. C. and M. Alber. 2007. Temperature controls a latitudinal gradient in the proportion of watershed nitrogen exported to coastal ecosystems. *Biogeochemistry* 85:333-346.

Schindler, D. W. and R. E. Hecky. 2009. Eutrophication: More nitrogen data needed. *Science* 324:721-722.

Sebilo, M., G. Billen, M. Grably, and A. Mariotti. 2003. Isotopic composition of nitrate-nitrogen as a marker of riparian and benthic denitrification at the scale of the whole Seine River system. *Biogeochemistry* 63:35-51.

Seitzinger, S. P. 1988. Denitrification in freshwater and coastal marine ecosystems: Ecological and geochemical significance. *Limnology and Oceanography* 33:702-724.

Seitzinger, S. P. and R. W. Sanders. 1997. Contribution of dissolved organic nitrogen from rivers to estuarine eutrophication. *Marine Ecology-Progress Series* 159:1-12.

Seitzinger, S. P., R. V. Styles, E. W. Boyer, R. B. Alexander, G. Billen, R. W. Howarth, B. Mayer, and N. Van Breemen. 2002. Nitrogen retention in rivers: model development and application to watersheds in the northeastern USA. Biogeochemistry 57:199-237.

Siemens, J. and M. Kaupenjohann. 2002. Contribution of dissolved organic nitrogen to N leaching from four German agricultural soils. *Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde* 165:675-681.

Silver, W. L., D. J. Herman, and M. K. Firestone. 2001. Dissimilatory nitrate reduction to ammonium in upland tropical forest soils. *Ecology* 82:2410-2416.

Smith, R. A., G. E. Schwarz, and R. B. Alexander. 1997. Regional interpretation of water-quality monitoring data. *Water Resources Research* 33:2781-2798.

Smith, S. V. 1984. Phosphorus Versus Nitrogen Limitation in the Marine-Environment. *Limnology and Oceanography* 29:1149-1160.

Spoelstra, J., S. L. Schiff, R. J. Elgood, R. G. Semkin, and D. S. Jeffries. 2001. Tracing the sources of exported nitrate in the turkey lakes watershed using N-15/N-14 and O-18/O-16 isotopic ratios. *Ecosystems* 4:536-544.

Starr, J. L., F. E. Broadbent, and D. R. Nielsen. 1974. Nitrogen transformations during continuous leaching. *Soil Science Society of America Journal* 38:283-289.

Staver, K. W. and R. B. Brinsfield. 1998. Using cereal grain winter cover crops to reduce groundwater nitrate contamination in the mid-Atlantic coastal plain. *Journal of Soil and Water Conservation* 53:230-240.

Strauss, E. A. and G. A. Lamberti. 2000. Regulation of nitrification in aquatic sediments by organic carbon. *Limnology and Oceanography* 45:1854-1859.

Strauss, E. A., N. L. Mitchell, and G. A. Lamberti. 2002. Factors regulating nitrification in aquatic sediments: effects of organic carbon, nitrogen availability, and pH. *Canadian Journal of Fisheries and Aquatic Sciences* 59:554-563.

Strauss, E. A., W. B. Richardson, L. A. Bartsch, J. C. Cavanaugh, D. A. Bruesewitz, H. Imker, J. A. Heinz, and D. M. Soballe. 2004. Nitrification in the Upper Mississippi River: patterns, controls, and contribution to the NO_3^- budget. *Journal of the North American Benthological Society* 23:1-14.

Sugimoto, R., A. Kasai, T. Miyajima, and K. Fujita. 2008. Nitrogen isotopic discrimination by water column nitrification in a shallow coastal environment. *Journal of Oceanography* 64:39-48.

Tank, J. L., J. L. Meyer, D. M. Sanzone, P. J. Mulholland, J. R. Webster, B. J. Peterson, W. M. Wollheim, and N. E. Leonard. 2000. Analysis of nitrogen cycling in a forest stream during autumn using a N-15-tracer addition. *Limnology and Oceanography* 45:1013-1029.

Tesoriero, A. J., T. B. Spruill, H. E. Mew, K. M. Farrell, and S. L. Harden. 2005. Nitrogen transport and transformations in a coastal plain watershed: Influence of geomorphology on flow paths and residence times. *Water Resources Research* 41.

Thamdrup, B., T. Dalsgaard, M. M. Jensen, O. Ulloa, L. Farias, and R. Escribano. 2006. Anaerobic ammonium oxidation in the oxygen-deficient waters off northern Chile. *Limnology and Oceanography* 51:2145-2156.

Van Breemen, N., E. W. Boyer, C. L. Goodale, N. A. Jaworski, K. Paustian, S. P. Seitzinger, K. Lajtha, B. Mayer, D. van Dam, R. W. Howarth, K. J. Nadelhoffer, M. Eve, and G. Billen. 2002. Where did all the nitrogen go? Fate of nitrogen

inputs to large watersheds in the northeastern U.S.A. *Biogeochemistry* 57/58:267-293.

Vidon, P. G. F. and A. R. Hill. 2004. Landscape controls on nitrate removal in stream riparian zones. *Water Resources Research* 40.

Wankel, S. D., C. Kendall, C. A. Francis, and A. Paytan. 2006. Nitrogen sources and cycling in the San Francisco Bay Estuary: A nitrate dual isotopic composition approach. *Limnology and Oceanography* 51:1654-1664.

Waser, N. A. D., P. J. Harrison, B. Nielsen, S. E. Calvert, and D. H. Turpin. 1998. Nitrogen isotope fractionation during the uptake and assimilation of nitrate, nitrite, ammonium, and urea by a marine diatom. *Limnology and Oceanography* 43:215-224.

Weston, N. B., J. T. Hollibaugh, and S. B. Joye. 2009. Population growth away from the coastal zone: Thirty years of land use change and nutrient export in the Altamaha River, GA. *Science of the Total Environment* 407:3347-3356.

Wollheim, W. M., B. J. Peterson, L. A. Deegan, M. Bahr, J. E. Hobbie, D. Jones, W. B. Bowden, A. E. Hershey, G. W. Kling, and M. C. Miller. 1999. A coupled field and modeling approach for the analysis of nitrogen cycling in streams. *Journal of the North American Benthological Society* 18:199-221.

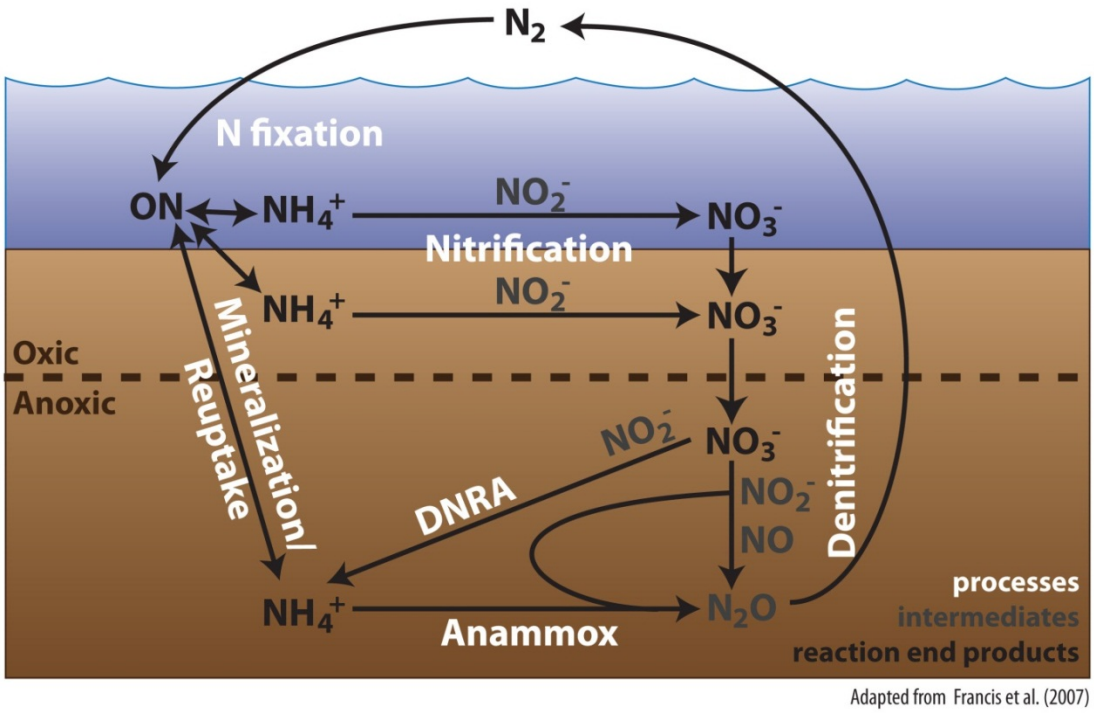
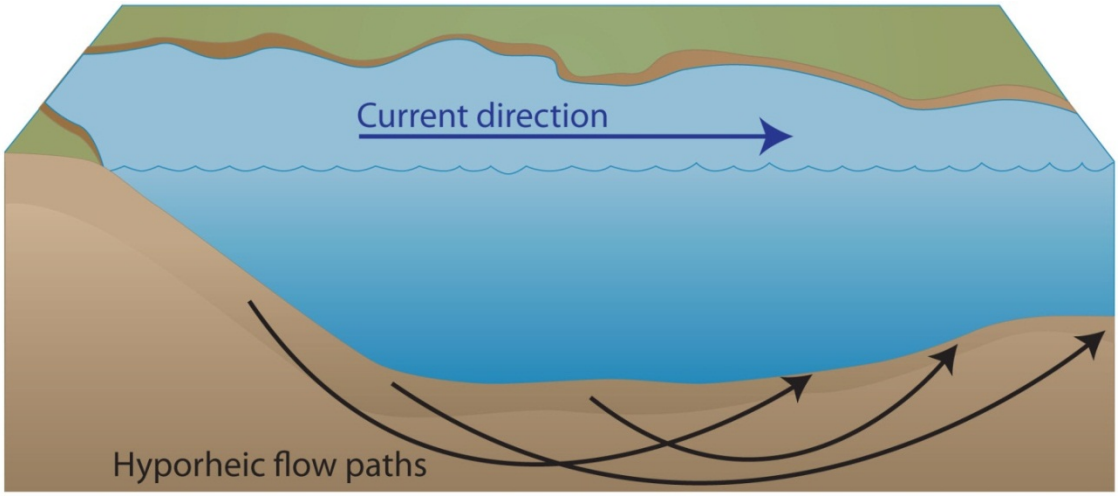


Figure 1.1. The nitrogen cycle. Adapted from Francis et al. (2007).



River cross-section credit: Tracey Saxby, Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/imagelibrary/)

Figure 1.2. Illustration of hyporheic flows.

CHAPTER 2

WATERSHED NITROGEN INPUT AND RIVERINE EXPORT ON THE WEST COAST OF THE US[1]

[1]Schaefer, S.C., J.T. Hollibaugh, and M. Alber. 2009. *Biogeochemistry* 93:219-233. Reprinted here with permission of the publisher.

ABSTRACT

This study evaluated the sources, sinks, and factors controlling net export of nitrogen (N) from watersheds on the west coast of the US. We calculated input of new N to 22 watersheds for 1992 and 2002. 1992 inputs ranged from 541 to 11,644 kg N km^{-2} $year^{-1}$, with an overall area-weighted average of 1,870 kg N km^{-2} $year^{-1}$. In 2002, the range of inputs was 490–10,875 kg N km^{-2} $year^{-1}$, averaging 2,158 kg N km^{-2} $year^{-1}$. Fertilizer was the most important source of new N, averaging 956 (1992) and 1,073 kg N km^{-2} $year^{-1}$ (2002). Atmospheric deposition was the next most important input, averaging 833 (1992) and 717 kg N km^{-2} $year^{-1}$ (2002), followed by biological N fixation in agricultural lands. Riverine N export, calculated based on measurements taken at the furthest downstream USGS water quality monitoring station, averaged 165 (1992) and 196 kg N km^{-2} $year^{-1}$ (2002), although data were available for only 7 watersheds at the latter time point. Downstream riverine N export was correlated with variations in streamflow (export = 0.94 × streamflow − 5.65, $R^2 = 0.66$), with N inputs explaining an additional 16% of the variance (export = 1.06 × streamflow + 0.06 × input − 227.78, $R^2 = 0.82$). The percentage of N input that is exported averaged 12%. Percent export was also related to streamflow (%export = 0.05 × streamflow − 2.61, $R^2 = 0.60$). The correlations with streamflow are likely a result of its large dynamic range in these systems. However, the processes that control watershed N export are not yet completely understood.

2.1 INTRODUCTION

Excess loading of nutrients to the coastal zone is posited to be a primary cause of eutrophication-related problems. As nutrient loading (particularly nitrogen loading) increases, coastal marine systems show symptoms such as increased frequency and severity of algal blooms, decreased dissolved oxygen concentrations, and loss of submerged aquatic vegetation (Bricker et al. 2007). N export from watersheds to the coastal zone has been shown to be directly related to watershed input, such that increased input of new N results in a predictable increase in export to downstream receiving waters (e.g., Howarth et al. 1996; Boyer et al. 2006). Controls on the percentage of watershed N input that reaches the coast are less clear. Different studies have shown that percent N export can be related to streamflow (Dumont et al. 2005), temperature (Schaefer and Alber 2007), and residence time (Howarth et al. 2006).

Watershed nitrogen budgets have been constructed for a variety of different regions, including much of the US (Boyer et al. 2002; McIsaac et al. 2002; Schaefer and Alber 2007); China (Liu et al. 2006); and New Zealand (Parfitt et al. 2006), but to date there are no large-scale studies of the west coast of the United States. The climatology and geomorphology of US west coast watersheds differs significantly from those on the US east coast, yet water quality and land use data sets are collected by the same agencies, suggesting that a comparison of the two might yield insights into the factors affecting nitrogen processing in watersheds. In this study we estimated watershed N input and export of that N to the coastal zone for 22 west coast watersheds (Fig. 2.1) for two periods: the early 1990s, which is comparable to the period of budgeting exercises for the eastern US (Boyer et al. 2002; Schaefer and Alber 2007), and the early 2000s. We

examined the relationship between watershed N input and export to the coast for these systems, and tried to elucidate the factors that explained both absolute and proportionate N export.

2.2 METHODS

Nitrogen budgets were constructed for the early 1990s (with a target year of 1992) and for the early 2000s (target year 2002) for 22 west coast watersheds (Fig. 2.1). The methodology used in constructing these budgets was very similar to that reported in Schaefer and Alber (2007), which was in turn based on the methods developed by Boyer et al. (2002) as part of the International SCOPE N project. We considered atmospheric deposition, fertilizer, net food and feed import, and biological nitrogen fixation as sources of new N to watersheds and compared these to riverine export at the most downstream USGS water quality monitoring station.

2.2.1 Atmospheric deposition

Wet and dry atmospheric N deposition were calculated by constructing Thiessen polygons from data collected at monitoring stations in the National Atmospheric Deposition Program/National Trends Network (NADP 2006) and the Clean Air Status and Trends Network (USEPA 1995), respectively. Organic N deposition was assumed to account for 30% of total atmospheric deposition (Neff et al. 2002), half of which was considered a new input (Boyer et al. 2002). 25% of N volatilized from animal manures and fertilizer (Battye et al. 1994) was assumed to be exported from each watershed and was subtracted from total atmospheric deposition (Boyer et al. 2002).

2.2.2 Fertilizer

Fertilizer use was calculated from county-by-county fertilizer sales data (Battaglin and Goolsby 1994; Ruddy et al. 2006) by weighting the amount of fertilizer sold in each county by the proportion of agricultural land in that county that was located within the watershed. Watershed totals were obtained by summing over all counties in the watershed. Land use was obtained from 1992 and 2001 national land cover data (USGS 1999a–c, 2000a–f; Vogelmann et al. 2001).

2.2.3 Net food and feed import

Net food and feed import is defined as total N consumption (by livestock and humans) minus total N production (by crops and livestock). This quantity will be negative (and hence represent an export) when N production exceeds consumption. Crop production in each county (USDA–NASS 1992, 2002) was multiplied by N content (Lander and Moffitt 1996; USDA–NRCS 2005) and weighted by the proportion of agricultural land in that county that was inside the watershed, then summed over all counties to obtain a watershed estimate. This represents a deviation from the methodology of the SCOPE Project (Boyer et al. 2002), which assumes that agricultural land is distributed evenly throughout a county and therefore weights these data by the proportion of each county inside the watershed. On the west coast of the US, agricultural activity tends to be concentrated in valleys, such that weighting by the proportion of agricultural land within a watershed gives a more accurate estimate of crop and animal production. (Weighting by land area results in estimates ranging from 18 to 716% of the livestock N consumption and 15–554% of the crop production calculated by weighting by agricultural area.) Only crops accounting for 1% or more of harvested cropland in a

watershed were considered, which could result in an underestimate of crop production in those watersheds where a wide variety of crops are grown. Vegetable crop yields were not available as part of the census of agriculture. However, the census did report crop acreages, which were multiplied by reported 1992 or 2002 vegetable yields per acre (CDFA 2002) in order to obtain estimates of vegetable production. Livestock production and consumption were similarly calculated by adjusting for the proportion of agricultural land inside the watershed and multiplying by published consumption and excretion factors (Van Horn 1998).

To estimate human consumption, Boyer et al. (2002) weighted county populations by the proportion of that county located inside the watershed and multiplied by a per-capita annual N consumption rate (5 kg N person^{-1} year^{-1}, Garrow et al. 2000). Because county sizes in the western United States tend to be substantially larger than those in the eastern US, we used the finer-scale data at the census tract level (USBoC 1990, 2000) to estimate population. Weighting by census tract results in human population estimates ranging from 4 to 760% of the estimates obtained from weighting by county; the largest differences were for smaller watersheds that include fewer whole counties. The Willamette watershed constituted a special case because the downstream boundary of this watershed intersects the city of Portland, Oregon. Portland is serviced in part by the Columbia Boulevard Wastewater Treatment Plant, which discharges downstream of the USGS gauging station used to calculate riverine export (see below). We therefore did not include the treatment plant's service area when calculating the population of this watershed.

2.2.4 Biological N fixation

Biological N fixation in agricultural land was calculated by multiplying acreages of leguminous crops in each watershed by published N fixation rates. Forest N fixation included both symbiotic and non-symbiotic components. Boyer et al. (2002) assumed that east coast species of alder cover 10% of wetland area as estimated from land cover data. We applied this same assumption to thinleaf alder (Alnus incana ssp. tenuifolia) on the west coast, with an N fixation rate of 5,000 kg N km^{-2} $year^{-1}$ (Uliassi and Ruess 2002). Red alder (Alnus rubra) is an important nitrogen-fixing tree in coastal areas of the Pacific northwest that can contribute substantially to in-stream nutrient loads (Compton et al. 2003), especially where anthropogenic nitrogen inputs are small (Cairns and Lajtha 2005). We used the Forest Inventory and Analysis (FIA) Program's (USDA–FS 2006) "red alder" class to estimate the coverage of red alder in each watershed and assumed a fixation rate of 8,000 kg N km^{-2} $year^{-1}$ (Binkley et al. 2002). Given that even low levels of alder coverage can result in elevated stream N exports (Cairns and Lajtha 2005), this may be an underestimate of the true input from biological nitrogen fixation due to red alder. There are a number of additional non-agricultural nitrogen-fixing plant species that grow in western North America, including plants of the genera Ceanothus, Cercocarpus, Comptonia, Elaeagnus, Myrica, Purshia, and Shepherdia (Torrey 1978). Given the difficulty of estimating their distribution and prevalence, and the generally small contribution of biological fixation in forest lands to total new nitrogen input, fixation by these species was not considered here. Non-symbiotic N fixation in forest soils was assumed to be 40 kg N km^{-2} $year^{-1}$ (Boyer et al. 2002) and calculated from forest areas reported in the FIA (USDA–FS 2006).

2.2.5 Other sources and sinks of N

Pacific salmon spawning runs can be an important source of marine-derived nutrients to both terrestrial and aquatic environments in many western US stream ecosystems (Gende et al. 2002; Naiman et al. 2002). However, runs have declined by more than 95% from their historical highs (Gresh et al. 2000). An order-of-magnitude estimation for the Siuslaw River, one of the more important salmon streams included in this study, suggests that N derived from current salmon runs would be minimal. The Chinook salmon run is the healthiest run in this river, numbering approximately 11,000 individuals (Siuslaw Basin Council 2002). Based on an average weight of 3.76 kg per fish (Bigler et al. 1996) and an N content of 2.5% (Drake et al. 2006), Chinook salmon would contribute less than 1 kg N km^{-2} $year^{-1}$ to the watershed inputs. This would account for less than 0.1% of the total inputs. We therefore did not include marine-derived nutrients from spawning salmon in these budgets. Finally, we assumed that essentially all cotton grown in a watershed was exported and subtracted this as a non-food crop export. This is in keeping with our treatment of both tobacco and cotton in the southeastern US (Schaefer and Alber 2007).

2.2.6 Total N inputs

Data are presented as the annual average input (in kg km^{-2} $year^{-1}$) of each source of N to a watershed. In addition to the sum of all new N inputs (atmospheric deposition, fertilizer, net food and feed import, and biological N fixation), we also present gross N inputs, which includes net food and feed import only when it represents an import rather than an export. We also calculated an area-weighted value for each input by multiplying inputs by the area of the corresponding watershed and then dividing by the total area of all watersheds.

2.2.7 Riverine N export

N export to the coast was calculated from water quality data collected by the USGS National Water Information System (USGS 2006) at the most downstream water quality gauge. We used the USGS's LOADEST model (Runkel et al. 2004; Booth et al. 2007) to estimate loads. For the 1990s, we did not calculate export for four of the 22 watersheds: the Cuyama, John Day, and Santa Ana, due to insufficient water quality data, and the San Joaquin, due to the difficulty of accounting for N in the many water transfers in this basin. Unfortunately, water quality sampling in the 2000s was very limited and we were only able to calculate riverine export for seven watersheds (Sacramento, Salinas, Santa Ana, Snake, Spokane, Willamette, and Yakima).

We used measurements of unfiltered TKN to obtain estimates of dissolved organic N plus NH_4 for all watersheds. Because the monitoring program took filtered samples for NO_X analysis (USGS parameter 631) more often than unfiltered samples (parameter 630), we used data from filtered samples. Although this may result in an underestimate of NO_X-N loading, NO_X concentrations in filtered samples were often higher than those observed in unfiltered samples and the two were well correlated. Where only unfiltered samples were available (the Spokane River), these were used in the calculations.

Additional adjustments were necessary in three cases. Stream flow measurements were not available for the most downstream water quality station on the Snake River (USGS station no. 13353200; Snake River at Burbank, Washington), so we used stream flow data from a station located approximately 11 km upstream (USGS station no. 13353000; Snake River below Ice Harbor Dam, Washington). This distance should not result in a substantial change in flow, and therefore difference in N export, for a river as

large as the Snake. Similarly, for the Merced River we used a streamflow station (USGS station no. 11272500; Merced River near Stevinson, CA) approximately 6 km upstream of the water quality station (USGS station no. 11273500; Merced River at River Road Bridge near Newman, CA). For the most downstream water quality station on the John Day River (USGS station no. 14048000; John Day River at McDonald Ferry, Oregon), stream flow measurements were only available beginning in April 1994. In order to be able to use water quality data taken prior to this, we developed a relationship between stream flow at this station and at the nearest upstream station (USGS station no. 14046500; John Day River at Service Creek, Oregon), which was then used to estimate stream flow for dates before April 1994 (McDonald Ferry flow = 1.06 × Service Creek flow + 34.59, $R^2 = 0.95$, $P < 0.0001$).

2.2.8 Relationship between export and input

New N input is often considered the primary determinant of N export to the coast (e.g., Howarth et al. 1996; Boyer et al. 2002), although streamflow can also be important (e.g., Lewis 2002). Factors that have been suggested to control percent N export include streamflow (Dumont et al. 2005), temperature (Schaefer and Alber 2007), and residence time (Howarth et al. 2006). We compared both absolute and percent export from the watersheds to total new N input, average annual temperature, average annual streamflow, and average watershed slope (an indicator of residence time). These factors were selected to encompass the various explanations proposed to control N export. Although additional characteristics could also have been used in this analysis, many of them covary—for example, watershed population density and total N inputs; streamflow and precipitation. Streamflow was calculated as described above. Watershed temperatures were calculated

for the 1990s using the DAYMET database (Thornton et al. 1997) and for the 2000s using PRISM data (PRISM Group 2008). Precipitation was also obtained (Thornton et al. 1997, PRISM Group 2008) to allow us to calculate the percentage of precipitation that runs off as streamflow as an additional characteristic of each watershed. Watershed slopes were calculated from a digital elevation model (USGS 1999d).

2.3 RESULTS

2.3.1 Watershed characteristics

The watersheds included in this study ranged in size from 1,531 to 279,438 km^2 (Table 2.1) and were dominated by forest and shrub/grassland in 1992 (Table 2.2). Note that only 1992 land use is presented here, but there were only minor changes in land use in 2001. Northern watersheds tended to be dominated by forest, whereas southern watersheds tended to be dominated by shrub and grasslands. Together, these two categories on average accounted for 81.1% of land cover in western watersheds. The next most important land use was agriculture (area-weighted average = 13.8%), with the highest percentage observed in the San Joaquin (30.4%) and the Willamette (24.3%) River watersheds. Urban area generally accounted for only a small (≤4%) percentage of watershed area, except in the Santa Ana where it accounted for 19.7% of the watershed area. Human population density in 1992 ranged from very low (≤5 people km^{-2} in 10 of the 22 watersheds) to an extreme high of 432 people km^{-2} in the Santa Ana basin. Population densities were similar in 2002, though generally slightly higher. The area-weighted annual average watershed temperature across all watersheds was 8.5°C in 1992 and 9.2°C in 2002 (Table 2.1). Area-weighted annual average precipitation was

649 mm $year^{-1}$ in 1992 and 596 mm $year^{-1}$ in 2002, with the highest in the watersheds of the Nehalem, Willamette, and Eel Rivers. The average watershed slope was 11.7°.

2.3.2 Inputs

New N input to the 22 western US watersheds studied here ranged from 541 to 11,644 kg N km^{-2} $year^{-1}$ in 1992 and from 409 to 10,875 kg N km^{-2} $year^{-1}$ in 2002 (Table 2.3). The watershed with the highest input was the Santa Ana, followed by the San Joaquin. The high input to the Santa Ana was due to high imports of food and feed. This was primarily driven by the high number of dairy cattle and layer chickens in this watershed, which resulted in high animal production. Human N consumption was also high, as the greater metropolitan Los Angeles area takes up a large proportion of this watershed. The San Joaquin watershed had large livestock populations, resulting in high net food and feed import, but also very high fertilizer use due to the large proportion of agricultural area in that watershed. Inputs to all other watersheds were less than 5,000 kg N km^{-2} $year^{-1}$ in both years.

In both periods, fertilizer was the largest source of new N to the region, followed by atmospheric deposition and N fixation in croplands. Fertilizer accounted for 956 kg N km^{-2} $year^{-1}$ (38.6% of gross N inputs) in 1992 and 1,073 kg N km^{-2} $year^{-1}$ (43.6% of gross N inputs) in 2002. Atmospheric deposition averaged 833 kg N km^{-2} $year^{-1}$ (33.7% of gross N inputs) in 1992. In 2002, this source remained second in importance but had decreased to 717 kg N km^{-2} $year^{-1}$ (29.2% of gross N inputs). This was primarily the result of a decline in dry deposition, which was the major component of atmospheric deposition in all watersheds. Atmospheric deposition was higher in southern than in northern watersheds. N fixation in croplands was the third-

largest source of new N to the region in both years and was positively related to the amount of pastureland. This source accounted for an average of 628 kg N km^{-2} $year^{-1}$ (25.4% of gross N inputs) in 1992 and 613 kg N km^{-2} $year^{-1}$ (24.9% of gross N inputs) in 2002. N export due to volatilization was most important in watersheds located in southern California, particularly those of the Salinas, Pajaro, Cuyama, and Santa Ana Rivers.

Net food and feed import was often negative (indicating a net export) because crop production was high in many of the watersheds. Crop production tended to be highest in California, and was generally dominated by hay and pastureland. However, high crop production values did not necessarily result in low N import, since watersheds with high crop production also often had large populations of animals, and hence high consumption of N by animals. In 1992, net food and feed import averaged −578 kg N km^{-2} $year^{-1}$, with half the watersheds exporting N. By 2002, import had increased in many watersheds, primarily as a result of increases in animal production, with an average of −261 kg N km^{-2} $year^{-1}$.

Forest N-fixation generally accounted for only a small percentage of overall N input. The one exception was the Nehalem watershed, where forest N-fixation accounted for 57.5 and 67.7% of gross N inputs in 1992 and 2002, respectively. This was a result of the very high density of red alder in this area, which has a high rate of N-fixation (8,000 kg N km^{-2} $year^{-1}$, Binkley et al. 2002).

2.3.3 Export

Streamflow from the study watersheds ranged from 22 to 1,262 mm $year^{-1}$ in 1992 (Table 2.4), and from 41 to 1,344 mm $year^{-1}$ in 2002. Parts of the western US experienced a drought during the late 1980s and early 1990s, such that the 2000s were

slightly wetter than the 1990s, with streamflow averaging 210 mm $year^{-1}$ in 1992 and 278 mm $year^{-1}$ in 2002. Streamflow was expressed as a percentage of precipitation as a measure of the flushing rate of water in these systems (with a high percentage indicating a shorter residence time). Streamflow ranged from values as low as 5% of precipitation in the Salinas to greater than 65% in the Siuslaw, Willamette, and Nehalem (Table 2.4). Low values may indicate high consumptive use or other losses from the watershed (e.g., evapotranspiration) that decrease runoff. High values were observed in systems that are either entirely (Siuslaw and Nehalem) or partially (Willamette) in the Oregon Coast Range.

Riverine N export ranged from 80 kg N km^{-2} $year^{-1}$ in the Tuolumne to 1,670 kg N km^{-2} $year^{-1}$ in the Nehalem, with an overall average of 165 kg N km^{-2} $year^{-1}$ for the 18 watersheds with available export data in 1992 (Table 2.4). Export ranged from values less than 100 kg N km^{-2} $year^{-1}$ in the Tuolumne, Merced, and Deschutes, to greater than 1,000 kg N km^{-2} $year^{-1}$ in the Siuslaw, Nehalem, and Willamette. These watersheds with high export values are discussed further below. Although there were some differences between the two periods, 2002 export estimates agreed well with those from 1992 for the 7 watersheds for which measurements were available, averaging 165 kg N km^{-2} $year^{-1}$ in 1992 and 195 kg N km^{-2} $year^{-1}$ in 2002. We therefore did not analyze the 1990s and 2000s time periods separately, but rather included observations from both periods for those watersheds for which we had estimates of export in 2002.

Percent N export had an area-weighted average of 12% in 1992 (Table 2.4). The two Coast Range watersheds (the Siuslaw and Nehalem) had extremely high percent export (115% and 70%, respectively). A value greater than 100% would be unsustainable over

the long term and most likely reflects errors inherent in the calculation of both N input and export. Despite the potential error, however, it is apparent that percent export is much higher in these two watersheds than in any of the others we considered (all of which were calculated in the same manner). The Siuslaw and Nehalem are both particularly small watersheds that are primarily forested. As noted above, they receive a great deal of rainfall, a high percentage of which becomes streamflow. These systems may also be highly disturbed. Logging is common in the Coast Range (Ripple et al. 2000) and a high proportion of clearcut area has been shown to increase streamflows in the Western Cascades of Oregon (Jones and Grant 1996), which could result in increased export due to the landscape's inability to retain N for processing. When precipitation as a percentage of streamflow is plotted against percent N export, the Siuslaw and Nehalem are clear outliers (Fig. 2.2). We therefore present the following analysis of the relationships between inputs and riverine N export both with and without these watersheds.

2.3.4 Relationship between input and export

The best single predictor of N export was streamflow, which explained 66% of the variability in the observations (Fig. 2.3a; export = 0.94 × streamflow − 5.65, $R^2 = 0.66$, $P < 0.001$). When the Coast Range systems were excluded, streamflow was still the best predictor of export, but it explained only 41% of the variability (export = 0.62 × streamflow + 65.76, $R^2 = 0.41$, $P = 0.001$). Although N input alone was not important (Fig. 2.3b), including it in the regression with streamflow increased the predictive power of both relationships, but input was more important when the Coast Range systems were excluded (all watersheds, export = 1.06 × streamflow + 0.06 × input − 227.78, $R^2 = 0.82$, $P < 0.001$; excluding

Coast Range, export = 0.76 × streamflow + 0.06 × input − 149.29, $R^2 = 0.77$, $P < 0.001$). The other two variables (temperature and slope) were not related to N export (Fig 2.3c, d) when all watersheds were considered, but slope accounted for another 5% of variability when the Coast Range watersheds were excluded (export = 0.82 × streamflow + 0.05 × input − 22.29 × slope + 137.37, $R^2 = 0.82$, $P < 0.001$).

Streamflow was also the best predictor of percent nitrogen export from these watersheds (all watersheds, % export = 0.05 × streamflow − 2.61, $R^2 = 0.60$, $P < 0.001$; excluding Coast Range, % export = 0.03 × streamflow + 3.18, $R^2 = 0.77$, $P < 0.001$; Fig. 2.4a). In this case, none of the other factors (input, temperature, or slope) were significant alone (Fig. 2.4b–d) or improved the relationship with streamflow any further with or without Coast Range watersheds. Although precipitation was not used in this analysis, it is worth noting that regressions of both total N and %N export against streamflow explained more variance than regressions against precipitation.

2.4 DISCUSSION

Area-weighted average N input to study watersheds was 1,870 kg N km^{-2} $year^{-1}$ in 1992 (Table 2.3). Inputs were generally higher in southern than in northern watersheds. Inputs to most systems had increased by 2002, with an overall, area-weighted average of 2,158 kg N km^{-2} $year^{-1}$. However, 1992 inputs were well correlated with 2002 inputs ($R^2 = 0.94$), indicating that N input increased consistently across the entire study region.

Fertilizer was the most important source of N to the region in both decades, followed by atmospheric deposition. Fertilizer N inputs increased in importance from 1992 to 2002, accounting for 38.6 and 43.6% of gross inputs, respectively, whereas atmospheric

deposition accounted for 33.7% of gross inputs in 1992 and 29.2% in 2002. A simultaneous increase in fertilizer use and a decline in atmospheric deposition resulted in fertilizer becoming an increasingly dominant N source in 2002. The reduction in atmospheric deposition was most likely a result of increasingly stringent emissions standards and cleaner-burning fuels mandated in California, which succeeded in reducing NO_X emissions substantially over the same time period (CARB 2008).

The relative importance of fertilizer as an N source to these watersheds is in agreement with observations in other regions of the world. However, fertilizer input to the western US is generally lower and atmospheric deposition is higher than in other areas. In the Mississippi River basin (McIsaac et al. 2002), the most important source of new N was fertilizer (~2,000 kg N km^{-2} $year^{-1}$), followed by agricultural N fixation (~1,500 kg N km^{-2} $year^{-1}$), whereas atmospheric deposition was approximately 500 kg N km^{-2} $year^{-1}$. In the Changjiang River basin in China, fertilizer N supplied approximately 2,000 kg N km^{-2} $year^{-1}$ (86% of inputs) in 1990 and 4,500 kg N km^{-2} $year^{-1}$ (91% of new N) in 2000, whereas atmospheric deposition was negligible (Liu et al. 2006). In New Zealand (Parfitt et al. 2006), the contribution of fertilizer N to watershed loading was comparable to that reported here (887 kg N km^{-2} $year^{-1}$) but atmospheric deposition was lower (599 kg N km^{-2} $year^{-1}$).

The 1992 observations of watershed N inputs can be compared directly to those on the east coast of the US, which were calculated for the same year using comparable methodologies (Boyer et al. 2002; Schaefer and Alber 2007). Although the range of N inputs on the east coast was lower (835–5,717 kg N km^{-2} $year^{-1}$), the area-weighted average N input was nearly 2-fold greater, 3,136 vs. 1,880 kg N km^{-2} $year^{-1}$ in west coast

watersheds. This difference in area-weighted input was largely the result of differences in crop production, which resulted in an average net export of 568 kg N km^{-2} $year^{-1}$ in food and feed on the west coast compared to an import of 781 kg N km^{-2} $year^{-1}$ on the east coast. Biological N fixation also contributed more new N on the east coast than on the west coast (agricultural N fixation, 716 vs. 628 kg N km^{-2} $year^{-1}$; forest land N fixation, 134 vs. 57 kg N km^{-2} $year^{-1}$ on the east and west coasts, respectively). However, west coast watersheds received more N from both atmospheric deposition (833 vs. 790 kg N km^{-2} $year^{-1}$) and fertilizer (956 vs. 725 kg N km^{-2} $year^{-1}$). The greater fertilizer use is surprising, given that agricultural land use comprised an average of 13.8% of watershed area on the west coast (Table 2.2), compared to 21.2% on the east coast (calculated based on data in Boyer et al. 2002 and Schaefer and Alber 2007), and suggests that fertilizer use is more intensive on the west coast than on the east coast.

Riverine N export was better predicted by streamflow than by watershed N input (Fig. 2.3a). Streamflow has also been found to be a good predictor of N export in undisturbed watersheds, which experience a large range of annual runoff (Lewis et al. 1999; Lewis 2002). However, this finding contrasts with studies on the east coast of the US (Boyer et al. 2002; Schaefer and Alber 2007), in the watersheds surrounding the North Atlantic (Howarth et al. 1996), and in New Zealand (Parfitt et al. 2006), where N loading has been shown to be an excellent predictor of riverine N export. A possible explanation for this difference, at least in comparison to the east coast, is that west coast watersheds have a larger range of streamflow (annual averages ranged from 22 to 1,262 mm $year^{-1}$) than those on the east coast (275–672 mm $year^{-1}$, Boyer et al. 2002; Schaefer and Alber 2007). West coast watersheds also tend to have more pronounced

seasonality in rainfall, such that most of the nitrogen export from these watersheds occurs during the wet season (fall/winter). Unfortunately the studies in the watersheds of the North Atlantic and New Zealand did not report comparable streamflow data which could be used to evaluate the dynamic range of streamflow in comparison to that of inputs.

Although export was best predicted by streamflow, the relationship improved when input was added to the equation (streamflow only, $R^2 = 0.66$; streamflow and inputs, $R^2 = 0.82$). This agrees well with the findings of Smith et al. (2005), who were able to predict riverine N loads from a combination of streamflow and population for 165 watersheds with a range of streamflows roughly comparable to those observed here. For the west coast, we suggest that export of nutrients may be limited primarily by the availability of water to transport them, with the magnitude of inputs playing a secondary role.

The percentage of N input exported to the coast ranged widely, from 2% (Salinas, 2002) to 115% (Siuslaw, 1992). This range is greater than that observed on the east coast, where percent export ranged from 5 to 40% (Boyer et al. 2002; Schaefer and Alber 2007). However, when the Coast Range systems were excluded, the remaining watersheds all exported less than 20% of the N inputs (the Willamette, which is partially in the Coast Range, exported 31% in 1992 and 30% in 2002). The watershed-area-weighted average export was 12% in 1992 (excluding the Coast Range watersheds, which are very small, changed this only slightly and still rounded to 12%). This value, which is substantially below the generally accepted global average of 25% (Galloway et al. 2004; Boyer et al. 2006), is largely a consequence of the fact that some of the largest watersheds in this study, such as the Snake and the Sacramento, tended to export smaller

percentages of the load and so brought down the area-weighted average. Our results correspond well with the results of a modeling study by Dumont et al. (2005). Their Fig. 5 shows percent N export on the west coast of the US to generally be below 14% except in Coast Range watersheds, where percent export was between 27 and 59%.

Percent export was best predicted by streamflow, which could be a consequence of the very large range of streamflows in watersheds of the west coast. Watersheds with extreme values of streamflow may either (in the case of high streamflow) have such a short residence time that N is exported before any processing has taken place, or (in the case of low streamflow) water movement may be so slow that N is processed or stored in the watershed, so percent export is low. The large fraction of the load exported from Oregon Coast Range watersheds, in particular, may be the result of rapid flushing in these systems, which have high streamflows relative to precipitation. These watersheds are also quite small, which could further decrease the residence time of N in them. It would be instructive to know if more N is exported in other mountainous regions where watersheds are small and precipitation is high. Dumont et al. (2005), who also included watersheds with a wide range of streamflows, also found that percent N export was correlated with streamflow.

The west coast results presented here contrast with our east coast observations where we found that percent export was related to temperature (Schaefer and Alber 2007). As described above, US east coast watersheds are more homogenous with respect to streamflow than those on the west coast and thus the effect of temperature may be easier to discern in them. The temperature range on the east coast is also slightly greater (4.2–19.3°C; Boyer et al. 2002; Schaefer and Alber 2007) than on the west coast (6.0–15.5°C).

Although the percentage of N exported from watersheds in the present study did not correlate with temperature, it is worth noting that percent export was lower and less variable at temperatures greater than 12°C (average = 6 ± 4%) as compared to lower temperatures (average = 22 ± 28%). This is in keeping with our east coast observations that percent N export is lower in warmer watersheds.

The apparent difference in the factors controlling N export from west coast watersheds compared to those in other regions suggests that our understanding of the relationship between input and export is incomplete. Export is not always predicted by input, and there is no single relationship that can be used to predict N export based on input for all watersheds. This was most obvious for the small mountainous watersheds of the Oregon Coast Range, which behaved differently from the rest of the region. Comparisons of N budgets for watersheds from other mountainous areas or across watersheds representing a wide range of streamflow may provide the perspective needed to develop an improved understanding of the relative importance of the environmental factors that control N export.

ACKNOWLEDGMENTS

We thank Adrian Burd and Joan Sheldon for useful discussions on statistics, and the staff of the Portland Bureau of Environmental Services for providing shapefiles of wastewater treatment plant service areas. We also thank an anonymous reviewer for helpful comments which improved the manuscript. Funding was provided by the Georgia Coastal Ecosystems LTER Project (NSF Award OCE-0620959) and Coastal Incentive Grant #NA170Z2331.

REFERENCES

Battaglin WA, Goolsby DA (1994) Spatial data in geographic information system format on agricultural chemical use, land use, and cropping practices in the United States. USGS Water Resources Investigations Report 94-4176, US Geologic Survey, Reston, VA. http://water.usgs.gov/pubs/wri/wri944176/

Battye R, Battye W, Overcash C, Fudge S (1994) Development and selection of ammonia emission factors. Final report, EC/R incorporated for EPA Atmospheric Research Assessment Lab, EPA contract number 68-D3-0034

Bigler BS, Welch DW, Helle JH (1996) A review of size trends among North Pacific salmon (Oncorhynchus spp.). *Can J Fish Aquat Sci* 53:455–465.

Binkley D, Sollins P, Bell R, Sachs D, Myrold D (1992) Biogeochemistry of adjacent conifer and alder-conifer stands. *Ecology* 73(6):2022–2033.

Booth G, Raymond P, Oh N-H (2007) LoadRunner. Yale University, New Haven, Connecticut. Available online at http://environment.yale.edu/raymond/loadrunner/

Boyer EW, Goodale CL, Jaworski NA, Howarth RW (2002) Anthropogenic nitrogen sources and relationships to riverine nitrogen export in the northeastern USA. *Biogeochemistry* 57:137–169.

Boyer EW, Howarth RW, Galloway JN, Dentener FJ, Green PA, Vörösmarty CJ (2006) Riverine nitrogen export from the continents to the coasts. Global Biogeochem Cy 20:Art. No. GB1S91

Bricker S, Longstaff B, Dennison W, Jones A, Boicourt K, Wicks C, Woerner J (2007) Effects of nutrient enrichment in the nation's estuaries: a decade of change,

NOAA Coastal Ocean Program Decision Analysis Series No. 26. National Centers for Coastal Ocean Science, Silver Spring, p 328

Cairns MA, Lajtha K (2005) Effects of succession on nitrogen export in the west-central Cascades, Oregon. Ecosystems (NY, Print) 8:583–601CrossRef

CARB California Air Resources Board (2008) California's air quality history key events, www.arb.ca.gov/html/brochure/history.htm. Cited 14 May 2008

CDFA California Department of Food and Agriculture (2002) California Agricultural Resource Directory. California Department of Agriculture, Sacramento, CA

Compton JE, Church MR, Larned ST, Hogsett WE (2003) Nitrogen export from forested watersheds in the Oregon coast range: the role of N_2-fixing red alder. *Ecosystems* (NY, Print) 6(8):773–785.

Drake DC, Naiman RJ, Bechtold JS (2006) Fate of nitrogen in riparian forest soils and trees: an ^{15}N tracer study simulating salmon decay. *Ecology* 87(5):1256–1266.

Dumont E, Harrison JA, Kroeze C, Bakker EJ, Seitzinger SP (2005) Global distribution and sources of dissolved inorganic nitrogen export to the coastal zone: Results from a spatially explicit, global model. *Global Biogeochem* Cycles 19. Art S02(No. GB4)

Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW, Seitzinger SP, Asner GP, Cleveland CC, Green PA, Holland EA, Karl DM, Michaels AF, Porter JH, Townsend AR, Vörösmarty CJ (2004) Nitrogen cycles: past, present, and future. *Biogeochemistry* 70(2):153–226.

Garrow JS, James WPT, Ralph A (eds) (2000) *Human nutrition and dietetics*. Churchill Livingstone, Edinburgh

Gende SM, Edwards RT, Willson MF, Wipfli MS (2002) Pacific salmon in aquatic and terrestrial ecosystems. *Bioscience* 52(10):917–928.

Gresh T, Lichatowich J, Schoonmaker P (2000) An estimation of historic and current levels of salmon production in the northeast Pacific ecosystem: evidence of a nutrient deficit in the freshwater systems of the Pacific northwest. *Fisheries* 25(1):15–21.

Howarth RW, Billen G, Swaney D, Townsend A, Jaworski N, Lajtha K, Downing JA, Elmgren R, Caraco N, Jordan T, Berendse F, Freney J, Kudeyarov V, Murdoch P, Zhao-Liang Z (1996) Regional nitrogen budgets and riverine N and P fluxed for the drainages to the North Atlantic ocean: natural and human influences. *Biogeochemistry* 35:75–139.

Howarth RW, Swaney DP, Boyer EW, Marino R, Jaworski N, Goodale C (2006) The influence of climate on average nitrogen export from large watersheds in the northeastern United States. *Biogeochemistry* 79:163–186.

Jones JA, Grant GE (1996) Peak flow responses to clear-cutting and roads in small and large basins, western Cascades, Oregon. *Water Resour Res* 32(4):959–974.

Lander CH, Moffitt D (1996) Nutrient use in cropland agriculture (commercial fertilizers and manure): nitrogen and phosphorus. Working paper 14, RCAIII. NRCS, United States Department of Agriculture, Washington

Lewis WM Jr (2002) Yield of nitrogen from minimally disturbed watersheds of the United States. *Biogeochemistry* 57/58:275–385

Lewis WM Jr, Melack JM, McDowell WH, McClain M, Richey JE (1999) Nitrogen yields from undisturbed watersheds in the Americas. *Biogeochemistry* 46:149–162.

Liu C, Wang QX, Watanabe M (2006) Nitrogen transported to three Gorges dam from agro-ecosystems during 1980–2000. *Biogeochemistry* 81:291–312.

McIsaac GF, David MB, Gertner GZ, Goolsby DA (2002) Relating net nitrogen input in the Mississippi river basin to nitrate flux in the Lower Mississippi river: a comparison of approaches. *J Environ Qual* 31(5):1610–1622

Naiman RJ, Bilby RE, Schindler DE, Helfield JM (2002) Pacific salmon, nutrients, and the dynamics of freshwater and riparian ecosystems. *Ecosystems* (NY, Print) 5:399–417.

NADP National Atmospheric Deposition Program/National Trends Network (NRSP-3) (2006) NADP Program Office, Illinois State Water Survey, 2204 Griffith Dr., Champaign, IL. Available online at http://nadp.isws.illinois.edu/

Neff JC, Holland EA, Dentener FJ, McDowell WH, Russell KM (2002) The origin, composition and rates of organic nitrogen deposition: a missing piece of the nitrogen cycle? *Biogeochemistry* 57:99–136.

Parfitt RL, Schipper LA, Baisden WT, Elliott AH (2006) Nitrogen inputs and outputs for New Zealand in 2001 at national and regional scales. *Biogeochemistry* 80:71–88.

PRISM Group (2008) Oregon State University. http://www.prism.oregonstate.edu/Cited 5 Feb 2008

Ripple WJ, Hershey KT, Anthony RG (2000) Historical forest patters of Oregon's central coast range. *Biol Conserv* 93:127–133.

Ruddy BC, Lorenz DL, Mueller DK (2006) County-level estimates of nutrient inputs to the land surface of the conterminous United States, 1982–2001. USGS Scientific Investigations Report 2006–5012. US Geological Survey, Reston

Runkel RL, Crawford CG, Cohn TA (2004) Load estimator (LOADEST): A FORTRAN program for estimating constituent loads in streams and rivers. US geological survey techniques and methods book 4, Chap. A5, 69 pp

Schaefer SC, Alber M (2007) Temperature controls a latitudinal gradient in the proportion of watershed nitrogen exported to coastal ecosystems. *Biogeochemistry* 85(3):333–346.

Siuslaw Basin Council (2002) A watershed assessment for the Siuslaw basin. Available online at http://www.inforain.org/siuslaw/

Smith SV, Swaney DP, Buddemeier RW, Scarsbrook MR, Weatherhead MA, Humborg C, Eriksson H, Hannerz F (2005) River nutrient loads and catchment size. *Biogeochemistry* 75:83–107.

Thornton PE, Running SW, White MA (1997) Generating surfaces of daily meteorological variables over large regions of complex terrain. *J Hydrol* (Amst) 190:214–225.

Torrey JG (1978) Nitrogen fixation by actinomycete-nodulated angiosperms. *Bioscience* 28(9):586–592.

Uliassi DD, Ruess RW (2002) Limitations to symbiotic nitrogen fixation in primary succession on the Tanana river floodplain. *Ecology* 83(1):88–103

[USBoC] United States Bureau of the Census (1990) 1990 census of population: general population characteristics, United States. Washington, DC. http://factfinder.census.gov

[USBoC] United States Bureau of the Census (2000) 2000 census of population: general population characteristics, United States. Washington, DC. http://factfinder.census.gov

[USDA–NASS] United States Department of Agriculture—National Agricultural Statistics Service (1992) 1992 Census of Agriculture, Vol. 1, Geographic Area Series. National Agricultural Statistics Service, Washington, DC. http://www.nass.usda.gov/Census_of_Agriculture/1992/index.asp

[USDA–NASS] United States Department of Agriculture—National Agricultural Statistics Service (2002) 2002 Census of Agriculture. Washington, DC. http://www.agcensus.usda.gov/

[USDA-NRCS] United States Department of Agriculture—Natural Resources Conservation Service (2005) PLANTS Database Crop Nutrient Tool. Washington, DC. http://npk.nrcs.usda.gov/

[USDA–FS] United States Department of Agriculture–Forest Service (2006) Forest Inventory and Analysis National Program. Arlington, VA, 2006. http://www.fia.fs.fed.us/tools-data/data/

[USEPA] United States Environmental Protection Agency (1995) Clean Air Status and Trends Network. Washington, DC. http://www.epa.gov/castnet/

[USGS] United States Geological Survey (2006) National Water Information System. Reston, VA. http://waterdata.usgs.gov/nwis/

[USGS] USGS United States Geological Survey (1999a) Idaho land cover data set. US Geological Survey, Sioux Falls

[USGS] USGS United States Geological Survey (1999b) Oregon land cover data set. US Geological Survey, Sioux Falls

[USGS] USGS United States Geological Survey (1999c) Washington land cover data set. US Geological Survey, Sioux Falls

[USGS] United States Geological Survey (1999d) National elevation dataset. USGS EROS Data Center, Sioux Falls, SD. http://gisdata.usgs.net/ned/

[USGS] USGS United States Geological Survey (2000a) Montana land cover data set. US Geological Survey, Sioux Falls

[USGS] USGS United States Geological Survey (2000b) Nevada land cover data set. US Geological Survey, Sioux Falls

[USGS] USGS United States Geological Survey (2000c) California-north land cover data set. US Geological Survey, Sioux Falls

[USGS] USGS United States Geological Survey (2000d) California-south land cover data set. US Geological Survey, Sioux Falls

[USGS] USGS United States Geological Survey (2000e) Utah land cover data set. US Geological Survey, Sioux Falls

[USGS] USGS United States Geological Survey (2000f) Wyoming land cover data set. US Geological Survey, Sioux Falls

Van Horn HH (1998) Factors affecting manure quantity, quality, and use. In: Proceedings of the Mid-South ruminant nutrition conference, Dallas-Ft. Worth, Texas Animal Nutrition Council, 7–8 May 1998, pp 9–20

Vogelmann JE, Howard SM, Yang L, Larson CR, Wylie BK, Van Driel N (2001) National land cover database 2001 (NLCD 2001). US Geological Survey, EROS data center, MRLC project, Sioux Falls, SD, http://seamless.usgs.gov/

Table 2.1. General characteristics of western US watersheds for 1992 and 2002.

Watershed	Abbrev.	Area (km^2)	Temperature (°C)		Precipitation (mm $year^{-1}$)		Slope (°)	Persons km^{-2}	
			1992	2002	1992	2002		1992	2002
Spokane	SPO	9,932	6.3	7.0	1,135	870	19.2	7	10
Yakima	YAK	14,542	7.6	7.7	653	640	11.7	15	18
Snake	SNA	279,438	6.0	6.8	537	444	11.6	4	5
Nehalem	NEH	1,747	9.0	9.5	1,862	2,071	13.9	5	6
Deschutes	DES	27,787	7.2	7.6	549	422	6.9	4	6
John Day	JDY	19,764	7.3	8.2	447	349	13.3	1	1
Willamette	WIL	28,992	9.6	10.0	1,499	1,380	12.0	11	60
Siuslaw	SIU	1,531	10.7	11.8	1,584	1,524	19.1	4	4
Rogue	ROG	10,188	9.5	9.7	959	877	15.7	20	24
Klamath	KLA	40,356	8.1	8.9	786	789	13.0	3	3
Eel	EEL	8,058	10.9	12.1	1,205	1,420	18.2	4	4
Russian	RUS	3,470	13.6	14.8	932	1,045	13.8	86	102
Sacramento	SCR	68,332	11.5	12.2	802	854	10.1	28	32
Stanislaus	STN	2,485	10.0	10.0	822	861	14.4	5	19
San Joaquin	SJQ	72,129	13.7	14.0	417	432	10.1	31	38
Tuolumne	TUO	4,307	9.8	10.6	704	796	14.1	15	23
Merced	MER	2,876	10.5	11.8	697	747	15.5	4	7
Pajaro	PAJ	3,063	14.0	14.8	406	429	13.6	33	44
Salinas	SAL	10,568	13.9	14.9	478	378	13.5	10	12
Cuyama	CUY	2,279	13.4	13.8	497	313	16.2	3	1
Santa Clara	STC	1,694	15.5	16.3	460	343	17.4	96	125
Santa Ana	STA	3,881	15.2	15.7	536	372	12.0	432	518
Area-weighted average			8.5	9.2	649	596	11.7	14	19

Table 2.2. Land use in western US watersheds for 1992.

Watershed	Forest (%)	Shrub and grasslands (%)	Agriculture (%)	Urban (%)	Wetland (%)	Water (%)	Other (%)
SPO	81.2	6.1	3.7	1.0	0.1	2.0	5.9
YAK	36.4	41.9	15.2	1.8	0.2	0.8	3.8
SNA	27.2	55.6	12.6	0.3	0.6	0.7	3.0
NEH	93.6	0.2	1.1	0.1	0.0	0.2	4.8
DES	43.4	48.1	5.4	0.4	0.4	0.6	1.6
JDY	46.2	48.0	5.0	0.1	0.2	0.1	0.6
WIL	65.3	3.9	24.3	3.0	0.3	0.9	2.2
SIU	94.7	2.4	1.7	0.0	0.0	0.1	1.0
ROG	80.6	9.6	6.9	1.1	0.2	0.4	1.2
KLA	66.2	22.4	5.9	0.2	2.3	1.6	1.3
EEL	66.6	31.9	0.4	0.2	0.0	0.3	0.6
RUS	47.4	32.7	15.5	3.2	0.1	0.8	0.3
SCR	51.0	29.9	13.7	1.7	0.8	1.9	1.0
STN	64.1	25.9	4.8	1.2	0.1	1.7	2.3
SJQ	26.1	36.7	30.4	1.8	0.4	0.7	3.9
TUO	48.0	38.6	4.9	1.4	0.1	1.9	5.2
MER	53.1	36.5	5.6	0.4	0.7	0.6	3.0
PAJ	23.9	58.6	13.9	1.9	0.0	0.1	1.6
SAL	17.6	65.8	12.0	0.8	0.0	0.2	3.5
CUY	18.0	68.0	5.9	0.2	0.0	0.0	8.0
STC	15.4	74.9	1.9	4.0	0.0	0.8	3.0
STA	20.6	48.3	8.5	19.7	0.4	0.6	2.0
Area-weighted average	38.4	42.7	13.8	1.0	0.6	0.9	2.6

Table 2.3. Inputs to watersheds on the west coast of the US in 1992 and 2002. All values in kg N km^{-2} $year^{-1}$.

Watershed	Atmospheric deposition		Fertilizer		Net food and feed import		Biological N fixation in ag. lands		Biological N fixation in forest lands		Non-food crop export		Total	
	1992	2002	1992	2002	1992	2002	1992	2002	1992	2002	1992	2002	1992	2002
SPO	379	496	165	75	−82	−53	83	41	41	52	0	0	587	612
YAK	768	469	1,002	1,313	−255	−54	940	895	23	67	0	0	2,478	2,691
SNA	538	535	652	713	−907	−740	633	598	44	33	0	0	960	1,140
NEH	838	839	19	75	79	88	83	48	1,378	2,199	0	0	2,398	3,249
DES	716	555	265	240	−563	−655	547	542	37	22	0	0	1,001	704
JDY	604	497	114	67	−775	−787	637	614	19	18	0	0	597	409
WIL	723	548	1,932	1,787	78	341	399	154	270	362	0	0	3,402	3,192
SIU	689	480	32	204	33	28	40	13	153	208	0	0	947	933
ROG	647	511	119	120	111	100	264	175	44	107	0	0	1,185	1,012
KLA	789	513	207	188	−378	−498	458	511	160	52	0	0	1,236	766
EEL	1,650	598	59	99	−160	−80	199	73	66	67	0	0	1,815	757
RUS	1,566	501	388	1,346	1,281	592	513	1,204	34	34	0	0	3,782	3,676
SCR	1,112	627	972	1,214	−2,126	−945	561	544	21	40	0	−17	541	1,463
STN	1,255	1,157	401	620	382	524	439	396	26	37	0	−2	2,473	2,731
SJQ	1,392	1,607	2,962	3,219	1,044	1,933	1,006	1,152	10	18	−224	−335	6,190	7,594
TUO	1,263	1,176	510	711	642	917	270	343	20	32	0	−3	2,704	3,175
MER	1,314	1,193	338	721	153	639	164	440	68	27	−14	−56	2,023	2,963
PAJ	1,838	1,018	1,019	1,369	−573	−929	1,332	1,374	19	27	0	0	3,635	2,859
SAL	1,708	1,160	2,127	3,378	−1,337	−1,360	815	689	15	41	0	0	3,328	3,908
CUY	819	1,179	994	1,179	−350	−694	495	547	7	18	−5	0	1,961	2,229
STC	3,920	3,287	83	290	459	592	41	24	5	94	0	0	4,509	4,287
STA	3,967	3,064	658	949	5,711	6,266	1,286	597	25	7	−4	−8	11,644	10,875
Area-weighted average	833	717	956	1,073	−578	−261	628	613	57	57	26	41	1,880	2,160

Table 2.4. N export and related characteristics for US west coast watersheds in 1992 and 2002. '–' indicates that data were not available.

Watershed	Streamflow (mm year^{-1})		Streamflow as a % of precipitation		Riverine N export (kg N km^{-2} year^{-1})		Riverine N export as a % of input	
	1992	2002	1992	2002	1992	2002	1992	2002
SPO	516	550	45	63	117	106	20	17
YAK	183	209	28	33	194	185	8	7
SNA	136	209	25	47	93	137	10	12
NEH	1,262	1,344	68	65	1,670	–	70	–
DES	170	190	31	45	71	–	7	–
WIL	987	1,021	66	74	1,065	959	31	30
SIU	1,026	1,165	65	76	1,086	–	115	–
ROG	405	507	42	58	114	–	10	–
KLA	290	400	37	51	115	–	9	–
EEL	704	953	58	67	334	–	18	–
RUS	466	637	50	61	329	–	9	–
SCR	252	344	31	40	104	119	19	8
STN	205	382	25	44	106	–	4	–
TUO	133	281	19	35	80	–	3	–
MER	110	225	16	30	99	–	5	–
PAJ	34	71	8	17	460		13	
SAL	22	41	5	11	88	95	3	2
STA	77	90	14	24	512	501	4	5
Area-weighted average	235	291	30	47	165	195	12	12

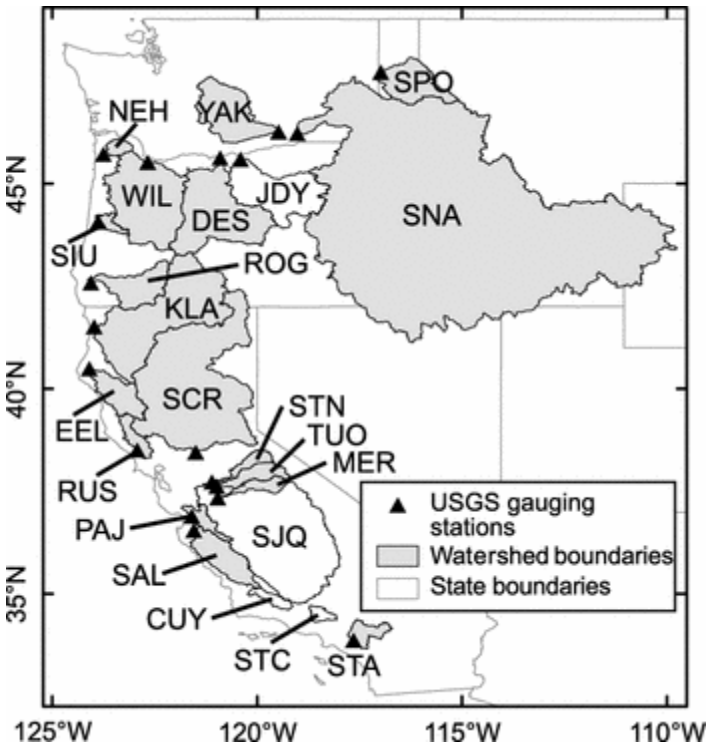


Figure 2.1. US west coast watersheds considered in this study. Abbreviations are as in Table 2.1. Watersheds not shaded (CUY, JDY, SJQ, and STC) were not included in the analysis of export (see text).

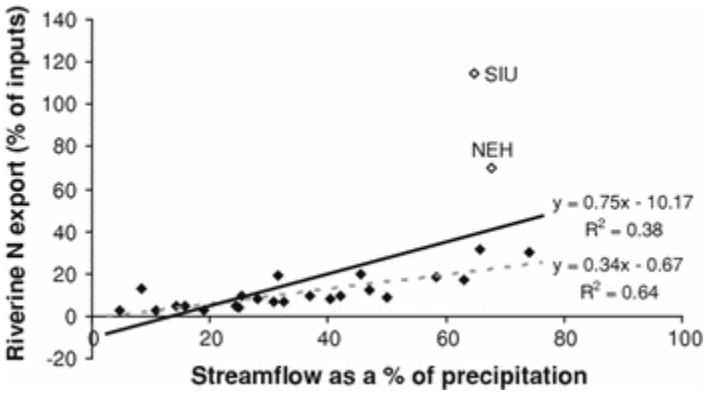


Figure 2.2. Streamflow as a percentage of precipitation versus riverine N export as a percentage of N inputs for watersheds on the west coast of the US. Open symbols: Coast Range watersheds (SIU, Siuslaw; NEH, Nehalem). Solid regression line includes all data; dashed regression line excludes the Coast Range systems.

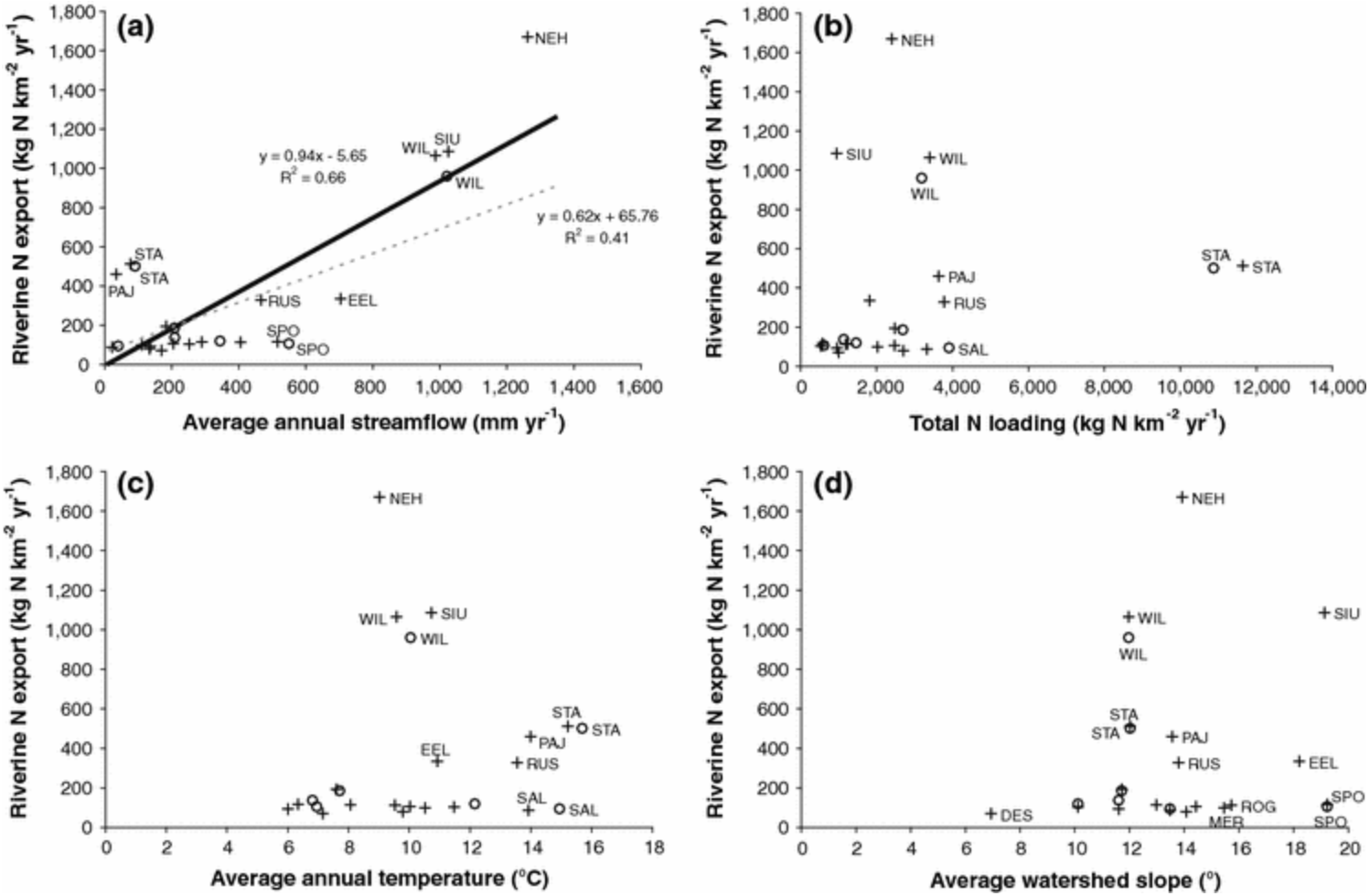


Figure 2.3. (a) Average annual streamflow, (b) total N inputs, (c) average annual temperature, and (d) average slope of watersheds on the west coast of the US versus riverine N export at the most downstream USGS water quality gauging station. Crosses 1990s data; circles 2000s data. Solid regression line includes all data; dashed regression line excludes the Coast Range systems (Siuslaw and Nehalem). Abbreviations (as in Table 2.1) are provided for selected watersheds.

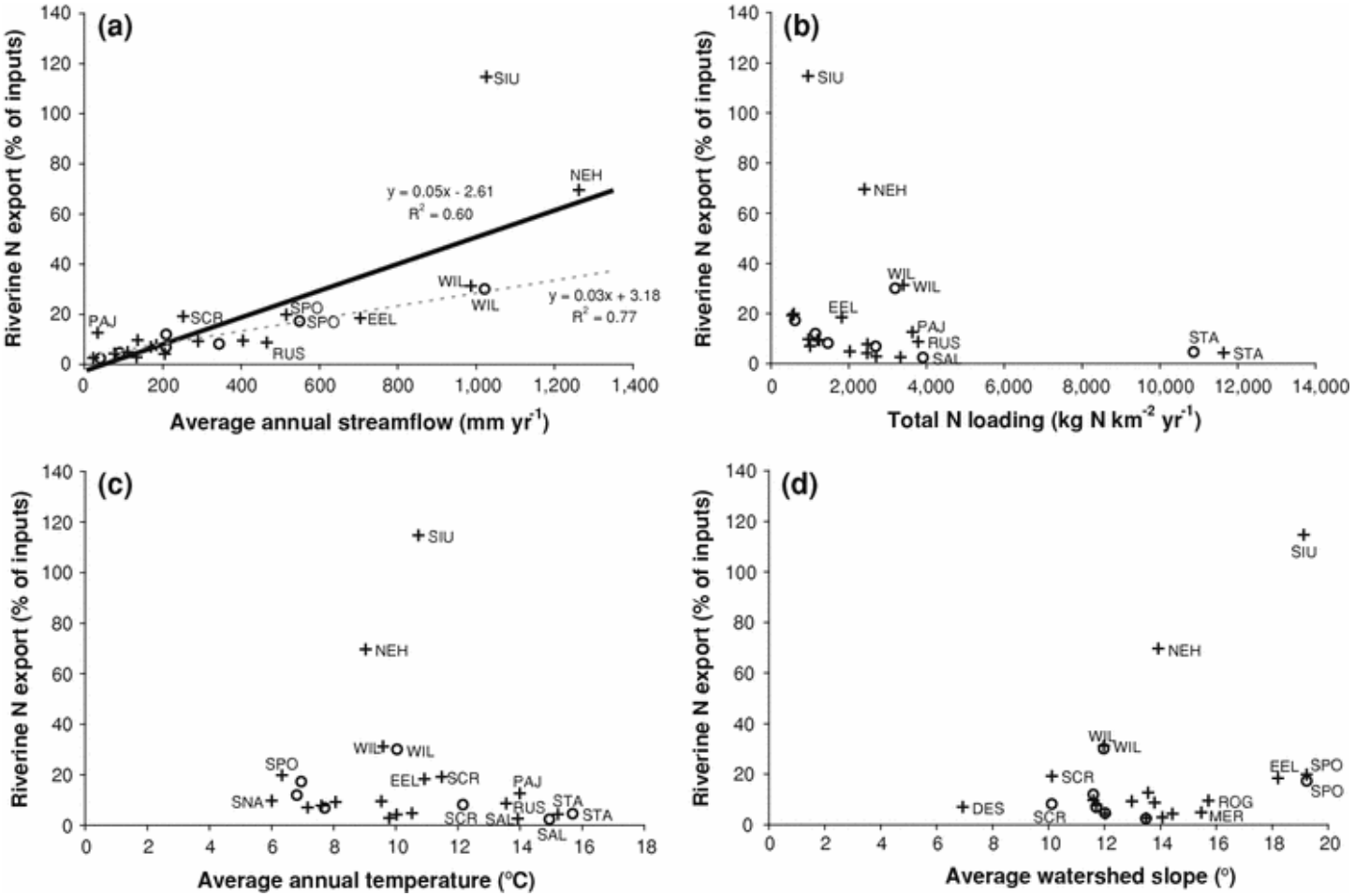


Figure 2.4. (a) Average annual streamflow, (b) total N inputs, (c) average annual temperature, and (d) average slope of watersheds on the west coast of the US versus riverine N export expressed as a percentage of watershed input. Crosses 1990s data; circles 2000s data. Solid regression line includes all data; dashed regression line excludes the Coast range systems (Siuslaw and Nehalem). Abbreviations (as in Table 2.1) are provided for selected watersheds.

CHAPTER 3

INVESTIGATING SOURCES OF NITROGEN IN THE ALTAMAHA RIVER, GEORGIA BASED ON CONCENTRATIONS AND STABLE ISOTOPES OF NITROGEN[1]

[1]Schaefer, S.C., J.A. Brandes, and M. Alber. To be submitted to *Biogeochemistry*.

ABSTRACT

Excess nitrogen in aquatic systems is a major problem worldwide, and an understanding of its sources is crucial. We evaluated N inputs to the 7 subwatersheds of the Altamaha River, Georgia, and N concentrations and loads in the corresponding rivers. Cumulative upstream N inputs to the land surface were calculated based on the SCOPE methodology (Boyer et al. 2002) for base year 2007. Inputs in the upper reaches of the greater Altamaha watershed were dominated by high populations of both humans and animals, whereas those in the lower portion of the watershed were dominated by agricultural sources. In-stream nitrogen concentrations (NH_4^+, NO_2^-, NO_3^-, DON, and PON), isotope composition (NO_3^-, PON), and associated parameters (temperature, conductivity, pH, and anions) in the rivers within each subwatershed were measured 13 times between July 2010 and July 2011. No seasonality was observed in in-stream nitrogen concentrations, but loads were highest in late winter and spring when streamflow was highest. Concentrations tended to be highest in the Ocmulgee River and lowest in the Little Ocmulgee River, one of two blackwater streams in the system. Concentrations were also low in the Upper Oconee River, where the sampling site was located directly below a hydropower dam. There were a number of significant relationships between specific cumulative upstream input factors and both concentrations and loads in the Altamaha subwatersheds. Local inputs were not well-related to in-stream N, suggesting that N inputs to the lower portion of the greater Altamaha watershed come from well upstream. However, both NO_3^- and TN concentrations and loads were best related to upstream human population density. The isotopic signature of nitrate generally was similar to literature values of isotopes in manure and sewage. In the blackwater Little

Ocmulgee tributary, where NO_3^- concentrations were low, nitrate with an isotopic signature indicating atmospheric origin predominated, suggesting that background nitrate in this system could be of atmospheric origin. An isotope mixing model with atmospheric and sewage/manure end members provided some additional evidence for this conclusion. Samples taken in headwater streams with watersheds of uniform land use exhibited a similar pattern, with undisturbed forest streams also containing nitrate of atmospheric origin. This suggested that in this system there is a background of atmospheric nitrate that is overwhelmed at higher concentrations by sewage and manure. While N processing was not measured directly in this study, there was little evidence of in-stream loss of N, particularly in the lower portions of the watershed where N was transported downstream fairly conservatively. Only a small proportion of the inputs to each subwatershed were exported in streamflow (generally less than 10%). Thus, N processing in this watershed is most likely occurring primarily on the land surface, rather than in the stream channel.

3.1 INTRODUCTION

Nitrogen (N) is an element important to all life, but oversupply of reactive N, which is available to organisms, is now causing problems in natural systems throughout the world (Howarth et al. 2002). Inputs of reactive N to the global N cycle are now nine times higher than in pre-industrial society as a result of the invention of the Haber-Bosch process (Galloway and Cowling 2002). Coastal systems are especially vulnerable to the effects of increased N (Bricker et al. 2007); however, rivers are also subject to the detrimental effects of excess nutrients, particularly the growth of undesirable algal species and decreased oxygen concentrations (Hilton et al. 2006). In order to address

eutrophication, it is essential to understand the relationships between watershed N inputs, N loads to rivers, and in-stream N concentrations.

Both watershed N inputs and human population densities are related to N loads in rivers. Studies such as the SCOPE Nitrogen Project (e.g. Howarth et al. 1996, Boyer et al. 2002) found riverine N export to be related to anthropogenic inputs to the land surface, although the percentage of N exported varies among regions (e.g. Schaefer and Alber 2007a), ranging from 0 to 100% in a worldwide survey (Green et al. 2004). Human population densities are also known to be correlated with the loading of both nitrate and total N to rivers (Caraco and Cole 1993, Vitousek et al. 1997).

Increased N concentrations in streamwater lead to increased N loads over longer time scales (Goolsby & Battaglin 2001, Justić et al. 2003); however, in-stream N concentrations can be highly variable both temporally and spatially as a result of changing water column conditions and spatial heterogeneity of the river channel, especially during low-flow periods when the exchange between the main channel and backwaters is constrained (Houser & Richardson 2010). As a result, N inputs from the land surface may not translate directly into changes in in-stream concentrations. Thus, less work has focused on the relationship between nitrogen concentrations and anthropogenic activity. However, a study of large rivers worldwide found a strong relationship between population density and nitrate concentration (Peierls et al. 1991). Total population has been shown to be related to in-stream N concentrations in the Changjiang River in China (Yan et al. 2003), and some broad-scale work suggests that NO_3^- concentrations, like loads, are related to human population densities (Cole et al. 1993). However, focus on N concentrations is important, as excess concentrations of

nutrients can push streams past a change point to eutrophication (Miltner 2010). Furthermore, once the streamwater reaches coastal ecosystems, concentrations rather than loads are the main control on phytoplankton biomass in estuaries (Monbet 1992).

Nitrate (NO_3^-) is a major constituent of in-stream N in systems affected by human activity (Meybeck 1982). Although ammonium is preferred as a substrate by phytoplankton, nitrate is nevertheless biologically available and can fuel high growth rates (Dortch 1990). It is also an important control on bacterial production, especially at high concentrations (Middelburg & Nieuwenhuize 2000). However, a number of transformations can take place to change NO_3^- concentrations, making it difficult to link concentrations to inputs (Kellman & Hillaire-Marcel 2003). In particular, denitrification can remove a significant portion of in-stream NO_3 (Seitzinger et al. 2002, Ostrom et al. 2002), whereas nitrification, also an important process in streams, creates new NO_3 from ammonium.

Stable isotopes are a useful tool for identifying the source of compounds upon which fractionation processes have acted. Recent advances have made the measurement of stable isotopes of both N and O at environmental levels of NO_3^- possible (Kendall et al. 2007). These types of dual isotope techniques, which examine both $\delta^{15}N$ and $\delta^{18}O$, can be useful in determining the origin of in-stream NO_3^-, with anthropogenic sources typically exhibiting enriched $\delta^{15}N$ and $\delta^{18}O$ (Kendall et al. 2007). A dual-isotope approach to the analysis of NO_3^- has been used to distinguish between atmospheric N, fertilizer, soil nitrification, and manure and sewage (Mayer 2005). Studies have also shown a link between land use and nitrate concentrations and isotopic composition (Chang et al. 2002, Lefebvre et al. 2007). Isotopes can also provide some inferences on N

processing within the watershed. For example, Spoelstra et al. (2001) used N and O isotopes to determine that microbially nitrified NO_3 was a source of instream NO_3^-.

Data from sub-watersheds of a large system can provide information on the relative importance of different N sources and on the relative contribution of different sub-watersheds to downstream flow, as well as information on differences between seasons. For example, Battaglin et al. (2001) used $\delta^{15}N$ and $\delta^{18}O$ in nitrate to investigate the sources of N in the watershed of the Mississippi River, finding differences among sites as well as evidence for seasonal shifts in isotope abundance. $\delta^{15}N$ and $\delta^{18}O$ values on the main stem of the Mississippi increased from spring to fall while flow decreased, suggesting that the proportions of water and nitrate deriving from sources such as groundwater and agricultural tile drainage were not the same over the course of the year.

This study used a simple mass balance watershed nitrogen budget similar to that of the SCOPE studies (e.g. Boyer et al. 2002) combined with in-stream concentration and isotopic measurements throughout the watershed in order to elucidate N processes in the Altamaha River, Georgia, watershed. The broad goals of this research are to understand which sources of N to a watershed reach the water and whether there are relationships between inputs and concentrations or loads.

3.2 METHODS

3.2.1 Site Description

The greater Altamaha River watershed, comprising approximately 36,000 km^2, is one of the largest watersheds on the east coast of the United States and the largest watershed contained solely within the state of Georgia. The watershed is approximately 50%

forested (much of it in managed pine plantations). 20% is agricultural land, and 11% is urban (NARSAL 1998). The urban areas (and hence the population) are concentrated in the upper reaches of the watershed, particularly the Upper Ocmulgee subwatershed, which contains a significant portion of the city of Atlanta. The lower portion of the watershed is more heavily dominated by agricultural land use, and the proportion of wetland area is also higher in this region.

The mainstem Altamaha River is formed by the confluence of its two main tributaries, the Oconee and Ocmulgee Rivers. Two smaller blackwater streams, the Little Ocmulgee and the Ohoopee, are also part of the watershed. The Little Ocmulgee joins the Altamaha just above the confluence of the Oconee and the Ocmulgee, and the Ohoopee joins the mainstem Altamaha.

There are three large reservoirs on the Oconee and Ocmulgee Rivers formed by major (greater than 50 feet) dams, which are used primarily for hydropower generation (National Atlas of the United States 2006). Of these, Wallace Dam (forming Lake Oconee) on the Upper Oconee River is the largest. Along with Sinclair Dam just downstream, these two impoundments form a nearly continuous reservoir along a substantial stretch of the Upper Oconee River.

3.2.2 Watershed N inputs

USGS 8-digit HUC units were used to subdivide the greater Altamaha watershed into seven subwatersheds. These subwatersheds were the Upper and Lower Ocmulgee, Upper and Lower Oconee, Little Ocmulgee, and mainstem Altamaha. Total N inputs were calculated for each subwatershed for the base year of 2007. The inputs to the land surface that were considered were atmospheric deposition, net food and feed import, fertilizer

use, and biological N fixation. These inputs were calculated using methods previously described in Schaefer and Alber 2007a, with the exception of atmospheric deposition, which was calculated using county values derived from the National Atmospheric Deposition Program by Ruddy et al. (2006) and multiplying by the fraction of the county located within each subwatershed.

Although inputs were calculated for each individual subwatershed ("local inputs"), the entire area can be viewed as a set of nested subwatersheds that all drain to the lower Altamaha. N input was therefore also calculated on a cumulative basis, thereby including upstream inputs as one of the inputs to a lower system. These summed estimates were used to evaluate inputs to the entire upstream area that potentially contributing to observed in-stream physical and chemical parameters. For example, while inputs to the Upper Oconee included only inputs within that subwatershed, cumulative total inputs to the Lower Oconee included inputs within both the Upper and the Lower subwatersheds, whereas inputs to the mainstem Altamaha included inputs to the entire watershed.

3.2.3 Sampling sites

Each of the seven subwatersheds was sampled monthly between July 2010 and July 2011 (Figure 3.1). For each sampling event, water from all 7 locations was collected over the course of approximately 8 hours. Rivers were sampled at the accessible highway bridge nearest to the outlet of each HUC (Figure 3.2), except for the mainstem Altamaha, which was sampled above the extent of tidal influence at the highway bridge nearest the final USGS stream gauge. Note that The Upper Oconee sampling station was located 6.5 kilometers below Lake Sinclair.

Surface water was collected by lowering a bucket on a rope from the highway bridge to the approximate middle of the channel. One sample was collected at each site. Care was taken to avoid any obvious surface film. 1-2 liters of whole water were collected in large Nalgene bottles for particulate analysis and stored on ice until filtration. For dissolved constituents, water was filtered through an ashed 49 mm Whatman GF/F filter in the field and filled into 30-60 ml Nalgene bottles which were stored on ice until return to the laboratory (a maximum of ~8 hours), whereupon they were frozen at -4°C. pH, temperature, and conductivity were measured in the field using a Fisher Scientific Accumet AP85 waterproof handheld meter.

In order to shed additional light on the potential sources of in-stream N, headwater streams with watersheds containing uniform land use were targeted for one-time sampling. Three types of land uses were targeted: row crop agriculture, forested, and urban. Sampling sites for small streams were chosen by overlaying a GIS land use coverage over road and stream networks. Areas of relatively uniform land use were identified and sample streams that intersected with public roads were chosen, thereby permitting access from the public right-of-way (Figure 3.3). Sampling was conducted during April 2013 in four streams of each land use.

3.2.4 Particulate Analyses

Raw water collected in the field was filtered through ashed and pre-weighed 25 mm GF/F filters under low pressure using a filter manifold. Filtering continued until the filters were clogged, and the total volume passed through each filter was recorded. Filters were then folded, wrapped in pre-ashed aluminum foil, and frozen until analysis of TSS, percent C and N, and $\delta^{15}N$.

For percent carbon and nitrogen analysis, triplicate filters were placed in aluminum dishes and dried overnight at 65°C, then weighed to allow calculation of total suspended sediment concentrations. Inorganic C was volatilized by placing filters in a dessicator overnight along with a beaker of approximately 100 ml of concentrated fuming HCl. Following this treatment, filters were again placed in a drying oven at 65°C overnight before being wrapped in tin cups and analyzed for percent N and C on a ThermoFinnigan Flash-EA elemental analyzer.

For particulate isotope analysis, filters were also dried overnight in a drying oven. Because particulate N isotopes were the primary analyte of interest, inorganic C was not volatilized in this case; rather, the filters were immediately wrapped in tin foil cups and analyzed on a Flash-EA 1112 Series connected to a Delta V Plus isotope ratio mass spectrometer along with a chitin standard.

3.2.5 Dissolved Constituents

3.2.5.1 Total Dissolved Nitrogen

Samples were analyzed for total dissolved nitrogen (TDN) on a Shimadzu Instruments TOC 5000 which was coupled to an Antek Instruments model 7020 NO analyzer. Samples were run in triplicate with amino acetic acid standards (Joye 2009). Dissolved organic nitrogen (DON) was calculated by subtracting inorganic N (measured as described below) from TDN.

3.2.5.2 Streamwater anions

Anions in streamwater, including NO_3^-, NO_2^-, PO_4^{3-}, SO_4^{2-}, and Cl^-, were measured by ion chromatography. Standard solutions and water samples were filled into 1.5 ml plastic vials with split septum caps (Environmental Express, Inc.). Samples were run on a

Dionex ICS-2000 Ion Chromatography System with a 2 cm analytical column, controlled by Chromeleon Chromatography Management System.

3.2.5.3 Ammonium

Ammonium concentrations in the samples were measured based on the colorimetric method of Koroleff (1983) as modified by Sheldon and Wiebe (pers. comm). Test tubes containing residual material from previous analyses were emptied and rinsed four times with DI water, then shaken vigorously until only small and relatively uniform water droplets remained. 5 ml of each sample was added to triplicate 20 x 150 mm test tubes. Three reagents were added to each test tube: 0.1 ml of a magnesium reagent (45 g NaCl and 20 g $MgSO_4 \bullet 7H_2O$ in 200 ml water with several drops of NaOH, boiled to drive off any ammonia), 250 µl of phenol/nitroprusside (0.2 g nitroprusside and 19 g phenol in 500 ml water), and 250 µl hypochlorite (750 mg available Cl^- [Clorox bleach] in 500 ml of 0.5 N low-nitrogen NaOH). Test tubes were vortexed after each reagent addition, then capped with Teflon-lined screw caps and allowed to react overnight in the dark. Absorbance was measured at 630 nm on a Shimadzu UV-1601 spectrophotometer.

3.2.5.3 NO_3 isotopes

Nitrogen and oxygen isotopes in dissolved NO_3^- were analyzed following the methods of McIlvin and Altabet (2005) and Ryabenko et al. (2009). This chemical method uses a two-step conversion process, from NO_3^- to NO_2^- and from NO_2^- to N_2O, which can then be analyzed on a gas chromatograph coupled to a mass spectrometer:

$$NO_3^- + Cd + H_2O \rightarrow NO_2^- + Cd(OH)_2 \qquad \text{(Eq. 1)}$$

$$HNO_2 + HN_3 \rightarrow N_2O + N_2 + H_2O \qquad \text{(Eq. 2)}$$

3.2.5.3.1 NO_3^- to NO_2^- conversion

Nitrite was reduced to nitrate using spongy cadmium. To make spongy cadmium, zinc sticks were acid washed in 10% HCl and rinsed with DI water. The zinc sticks were then placed into test tubes, covered with a solution of 20% $CdSO_4$, and allowed to react 6-8 hours. Zinc sticks were then removed, the remaining solution acidified with 6 N HCl, and the spongy cadmium transferred from the test tubes into a larger container. Cadmium was covered with 6 N HCl, then rinsed approximately 10 times with DI water. The spongy cadmium was stored under DI water to prevent formation and inhalation of cadmium dust, and was recovered and regenerated with HCl and DI water rinses before every use.

Reaction yield was determined to be best at concentrations of 20 µM NO_3; therefore, samples with concentrations greater than 20 µM were diluted to 20 µM. Samples with concentrations less than 20 µM were used undiluted.

5 ml of each sample was pipetted into triplicate 15 ml polypropylene centrifuge tubes. 1.49 g of NaCl was added to each tube to bring the salt concentration to 5 M. The addition of chloride ions is useful both in increasing the efficiency of NO_3^- to NO_2^- reduction (Ryabenko et al. 2009) and in catalyzing the conversion of NO_2^- to N_2O (McIlvin and Altabet 2005). 100 µl of 1M imidazole buffer and 0.4 g of spongy cadmium were added to each tube. Tubes were capped with plug seal caps and shaken on a horizontal shaker table at 150 rpm for 20 hours. Reaction yield was checked on a Technicon Autoanalyzer II.

3.5.2.3.2 NO_2^- to N_2O conversion

For the conversion to nitrous oxide, samples were transferred to gas-tight 12 ml borosilicate exetainers (538W, Labco Limited, High Wycombe, UK). In order to keep N_2O volumes as consistent as possible, the lesser of 5 ml (the maximum possible while

still maintaining sufficient headspace in the vial) or a sample volume containing 20 nmol N was transferred to each vial. Vials were capped and the caps tightened until the septa just began to depress in the center. Each vial was purged for approximately 10 minutes with a gentle flow of high-purity compressed N_2 gas using 22-gauge deflected point non-coring septum penetration needles.

NO_2 reacts with azide to form N_2O under acidified conditions (McIlvin and Altabet 2005):

$$H_2NO_2^+ + HN_3 \rightarrow N_3NO + H_2O + H^+ \qquad \text{(Eq. 1)}$$

$$N_3NO \rightarrow N_2O + N_2 \qquad \text{(Eq. 2)}$$

A 20% acetic acid solution (1 ml acetic acid in 4 ml DI water) and a 2 M sodium azide solution (0.6501 g NaN_3 in 5 ml) were prepared separately and then combined in a 12 ml glass vial capped with a lid with a PTFE silicone septum. The headspace of this vial was purged for approximately 10 minutes with a gentle flow of high-purity compressed N_2 to remove any N_2O from atmospheric sources or that may have been created during reagent preparation.

Using a disposable 1 ml graduated syringe and a 22-gauge BD Precision Glide needle, each vial was injected with a volume of reagent corresponding to 0.04 ml per milliliter of sample. Vials were vortexed to mix well and allowed to react overnight. Vials were stored upside-down with the liquid sample covering the septum to minimize any potential leakage.

The following morning, the reaction was neutralized using a 6 M NaOH solution. 10 ml of 6 M NaOH were placed in a 12 ml glass vial, capped with a PTFE silicone septum lid, and the headspace purged with a gentle flow of high-purity compressed N_2 for

approximately 10 minutes. Each vial was injected with a volume of reagent corresponding to 0.02 ml per milliliter of sample. Vials were vortexed to mix well, and stored upside-down and refrigerated until the time of analysis.

3.2.5.3.3 Isotope analysis

Isotope samples were analyzed on a Finnigan Gas Bench II gas chromatograph (GC) coupled to a Delta V Plus continuous flow isotope ratio mass spectrometer (CF-IRMS, Thermo Fisher Scientific). The system is controlled by Isodat 2.5 Gas Isotope Ratio MS Software (Thermo Electron, 1991-2005).

Vials were placed on a GC Pal autosampler equipped with a two-port needle that samples the vial headspace by flushing with helium (Internal Method No. 8). The analyte then passed through an organic material trap filled with granulated sodium hydroxide on support (Merck 1.01567.0250) and quartz wool at both ends. This trap proved to be very important in preventing the development of unidentified peaks in the sample, and lasted at most one week before needing to be refilled with fresh material. At this time the GC column was also baked out at 100°C for a minimum of one hour to remove any organic material that was not caught in the trap.

After passage through the organics trap, the analyte stream entered a dual cryotrap. These two liquid nitrogen traps focused the N_2O gas before introduction to the gas chromatograph.

Once the analyte stream entered the GC, it passed through an initial water removal trap before being injected onto the GC column at 30°C. After passing through the column, which separates N_2O from gaseous CO_2 of the same molecular weight, it was

routed through a final water removal trap before entering the CF-IRMS. Total sample run time was 1200 seconds (Table 3.1).

3.2.5.4 H_2O isotopes

Water isotopes were analyzed using a high-temperature reduction technique on a dual inlet mass spectrometer following the method of Gehre et al. (2004).

3.2.6 Statistics

Differences between sampling stations were analyzed using a 1-way ANOVA. In-stream N concentrations and isotopic ratios of water, NOx, and particulate matter were included in a principal components analysis.

3.3 RESULTS

3.3.1 Stream Chemistry

Most of the streams included in this study had conductivities of approximately 150 $\mu S\ cm^{-1}$, and slightly alkaline pH. Total suspended solids were highest in the mainstem Altamaha, Lower Oconee, and Upper and Lower Ocmulgee Rivers. Anion concentrations were largely comparable, with the highest concentrations in the Upper Ocmulgee River. Aside from water temperature, none of the physical parameters or anion concentrations showed any obvious or consistent seasonal cycles (Figures 3.4, 3.5).

The Ohoopee and Little Ocmulgee are both blackwater streams, and displayed characteristics typical of these types of systems, including lower pH, low concentrations of nutrients and other inorganic ions, and high concentrations of organic matter (Janzen 1974). Samples taken at the Upper Oconee site also displayed some characteristics similar to the two blackwater streams, most likely due to the fact that this sampling site

was located immediately below a reservoir. Conductivity, total suspended solids, and sulfate concentrations were significantly lower at these three sites than at the other sampling locations (Figures 3.6, 3.7).

3.3.2 N inputs

Local watershed inputs ranged from 766 kg km^{-2} yr^{-1} (Lower Oconee) to 3,076 kg km^{-2} yr^{-1} (Upper Ocmulgee; Table 3.2, Figure 3.8). Net food and feed predominated in the upper portion of the watershed, while in the lower portion of the watershed agricultural inputs predominated and net food and feed import was low or even negative due to large amounts of row crop agriculture.

Cumulative upstream nitrogen inputs to the surface of the subwatersheds ranged from 928 kg km^{-2} yr^{-1} (Ohoopee) to 3,076 kg km^{-2} yr^{-1} (Upper Ocmulgee; Tables 3.3-3.4, Figure 3.8), with the highest total inputs observed in the upper portion of the greater Altamaha watershed. These high inputs were due largely to net food and feed import, which are driven by high human population in the Upper Ocmulgee (a portion of the city of Atlanta is within this watershed) and high animal populations in the Upper Oconee (home to many chicken operations). The inputs from net food and feed import propagated downward to the lower portion of the watershed (particularly the Oconee, Little Ocmulgee, and mainstem Altamaha subwatersheds), but these systems also received high agricultural inputs in the form of both fertilizer and biological N fixation by crops.

3.3.3 N Concentrations

Nitrogen in the rivers studied consisted primarily of nitrate and dissolved organic nitrogen, with particulate matter also playing a role in some rivers. No seasonal cycles were evident in total N or any of its components (Figure 3.9), although additional data

would be useful in this regard as only one year's worth of samples were taken during this study.

Total in-stream N concentrations were highest at the sites sampled in the Upper and Lower Ocmulgee Rivers. DIN (mostly NO_3^-) was the dominant form of N at these sites, which had significantly higher concentrations of both NO_3^- and total DIN than the other rivers. Total N concentrations were lowest in the sampling sites in the Oconee River and the Little Ocmulgee River. The DIN:DON ratio usually ranged between 0 and 2 in most rivers, although it was occasionally higher, particularly in the Upper Ocmulgee River (Figure 3.9).

The blackwater rivers (Little Ocmulgee and Ohoopee) were dominated by organic nitrogen (Figure 3.11). In the Little Ocmulgee in particular, NO_3^- played only a very minor role. The percent organic nitrogen of particulate matter in the Little Ocmulgee and Ohoopee was significantly higher than in any other river except the Upper Oconee. However, since suspended sediment concentrations in these rivers were also significantly lower than in the other rivers (again except the Upper Oconee), the total amount of organic N was no higher, and the C/N ratio of particulate matter was similar in all rivers.

In the headwater streams, those with agricultural land use exhibited the highest average concentrations of N, although there was large variability among streams (Figure 3.12). Forested streams were uniformly low in all forms of N, and urban streams generally exhibited moderate levels of N. Ammonium concentrations in agricultural streams ranged from 9.1 to 19.9 μM. These concentrations were the highest seen in any stream or river in this study. Nitrate concentrations in some agricultural streams were also extremely high (up to 257 μM), and extremely high concentrations of DON were also

observed in some cases. Concentrations of NH_4^+ in agricultural streams were significantly higher than those in either forested or urban. Both DON and total N were significantly higher in agricultural streams than in forested streams, but urban streams were not significantly different from either of the other two land use classes. Particulate organic N was comparable across all streams.

3.3.4 N loads

The percentage of cumulative upstream N inputs that were exported in stream runoff was calculated by dividing the in-stream N load by the inputs to the watershed (here, cumulative inputs were used). The percentage exported was relatively consistent throughout the watershed, with 7% of total watershed inputs exported in the Altamaha River. Only 3% of watershed N inputs were exported from the Upper Oconee and Little Ocmulgee watersheds, and 8% from the Ohoopee. Watershed N export was highest from the Ocmulgee River, with 13% of inputs exported from the Upper Ocmulgee and 12% from the Lower Ocmulgee.

In-stream loads of all species of N showed a clear seasonal pattern, with load highest between December and April, and lowest between May and November (Figure 3.13). As N load is calculated by multiplying concentration by streamflow, this result is a consequence of the seasonal cycle of streamflow, which is highest during winter and early spring and lowest in summer and early fall.

To compare loads amongst different subwatersheds, the total (not area-normalized) average loads of TN were calculated for each watershed. Loads of all these constituents were lowest in the Little Ocmulgee, Ohoopee, and Upper Oconee Rivers and highest in the mainstem Altamaha (Table 3.5). Loads increased from the Upper to the Lower

Oconee and Ocmulgee Rivers. The Ocmulgee River contributes more than half of the total N load (198 Mmol N) in the Altamaha, confirming that this river is the most important contributor of nitrogen to the overall system. Loads were lowest from the small blackwater rivers (Ohoopee and Little Ocmulgee).

The load of TN in the Altamaha River can be compared to the sum of loading from the Ocmulgee, Oconee, Little Ocmulgee, and Ohoopee Rivers. If the load of TN in the Altamaha is lower than the summed loading from the tributaries, this indicates a loss. The loading of TN from the Altamaha River at the most downstream station is comparable to the sum of TN loading from all the tributary rivers (328 Mmol N vs. 351 Mmol N), suggesting limited loss of N.

A comparison of total load in the Altamaha River and summed loads of the tributaries was also undertaken for Cl^-, and Br^-, which act as conservative tracers. If the behavior of N in the system is similar to that of the conservative tracers, this suggests that N is also acting conservatively. When compared to the sum of the tributary load, 93.3% of chloride and 99.6% of the bromide were accounted for in the Altamaha River (Table 3.5). This is similar to the 93.4% of TN accounted for in this manner.

3.3.5 Isotopes

Amongst headwater streams, the water in agricultural streams was significantly more enriched in both D/H and $\delta^{18}O$ than the water in the other stream types sampled (Figure 3.13). This is surprising, and may indicate a different water source for these streams. $\delta^{15}N$-NOx was significantly more enriched in both agricultural and urban streams than in forested streams. In contrast, forested streams were significantly more enriched in ^{18}O-NOx than urban streams (and also higher than agricultural streams). The isotopic

signatures in agricultural and urban streams were similar to literature values reported for manure or sewage, although many of these values also overlapped with the reported range of nitrate derived from soil nitrification. The signature in forested streams suggested a mixture of NO_3^- from soil nitrification and from atmospheric deposition. Particulate N isotopes were not measured in headwater streams as TSS was very low in these streams and material collected on filters was insufficient for analysis.

With the exception of the Little Ocmulgee, NOx isotope values in all of the rivers were relatively constant over the course of the year and were narrowly constrained: average δ^{15}N-NOx values ranged from +7.5‰ to +10.2‰ and average δ^{18}O-NOx ranged from +5.2‰ to +9.5‰ (Figure 3.15). There was also a strong negative relationship between δ^{15}N-NOx and δ^{18}O-NOx (Figure 3.16). These values are similar to those of manure and septic NO_3^- and, in some cases, soil nitrification. The isotopic signatures also mostly overlapped with marine NO_3^- , but this is an unlikely source as all sampling locations were well upstream of any tidal influence. The isotopic signature in the Little Ocmulgee tended to be depleted in ^{15}N-NOx (+1.67‰) and enriched in ^{18}O-NOx (+22.8‰), and showed a decline in δ^{15}N-NOx and an increase in δ^{18}O-NOx over the course of the year (Figure 3.17). These values, which are similar to that of atmospheric deposition, were significantly different from those observed in other rivers.

δ^{15}N values of particulate matter were highest at the Upper Oconee sampling station and lowest in the Little Ocmulgee and Ohoopee (Figure 3.15). δ^{15}N in particulate matter was only measured between September 2010 and July 2011 and held relatively constant, although there may have been a slight decrease in δ^{15}N during the winter (Figure 3.17).

3.3.6 Relationships with inputs

The relationships between both the concentrations and stable isotope compositions of the various N species and inputs to the watershed were analyzed by averaging measured values over the sampling period and comparing these values to both local and cumulative upstream inputs. When only local inputs were considered, there were very few significant relationships between concentrations and watershed inputs to the subwatersheds (Table 3.6). Ammonium concentrations were related to human consumption, crop production, and forest fixation; nitrite concentration was related to human consumption. Although there were some significant relationships, nothing was related to nitrate, DON, or PON, which were the major components of total nitrogen in this study.

In comparison to local inputs, there were a number of significant relationships when total upstream inputs were considered (Table 3.7, Figure 3.18). Total N, TDN, and NO_2^- and NO_3^- concentrations were all positively related to population density. Total N concentration was also negatively related to crop N fixation. Dissolved organic N concentration was significantly related to a number of factors, including positive relationships with crop production and fertilizer use and negative relationships with net food and feed import and forest N fixation. $\delta^{15}N$ in particulate matter was positively related to net food and feed import, atmospheric deposition, and total N inputs. Both $\delta^{18}O$-H_2O and D/H- H_2O were also related to a number of watershed inputs, although there is no obvious mechanism for inputs to influence water isotopes.

As discussed above, the Upper Oconee sampling site was located only a few miles downstream of Sinclair Dam. Because man-made reservoirs can be sites of substantial nitrogen processing (Tomaszek & Koszelnik 2003) and thus the in-stream measurements do not necessarily reflect N behavior further upstream or on the landscape, the data set

was also analyzed excluding the Upper Ocmulgee sampling site. Without the Upper Ocmulgee, a substantial number of additional significant relationships between N concentrations and isotope composition and budget inputs were found (Table 3.8; starred panels of Figure 3.18). In particular, both δ^{15}N-NOx and δ^{18}O-NOx were positively related to total N inputs.

In addition to considering concentrations, we also evaluated relationships between N loads and cumulative watershed inputs. Loads of inorganic N were all very strongly related to population density (Figure 3.19). Most notably, there was a near-perfect relationship between NO_3^- load and population density (R^2=0.99). NO_3^- load was also related, albeit less strongly, to crop N production and fixation and forest N fixation. TDN and total N loads were strongly related to population density as well as to crop production and crop fixation, whereas dissolved and particulate organic N loads were not related to population density or any other factor.

3.3.7 Principal Components Analysis

We performed a PCA analysis that included NOx and PN isotope compositions and N concentrations (DIN, DON, and PON; Figure 3.20). Samples mostly separated along the first component, which was positively related to DIN concentration and δ^{15}N-NOx, and negatively related to DON concentration and δ^{18}O-NOx abundance (see Chapter 1 for a discussion of processes affecting nitrate isotopes). The second axis was positively related to particulate N (both concentration and isotopic signature). Observations in one of the blackwater streams, the Little Ocmulgee, separated from the other sampling sites, whereas observations in the Ohoopee sometimes clustered with the Little Ocmulgee samples and sometimes with the other sites, suggesting that this stream does not always

act as a blackwater stream. The Upper and Lower Ocmulgee observations were generally at the opposite extreme of the first axis, and observations in the Upper Oconee, which was located below a dam, were generally higher on the second axis than the other rivers studied.

3.4 DISCUSSION

The N concentrations measured in the Altamaha River in this study were generally comparable to previous measurements in this river system (Weston et al. 2009, Fisher et al. 2000). Rivers flowing through the southeastern coastal plain of Georgia tend to be low in pH and TSS and dominated by organic rather than inorganic constituents (Beck et al. 1974). In this study, these characteristics were observed particularly in those tributaries originating in the coastal plain (Little Ocmulgee and Ohoopee), where organic N concentrations were highest, while the Oconee and Ocmulgee tributaries were dominated by inorganic N. Natural background nutrient loads for large rivers in the southeastern temperate forest ecoregion are on the order of 50 kg km^{-2} yr^{-1}, which translates to a concentration of approximately 0.15 mg/L (10 μM) (Smith et al. 2003). N concentrations in all rivers were above this level, suggesting they were all affected by anthropogenic activity.

NO_3^- is typically the largest constituent of riverine N concentrations in systems with substantial anthropogenic impact, accounting for around 77% of total in-stream N globally (Turner et al. 2003). In the Altamaha River system, organic N plays a more important role than would be expected based on these global averages. Among the rivers, concentrations of inorganic and total nitrogen were highest in the Upper Ocmulgee River,

whereas concentrations of organic nitrogen were highest in the Little Ocmulgee and Ohoopee Rivers. (The ratio of DIN to DON generally remained fairly constant.) While nitrate is dominant in the Ocmulgee River, it accounts for less than half of instream N concentrations in the rest of this system, including the mainstem Altamaha River. This is in agreement with the results found by Takagi et al. (*in review*). The high organics in the Little Ocmulgee and Ohoopee rivers are consistent with the characteristics observed in blackwater rivers (Janzen 1974).

N concentrations at the Upper Oconee sampling station were among the lowest observed. This contrasts with previous studies, which have reported that concentrated animal feeding operations and human populations in the Upper Oconee are both significant contributors to increased in-stream N concentrations in this system (Fisher et al. 2000). However, in this case the sampling station was located directly below a dam. Dams have been shown to retain nitrogen and phosphorus (Friedl & Wüest 2001), and nutrient concentrations downstream of a dam are often lower than in the water entering the reservoir (Stanley & Doyle 2002).

The highest N concentrations in this study were observed in headwater agricultural streams, although as a group these streams were exceedingly variable, with a large range in both NO_3^- and organic nitrogen concentrations. Forested streams had the lowest N concentrations, with urban streams in between. This pattern has been observed in other systems (Battaglin et al. 2001b, Inwood et al. 2005). Agricultural headwater streams in other areas have shown even higher concentrations of NO_3 than was observed here (Battaglin et al. 2001b, Royer et al. 2004), whereas undeveloped forested streams tend to be low (Mulholland 1992), particularly in the southeastern US (Binkley et al. 2004).

Although the NO_3^- concentrations observed in agricultural streams were not significantly different from urban or forested streams, the very high concentrations observed at some agricultural sampling sites suggest that these types of streams may be important contributors to the N observed in the rivers. This agrees with the findings by Chang et al. (2002), who found agricultural sites within the Mississippi River watershed to have the highest NO_3^- concentrations, and the isotopic composition of NO_3^- in large rivers to resemble these agricultural sites, suggesting that agricultural streams were major contributors to N present in the rivers.

No obvious seasonality was observed in any of the N species measured. The lack of any sort of seasonal trend in the concentration data is not surprising, given that observations of seasonality are inconsistent among and even within studies. Scottish rivers have been found to have higher NO_3^- concentrations in late fall and winter (Clark et al. 2004), whereas the Mississippi River has exhibited a spring peak in NO_3^- due to fertilizer application to the land surface (Turner and Rabalais 1991). Even within a larger watershed, the timing of peak NO_3^- concentrations can vary among tributaries: a study of the Yangtze River and its tributaries found that in some locations, the period of highest N concentration was during the flood season, in some locations it was during the dry season, and in some locations there was no such period at all (Jingsheng et al. 1999).

Loads were highest during winter and early spring, the time when streamflow is highest. This is typical—globally, the period of maximum flux varies widely among watersheds but is dependent on the period of maximum streamflow (Green et al. 2004). However, Takagi et al. (*in review*) also found that DIN loading from the tributaries to the mainstem Altamaha is most conservative during high-flow periods, compared to low-

flow periods when a greater proportion of tributary DIN load is lost before the downstream reach of the Altamaha River. As with concentrations, NO_3^- and DON were the dominant constituents of N loading, followed by particulate N.

DIN loads have been shown to decrease during low-flow conditions (Takagi et al. *in review*), and the samples taken during this study were taken during a particularly low-flow period. In particular, the first half of the sampling period was dry, as evidenced by the isotopic composition of water (Figure 3.21). Samples taken between July and December 2010 were subject to more evaporation than samples taken between January and July 2011.

Based on the principal components analysis, the primary differences between rivers had to do with the concentrations of organic vs. inorganic N. This resulted in the separation of the Little Ocmulgee and some Ohoopee samples from the other streams. This is consistent with the generally accepted stream chemistry of blackwater rivers, which tend to be high in organic matter and low in inorganic nutrients (Janzen 1974). Other aspects of stream chemistry, including low conductivity, pH, TSS, and sulfate, support the characterization of both these streams as blackwater rivers.

The second component of the principal components analysis reflected differences in the concentration and $\delta^{15}N$ of particulate organic matter. Except for one particularly high sample in the Little Ocmulgee, samples taken in the Upper Oconee were generally highest on this axis. This confirms that the Upper Oconee sampling station, located directly beneath the manmade Lake Sinclair, was anomalous in its low particulate matter concentrations. Dams are well known to decrease downstream sediment delivery by allowing sediment to settle and thus trapping it in the reservoir (Baxter 1977, Meade

1982, Ligon et al. 1995, Stanley & Doyle 2003, and many others), and Sinclair Dam is no exception (Ligon et al. 1995).

3.4.1 Isotopes

The range of isotopic values observed in this study is similar to that reported in the Mississippi River basin (Chang et al. 2002) but broader than isotopic values reported for the northeastern United States (Mayer et al. 2002). The NO_3 in most samples from the greater Altamaha river system showed a manure/sewage signature, which has been observed in these other studies as well (Mayer et al. 2002, Chang et al. 2002), although many samples also overlapped with typical isotopic signature of nitrate derived from soil nitrification.

Isotopic values did not exhibit any obvious seasonal patterns, although there were some trends in NO_3^- isotope composition over the course of the sampling period. Most sites, with the exception of the Little Ocmulgee, saw a decline in $\delta^{18}O$ and possibly a slight increase in $\delta^{15}N$ over the course of the sampling period. The trends in the Little Ocmulgee, on the other hand, were reversed. This contrasts with the findings of Battaglin et al. (2001) in the Mississippi, where a pattern of increasing isotopic values of both ^{15}N and ^{18}O was observed from spring to fall, concurrent with decreasing streamflow. However, that study as well as this one represented only one year of monthly or less frequent sampling, and sampling for a longer time period or more often could have uncovered patterns not apparent in the existing sample sets.

In addition to providing information about potential sources, isotopic values can be used to gain insights on transformations of N such as uptake or denitrification. There was limited evidence for uptake of NO_3^- in this system. $\delta^{15}N$-PN was positively related to

$\delta^{15}N\text{-}NO_3^-$, and in almost all cases PN tended to be more depleted than NO_3^- isotopes. This would be an expected consequence of NO_3^- uptake. However, the relationship between NO_3^- concentration and $\delta^{15}N\text{-}NO_3^-$, which is positive, does not indicate any drawdown of NO_3^- through uptake. Unfortunately, the concentrations of NH_4^+ in the rivers were too low to analyze the isotopic composition of this compound, thus leaving the question of uptake open.

There was a positive relationship between NO_3^- concentration and $\delta^{15}N\text{-}NO_3^-$. Where such a positive relationship occurs, mixing rather than denitrification is generally taking place, since denitrification would be associated with a decreasing NO_3^- concentration and a concurrent increase in $\delta^{15}N\text{-}NO_3^-$ (Kendall et al. 2007, Mayer et al. 2002).

A simple mixing model following the equation y=a/x+b was fit for both $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ (Figure 3.22). When these two best fit equations are solved for theoretical end members, at extremely low concentration (0.25 μM), $\delta^{15}N\text{-}NO_3^- = 0$ and $\delta^{18}O\text{-}NO_3^- = 27$; at high concentration (80 μM), $\delta^{15}N\text{-}NO_3^- = 9.0$ and $\delta^{18}O\text{-}NO_3^- = 7.5$. At slightly higher concentrations the low end member could reflect nitrate produced through soil nitrification (at 1 μM, $\delta^{15}N\text{-}NO_3^- = 6.8$; $\delta^{18}O\text{-}NO_3^- = 12.5$), and the septic/manure end member is close to the upper limit of typical $\delta^{15}N\text{-}NO_3^-$ signatures observed in soil nitrification, suggesting that this source cannot be ruled out. The $\delta^{15}N\text{-}NO_3^-$ of some observations was higher than the septic/manure end member of the model, which may suggest processing or additional highly enriched inputs. Since the $\delta^{18}O\text{-}NO_3^-$ observations were generally less enriched than predicted by the model, the latter appears more likely in this case. .While the overall isotopic composition of nitrate was comparable across most rivers, there were a number of occasions on which the isotopic signature appeared to

reflect atmospheric inputs. Overall, the isotopic composition of nitrate in the blackwater Little Ocmulgee River was significantly different from the other rivers. NO_3^- was consistently more depleted in ^{15}N and more enriched in ^{18}O than at other sites, suggesting that atmospheric nitrogen is the dominant source of NO_3^- in this river. On two occasions, when in-stream NO_3^- concentrations were very low in the Ohoopee River, the isotopic values were similar to those observed regularly in the Little Ocmulgee River. Isotopic values similar to the Little Ocmulgee were also observed in the Altamaha River in June 2011, when in-stream NO_3^- concentration was unusually low. The distinctly different isotopic signature at low NO_3^- concentrations suggests that at these times, background NO_3^- of atmospheric origin is being observed, while at higher concentrations the inputs of manure and sewage swamp the atmospheric signal. This interpretation is also supported by the measurements taken in small watersheds, where low-N forested streams were less enriched in ^{15}N and more enriched in ^{18}O than agricultural and urban streams.

Although the Ohoopee River can also be categorized as a blackwater river and shows many of the same characteristics as the Little Ocmulgee, both its NO_3^- concentration and isotopic composition were more in line with the whitewater rivers (used here to describe the physical and chemical quality of the water, as opposed to its turbulence). The similar isotopic signature may be a consequence of the higher NO_3^- concentration in this river. Higher concentrations of NO_3^- may potentially overwhelm a low-level atmospheric signal. The isotopic composition of NO_3^- in blackwater rivers specifically has not been previously reported, and this is an area ripe for future study.

Measurements made in the headwater streams provided support for the notion that background NO_3^- in this system is of atmospheric input. Although the isotopic signature

of forested streams was similar to that of fertilizer, this is unlikely since these forested watersheds were not recently disturbed. Rather, since the ^{18}O values are halfway between those observed in NO_3^- from atmospheric and soil nitrification sources, it is most likely that NO_3^- in forested streams is from a mixture of these sources. The isotopic values observed in the forested watersheds are similar to those found in other forested headwaters by Spoelstra et al. (2001), where NO_3^- was found to derive primarily from microbial sources. The isotope signatures of the agricultural and urban streams were similar to manure and septic sources, which is in keeping with their likely inputs.

3.4.2 N inputs

Local N inputs for this system have been previously reported (Schaefer & Alber 2007b) for 1954-2002, and the 2002 inputs are comparable to those calculated in this study, which were for 2007. Fertilizer use declined across all watersheds. Changes in human and animal populations led to an increase in net food and feed import in the Upper Ocmulgee (containing part of the city of Atlanta and thus dominated by human population) but declined in the Upper Oconee, Little Ocmulgee, and Ohoopee. N fixation and atmospheric N inputs were also lower than in previous years. The upper watersheds, which are highest in human and animal populations, still have the highest N inputs while the lower portion of the watershed continues to be dominated by agricultural sources. Overall inputs increased in the Upper Oconee and Ocmulgee watersheds and declined in all other subwatersheds. When cumulative upstream inputs were considered, the high inputs from the upper watershed propagated downstream, resulting in higher inputs for the more sparsely populated downstream watersheds. Overall, inputs continue to be

comparable to those reported in other areas (Boyer et al. 2002, Schaefer and Alber 2007a, others).

There are of course errors associated with these types of budgets due to the many data sources and conversion factors used. However, these budgets were calculated in a consistent manner, such that they are comparable not only across watersheds in this study, but also comparable to other studies that have used this approach (e.g. Boyer et al. 2002). Thus, errors should be consistent across budgets, such that comparisons between watersheds and analysis of relationships are still possible.

3.4.3 Relationships with inputs

Local inputs were not well related to in-stream loads or concentrations, suggesting that N inputs to the immediate environs of a stream do not necessarily predict in-stream conditions and that upstream conditions must be considered. The best predictor of both inorganic N concentrations and loads was cumulative upstream population density, rather than any of the estimated N inputs (except human consumption, which is simply population density multiplied by a consumption factor). The slope of the TN-load-versus-population-density regression line was 0.995 with an intercept of 52.4, suggesting that 52 kg km^{-2} yr^{-1} is the background N load in this system, and each additional person adds 1 kg km^{-2} yr^{-1}. Population density has been shown to be a good predictor of nitrogen loads in a number of studies (Howarth et al. 1996, Caraco & Cole 1998, Yan et al. 2003), with dry systems exporting significantly less N (Caraco & Cole 2001).

The relationship of population density to N concentrations has not been well-studied, although Peierls et al. (1991) found a relationship between the two in a study of large rivers. Total population rather than population density has been shown to be related to in-

stream N concentrations in the Changjiang River in China (Yan et al. 2003). In this study, the relationship was excellent, with R^2=0.88. This observation, taken in conjunction with the fact that the isotopic signature of NO_3^- was similar to that of manure or sewage in most rivers in the system, suggests that most NO_3^- in the Altamaha River system originates as human wastewater. This may be due to the fact that a number of the larger population centers in Georgia (including Athens, Milledgeville, and Macon) are located adjacent to the Oconee and Ocmulgee Rivers, providing little opportunity for transformation of NO_3^- before reaching the stream.

There were also some relationships between both concentrations and loads and the percentage of particular forms of land use within each watershed. NO_X and TDN concentrations and loads, as well as TN loads, were related to urban area within the watershed whereas DON loads were related to agricultural land. (Some other measured indicators of water quality, including conductivity, TSS, and SO_4, were also related to agricultural land percentage.) Unlike previous studies (Mayer et al. 2002), no relationships between $\delta^{15}N\text{-}NO_3^-$ or $\delta^{18}O\text{-}NO_3^-$ and any form of land use were observed. These results also differ from those of Chang et al. (2002), who found that large amounts of urban area in watersheds resulted in an atmospheric $\delta^{18}O\text{-}NO_3^-$ signal, whereas $\delta^{15}N\text{-}NO_3^-$ was highest in watersheds dominated by crops and livestock agriculture (see also Lefebvre et al. 2007).

There were a limited number of relationships observed between N inputs and in-stream concentrations or loads. DON was related to crop production and fertilizer use as well as to percent agricultural and wetland area in a watershed. Although a relationship between increased fertilizer use and higher in-stream NO_3^- levels is well-established

(Shuiwang et al. 2000, Turner & Rabalais 1999), the impact of fertilizers on organic N is less clear (Rabalais et al. 2002). The positive relationship between organic N and agricultural activity observed in this study may be an artifact of land use distribution within the watershed. Pellerin et al. (2004) found organic N concentrations within streams to be dependent on the percentage of wetland area within their watersheds. Wetland areas in the greater Altamaha watershed are concentrated in the lower portion of the watershed, where agricultural activity and fertilizer use also happen to be highest. In addition, rivers originating in this area tend to be blackwater rivers that are naturally high in organic nitrogen (Beck et al. 1974, Janzen 1974).

When the Upper Oconee sampling station, located directly below the reservoir, was excluded, total N inputs were related to both increased $\delta^{15}N\text{-}NO_3^-$ and decreased $\delta^{18}O\text{-}NO_3^-$. Increased $\delta^{15}N\text{-}NO_3^-$ as a result of increased agricultural and urban land has been found in previous studies (Mayer et al. 2002, Lefebvre et al. 2007). The time period during which this study was conducted was particularly dry, and therefore atmospheric N reaching the stream through runoff from the land surface could have been limited. However, isotopic composition was not markedly different during winter and early spring when runoff in the Altamaha watershed was highest.

In order to explore the relationship between nitrogen inputs and in-stream N further, we calculated predicted isotopic values of nitrate for each watershed based on the inputs estimated here and isotopic signatures for atmospheric deposition, fertilizer, sewage/manure, and soil nitrification reported in the literature (Tables 3.9-3.10, Figure 3.23). This approach allows an examination of the differences between the expected and the observed isotopic measurements in a stream, thus potentially shedding light on

transformations or losses that occur before nitrogen reaches the stream. Literature averages were multiplied by the proportion of the source's contribution to the total N input budget in each watershed. $\delta^{15}N$ predictions were further broken down by sewage and types of animal manure.

Measured values of $\delta^{15}N\text{-}NO_3^-$ were not well related to predicted values. This may suggest either that N does not reach streamwater in the same proportions as it is applied to the land surface, or that N is being transformed. The former is most likely in this study. In most subwatersheds, less than 10% of inputs reached the stream. The other 90% may have been transformed on the land surface and thereby been prevented from reaching streamwater, but there was little evidence for in-stream N loss. Predicted values of $\delta^{18}O\text{-}NO_3^-$ were positively related to observations (R^2=0.67, p=0.02); however, the slope differed substantially from 1 (m=1.54; Figure 3.23).

NO_3^- was enriched in ^{15}N and depleted in ^{18}O compared to predicted values in the Ocmulgee, Altamaha, and Ohoopee Rivers. The Little Ocmulgee exhibited the opposite pattern. The deviation from expected $\delta^{15}N\text{-}NO_3^-$ values could be due to in-stream NO_3^- production in the Little Ocmulgee and uptake in other rivers; however, since $\delta^{18}O\text{-}NO_3^-$ was not similarly enriched or depleted, the difference between predicted and observed isotopic values is most likely due to differences in which inputs reach the stream. There was a relationship between $\delta^{18}O\text{-}H_2O$ and $\delta^{18}O\text{-}NO_3^-$; however, the fit was quite poor (R^2=0.05, p=0.04). In particular, the high $\delta^{18}O\text{-}NO_3^-$ samples taken in the Little Ocmulgee River corresponded very poorly with ^{18}O isotopes in the water. This lack of correspondence suggests that there was little exchange between oxygen atoms of water and nitrate and does not explain the $\delta^{18}O\text{-}NO_3^-$ values.

Taken together, these data suggest that inputs to the watershed, while useful as a large-scale predictor of riverine N loads, do not adequately describe which inputs are actually reaching the stream. The fact that apparently not all inputs reach the stream equally may help to explain the disparities in the percentage of inputs reaching the stream that have been observed in different regions (Boyer et al. 2002, Schaefer & Alber 2007a, others). For example, agricultural regions may have high fertilizer inputs but this may not be reflected in in-stream N due to differential processing driven by such variations. The location of inputs in relation to the stream network may also play a role in this regard. Headwater streams are more efficient than higher-order rivers in removing excess nitrogen (Seitzinger et al. 2002), and thus nutrients that are input to headwaters are more likely to be removed by the time the water reaches the river.

While processing was not directly measured in this study, there was little indirect evidence for in-stream N loss. Although denitrification is extremely spatially variable (e.g. Folorunso & Rolston 1984) and may in fact be occurring in-stream, the data here did not show evidence for this process. This is consistent with results from the northeastern U.S.: Mayer et al. 2002 did not see evidence of in-stream denitrification in isotopic nitrate data despite conflicting evidence from other studies (Seitzinger et al. 2002). The relationship observed here between nitrate concentration and isotopes, and the model fit to these data, support mixing rather than denitrification. In the Altamaha, the mass balance of loading data from the tributaries also suggested that little nitrogen is being lost from the lower portion of the system. Thus, N loss that is occurring in this system is most likely taking place on the watershed surface, in places such as soils and riparian zones.

The low percentage of inputs to the watershed surface that reach the stream lend further credence to this idea.

3.5 CONCLUSIONS

Nitrogen concentrations in this study were highest in the most populated watersheds and lowest in the blackwater rivers and below a manmade impoundment. There was no seasonality in the observed N concentrations, but loads were highest in the winter and early spring as a result of increased streamflow during that time of year. The Ocmulgee River contributed the majority of TN loading to the mainstem Altamaha. The isotopic signature of the nitrate was consistent with sewage and manure inputs in rivers where NO_3^- concentrations were high, although contributions from soil nitrification could not be ruled out. Rivers low in NO_3^- exhibited an isotopic signature consistent with inputs from atmospheric deposition, suggesting that these are the background inputs. The inputs to the watershed were highest in the upper portion of the watershed, where there were high human and animal populations, whereas the lower portion of the watershed was dominated by agricultural inputs. Overall, the best predictor of N loads was upstream population density rather than any of the calculated inputs. Local inputs were very poorly related to in-stream N, suggesting that despite differences among subwatersheds, the lower subwatersheds are affected primarily by N from well upstream. We also found that population density was the best predictor of concentrations, a relationship that has not been well-studied and deserves further attention. Only a small proportion of watershed N inputs reached the streams (generally less than 10%). N behaved conservatively in the lower portion of the watershed, and an isotope mixing model fit the data well. Taken

together, this suggests that losses of N in this system are primarily occurring on the land surface, rather than after reaching the stream.

ACKNOWLEDGMENTS

We thank Joan Sheldon and Natalie McLenaghan for assistance with field work and Mandy Joye, Chuck Hopkinson, Kim Hunter, Emily Davenport, and Adrian Burd for assistance with lab work. This research was supported by the Georgia Coastal Ecosystems LTER Project (NSF Awards OCE-0620959 and OCE-1237140).

REFERENCES

Battaglin, W.A., C. Kendall, C.C.Y. Chang, S.R. Silva, and D.H. Campbell. 2001. Chemical and isotopic evidence of nitrogen transformation in the Mississippi River, 1997-98. Hydrological Processes 15:1285-1300.

Battaglin, W.A., C. Kendall, C.C.Y. Chang, S.R. Silva, and D.H. Campbell. 2001b. Isotopic and Chemical Composition of Inorganic and Organic Water-Quality Samples from the Mississippi River Basin, 1997-98. U.S. Geological Survey Water-Resources Investigations Report 01-4095, Denver, Colorado.

Baxter, R.M. 1977. Environmental effects of dams and impoundments. *Annual Review of Ecology and Systematics* 8: 255-283.

Beck, K.C., J.H. Reuter, and E.M. Perdue. 1974. Organic and inorganic geochemistry of some coastal plain rivers of the southeastern United States. *Geochimica et Cosmochimica Acta* 38: 341-364.

Binkley, D., G.G. Ice, J. Kaye, and C.A. Williams. 2004. Nitrogen and phosphorus concentrations in forest streams of the United States. *Journal of the American Water Resources Association* 40(5): 1277-1291.

Boyer, E.W., C.L. Goodale, N.A. Jaworski, and R.W. Howarth. 2002. Anthropogenic nitrogen sources and relationships to riverine nitrogen export in the northeastern U.S.A. *Biogeochemisty* 57/58: 137-169.

Bricker, S., B. Longstaff, W. Dennison, A. Jones, K. Boicourt, C. Wicks, and J. Woerner. 2007. Effects of Nutrient Enrichment in the Nation's Estuaries: A Decade of Change. National Centers for Coastal Ocean Science, Silver Spring, MD.

Caraco, N.F., and J.J. Cole. 1998. Human impacts on nitrate export: An analysis using major world rivers. *Ambio* 28(2): 167-170.

Caraco, N.F., and J.J. Cole. 2001. Human influence on nitrogen export: a comparison of mesic and xeric catchments. *Mar. Freshwater Res.* 52: 119-125.

Chang, C.C.Y., C. Kendall, S.R. Silva, W.A. Battaglin, and D.H. Campbell. 2002. Nitrate stable isotopes: tools for determining nitrate sources among different land uses in the Mississippi River Basin. Canadian Journal of Fisheries and Aquatic Sciences 59:1874-1885.

Clark, M.J., M.S. Cresser, R. Smart, P.J. Chapman, and A.C. Edwards. 2004. The influence of catchment characteristics on the seasonality of carbon and nitrogen species concentrations in upland rivers of northern Scotland. *Biogeochemistry* 68(1): 1-19.

Cole, J.J., B.L. Peierls, N.F. Caraco, M.L. Pace. 1993. Nitrogen loading of rivers as a human-driven process. In M. J. McDonnell and S. T. A. Pickett (eds.), *Humans as*

Components of Ecosystems: The Ecology of Subtle Human Effects and Populated Areas, pp. 141-157. Springer, New York, NY

Dortch, Q. 1990. The interaction between ammonium and nitrate uptake in phytoplankton. *Marine Ecology Progress Series* 61: 183-201.

Fisher, D.S., J.L. Steiner, D.M. Endale, J.A. Stuedemann, H.H. Schomberg, A.J. Franzluebbers, and S.R. Wilkinson. 2000. The relationship of land use practices to surface water quality in the Upper Oconee Watershed of Georgia. *Forest Ecology and Management* 128: 39-48.

Folorunso, O.A., and D.E. Rolston. 1984. Spatial variability of field-measured denitrification gas fluxes. *Soil Science Society of America Journal* 48(6): 1214-1219.

Friedl, G., and A. Wüest. 2001. Disrupting biogeochemical cycles—Consequences of damming. *Aquatic Science* 64: 55-65.

Galloway, J.N. and E.B. Cowling. 2002. Reactive nitrogen and the world: 200 years of change. Ambio 31:64-71.

Gehre, M., H. Geilmann, J. Richter, R.A. Werner, and W.A. Brand. 2004. Continuous flow $^{2}H/^{1}H$ and $^{18}O/^{16}O$ analysis of water isotopes with dual inlet precision. *Rapid Communications in Mass Spectrometry* 18: 2650-2660.

Goosby, D.A., and W.A. Battaglin. 2001. Long-term changes in concentrations and flux of nitrogen in the Mississippi River Basin, USA. *Hydrological Processes* 15: 1209-1226.

Green, P.A., C.J. Vörösmarty, M. Meybeck, J.N. Galloway, B.J. Peterson, and E.W. Boyer. 2004. Pre-industrial and contemporary fluxes of nitrogen through rivers: A global assessment based on typology. *Biogeochemistry* 68(1): 71-105.

Hilton, J., M. O'Hare, M.J. Bowes, and J.I. Jones. 2006. How green is my river? A new paradigm of eutrophication in rivers. *Science of the Total Environment* 365: 66-83.

Houser, J.N., and W.B. Richardson. 2010. Nitrogen and phosphorus in the Upper Mississippi River: transport, processing, and effects on the river ecosystem. *Hydrobiologia* 640: 71-88.

Howarth, R.W., G. Billen, D. Swaney, A. Townsend, N. Jaworski, K. Lajtha, J.A. Downing, R. Elmgren, N. Caraco, T. Jordan, F. Berendse, J. Freney, V. Kudeyarov, P. Murdoch, and Z.L. Zhu. 1996. Regional nitrogen budgets and riverine N&P fluxes for the drainages to the North Atlantic Ocean: Natural and human influences. Biogeochemistry 35:75-139.

Howarth, R.W., A. Sharpley, and D. Walker. 2002. Sources of nutrient pollution to coastal waters in the United States: Implications for achieving coastal water quality goals. Estuaries 25:656-676.

Inwood, S.E., J.L. Tank, and M.J. Bernot. 2005. Patterns of denitrification associated with land use in 9 midwestern headwater streams. *Journal of the North American Benthological Society* 24(2): 227-245.

Janzen, D.H. 1974. Tropical blackwater rivers, animals, and mast fruiting by the Dipterocarpaceae. *Biotropica* 6(2): 69-103.

Jingsheng, C., G. Xuemin, H. Dawei, and X. Xinghui. 1999. Nitrogen contamination in the Yangtze River system, China. *Journal of Hazardous Materials* A73: 107-113.

Joye, S.B. 2009. Long-term water quality monitoring in the Altamaha, Doboy, and Sapelo sounds and the Duplin River near Sapelo Island, Georgia from May 2001 to August 2009. Georgia Coastal Ecosystems LTER Data Catalog (data set NUT-

GCEM-0909a; http://gce-lter.marsci.uga.edu/public/app/dataset_details.asp?accession=NUT-GCEM-0909a)

Justić, D., R.E. Turner, and N.N. Rabalais. 2003. Climatic influences on river nitrate flux: Implications for coastal marine eutrophication and hypoxia. *Estuaries* 26(1): 1-11.

Kellman, L., and C. Hillaire-Marcel. 2003. Evaluation of nitrogen isotopes as indicators of contamination sources in an agricultural watershed. *Agriculture, Ecosystems and Environment* 95: 87-102.

Kendall et al. 2007 in Michener, R., and K. Lajtha. 2007. *Stable Isotopes in Ecology and Environmental Science*. Blackwell Publishing, Malden, MA. 592pp.

Koroleff, F. 1983. Determination of ammonia. In Grasshoff, K., M. Ehrhardt, and K. Kremling, eds. Methods of seawater analysis: second, revised and extended edition. pp. 150-157. Verlag Chemie, Weinheim.

Lefebvre, S., J.C. Clement, G. Pinay, C. Thenail, P. Durand, and P. Marmonier. 2007. N-15-nitrate signature in low-order streams: Effects of land cover and agricultural practices. Ecological Applications 17:2333-2346.

Ligon, F.K., W.E. Dietrich, and W.J. Trush. 1995. Downstream ecological effects of dams. *BioScience* 45(3): 183-192.

Mayer, B., E.W. Boyer, C. Goodale, N.A. Jaworski, N. van Breemen, R.W. Howarth, S. Seitzinger, G. Billen, K. Lajtha, N. Nadelhoffer, D. van Dam, L.J. Hetling, M. Nosal, and K. Paustian. 2002. Sources of nitrate in rivers draining sixteen watersheds in the northeastern U.S.: Isotopic constraints. *Biogeochemistry* 57/58: 171-197.

Mayer, B. 2005. Assessing sources and transformations of sulphate and nitrate in the hydrosphere using isotope techniques. Pages 67-89 in P. K. Aggarwal, J. R. Gat, and

K. F. O. Froehlich, editors. Isotopes in the Water Cycle: Past, Present and Future of a Developing Science. IEA, Netherlands.

Meade, R.H. 1982. Sources, sinks, and storage of river sediment in the Atlantic drainage of the United States. *The Journal of Geology* 90(3): 235-252.

Meybeck, M. 1982. Carbon, nitrogen, and phosphorus transport by world rivers. *American Journal of Science* 282: 401-450.

McIlvin, M.R., and M.A. Altabet. 2005. Chemical conversion of nitrate and nitrite to nitrous oxide for nitrogen and oxygen isotopic analysis in freshwater and seawater. *Anal. Chem.* 77, 5589-5595.

Middelburg, J.J., and J. Nieuwenhuize. 2000. Nitrogen uptake by heterotrophic bacteria and phytoplankton in the nitrate-rich Thames estuary. *Marine Ecology Progress Series* 203: 13-21.

Miltner, R.J. 2010. A method and rationale for deriving nutrient criteria for small rivers and streams in Ohio. *Environmental Management* 45: 842855.

Monbet, Y. 1992. Control of phytoplankton biomass in estuaries: A comparative analysis of microtidal and macrotidal estuaries. *Estuaries* 15(4): 536-571.

Mulholland, P.J. 1992. Regulation of nutrient concentrations in a temperate forest stream: Roles of upland, riparian, and instream processes. *Limnology and Oceanography* 37(7): 1512-1526.

National Atlas of the United States. 2006. Major Dams of the United States. Earth Science Information Center, U.S. Geological Survey, Reston, VA

Natural Resource Spatial Analysis Laboratory (NARSAL). 1998. 1998 land cover map of Georgia. University of Georgia, Athens, GA. http://narsal.uga.edu/glut

Ostrom, N.E., L.O. Hedin, J.C. von Fischer, and G.P. Robertson. Nitrogen transformations and NO_3^- removal at a soil-stream interface: A stable isotope approach. *Ecological Applications* 12(4): 1027-1043.

Pelleri, B.A., W.M. Wollheim, C.S. Hopkinson, W.H. McDowell, M.R. Williams, C.J. Vörösmarty, and M.L. Daley. 2004. Role of wetlands and developed land use on dissolved organic nitrogen concentrations and DON/TDN in northeastern U.S. rivers and streams. *Limnology and Oceanography* 49(4): 910-918.

Peierls, B.L., N.F. Caraco, M.L. Pace, and J.J. Cole. 1991. Human influence on river nitrogen. *Nature* 350: 386-387

Pionke, H.B., W.J. Gburek, and A.N. Sharpley. 2000. Critical source area controls on water quality in an agricultural watershed located in the Chesapeake Basin. *Ecological Engineering* 14: 325-335.

Rabalais, N.N., R.E. Turner, and D. Scavia. 2002. Beyond science into policy: Gulf of Mexico hypoxia and the Mississippi River. *BioScience* 52(2): 129-142.

Royer, T.V., J.L. Tank, and M.B. David. 2004. Transport and fate of nitrate in headwater agricultural streams in Illinois. *Journal of Environmental Quality* 33: 1296-1304.

Ruddy, B.C., D.L. Lorenz, and D.K. Mueller. 2006. County-level estimates of nutrient inputs to the land surface of the conterminous United States, 1982-2001. USGS Scientific Investigations Report 2006-5012, Reston, VA.

Ryabenko, E., M.A. Altabet, and D.W.R. Wallace. Effect of chloride on the chemical conversion of nitrate to nitrous oxide for $\partial^{15}N$ analysis. *Limnol. Oceanogr.: Methods* 7, 545-552.

Schaefer, S.C., and M. Alber. 2007a. Temperature controls a latitudinal gradient in the proportion of watershed nitrogen exported to coastal ecosystems. *Biogeochemistry* 85: 333-246.

Schaefer, S.C., and M. Alber. 2007b. Temporal and spatial trends in nitrogen and phosphorus inputs to the watershed of the Altamaha River, Georgia, USA. *Biogeochemistry* 86: 231-249.

Seitzinger, S.P., R.V. Styles, E.W. Boyer, R.B. Alexander, G. Billen, R.W. Howarth, B. Mayer, and N. van Breemen. 2002. Nitrogen retention in rivers: model development and application to watersheds in the northeastern U.S.A. *Biogeochemistry* 57/58: 199-237.

Seitzinger, S.P., and R.W. Sanders. 1997. Contribution of dissolved organic nitrogen from rivers to estuarine eutrophication. *Marine Ecology Progress Series* 159: 1-12.

Shuiwang, D., Z. Shen, and H. Hongyu. 2000. Transport of dissolved inorganic nitrogen from the major rivers to estuaries in China. *Nutrient Cycling in Agroecosystems* 57: 12-22.

Smith, R.A., R.B. Alexander, and G.E. Schwarz. 2003. Natural background concentrations of nutrients in streams and rivers of the continental United States. *Environmental Science and Technology* 37(14): 3039-3047.

Spoelstra, J., S.L. Schiff, R.J. Elgood, R.G. Semkin, and D.S. Jeffries. 2001. Tracing the sources of exported nitrate in the Turkey Lakes watershed using $^{15}N/^{14}N$ and $^{18}O/^{16}O$ isotopic ratios. *Ecosystems* 4: 536-544.

Stanley, E.H., and M.W. Doyle. 2002. A geomorphic perspective on nutrient retention following dam removal. *BioScience* 52(8): 693-701.

Takagi, K.K., K.S. Hunter, and S.B. Joye. Agents of change and temporal shifts in the Altamaha River nutrient dynamics. *Biogeochemistry*, in review.

Tomaszek, J.A., and P. Koszelnik. 2003. A simple model of nitrogen retention in reservoirs. *Hydrobiologia* 504: 51-58.

Turner, R.E., and N.N. Rabalais. 1991. Changes in Mississippi River water quality this century. *BioScience* 41(3): 140-147.

Turner, R.E., N.N. Rabalais, D. Justic, Q. Dortch. 2003. Global patterns of dissolved N, P and Si in large rivers. *Biogeochemistry* 64: 297-317.

Weston, N.B., J.T. Hollibaugh, and S.B. Joye. 2009. Population growth away from the coastal zone: Thirty years of land use change and nutrient export in the Altamaha River, GA. *Science of the Total Environment* 407: 3347-3356.

Yan, W., S. Zhang, P. Sun, S.P. Seitzinger. 2003. How do nitrogen inputs to the Changjiang basin impact the Changjiang River nitrate: A temporal analysis for 1968-1997. *Global Biogochemical Cycles* 17(4): 1091.

Table 3.1. Method for extracting N_2O from vials and analyzing on a coupled gas chromatograph and continuous flow isotope ratio mass spectrometer.

Time (s)	Reference 1	Reference 2	Reference 3	Split	Valco Inject	Trap	Trap 2	Flush Fill
0				off	on	off	off	
1				off	on			
5					off	on		
30	off		on					
60	off		off					
90	off		on					
120	off		off					
540							on	
599				off	on			
600						off		
850	off		on				off	
880	off		off					
900	off		on	on				
930	off		off					
960			on					
990			off					
1020			on					
1050			off					
1199				off				on

Table 3.2. 2007 inputs to subwatersheds of the greater Altamaha River. Reported values are local watershed inputs. All values are in kg km^{-2} yr^{-1}.

	Net food & feed import	Crop N fixation	Non-food crop exports	Fertilizer use	Forest N fixation	Atmos-pheric deposition	Manure volatil-ization	Total inputs
Altamaha	576	540	67	912	32	265	414	1,836
Upper Oconee	2,482	640	1	298	44	306	692	3,076
Lower Oconee	-108	325	19	282	43	272	26	766
Upper Ocmulgee	1,620	273	1	155	49	286	162	2,220
Lower Ocmulgee	-252	490	127	799	34	270	153	1,055
Little Ocmulgee	-335	486	75	584	34	268	29	928
Ohoopee	99	549	37	760	33	266	211	1,451

Table 3.3. Components of net food and feed import factor in subwatersheds of the greater Altamaha River for 2007. Reported values are total upstream inputs. All values are in kg km^{-2} yr^{-1} unless otherwise indicated.

	Population density (persons/km^2)	Human consumption	Livestock consumption	Livestock production	Crop production	Net food & feed import
Altamaha	80	402	1,537	459	631	850
Upper Oconee	74	369	3,909	1,235	560	2,482
Lower Oconee	49	246	2,290	700	525	1,310
Upper Ocmulgee	235	1,175	962	271	246	1,620
Lower Ocmulgee	148	738	938	264	612	799
Little Ocmulgee	15	75	395	56	750	-335
Ohoopee	16	81	1,209	356	834	99

Table 3.4. 2007 cumulative upstream inputs to subwatersheds of the greater Altamaha River. All values are in kg km^{-2} yr^{-1}.

	Net food & feed import	Crop N fixation	Non-food crop exports	Fertilizer use	Forest N fixation	Atmospheric deposition	Manure volatilization	Total inputs
Altamaha	850	454	38	449	41	281	262	1,771
Upper Oconee	2,482	640	1	298	44	306	692	3,076
Lower Oconee	1,310	497	9	291	44	291	390	2,030
Upper Ocmulgee	1,620	273	1	155	49	286	162	2,220
Lower Ocmulgee	799	368	56	438	43	279	158	1,709
Little Ocmulgee	-335	486	75	584	34	268	29	928
Ohoopee	99	549	37	760	33	266	211	1,451

Table 3.5. Total loads of TN, Cl^-, and Br^- in subwatersheds of the Altamaha River. All values in kmol/year.

Subwatershed	**TN**	**Cl^-**	**Br^-**
Altamaha	328,307	145,936	253
Upper Oconee	49,246	23,008	46
Lower Oconee	120,829	55,337	104
Upper Ocmulgee	160,044	67,509	100
Lower Ocmulgee	198,206	85,840	139
Little Ocmulgee	4,795	2,578	2
Ohoopee	27,626	12,568	9

Table 3.6. Relationships between local watershed inputs and concentrations.

	NH_4^+	NO_2^-	PO_4^{3+}
Population density	m=0.0052 b=1.47 R^2=0.73 p=0.01	m=9.3e-8 b=0.085 R^2=0.65 p=0.03	m=0.0032 b=0.19 R^2=0.58 p=0.048
Crop production	m=-0.0013 b=2.70 R^2=0.64 p=0.03		
Forest fixation	m=0.062 b=-0.60 R^2=0.76 p=0.01		

Table 3.7. Significant relationships between inputs to the surface of Altamaha subwatersheds and in-stream parameters, including the Upper Oconee watershed.

	Population Density	Crop Production	Crop Fixation	Net Food & Feed Import	Non-food Crop Export	Fertilizer Use	Forest N Fixation	Atmospheric Deposition	Total Inputs
NH_4^+		p=0.033758 R^2=0.62728 m=-0.0020522 b=2.9898			p=0.013179 R^2=0.73857 m=-0.014526 b=2.2221				
NO_2^-	p=0.0092637 R^2=0.77161 m=0.00082795 b=0.049183								
NO_3^-	p=0.0016213 R^2=0.88411 m=0.21398 b=4.784		p=0.019654 R^2=0.6957 m=-0.12553 b=82.2461						
DIN	p=0.0014824 R^2=0.88812 m=0.21889 b=6.2431		p=0.021457 R^2=0.68544 m=-0.12718 b=84.9066						
TDN	p=0.020077 R^2=0.69323 m=0.16649 b=26.92								
DON		p=0.023946 R2=0.67217 m=0.024198 b=1.8515		p=0.049083 R^2=0.57216 m=-0.0044378 b=20.5576		p=0.0035595 R^2=0.84234 m=0.025114 b=5.5596	p=0.0014647 R^2=0.88865 m=-0.8882 b=52.7214		
%ON							p=0.020415 R^2=0.69129 m=-0.15413 b=7.8527		
%OC							p=0.018866 R^2=0.70037 m=-1.095 b=55.8477		

POC			p=0.033736 R^2=0.62737 m=-0.0017286 b=1.7238						
TN	p=0.025667 R^2=0.66351 m=0.1666 b=31.981		p=0.013427 R^2=0.7367 m=-0.1161 b=100.8611						
Cl^-	p=0.021598 R^2=0.68466 m=0.44689 b=131.9193		p=0.0050349 R^2=0.81956 m=-0.32335 b=322.2567						
Br^-	p=0.013777 R^2=0.73411 m=0.00071806 b=0.20252	p=0.022956 R^2=0.67733 m=-0.00029071 b=0.43859				p=0.033481 R^2=0.62842 m=-0.00025961 b=0.37615	p=0.0054681 R^2=0.81369 m=0.010172 b=-0.15205		
SO_4^{2-}		p=0.042225 R^2=0.59513 m=-0.26025 b=268.8939					p=0.037587 R^2=0.61214 m=8.4257 b=-231.9076		
PO_4^{3-}	p=0.016285 R^2=0.71662 m=0.0036559 b=0.062592		p=0.013932 R^2=0.73297 m=-0.0024452 b=1.5263						
$\delta^{18}O$-H_2O	p=0.022345 R^2=0.6806 m=-0.0019316 b=-2.6719	p=0.014092 R^2=0.7318 m=0.00084421 b=-3.3439				p=0.0083909 R^2=0.78014 m=0.00080811 b=-3.1856	p=0.0078353 R^2=0.78587 m=-0.027927 b=-1.6949		
D/H-H_2O		p=0.020751 R^2=0.68939 m=0.008478 b=-21.4596		p=0.014995 R^2=0.72538 m=-0.0017287 b=-14.7363		p=0.0024095 R^2=0.86463 m=0.0088025 b=-20.162	p=0.00062047 R^2=0.92063 m=-0.31276 b=-3.5725	p=0.021869 R^2=0.68317 m=-0.11375 b=15.6835	p=0.046872 R^2=0.57931 m=-0.0021712 b=-12.3327
$\delta^{15}N$-particulates				p=0.0059726 R^2=0.8072 m=0.0010801 b=4.5108				p=0.00225 R^2=0.86821 m=0.075952 b=-15.8725	p=0.01058 R^2=0.75965 m=0.0014726 b=2.7905

Table 3.8. Significant relationships between inputs to the surface of Altamaha subwatersheds and in-stream parameters, excluding the Upper Oconee watershed.

	Population Density	Crop Production	Crop Fixation	Net Food & Feed Import	Non-food Crop Export	Fertilizer Use	Forest N Fixation	Atmospheric Deposition	Total Inputs
Conductivity				p=0.010188 R^2=0.83981 m=0.04353 b=82.9495			p=0.021862 R^2=0.76863 m=4.878 b=-83.4165	p=0.017354 R^2=0.79285 m=3.1116 b=-751.3759	p=0.016258 R^2=0.79924 m=0.068152 b=-0.35576
pH								p=0.039511 R^2=0.69387 m=0.026111 b=-0.47466	
NH_4^+					p=0.020113 R^2=0.77767 m=-0.01645 b=2.3273				
NO_2^-	p=0.019813 R^2=0.77926 m=0.00083465 b=0.046113								
NO_3^-	p=0.0025372 R^2=0.91889 m=0.21025 b=6.4938		p=0.017611 R^2=0.79138 m=-0.16753 b=98.8919						
DIN	p=0.0025321 R^2=0.91897 m=0.21529 b=7.8915		p=0.01814 R^2=0.7884 m=-0.17122 b=102.3636						
TDN	p=0.024313 R^2=0.75661 m=0.16141 b=29.248								
DON		p=0.043489 R^2=0.67983 m=0.023846 b=2.3745		p=0.022849 R^2=0.7637 m=-0.0070623 b=21.7905		p=0.010617 R^2=0.83658 m=0.025444 b=5.3284	p=0.0053171 R^2=0.88333 m=-0.88968 b=52.7681	p=0.010919 R^2=0.83434 m=-0.54306 b=167.7911	

%ON				p=0.0087205 R^2=0.85147 m=-0.0014926 b=2.5548			p=0.018199 R^2=0.78806 m=-0.16821 b=8.2975	p=0.020551 R^2=0.77536 m=-0.10479 b=30.6331	p=0.013866 R^2=0.81405 m=-0.0023423 b=5.4203
%OC				p=0.011339 R^2=0.83129 m=-0.010444 b=18.1949			p=0.020011 R^2=0.77821 m=-1.1836 b=58.6493	p=0.016348 R^2=0.79871 m=-0.75313 b=220.2027	p=0.019825 R^2=0.7792 m=-0.016228 b=37.9734
POC						p=0.040296 R^2=0.69103 m=-0.00052647 b=1.2386	p=0.045276 R^2=0.67376 m=0.017689 b=0.28621	p=0.03316 R^2=0.71806 m=0.01147 b=-2.1877	
TN	p=0.016906 R^2=0.79543 m=0.15955 b=35.2082		p=0.048956 R^2=0.6617 m=-0.12495 b=104.3708						
Cl^-	p=0.0077871 R^2=0.85943 m=0.42666 b=141.1843		p=0.024374 R^2=0.75632 m=-0.34367 b=330.3076						
Br^-	p=0.029679 R^2=0.73245 m=0.00071707 b=0.20298	p=0.038488 R^2=0.69761 m=-0.00029493 b=0.44485		p=0.0081672 R^2=0.85613 m=9.1297e-005 b=0.20185		p=0.031144 R^2=0.72628 m=-0.00028946 b=0.39706	p=0.003499 R^2=0.90498 m=0.010995 b=-0.17807	p=0.032696 R^2=0.71993 m=0.0061592 b=-1.4459	p=0.018405 R^2=0.78692 m=0.00014047 b=0.031285
SO_4^{2-}		p=0.026792 R^2=0.74511 m=-0.2718 b=286.0476		p=0.0012436 R^2=0.94297 m=0.085439 b=61.1624		p=0.0084436 R^2=0.85378 m=-0.27986 b=247.8455	p=0.0023977 R^2=0.92112 m=9.8914 b=-278.2327	p=0.0016481 R^2=0.93445 m=6.2572 b=-1618.1282	p=0.0090043 R^2=0.84914 m=0.13012 b=-96.2029
PO_4^{3-}	p=0.010525 R^2=0.83726 m=0.0035181 b=0.1257	p=0.035609 R^2=0.70844 m=-0.0013639 b=1.2625	p=0.044195 R^2=0.67742 m=-0.0027171 b=1.6341						p=0.047769 R^2=0.66553 m=0.00059281 b=-0.55428
$\delta^{18}O$-H_2O	p=0.035687 R^2=0.70814 m=-0.001965 b=-2.6566	p=0.030188 R^2=0.73029 m=0.00084098 b=-3.3391	p=0.019098 R^2=0.78311 m=0.0017743 b=-3.6115			p=0.016506 R^2=0.79777 m=0.00084548 b=-3.2118	p=0.017205 R^2=0.7937 m=-0.028696 b=-1.6706		

D/H-H_2O		p=0.027062 R^2=0.7439 m=0.0082362 b=-21.1007		p=0.013815 R^2=0.81439 m=-0.002408 b=-14.4172		p=0.0079832 R^2=0.85772 m=0.0085068 b=-19.9549	p=0.0017958 R^2=0.9316 m=-0.30168 b=-3.9227	p=0.012544 R^2=0.82284 m=-0.17807 b=33.3897	p=0.043332 R^2=0.68037 m=-0.0035323 b=-10.2094
δ^{15}N-NOx									p=0.03944 R^2=0.69412 m=0.0055101 b=-1.7668
δ^{18}O-NOx				p=0.039575 R^2=0.69363 m=-0.0078029 b=14.8764					p=0.014736 R^2=0.80852 m=-0.01352 b=32.0054
δ^{15}N-particulates						p=0.034295 R^2=0.71356 m=-0.0032925 b=6.7138	p=0.020916 R^2=0.77346 m=0.11664 b=0.51348	p=0.034547 R^2=0.71257 m=0.070317 b=-14.3213	

Table 3.9. Literature source values used in the estimation of predicted isotopic values of in-stream NO_3^-.

Source	15N	18O	Reference
swine slurry	29.68		Curt et al. 2003
poultry manure	10.98		Curt et al. 2003
dairy cattle manure	12.19		Curt et al. 2003
beef cattle manure	16.21		Curt et al. 2003
sheep manure	17.14		Curt et al. 2003
sewage	7.35		Widory et al. 2004
Atmospheric deposition	3	56	Kendall et al. 2007
manure/sewage	12.5	0	Kendall et al. 2007
fertilizer	0.5	22	Kendall et al. 2007
natural soil organic N	1	--	Kendall et al. 2007
soil nitrification	-0.5	0	Kendall et al. 2007

Table 3.10. Predicted values of $\delta^{15}N\text{-}NO_3$ and $\delta^{18}O\text{-}NO_3$ in subwatersheds of the Altamaha River watershed, based on literature source values, and the average of measured isotopes of NO_3^-.

River	Predicted $\delta^{15}N\text{-}NO_3$	Measured $\delta^{15}N\text{-}NO_3$	Predicted $\delta^{18}O\text{-}NO_3$	Measured $\delta^{18}O\text{-}NO_3$
Altamaha	7.4	8.0	9.7	6.7
Upper Oconee	9.0	7.5	5.7	6.9
Lower Oconee	8.3	7.5	7.9	5.7
Upper Ocmulgee	9.4	10.2	7.7	5.2
Lower Ocmulgee	7.5	9.4	10.2	5.4
Little Ocmulgee	4.2	1.7	15.7	22.8
Ohoopee	5.5	8.3	12.7	9.5

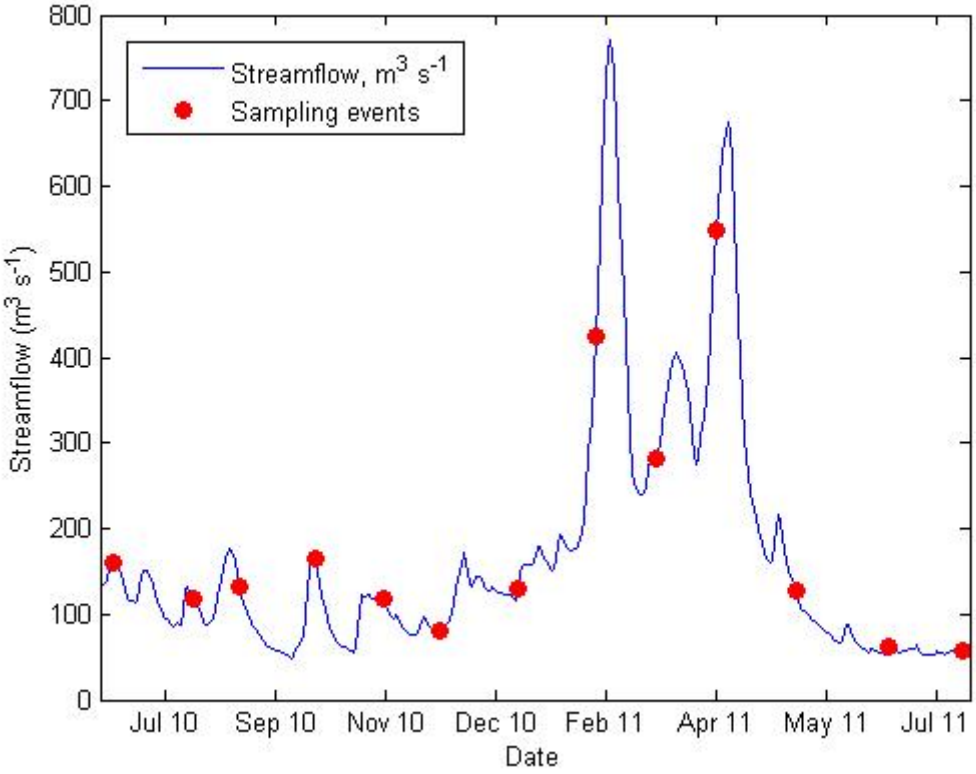


Figure 3.1. Streamflow in the mainstem Altamaha over the course of the sampling period, with sampling events marked in red.

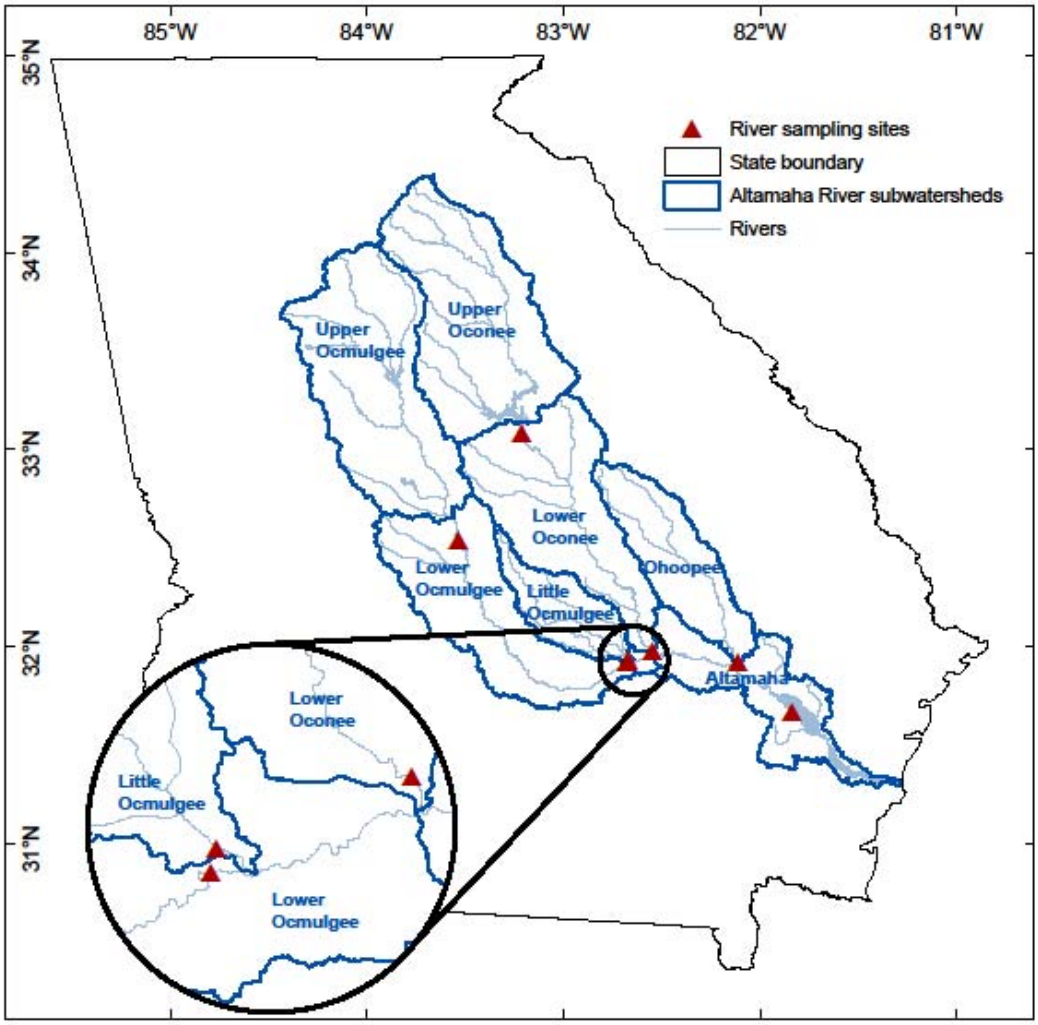


Figure 3.2. Sampling sites of subwatersheds of the greater Altamaha River within the state of Georgia.

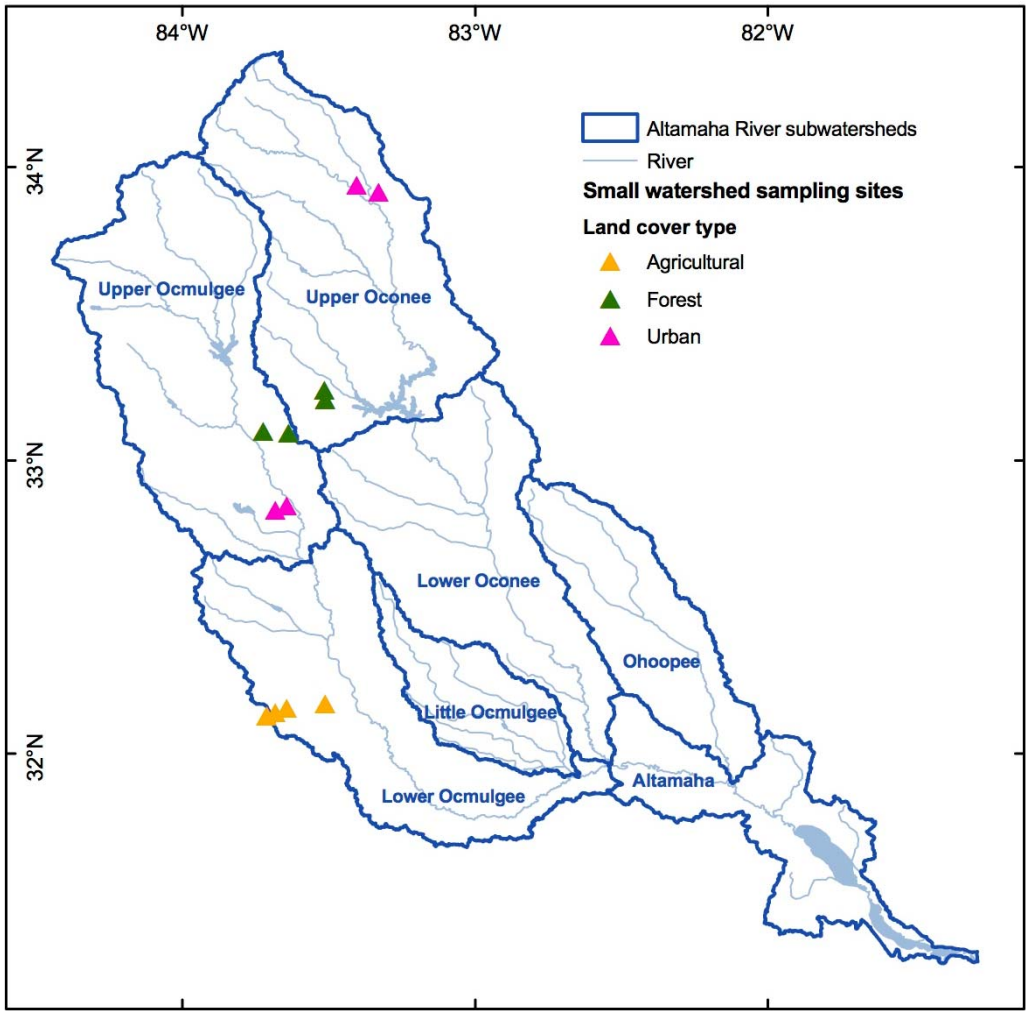


Figure 3.3. Sites of small watersheds with uniform land use sampled in relation to the Altamaha River watershed.

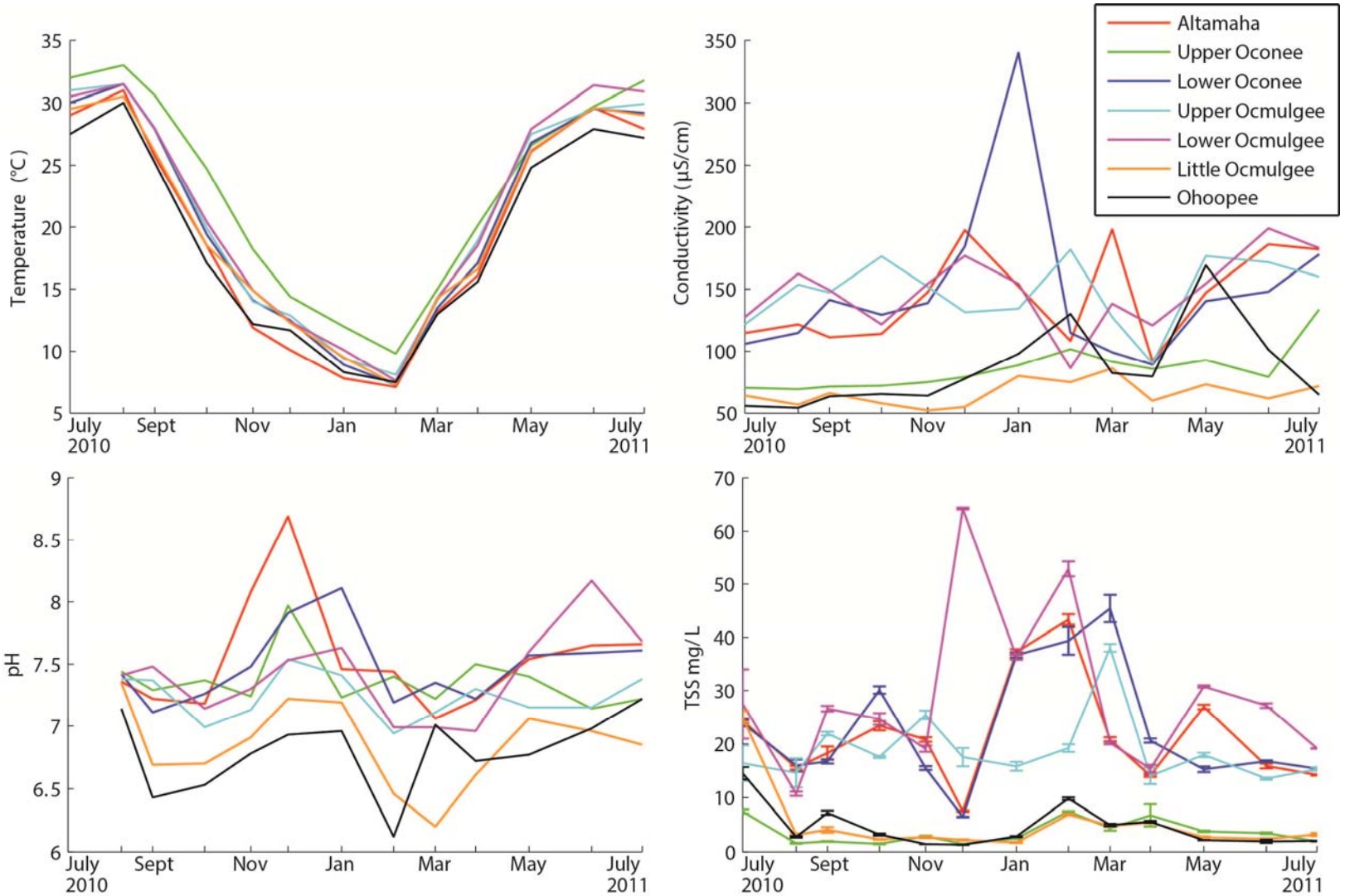


Figure 3.4. Time series of physical parameters measured in subwatersheds of the Altamaha River. Error bars in this figure represent analytical error.

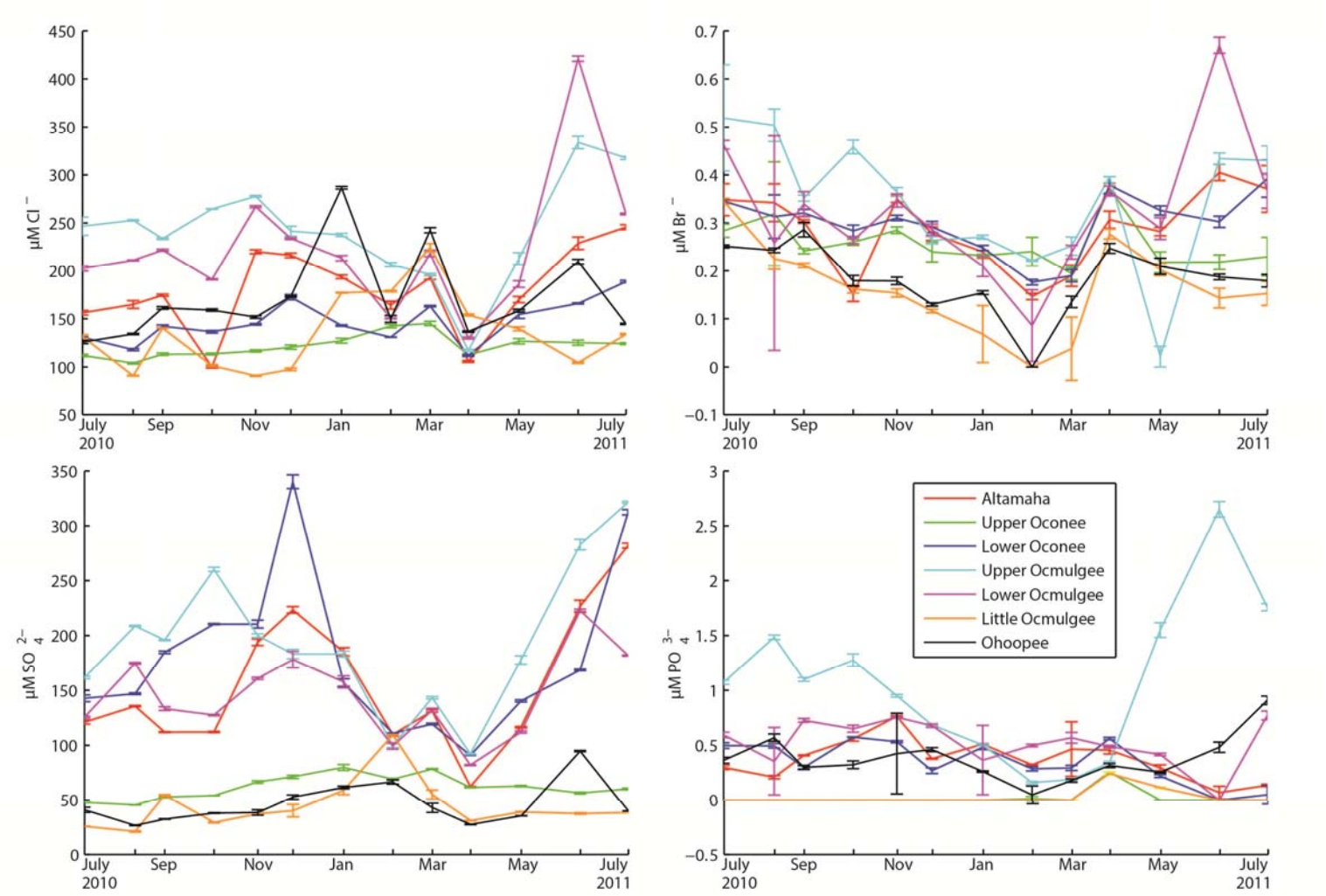


Figure 3.5. Time series of anions measured in subwatersheds of the Altamaha River. Error bars in this figure represent analytical error.

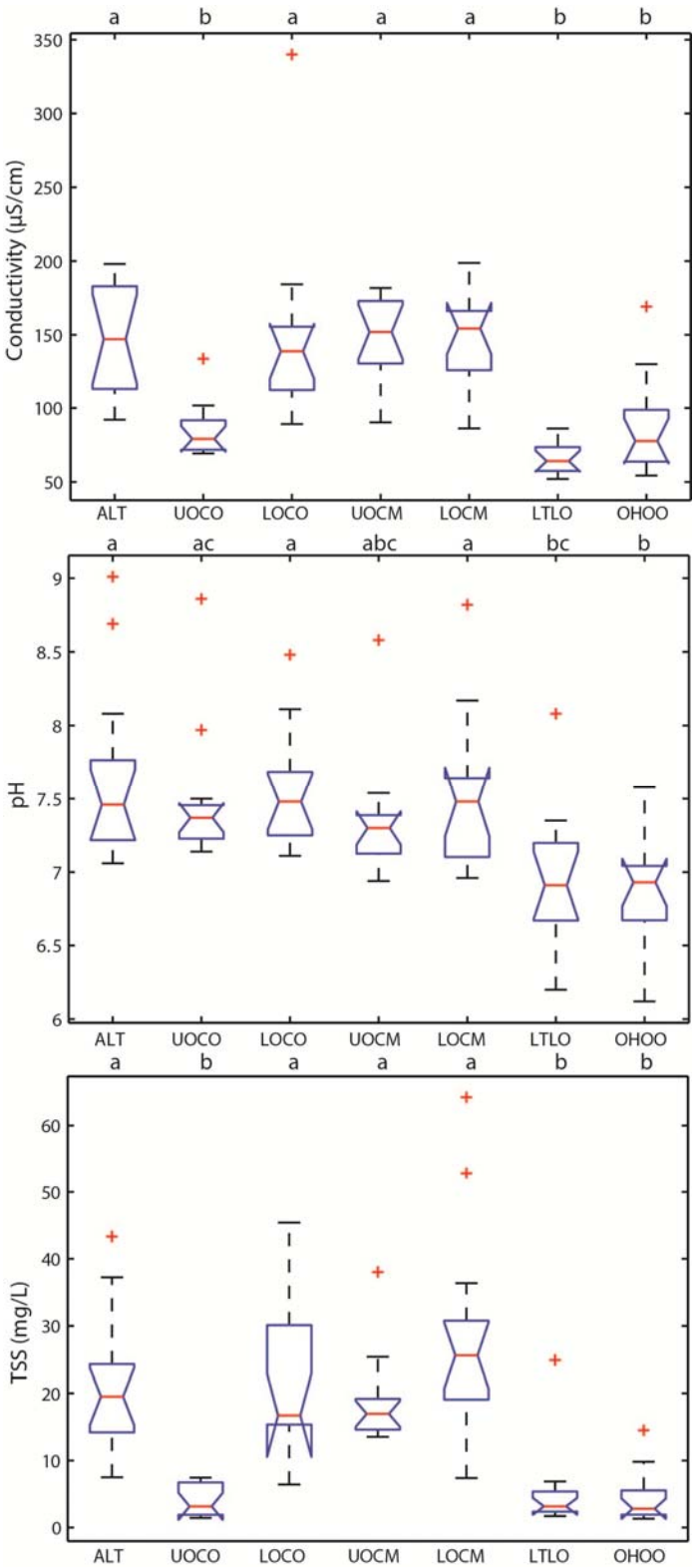


Figure 3.6. Box and whisker plot of physical parameters measured in Altamaha River subwatersheds. ALT, mainstem Altamaha; UOCO, Upper Oconee; LOCO, Lower Oconee; UOCM, Upper Ocmulgee; LOCM, Lower Ocmulgee; LTLO, Little Ocmulgee; OHOO, Ohoopee. Sample groups sharing a letter are not statistically different.

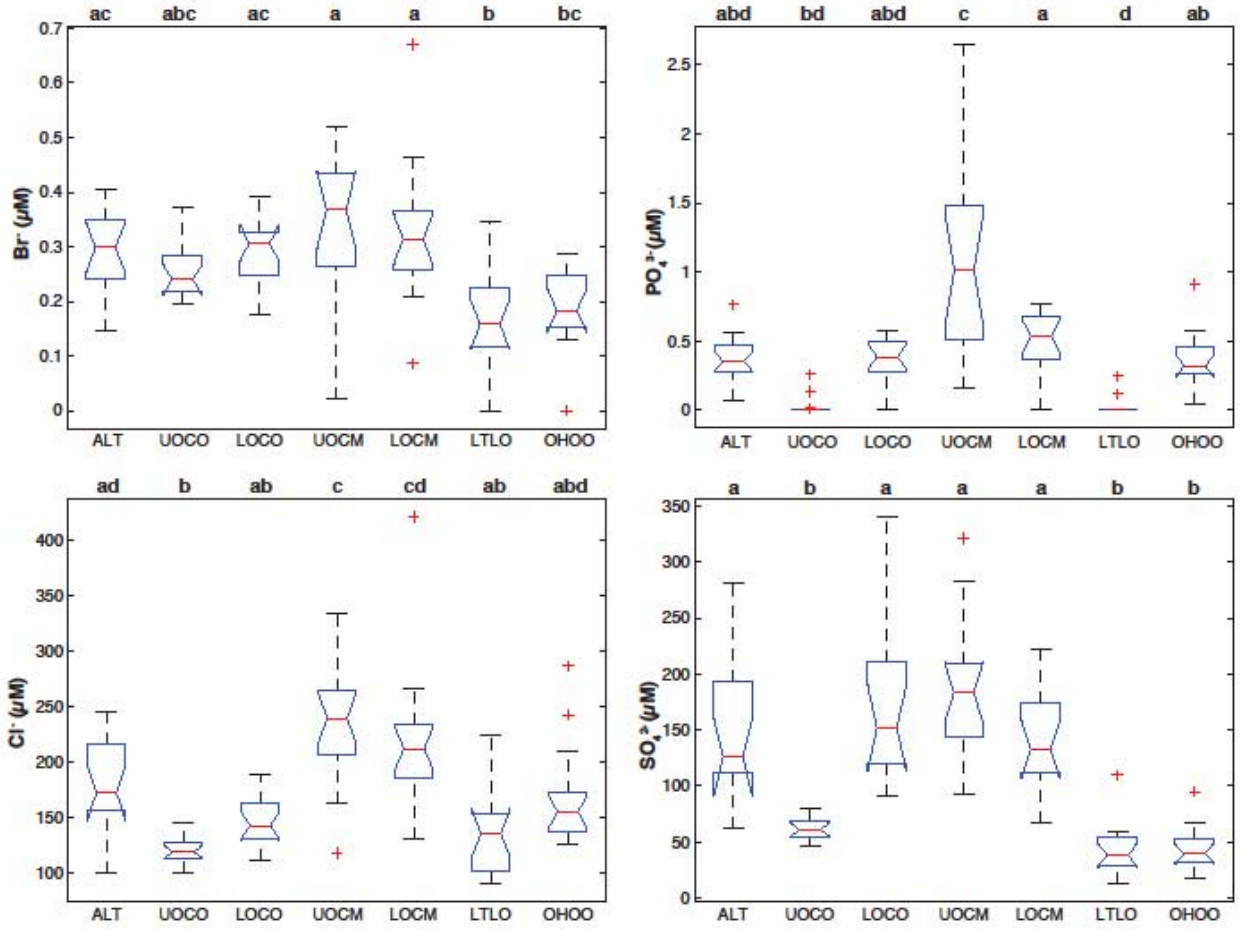


Figure 3.7. Box and whisker plot of anions measured in subwatersheds of the Altamaha River. ALT, mainstem Altamaha; UOCO, Upper Oconee; LOCO, Lower Oconee; UOCM, Upper Ocmulgee; LOCM, Lower Ocmulgee; LTLO, Little Ocmulgee; OHOO, Ohoopee. Sample groups sharing a letter are not statistically different.

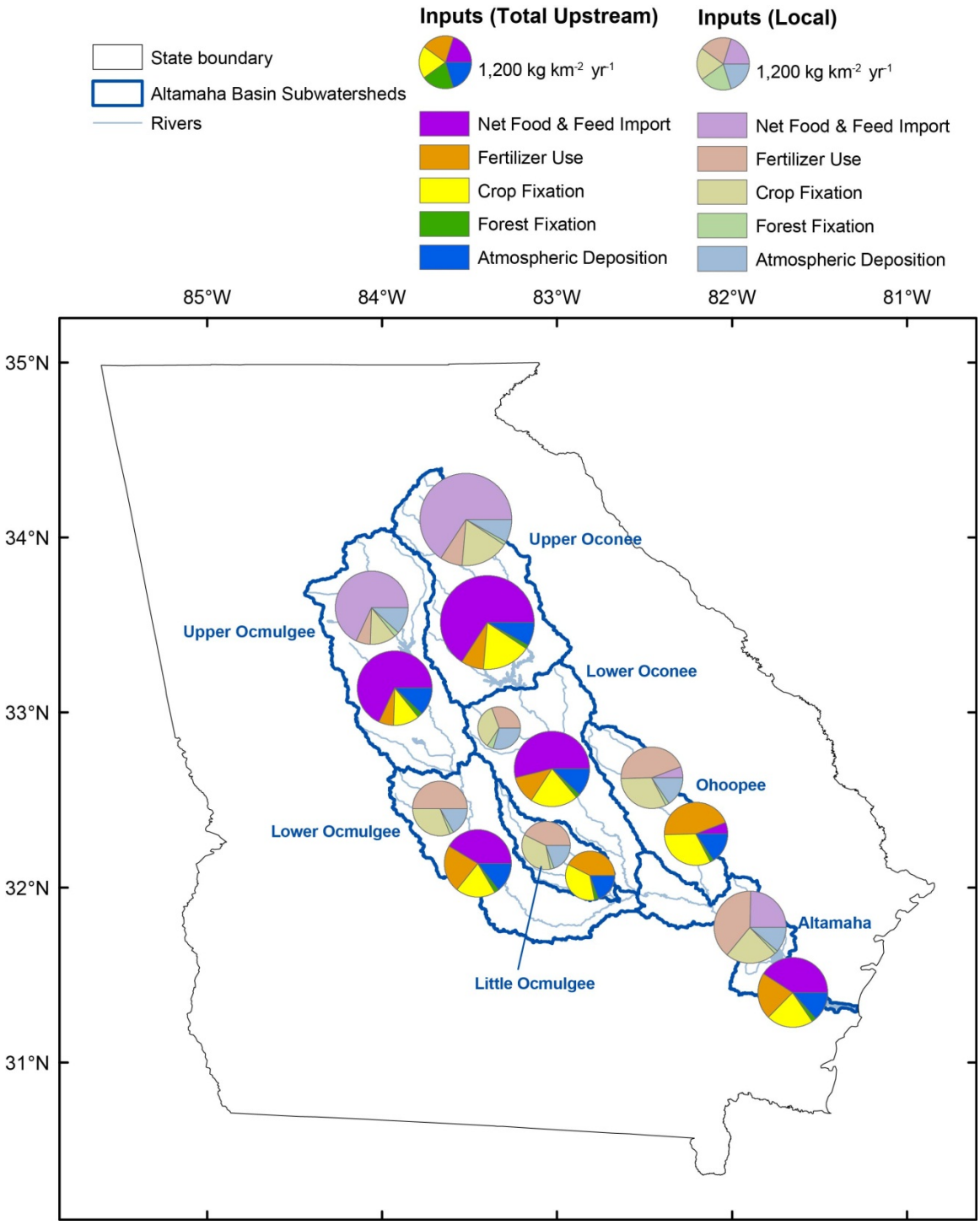


Figure 3.8. Local (subwatershed only) and cumulative upstream inputs to subwatersheds of the Altamaha River.

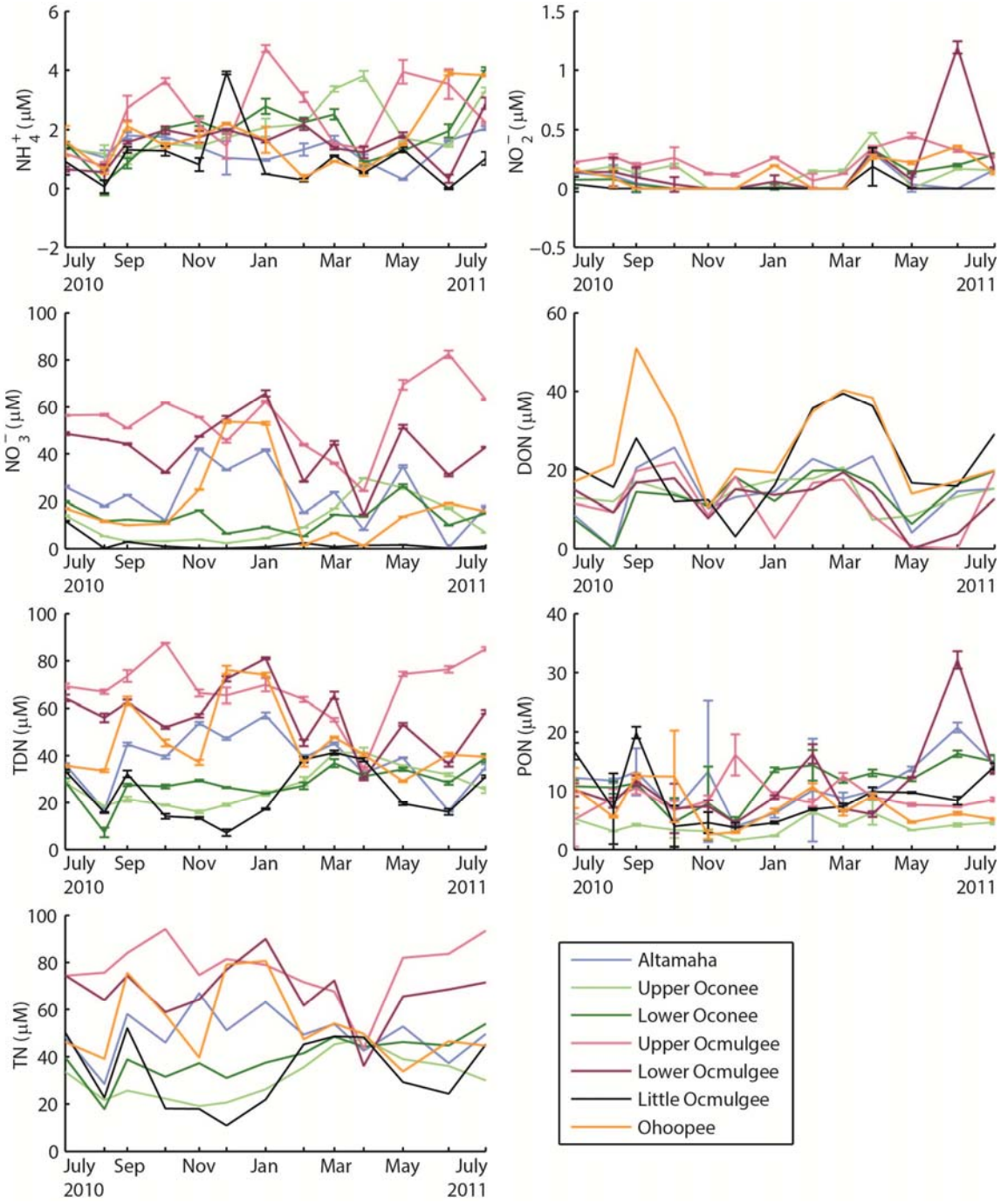


Figure 3.9. Time series of nitrogen concentrations measured in subwatersheds of the Altamaha River. Error bars in this figure represent analytical error.

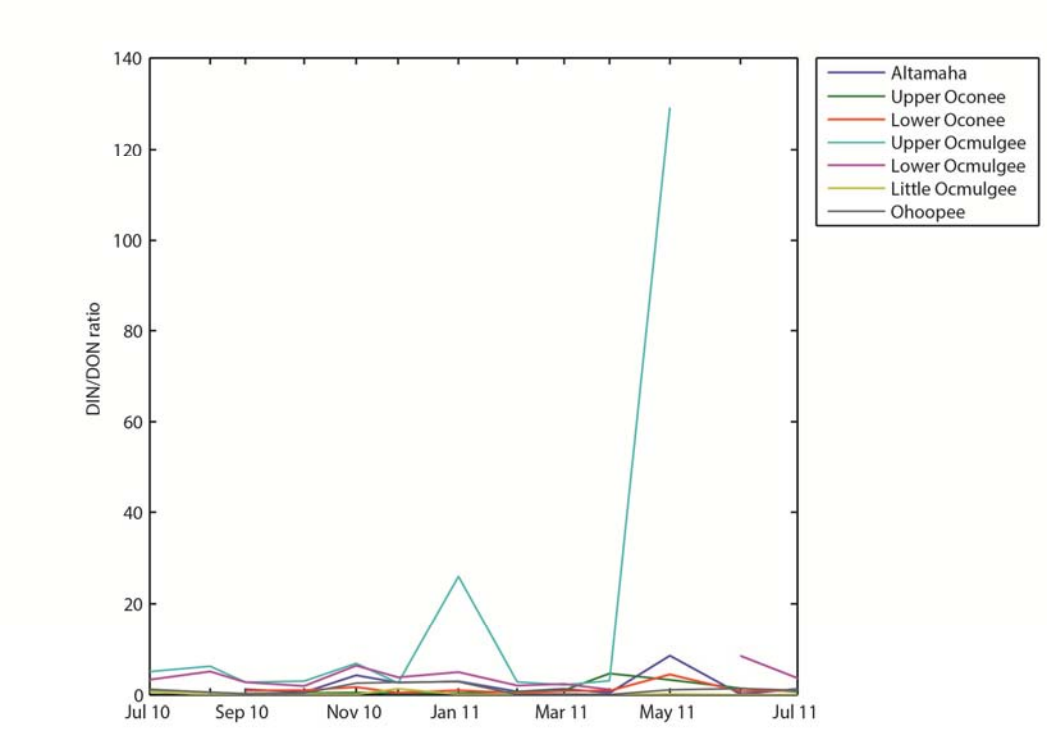


Figure 3.10. Ratio of DIN to DON over time in subwatersheds of the Altamaha River. Missing values indicate that measured DON concentration was 0.

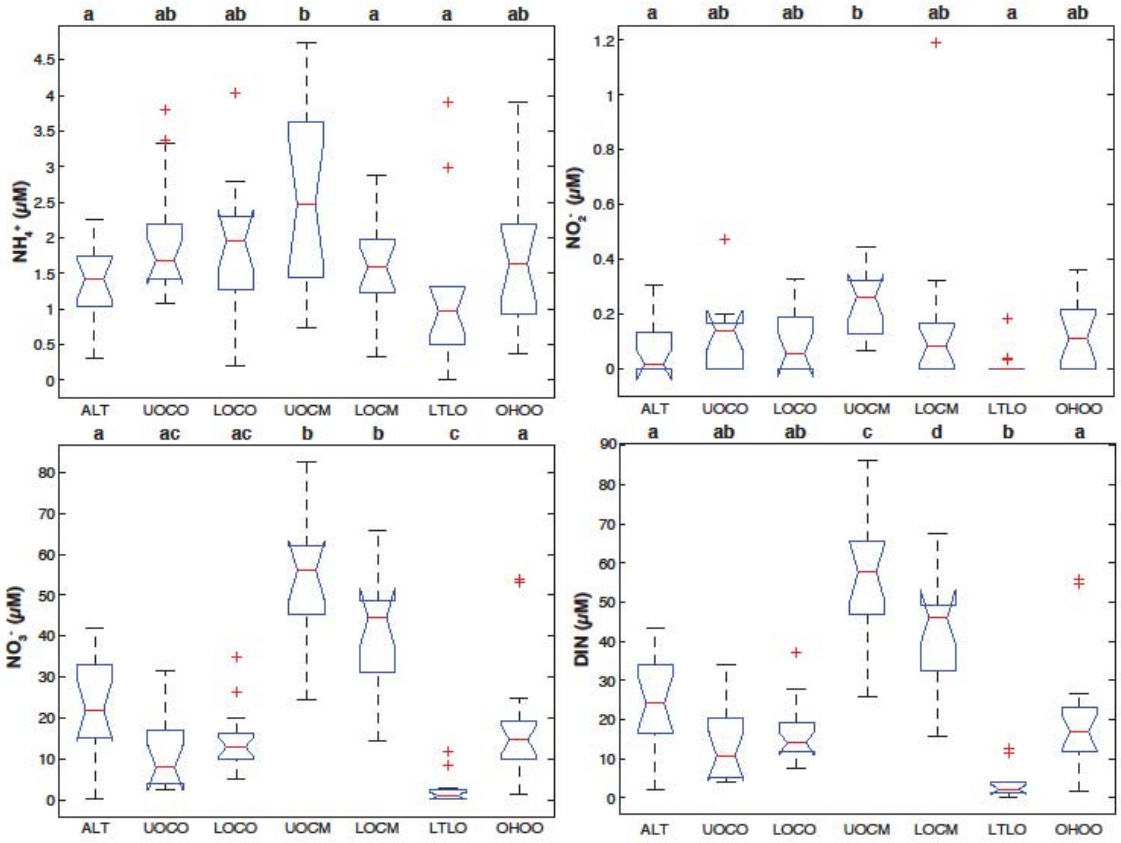


Figure 3.11. Box and whisker plot of concentrations of nitrogen measured in subwatersheds of the Altamaha River. ALT, mainstem Altamaha; UOCO, Upper Oconee; LOCO, Lower Oconee; UOCM, Upper Ocmulgee; LOCM, Lower Ocmulgee; LTLO, Little Ocmulgee; OHOO, Ohoopee. Sample groups sharing a letter are not statistically different.

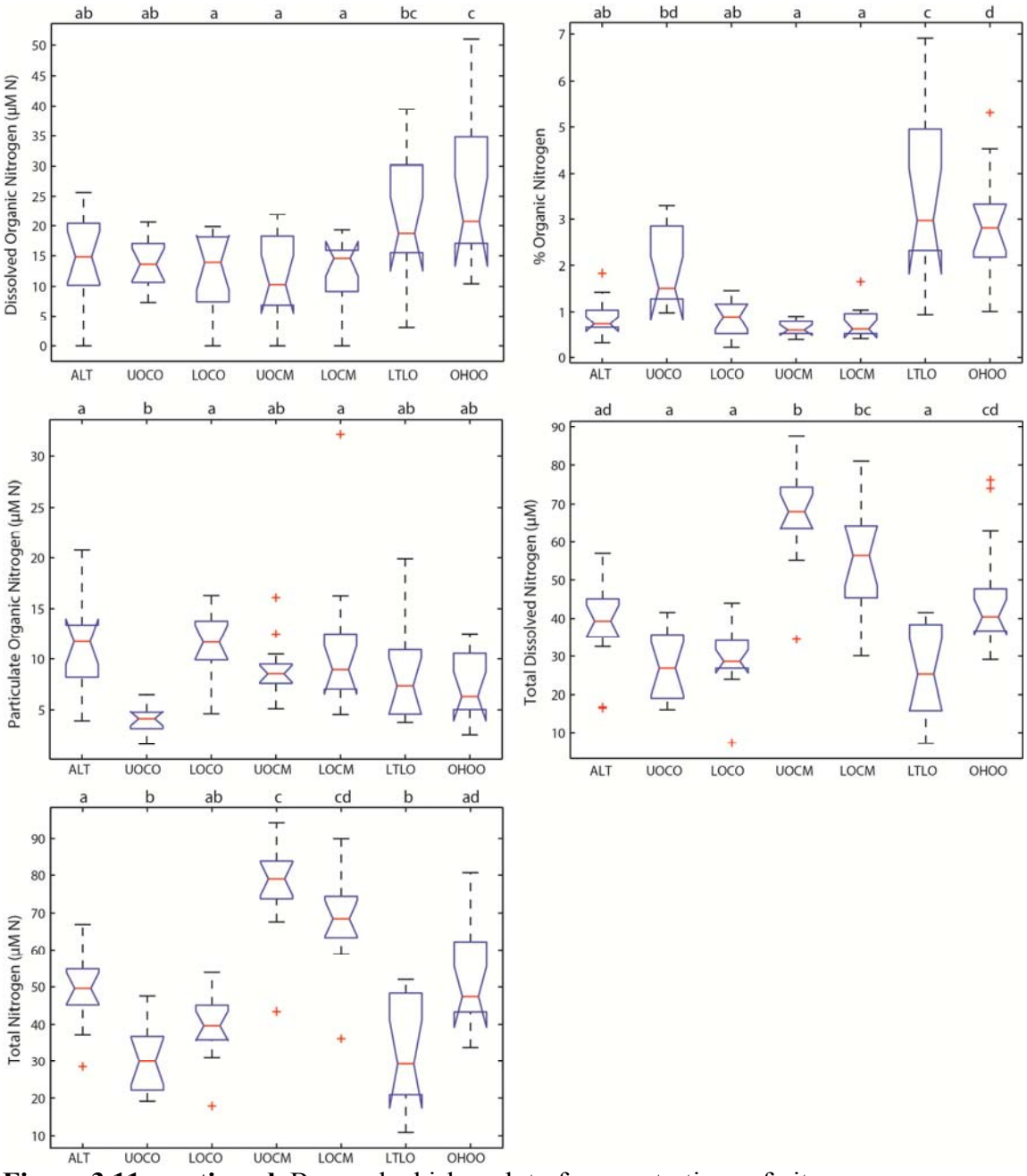


Figure 3.11, continued. Box and whisker plot of concentrations of nitrogen measured in subwatersheds of the Altamaha River. ALT, mainstem Altamaha; UOCO, Upper Oconee; LOCO, Lower Oconee; UOCM, Upper Ocmulgee; LOCM, Lower Ocmulgee; LTLO, Little Ocmulgee; OHOO, Ohoopee. Sample groups sharing a letter are not statistically different.

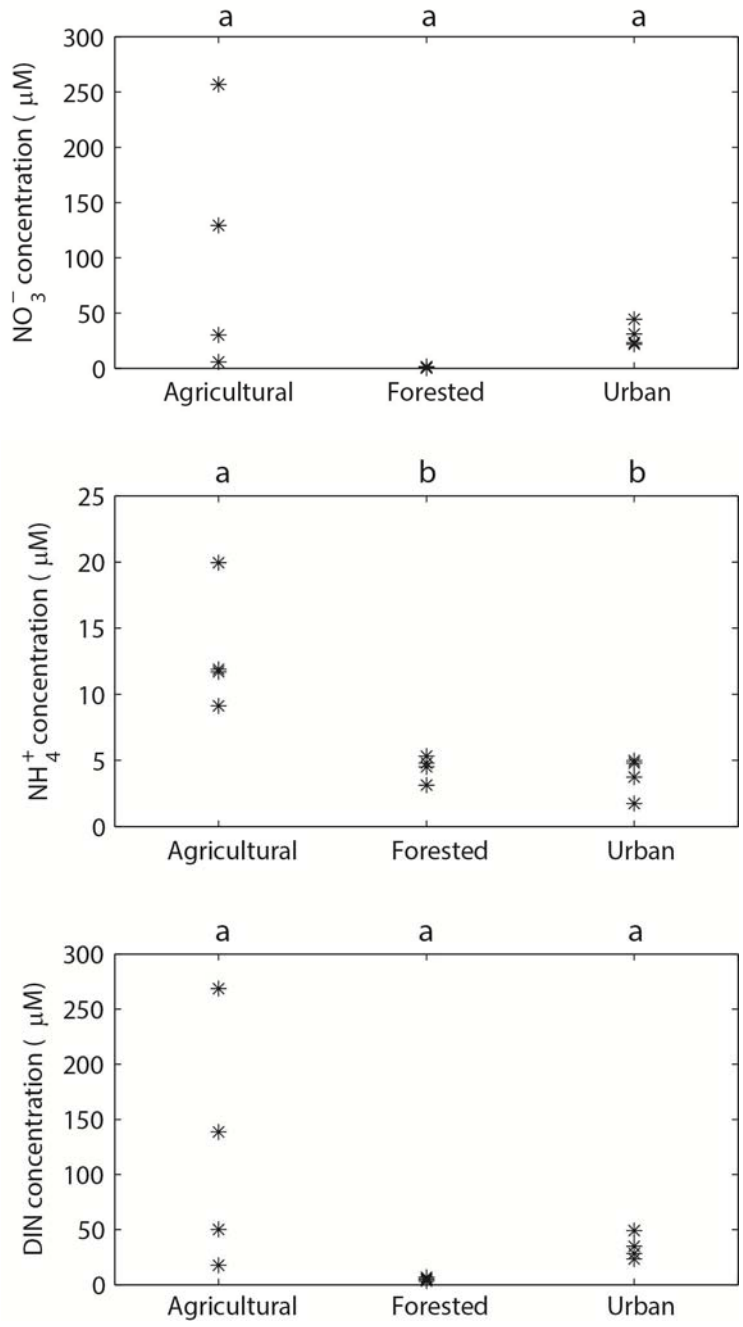


Figure 3.12. N concentrations measured in headwater streams with watersheds of uniform use. Sample groups sharing a letter are not significantly different.

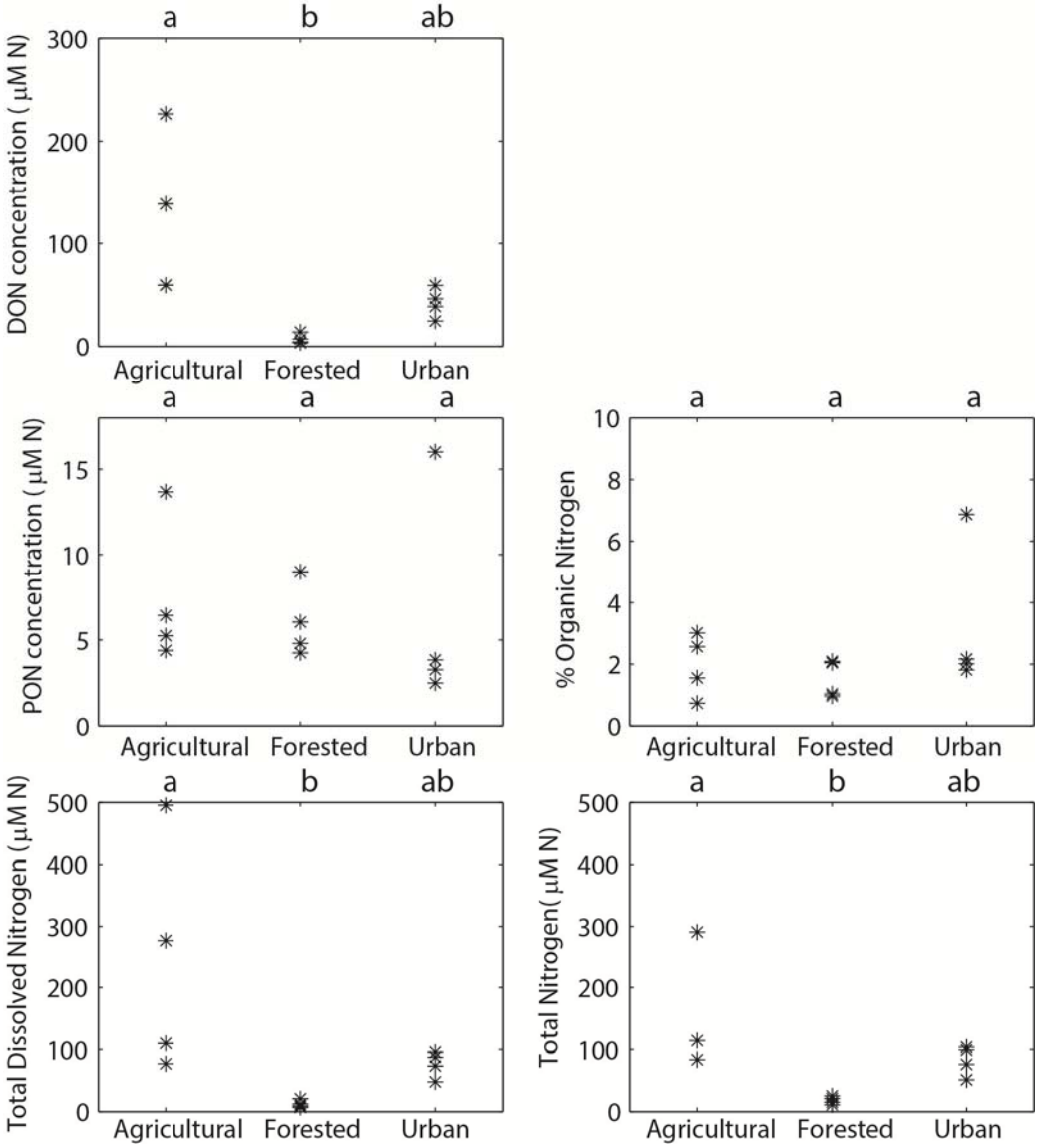


Figure 3.12, continued. N concentrations measured in headwater streams with watersheds of uniform use. Sample groups sharing a letter are not significantly different.

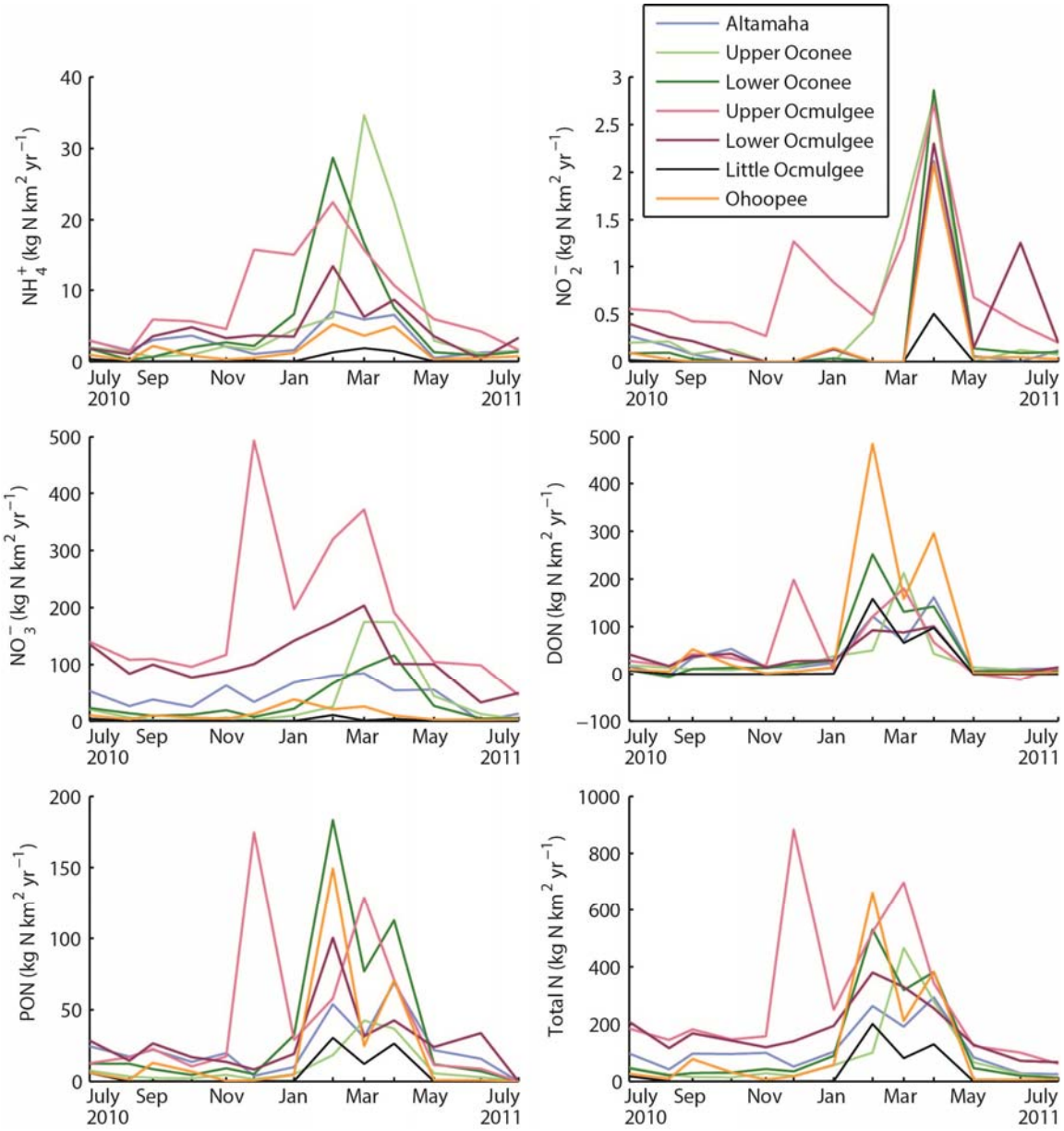


Figure 3.13. Time series of area-normalized N loads in subwatersheds of the Altamaha River.

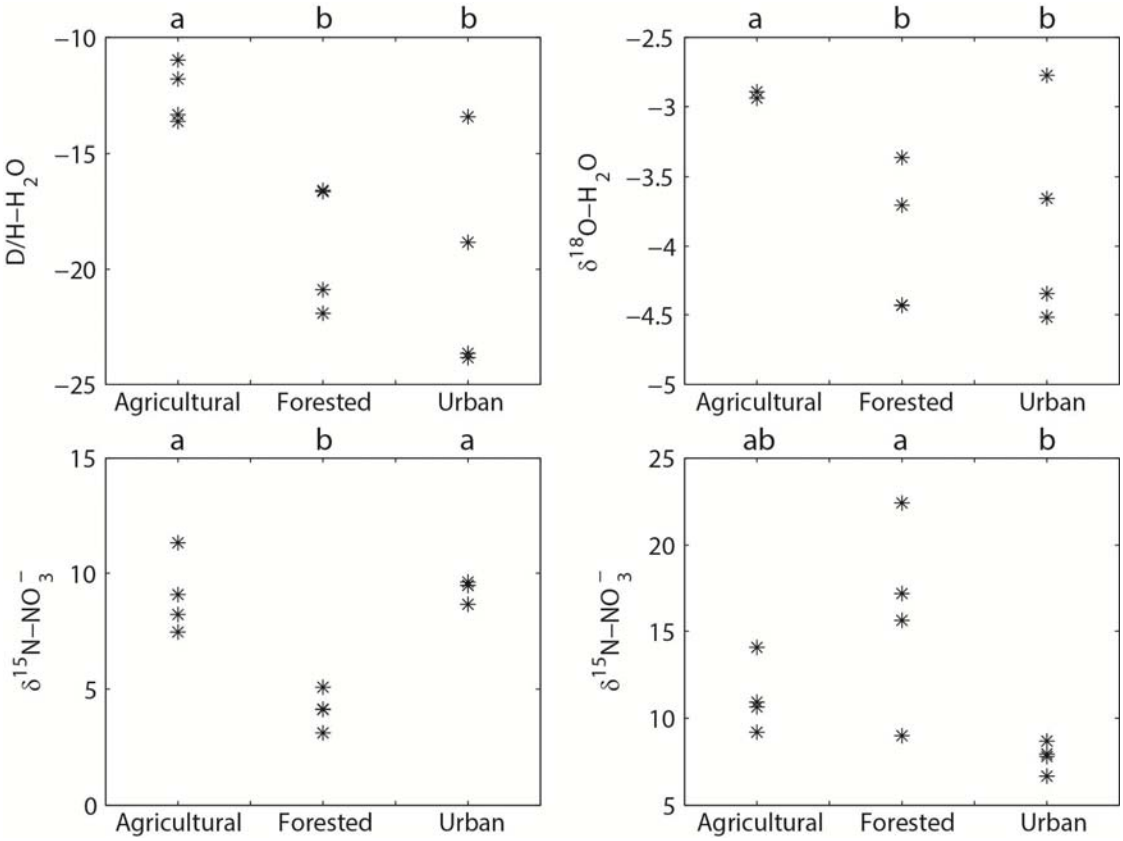


Figure 3.14. Isotope measurements in headwater streams with watersheds of uniform use. Sample groups sharing a letter are not significantly different.

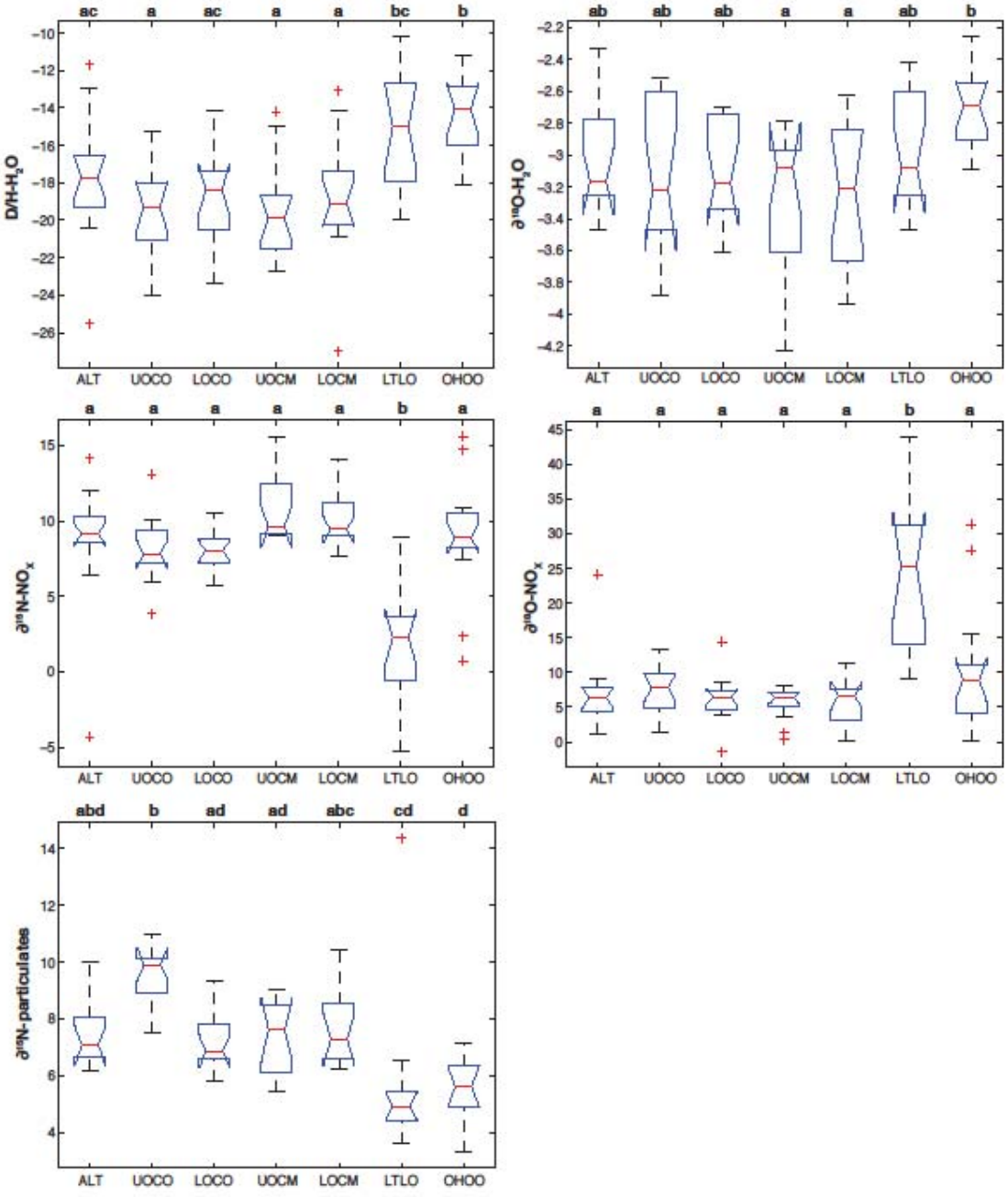


Figure 3.15. Box and whisker plot of isotopes measured in subwatersheds of the Altamaha River. Sample groups sharing a letter are not statistically different.

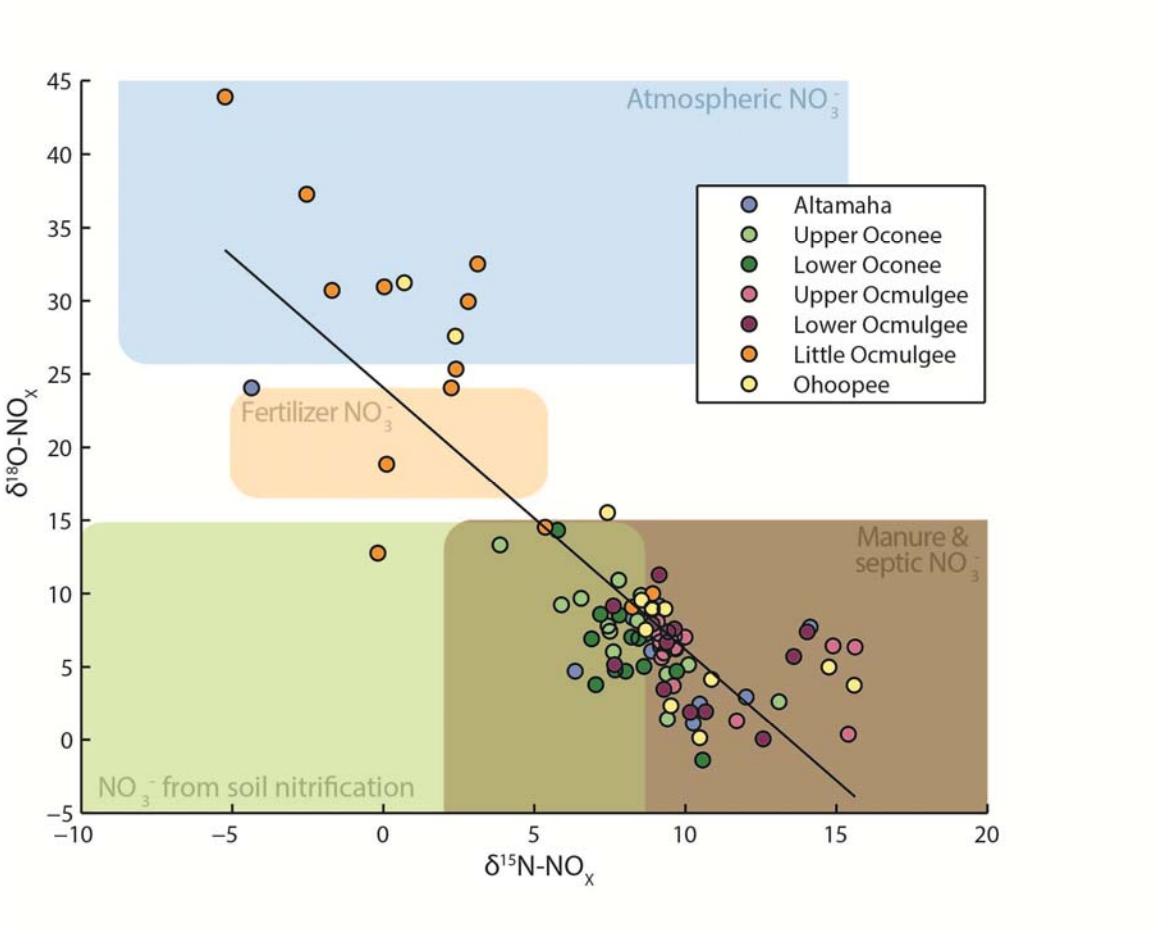


Figure 3.16. δ^{15}N-NOx versus δ^{18}O-NOx in subwatersheds of the Altamaha River. Source values from Kendall et al. (2007). Regression line: y = -1.79x + 24.07 (R^2=0.70, p<0.0001).

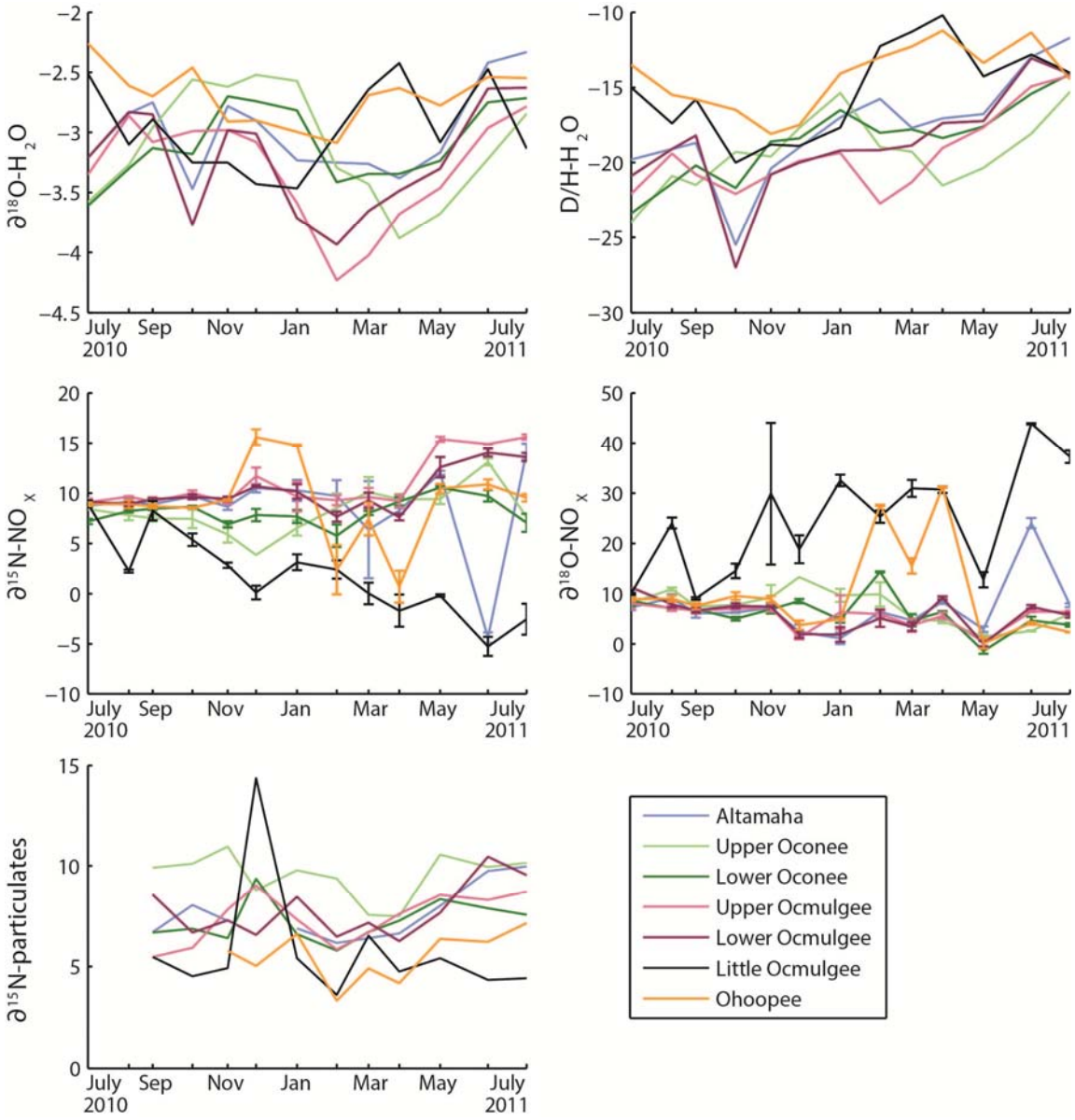


Figure 3.17. Time series of N isotopes in subwatersheds of the Altamaha River. Error bars in this figure represent analytical error.

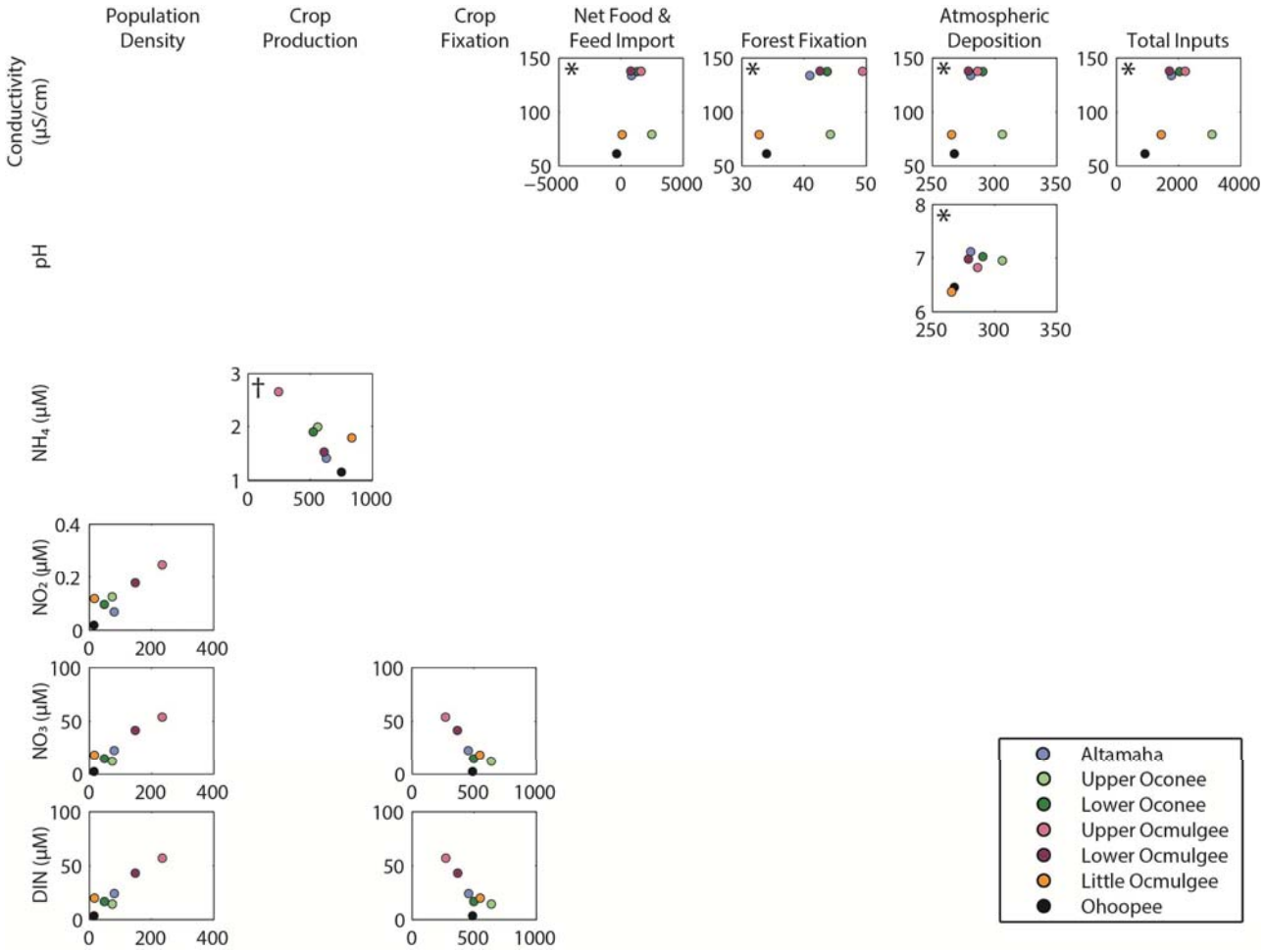


Figure 3.18. Plots of average in-stream measurements of studied parameters versus inputs to the surface of subwatersheds of the greater Altamaha. Asterisks (*) indicate results that are significant only when the Upper Oconee subwatershed is omitted; crosses (†) indicate results that are significant only when the Upper Oconee subwatershed in included. All other results are significant in both cases.

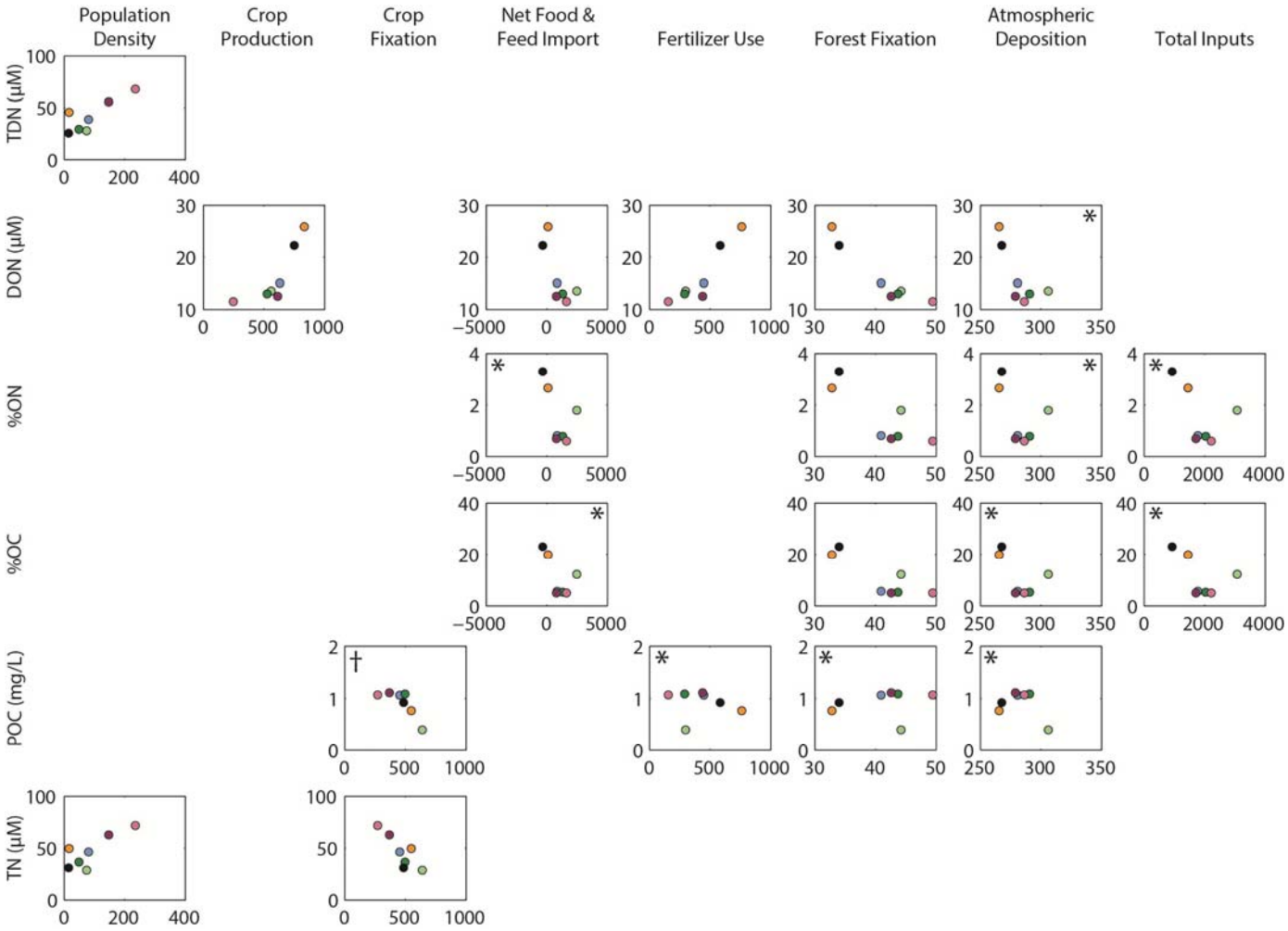


Figure 3.18, continued. Plots of average in-stream measurements of studied parameters versus inputs to the surface of subwatersheds of the greater Altamaha. Asterisks (*) indicate results that are significant only when the Upper Oconee subwatershed is omitted; crosses (†) indicate results that are significant only when the Upper Oconee subwatershed in included. All other results are significant in both cases.

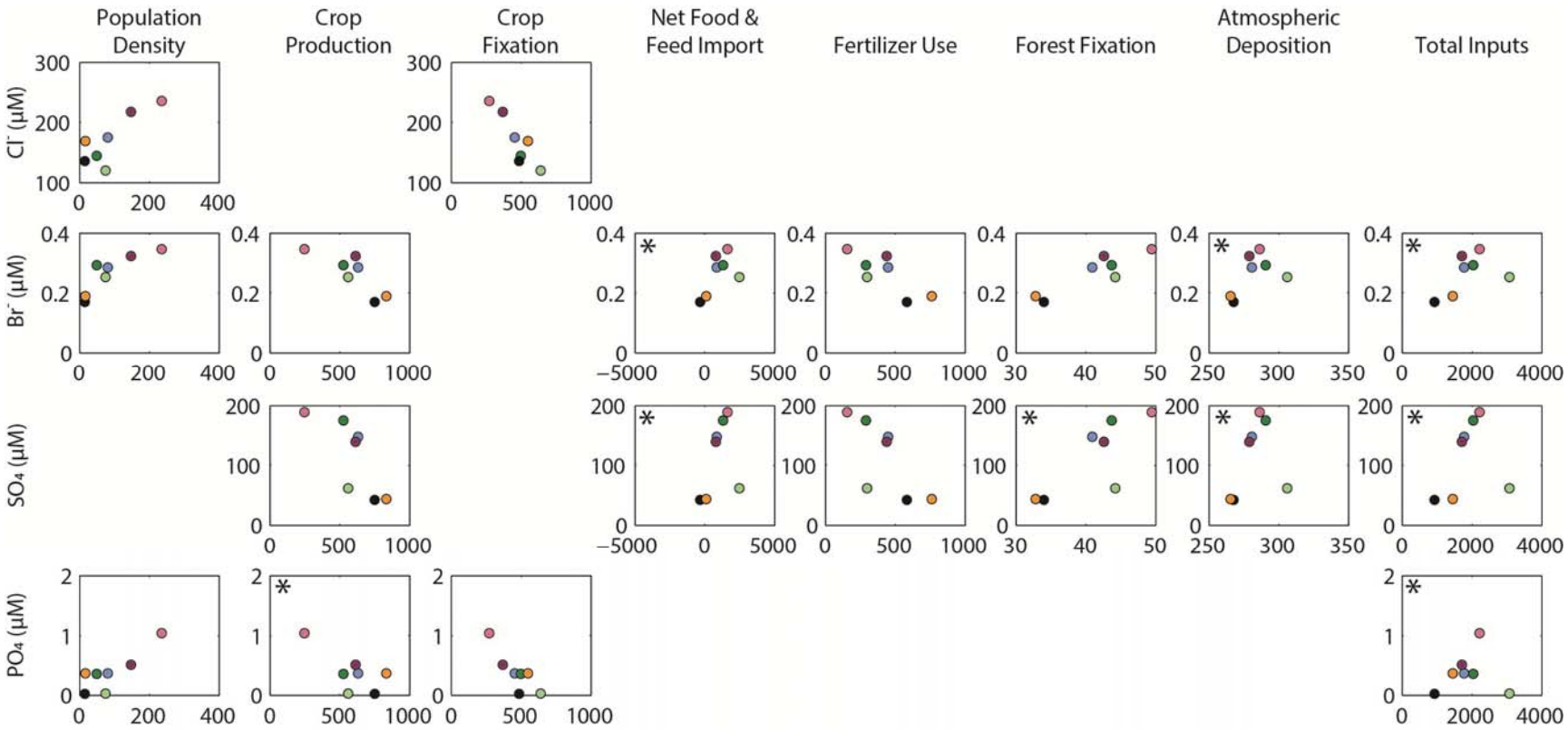


Figure 3.18, continued. Plots of average in-stream measurements of studied parameters versus inputs to the surface of subwatersheds of the greater Altamaha. Asterisks (*) indicate results that are significant only when the Upper Oconee subwatershed is omitted; crosses (†) indicate results that are significant only when the Upper Oconee subwatershed in included. All other results are significant in both cases.

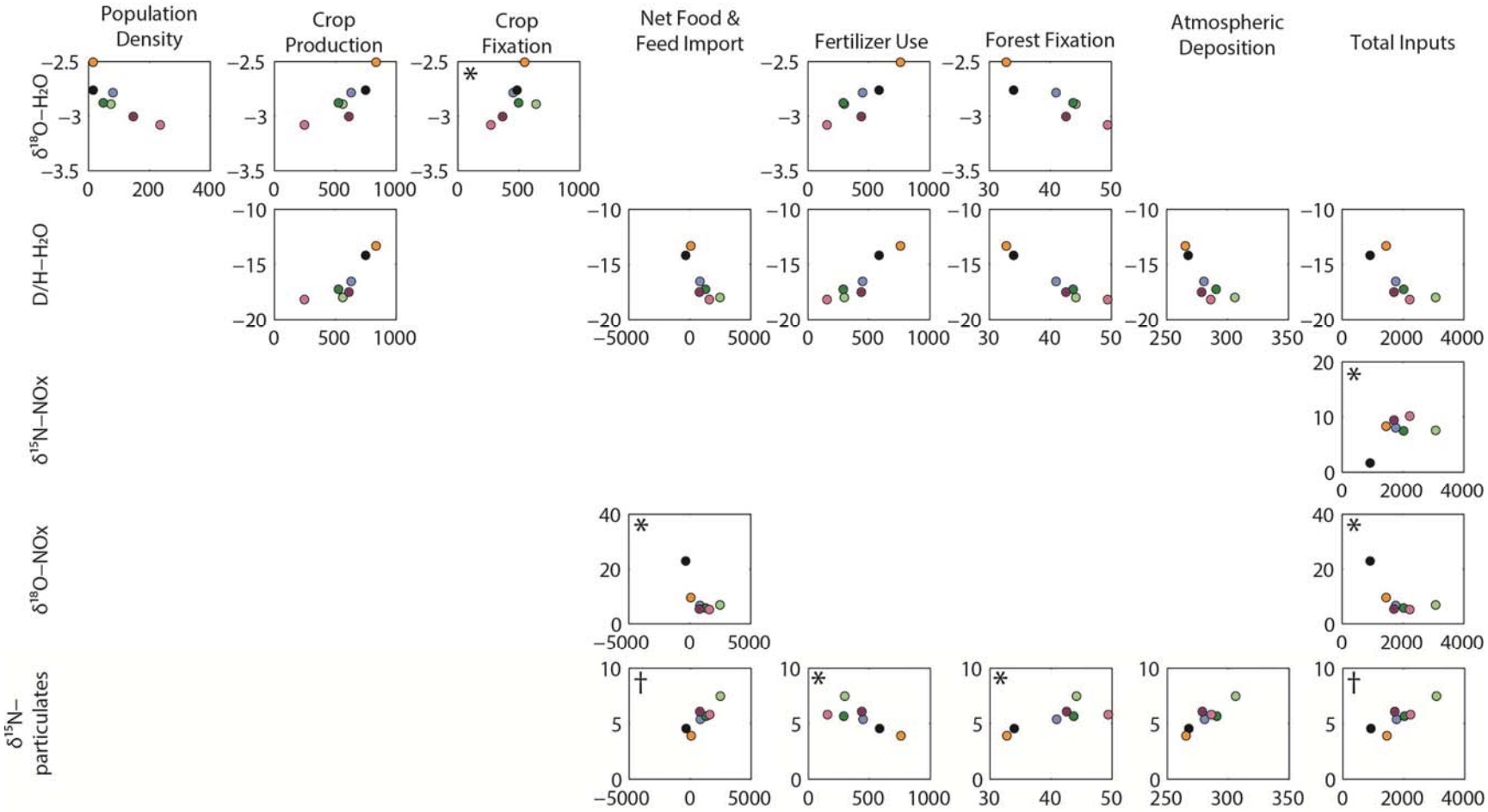


Figure 3.18, continued. Plots of average in-stream measurements of studied parameters versus inputs to the surface of subwatersheds of the greater Altamaha. Asterisks (*) indicate results that are significant only when the Upper Oconee subwatershed is omitted; crosses (†) indicate results that are significant only when the Upper Oconee subwatershed in included. All other results are significant in both cases.

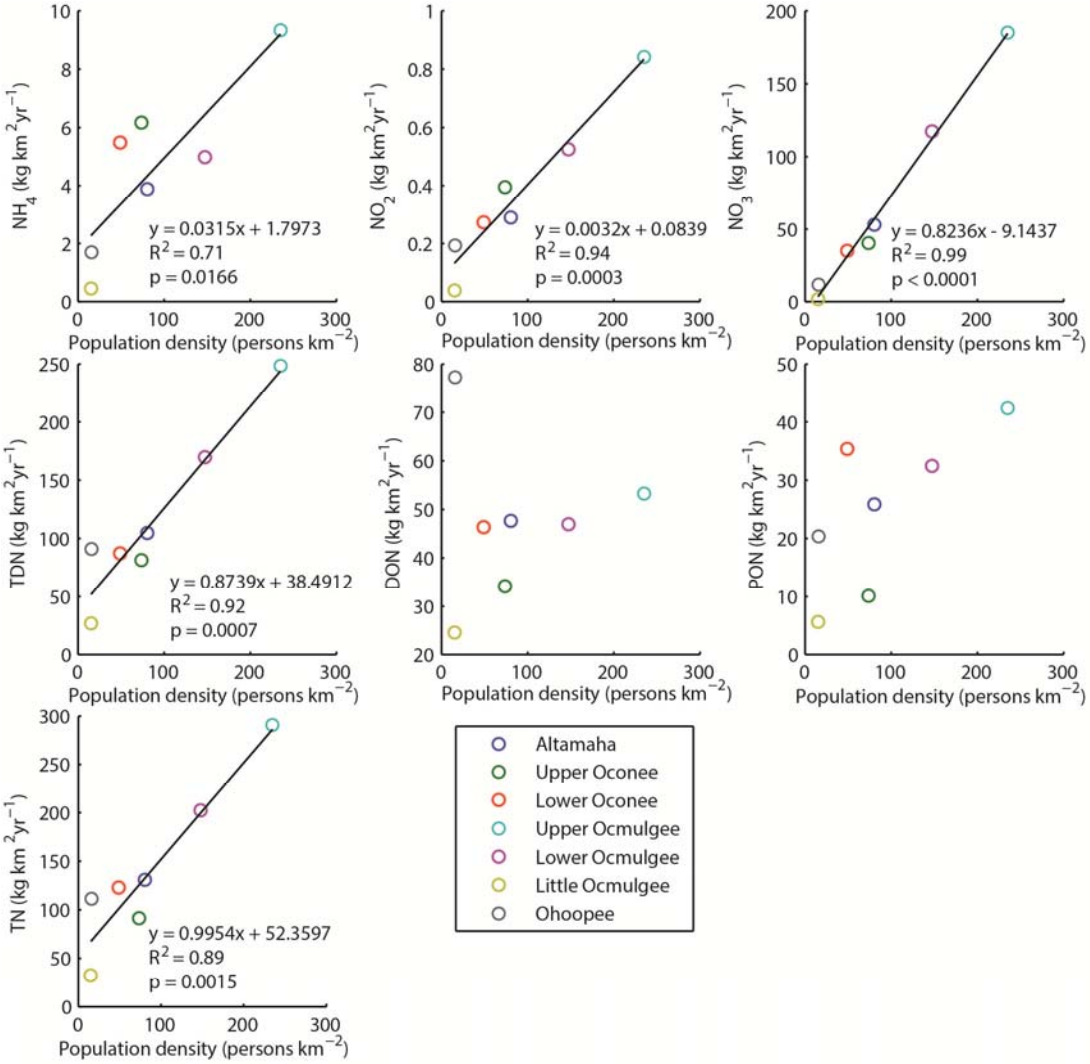


Figure 3.19. Relationships between loads of N and population density in subwatersheds of the Altamaha River.

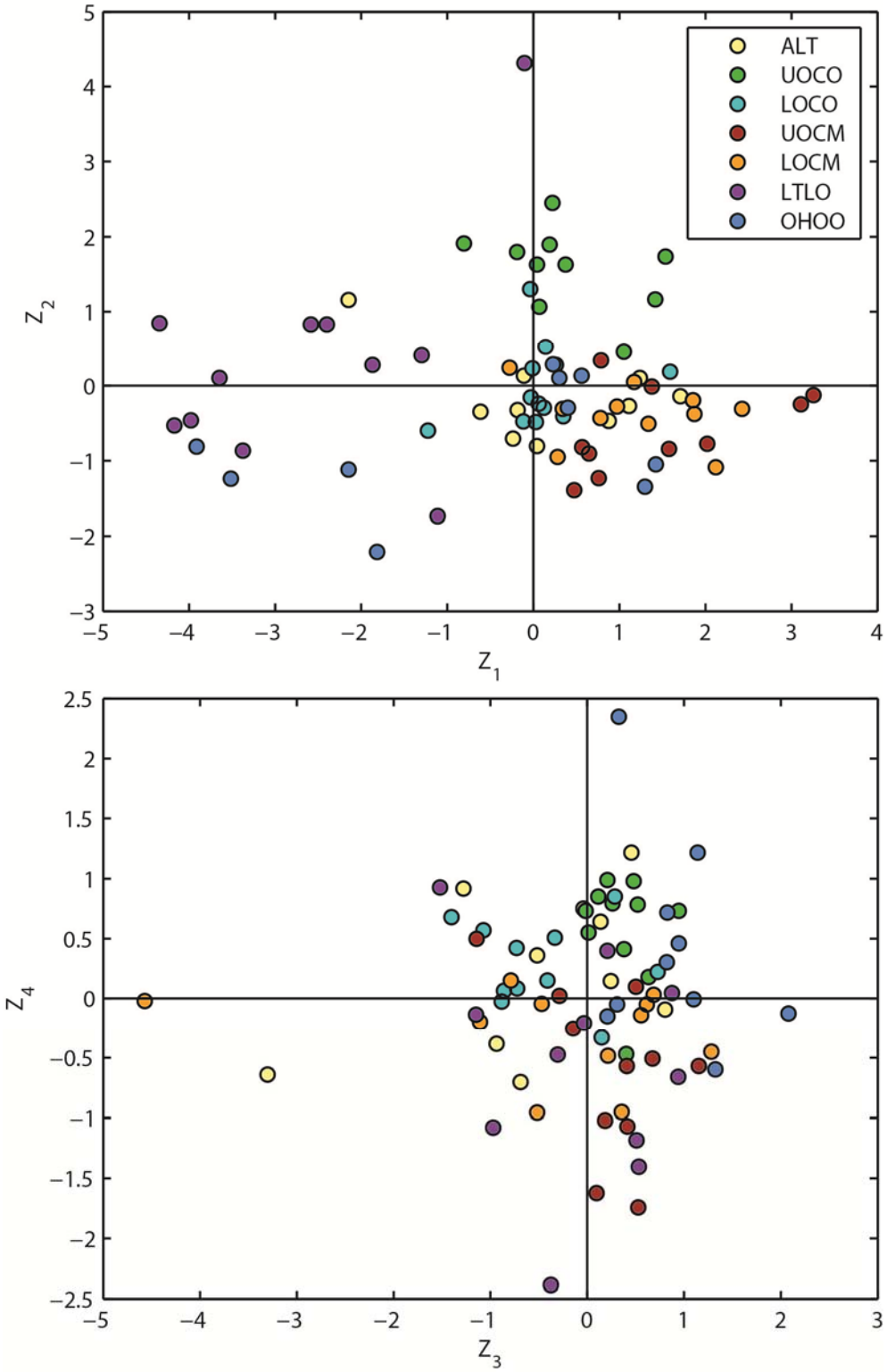


Figure 3.20. PCA analysis of N concentrations (DIN, DON, and PN) and isotopic values of NOx and particulate N.

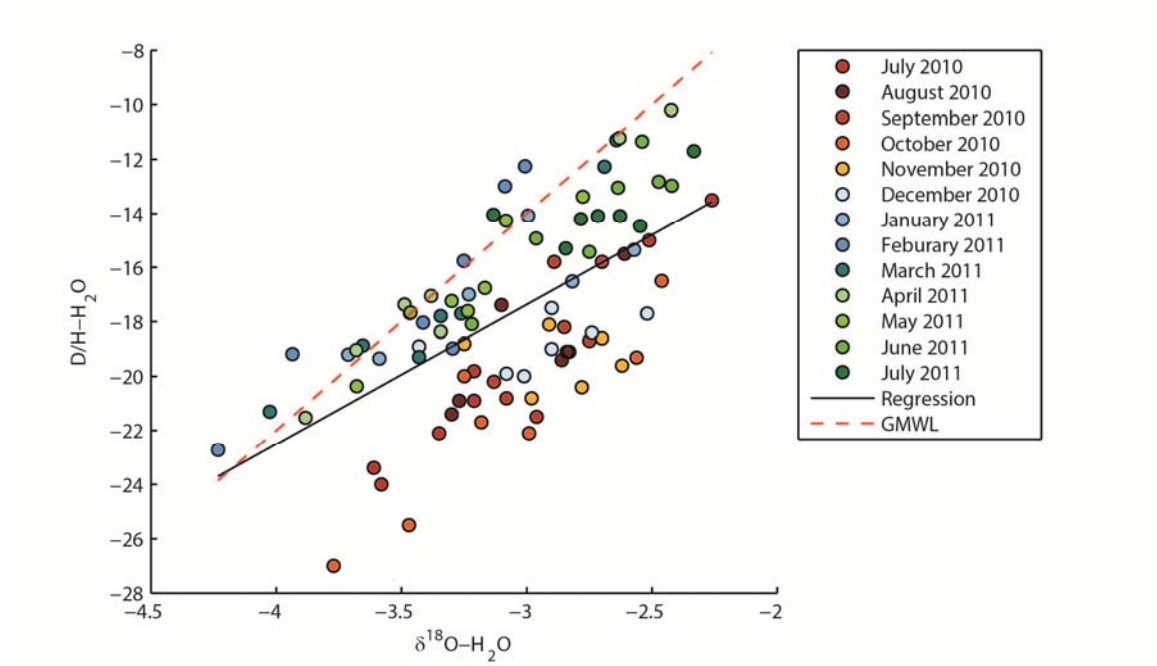


Figure 3.21. Isotopes of water collected in subbasins of the Altamaha River.

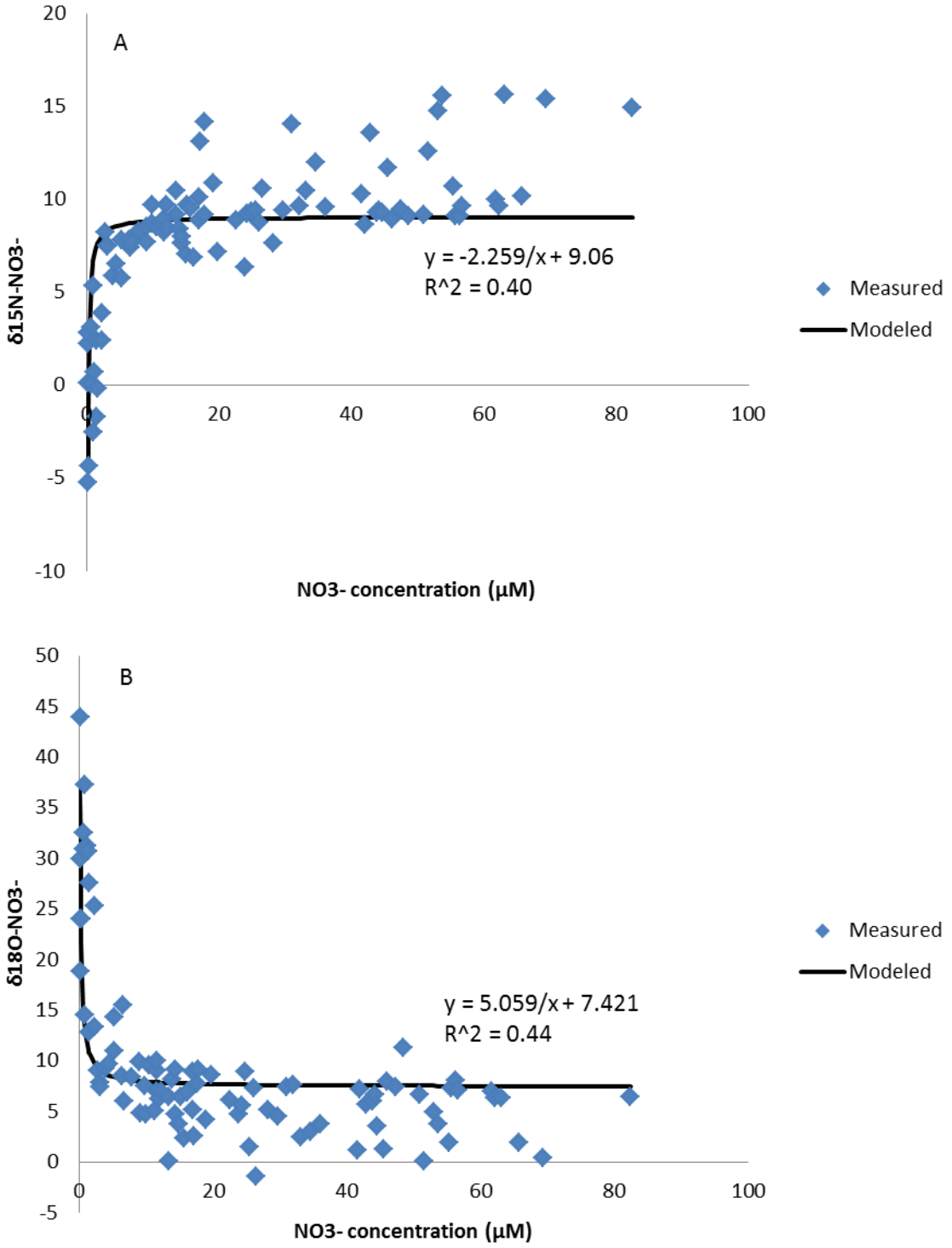


Figure 3.22. Simple stable isotope mixing model with two end-members. A, ^{15}N isotopes; B, ^{18}O isotopes.

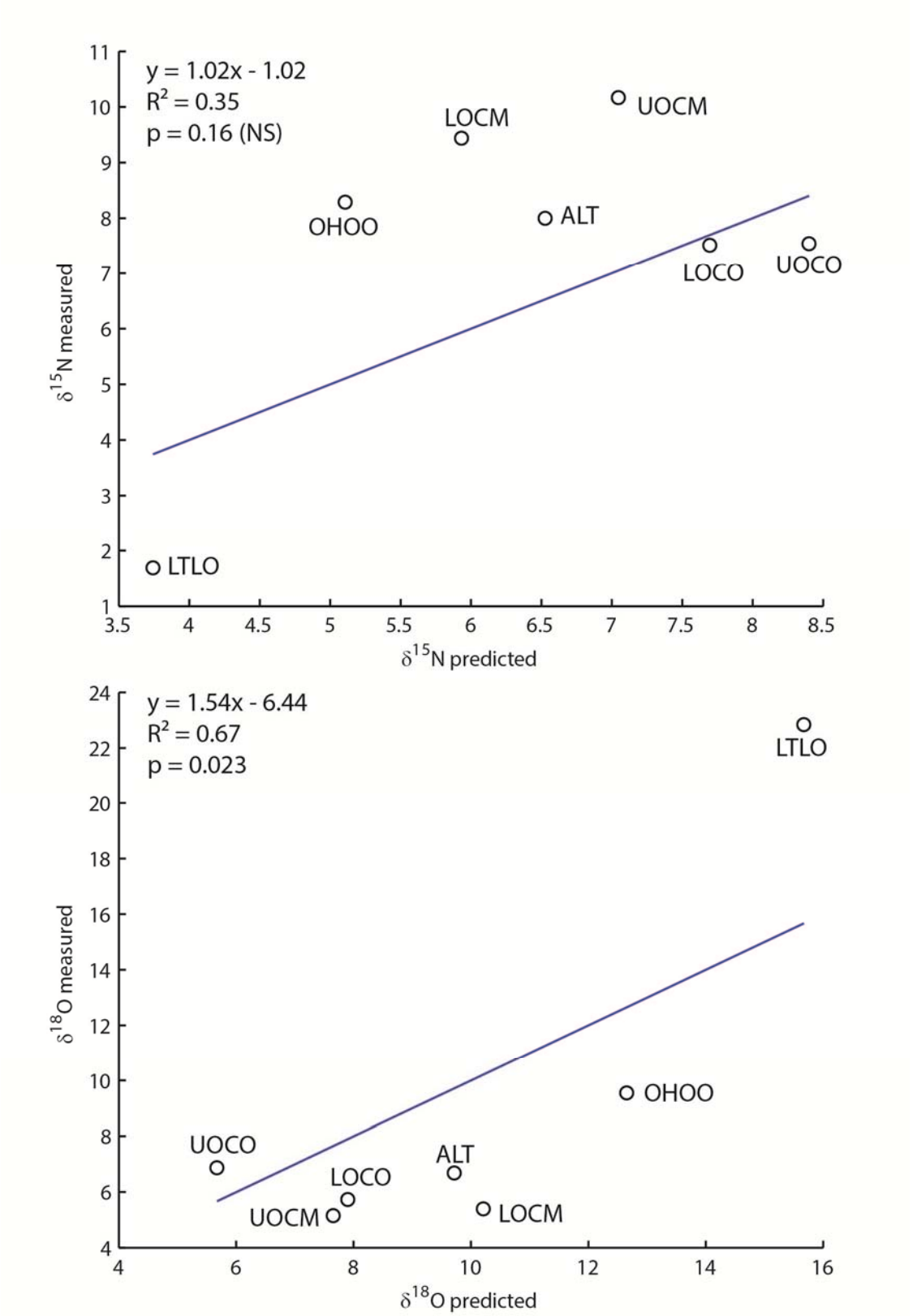


Figure 3.23. Predicted vs. measured isotopic values of NO_3^- for subwatersheds of the Altamaha River. Lines shown are 1:1.

CHAPTER 4

ANALYSIS OF NITRATE CONCENTRATIONS IN MEDIUM-SIZED RIVERS THROUGHOUT THE WORLD[1]

[1]Schaefer, S.C., and M. Alber. To be submitted to *Science of the Total Environment*.

ABSTRACT

We conducted a meta-data analysis of nitrate concentrations in "medium" sized rivers around the world, which have watersheds that range from 2,000 to 50,000 km^2. Data were collected from published measurements of in-stream nitrate concentrations in 232 medium-sized rivers throughout the world. A variety of watershed characteristics, including climatic variables, slope, population densities, manure and fertilizer use, and sanitation facilities, were also calculated for each system using spatial statistics in GIS. Although nitrate concentrations ranged as high as 671 μM, they were generally low (<25 μM). On a global basis, in-stream nitrate concentrations were best related to population density in the watershed (R^2=0.33). These relationships varied on individual continents, and were strongest in Europe (R^2=0.81). Nitrate concentrations were highest in tropical watersheds, but the slope of the relationship between population density and NO_3^- concentrations was very similar in both tropical and temperate regions. The slope was higher in polar rivers; however, there were only a few observations in this region and concentrations in polar areas were low. The worldwide relationship was improved by the addition of additional factors considered here (population density, population density without access to improved sanitation, temperature, precipitation, slope, and fertilizer use) to the relationship (R^2=0.42). Population density was also the best predictor (R^2=0.13) of nitrate loads. This relationship was improved by the addition of precipitation, temperature, slope, size, and fertilizer use (R^2=0.37). The explanatory power of the relationships in medium-sized rivers is lower than that previously reported for larger watersheds. We speculate that the increased variability among medium-sized

watersheds may be because they are more susceptible to variation in local controls on nitrogen processing.

4.1 INTRODUCTION

Nitrogen (N) pollution is now a crucial issue facing aquatic ecosystems worldwide (Howarth et al. 2002). Inputs of reactive N to the global cycle have increased nine-fold since the invention of the Haber-Bosch process (Galloway and Cowling 2002), and have doubled since 1970 alone (Galloway et al. 2008). These increased inputs have led to increases in-stream N concentrations, particularly in terms of nitrate, which is the is most clearly related to human activities as it mobilizes more easily in response to human disturbance (Howarth et al. 1996). This form of nitrogen is most sensitive to anthropogenic impacts and thus serves as a convenient indicator of disturbance (Caraco and Cole 1999). Nitrate or nitrite + nitrate (NOx) are also the most frequently reported species of nitrogen in the literature.

Numerous studies have examined N input to rivers. However, this research has tended to focus on large rivers. For example, the SCOPE N project looked at N inputs to the watersheds of large rivers that drained to the North Atlantic (Howarth et al. 1996). A study by Caraco and Cole (1999) also focused predominantly on large rivers. These studies have found relationships between riverine N loads and human population densities. However, other drivers such as temperature (Schaefer and Alber 2007) and streamflow (Howarth et al. 2012; Schaefer et al. 2009) were also related to N export.

Although most studies have focused on N loads rather than in-stream concentrations (e.g. Howarth et al. 1996, Caraco and Cole 1999), Peierls et al. (1991) did find that both

N concentrations and loads were significantly related to human population density. Understanding what drives in-stream nitrogen concentrations is critical, however, as it is high concentrations in receiving water bodies that result in phytoplankton blooms and other symptoms of eutrophication. Excess concentrations of nitrate in drinking water can also cause human health problems, particularly in infants (Fan et al. 1987). As such, regulatory water quality standards are usually set in terms of concentrations, rather than being concerned with overall loads.

Medium-sized rivers, which are often overlooked, can have different N dynamics than larger streams. Seitzinger et al. (2002) found that lower-order streams are more important in terms of nitrogen removal through denitrification than larger streams. Other factors that affect N retention in a catchment may also differ at smaller scales (Caraco and Cole 1999). For example, Wollheim et al. (2005) found N export from small catchments to be related to impervious surface cover. Thus, it is important to consider the characteristics of smaller streams separately.

This study focused on medium-sized stream catchments from throughout the world. The objectives of this study were to compile information on nitrate concentrations and compare these across continents and latitudes, and to evaluate relationships between NO_3^- concentrations and their potential drivers and compare these relationships to larger systems. We were interested in relationships with human population density, and other potential contributing drivers such as climatic factors.

4.2 METHODS

Watersheds with areas greater than 2,000 km^2 and less than 50,000 km^2 were identified from the USGS Hydro1k dataset (USGS EROS 1996), which covers most of the world. This dataset does not include watersheds in Australia, so a separate dataset was used for watersheds on that continent (Geoscience Australia 2013). A total of 4,024 watersheds meeting these size criteria were identified. Watersheds selected drained either to the coast or to a larger tributary. The centroid of each watershed was calculated in ArcMap using the polygon to point conversion tool. Watersheds whose midpoint was located below 23.5 degrees of latitude were considered tropical; watersheds with a midpoint above 66 degrees were considered polar. All others were considered temperate.

Recent (1990 or later) measurements of typical nitrate concentrations for the rivers associated with each watershed were compiled from the scientific literature by querying Google Scholar using the keywords "nitrogen concentration [river name] river". A search was run for each watershed meeting the size criteria. Studies were considered for inclusion if they reported a nitrate concentration measured in the main stem of the river in question. Some of these studies were overview studies that included multiple rivers. Such studies were particularly prevalent in the United States (e.g. Boyer et al. 2002, Goolsby et al. 2000). In those instances where loads were reported along with streamflows, concentrations were back-calculated. Data presented only in figures were digitized using a digitization application (Plot Digitizer, http://plotdigitizer.sourceforge.net/). For watersheds draining directly to the coast, estuarine measurements were not included since these could have been influenced by coastal ocean nitrate concentrations. However, if the study included a measurement taken in the freshwater portion of the river before it

reached the estuary, that measurement was used. In general, the most downstream measurement was used in order to correspond best with the GIS watershed boundaries.

Mean annual nitrate concentrations were estimated for each system. In some studies mean nitrogen concentrations were reported directly. In some cases, data were averaged from digitized data derived from figures. For seasonal studies that reported a measurement for several different seasons, such as wet and dry, the average of the reported seasons was used. In cases where a range of values was reported, the midpoint of the reported range was used. This most likely introduced some error, as the midpoint NO_3^- concentration is unlikely to be the same as the average concentration. Studies generally did not report streamflow measurements to correspond with individual nitrate data points, such that a flow-weighted approach was not possible.

The frequency with which rivers were sampled was noted and recorded. Studies reporting measurements only for specific unusual conditions, such as directly after a hurricane or during a stormflow event, were not included. However, many survey studies provided only one or a few observations. For tropical and temperate areas, rivers were flagged if they were sampled less than four times over the course of a year, or if samples were not taken during each season. In polar regions, where rivers are often frozen for a large portion of the year, during which time streamflow is minimal, four or more samples over the course of spring, summer, and fall were considered sufficient for these rivers and so they were not flagged.

Studies generally adhered to standard methods for measuring nitrate concentrations. Most commonly, standard methods laid out by the American Public Health Association were cited. These methods include nitrate measurement via ion chromatography,

capillary ion electrophoresis, nitrate probes, and cadmium reduction methods. Many studies also used monitoring data from the respective national governments of each river. Methods used by the monitoring programs were not immediately available but were assumed to also rely on standard methods.

For this analysis, all values were converted to μM NO_3^-. Although this is a more stoichiometrically logical unit for concentrations, most studies reported concentrations in mg/L. This exercise illustrated the problematic nature of reporting nitrate concentrations in units of mass per liter, as it was often unclear whether the measurements were reported in mg NO_3^--N/L or mg NO_3^-/L. For this analysis, nitrate concentrations reported with units of mg/L were assumed to be in mg/L NO_3^-, unless studies specifically reported nitrate concentrations in mg NO_3^--N/L. This may have been a faulty assumption in some cases and may have artificially depressed the NO_3^- concentrations in certain watersheds. Rivers in which the units of concentration were unclear were therefore flagged. Nitrite (NO_2^-) tends to be very low in riverine systems (Meybeck 1982, Turner et al. 2003), so NO_X measurements were lumped with NO_3^- measurements in this analysis.

In order to examine potential drivers of differences in NO_3^- concentrations, watershed characteristics were calculated in ArcMap. Human population density (2010; 2.5 arc-minute grid) was obtained from the NASA Socioeconomic Data and Applications Center (CIESIN et al. 2005). Climatic factors could also affect nitrate concentrations; for example, increased temperature could result in increased denitrification, reducing in-stream NO_3^-. Average annual temperature and rainfall data on 2.5 arc-minute grids were obtained from the WorldClim global climate data set (Hijmans et al. 2005). To evaluate whether level of unimproved sanitation within a watershed affected the NO_3

concentrations in a stream, 2011 data on sanitation (including pit latrines, hanging latrines, and bucket latrines as well as open defecation) was obtained from the WHO/UNICEF Joint Monitoring Programme for Water Supply and Sanitation (JMP 2014). In this analysis, the percent of the population using unimproved sanitation facilities was multiplied by the population density of a watershed to obtain the population density of individuals without access to improved sanitation. The slope of a watershed could affect the speed of the river and therefore the residence time, which in turn could reduce N loss through microbial transformations such as denitrification. Slopes were calculated using elevation data obtained from the SRTM 90m Digital Elevation Dataset (Jarvis et al. 2008). Global gridded N manure and fertilizer use were obtained from Potter et al. (2010). Watershed averages for all these parameters were then calculated using Zonal Statistics.

Although the main focus of this paper was nitrate concentrations we also made a rough estimate of loads for each study by multiplying by average discharge. Although some studies did report streamflow, we used the average watershed discharge using data from the World Water Development Report II to estimate loads for all systems (Fekete 2001). However, the discharge data used here was a large-scale grid so these estimates are not always precise. There were 39 cases where the estimated discharge was greater than precipitation, which is highly unlikely, so those watersheds were dropped from analyses requiring discharge information.

4.3 RESULTS

Concentration data were located for 271 rivers out of 1747 that met the watershed size criteria (Tables 4.1-4.2). The majority of these rivers were located in North America and Europe (86 and 76 watersheds, respectively). The larger number of rivers with available concentration data in these regions indicates that these areas receive more study. However, it may have also been partly due to the fact that there were also more overview studies on these continents, in which nitrate concentrations were reported for a large number of rivers. 63 measurements were flagged because of limited sampling and 38 were flagged due to questions about units of reporting. Removing these watersheds—a total of 83—generally improved relationships but did not change the results of the analysis. However, it also substantially reduced the number of measurements available, particularly in Africa and Asia. Thus, we included all 271 watersheds in the results reported below.

Reported nitrate concentrations were generally relatively low, and were log-normally distributed. Nearly half of systems (126) had NO_3^- concentrations between 0 and 25 μM (Figure 4.1), which is similar to concentrations observed in undisturbed systems (e.g. Lewis et al. 1999, Clark et al. 2000). Only 50 watersheds reported nitrate concentration above 100 μM and only 16 had a concentration above 300 μM. The highest value of NO_3 was reported in the Ogun watershed in Nigeria, with an average nitrate concentration of 671 μM, based on bimonthly observations over the course of a year (Jaji et al. 2007). The overall average NO_3^- concentration for all systems was 77 μM (Table 4.3) with an overall median value of 28 μM (limited dataset, mean=85 μM; median=27 μM). The highest NO_3^- concentrations when all data were considered were found in Europe (mean, 97 μM;

median 40 μM) and the lowest concentrations in Australia (mean, 3 μM; median, 2 μM). While a Wilcoxon rank sum test indicated significant differences in average NO_3^- concentrations between continents (Table 4.3), variability was very high, with the standard deviations for the different continents in all cases being more than 100% of the average. When sorted by latitude, concentrations were highest in tropical rivers (mean, 82 μM; median, 27 μM), intermediate in temperate rivers (mean, 78 μM; median, 31 μM), and lowest in the polar region (mean, 3 μM; median, 3 μM).

Watershed population densities were significantly related to in-stream NO_3^- concentrations, although there was a large amount of scatter in the observations. For all of the data together, the relationship had an R^2 value of 0.15. When log-transformed, the R^2 improved to 0.32 (Table 4.4). The strength of the relationships varied by continent (Table 4.5, Figure 4.3). The strongest relationship (log-transformed) was observed in Europe (R^2=0.75). There were weaker relationships on the other continents (R^2 between 0.11 and 0.39; log-transformed). When observations were separated by latitude, the log-transformed relationship between population density and nitrate concentration was steeper for tropical systems than for temperate systems (temperate, log NO_3^- concentration = 0.50*log population density + 0.75, R^2 = 0.34, p = 1e-21; tropical, log NO_3^- concentration = 0.64* log population density + 0.08, R^2 = 0.27, p = 0.0007). The relationship in polar systems had a much steeper slope (log NO_3^- concentration = 1.23* log population density + 0.15, R^2 = 0.88, p = 0.002), but this was only based on 7 polar rivers.

Both manure and fertilizer use within a watershed were significantly related to nitrate concentrations, although not as well as population density (R^2=0.12 and 0.22,

respectively). Considering both manure and fertilizer N use together improved the relationship slightly (R^2=0.23). Relationships were best in Europe (log [NO_3^-] = 0.003*fertilizer use - 1.03, R^2=0.43 and log [NO_3^-] = 0.002*manure use + 1.15, R^2=0.33).

Although several other potential drivers of in-stream nitrate concentrations were significant on individual continents, temperature was the only other driver that was significant overall. Average watershed temperature alone was a significant predictor of nitrate concentration (Figure 4.2), albeit with a far greater scatter than in the relationship with population density (R^2=0.02 (NO_3^- concentration log-transformed) versus 0.33 for population density (both factors log-transformed)). Density of population without access to improved sanitation, precipitation, slope, and discharge alone were not related to NO_3^- concentration. When evaluated on individual continents, North America, Europe, and Asia exhibited significant positive relationships between temperature and NO_3^- concentration, with the relationship in Europe being particularly strong (R^2=0.71). Other factors of importance on a continent-by-continent basis were precipitation, which was positively related in North America (R^2=0.17), and slope, which was positively related in North America (R^2=0.07) and Australia (R^2=0.44). Manure use was signficnatly related in Europe, North and South America, and fertilizer use wassignificantly related in Asia, Australia, Europe, and North America (Table 4.5).

In order to evaluate the best model fit for NO_3^- concentration data, an Akaike Information Criterion test (AICc) was run using the glm package in R software. The highest-scoring model included population density (both total population density in the watershed as well as the density of the population without access to improved sanitation), temperature, precipitation, slope, and fertilizer use. Using these factors, the best fit was

$\log[NO_3^-] = 0.52*\log(\text{population density}) - 0.16*\log(\text{population density without access to improved sanitation} + 1) - 0.03*\text{temperature} - 0.18*\log(\text{precipitation}) - 0.02*\text{slope} + 0.001*\text{fertilizer use} + 1.56$; $R^2=0.42$.

The estimated nitrate load had a worldwide average of 17 kmol NO_3^- km^{-2} yr^{-1} (median, 4 kmol NO_3^- km^{-2} yr^{-1}). Loads on individual continents ranged from an average of 1 kmol NO_3^- km^{-2} yr^{-1} (median, 0.06 kmol NO_3^- km^{-2} yr^{-1}) in Australia to 25 kmol NO_3^- km^{-2} yr^{-1} (median, 5 kmol NO_3^- km^{-2} yr^{-1}) in North America, with the highest load (935 kmol NO_3^- km^{-2} yr^{-1}) in North America's Tarcoles River (Silva-Benides 1996). The highest median load was observed in Europe (5.2 kmol NO_3 km^{-2} yr^{-1}). Variability amongst loads, both worldwide and within individual continents, was also very high. Although discharge was significantly related to load in all cases, this was not considered as a predictor since discharge was used to generate load estimates. The single factor best predicting nitrate loads was population density ($R^2=0.14$, both variables log-transformed; Table 4.4, Figure 4.4), Fertilizer and manure use were also positively related to NO_3^- loads ($R^2=0.12$ and 0.09, respectively). Loads were also significantly related to precipitation (Table 4.4). Using AICc, the best prediction of loads was $\log(NO_3^-\ \text{load}) = 0.43*\text{population density} - 0.07*\text{temperature} + 2.1*\log(\text{precipitation}) + 0.0064*\text{slope} - 1.1\text{e-}5*\text{size} - 5.5$ ($R^2=0.37$).

4.4 DISCUSSION

The river nitrate measurements compiled in this study were not evenly distributed across continents. The low number of watersheds in South America in particular was due in part to the fact that the GIS file used did not include subwatersheds for the Amazon

River, precluding the inclusion of any rivers in this basin. Additionally, large-scale studies that reported concentrations for multiple watersheds were largely limited to more developed countries and regions such as Europe, the United States, or South Africa (e.g. Goolsby et al. 2000; Stalnåcke et al. 1998; deViliers and Thiart 2007).

Nitrate concentrations in a survey of large rivers ranged from 1 to over 500 μM, with an average of 63 μM and a median of 29 μM (digitized from figure; Peierls et al. 1991). The observations for small- to medium-sized rivers included in this study had an even larger range, from a minimum detection of 0.03 μM to a maximum of 671 μM NO_3^-. The overall average NO_3^- concentration in this study, 77 μM, was similar to that in large rivers, and the medians were identical (29 μM). In both cases there were a large number of rivers at the lower end of the range. When the rivers in this study were plotted against watershed size, there was greater variability amongst the smaller watersheds (Figure 4.5a).

We found both similarities and differences with the relationship between population density and nitrate concentration reported by Peierls et al. (1991). The slope of our relationship (0.50 on a log-log plot) was similar to that reported by Peierls et al. (1991; 0.56). This suggests that the response of nitrate concentrations to increases in population in smaller watersheds is highly comparable to those in larger rivers. In this study, however, population density accounted for very little of the variability in nitrate concentrations. Peierls et al. (1991) found that population density explained 76% of the variation in nitrate concentration at the mouths of 42 large rivers draining to the ocean. In this study, population density explained far less of the variability in nitrate concentrations, accounting for only 33% on a log-log plot, although the relationships

were far better on some individual continents. Although the range of data in this study was similar to that in the study by Peierls et al. (1991), the fact that these watersheds were generally much smaller could have made them more susceptible to landscape variability in the transport of nitrate, whereas large watersheds integrate over a much larger area.

There is also some evidence that tropical and temperate rivers behave differently (Howarth et al. 1996), as do mesic and xeric catchments (Caraco and Cole 2001). In this study, the slope of the relationship between population density and nitrate concentration was steeper in tropical than in temperate rivers (0.64 vs. 0.50, respectively). Previous research has suggested that tropical watersheds and watersheds in warmer regions tend to export less nitrogen than those in cooler or temperate regions for a given population density (Peierls et al. 1991, Howarth et al. 1996, Schaefer and Alber 2007). This was not the case here, where the ranges of nitrate concentrations in the two areas were very similar, and the average concentration in tropical rivers was higher than that in temperate rivers. However, both tropical and temperate regions encompass a wide variety of ecosystems and climates. The slope of the relationship was steepest in the polar regions; however, there were only 7 rivers in this area included in this study, and nitrate concentrations in these rivers were all less than 10 μM. Thus, the slope calculated here may not reflect the true nature of the relationship. Since the poles will be particularly affected by climate change, rivers in this area are worth examining further, as increased temperatures will result in increased freshwater discharge (Peterson et al. 2002) which could potentially result in higher nitrate delivery to the Arctic Ocean, particularly if populations increase as well. Holmes et al. (2012) suggest that nitrogen dynamics in

smaller polar rivers may be substantially different from those in the large rivers draining into the Arctic Ocean since the headwaters of the large rivers are almost all located well within the temperate zone. In this study, which considered medium rivers, the headwaters tended to be located further north than in large rivers.

The positive relationships between temperature and NO_3^- concentrations on some continents were surprising. Higher temperatures promote microbial activity, and increases in denitrification with temperature have been found in a number of studies (e.g. Dawson and McMurphy 1972; Holmes et al. 1996; Pfennig and McMahon 1996). Thus, a negative relationship would have been expected. However, this relationship may be an artifact of warmer latitudes being more heavily populated, and in this data set there is a positive relationship between population density and average watershed temperature.

The background NO_3^- concentration for undeveloped watersheds in the United States has been reported to be 6.2 μM (0.087 mg/L NO_3^--N; Clark et al. 2000). Lewis et al. (1999) report tropical background concentrations on the order of 8.6 μM (120 μg/L). Meybeck (1982) reports background NO_3^- levels in unpolluted world rivers, including large rivers such as the Ob, to range between 1.1 and 17 μM (16-240 μg N/L). This suggests that many rivers in this study still exhibit nitrate concentrations representative of undisturbed watersheds. 113 of the watersheds in this study had NO_3^- concentrations less than 20 μM.

Some of the rivers with very low nitrate concentrations had very high population densities, which was surprising. In some cases, this may have been due to the difficulty in determining whether concentrations reported in mg/L were in mg/L NO_3^- or mg/L NO_3^--N. A number of such low-concentration-high-population watersheds fell into this

category. If a measurement was assumed to be in mg/L NO_3^- but was in fact mg/L NO_3^--N, this would have resulted in the estimated concentration being only 23% of the true concentration.

There were also a number of watersheds with high population but low concentrations that were not affected by this unit confusion. In particularly densely populated areas, governments may have made efforts to reduce nutrient pollution through tertiary wastewater treatment. For example, the most densely populated watershed in this study is that of the Arakawa River in Japan (3,170 persons km^{-2}). The Arakawa River runs through the city of Tokyo, whose wastewater treatment plants are advanced and whose effluent N concentrations are extremely low (Kitamura et al. 2013).

When considered in a multiple linear regression, we found that the best predictor of in-stream NO_3^- concentration was a combination of population density (both total and without access to improved sanitation), temperature, precipitation, slope, and fertilizer use. When considered together, these factors explained 45% of the variability in NO_3^- concentrations. Although this is still a substantially lower proportion of the variability than was explained in large watersheds by Peierls et al. (1991), these results confirm that anthropogenic activities and climatic factors are both important in determining N export from watersheds (Howarth et al. 2012). These results also suggest that the mechanisms controlling NO_3^- export are complex and not easily explained by a single factor when considered on a global scale.

A relationship between population density and nitrate load has been seen in multiple studies (Peierls et al. 1991, Howarth et al. 1996, Caraco & Cole 1999). However, the relationship between population density and load observed in this study was not as strong

as has been previously reported for large rivers, and the slope of the regression line was not as steep (R^2 = 0.11 and slope = 0.48 vs. R^2 = 0.76 and slope = 0.64 in Peierls et al. 1991; Figure 4.6). The difference in these relationships may be due in part to the rough nature of the load calculation in this study. We used a relatively large-scale global grid to calculate loads rather than discharges measured simultaneously with concentrations, which would be preferable. However, as discussed previously, there was more variability in concentrations among the smallest watersheds in this study (Figure 4.5a) and this translated to loads as well (Figure 4.5b). In this study, loads were as high as 935 kmol km^{-2} yr^{-1} in the smallest watersheds and were always less than 400 kmol km^{-2} yr^{-1} in watersheds larger than 15,000 km^2. In Peierls et al.'s (1991) study of larger rivers, loads were always less than 125 kmol km^{-2} yr^{-1}. There is also some evidence that N export in different regions behaves differently (Green et al. 2004; Schaefer and Alber 2007), which may explain why there is so much variability in this global study.

4.5 CONCLUSIONS

Nitrate concentrations in medium-sized (2,000-50,000 km^2) watersheds are generally comparable to those found in larger watersheds, with most watersheds having a low NO_3^- concentration (<25 μM). This may in some cases be due to advanced wastewater treatment that has returned at least nitrate concentrations back to background levels. This study confirms that population density is the major determinant of in-stream nitrate concentrations. We found this to be the case in medium-sized watersheds as well as the large watersheds that have been previously reported on. These relationships also varied on individual continents. When tropical and temperate rivers were compared, tropical

rivers tended to have higher nitrate concentrations, and the slope of the relationship between population density and NO_3^- concentration was steeper in tropical rivers. The slope was highest in polar rivers, but there were only a few observations in this area, and concentrations were uniformly low. There was more variability in the relationships noted here than in previous studies of larger watersheds, with population density accounting for far less of the variability in NO_3^- concentrations. When population density (both total and that without access to improved sanitation), temperature, precipitation, slope, and fertilizer use were considered together, only 42% of the variability was explained, still below that found for population density alone in other studies. There was also an indication that variability increased as watershed size decreased within the limited size range investigated here. This suggests that smaller watersheds may be more sensitive to other factors influencing nitrogen processing and retention than large watersheds.

ACKNOWLEDGMENTS

We thank Natalie McLenaghan for assistance with AIC analysis. This research was supported by the Georgia Coastal Ecosystems LTER Project (NSF Awards OCE-0620959 and OCE-1237140).

REFERENCES

Agrahari, M., and V.B. Kushwaha. 2012. Effect of domestic sewage on the physico-chemical quality of River Rapti at Gorakhpur. *The Bioscan* 7(1): 135-138

Akaike, H. 1974. A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19(6): 716-723

Alam, S., S. Ahmad, and F.K. Bangash. 2009. Drinking water quality of Swat district. *J. Chem. Soc. Pak.* 30(1): 1-10

Alvarez-Cobelas, M., R. Sánchez-Andrés, S. Sánchez-Carillo, and D.G. Angeler. 2010. Nutrient contents and export from streams in semiarid catchments of central Spain. *Journal of Arid Environments* 74: 933-945.

Ashton, P.J., D. Love, H. Mahachi, P.H.G.M. Dirks (2001). *An Overview of the Impact of Mining and Mineral Processing Operations on Water Resources and Water Quality in the Zambezi, Limpopo and Olifants Catchments in Southern Africa*. Contract Report to the Mining, Minerals and Sustainable Development (Southern Africa) Project, by CSIREnvironmentek, Pretoria, South Africa and Geology Department, University of Zimbabwe, Harare, Zimbabwe. Report No. ENV-P-C 2001-042. xvi + 336 pp.

Avtar, R., P. Kumar, C.K. Singh, and S. Mukherjee. 2011. A comparative study on hydrogeochemistry of Ken and Betwa Rivers of Bundelkhand using statistical approach. *Water Qual. Expo. Health* 2: 169–179

Barker, H.J. 2006. Physico-chemical characteristics and metal bioaccumulation in four major river systems that transect the Kruger National Park, South Africa. M.S. Thesis, University of Johannesburg, South Africa.

Boukari, Y., L.M. Bawa, and G. Djaneye-Boundjou. 1999. Characterization of some Togo surface waters. *Bull. Chem. Soci. Ethiop.* 13(1): 11-21

Bouraoui, F., S. Benabdallah, A. Jrad, and G. Bidoglio. 2005. Application of the SWAT model on the Medjerda river basin (Tunisia). *Physics and Chemistry of the Earth* 30: 497–507

Bowes, M.J., J. Hilton, G.P. Irons, and D.D. Hornby. 2005. The relative contribution of sewage and diffuse phosphorus sources in the River Avon catchment, southern England: Implications for nutrient management. *Science of the Total Environment* 344: 67– 81

Boyer, E.W., C.L. Goodale, N.A. Jaworski, and R.W. Howarth. 2002. Anthropogenic nitrogen sources and relationships to riverine nitrogen export in the northeastern U.S.A. *Biogeochemistry* 57/58: 137–169

Bricker, S., B. Longstaff, W. Dennison, A. Jones, K. Boicourt, C. Wicks, and J. Woerner. 2007. Effects of Nutrient Enrichment in the Nation's Estuaries: A Decade of Change. National Centers for Coastal Ocean Science, Silver Spring, MD.

Brodie, J.E., and A.W. Mitchell. 2005. Nutrients in Australian tropical rivers: changes with agricultural development and implications for receiving environments. *Marine and Freshwater Research* 56: 279-302

Caraco, N.F., and J.J. Cole. 1999. Human impact on nitrate export: an analysis using major world rivers. *Ambio*, *28*(2): 167-170

Caraco, N.F., and J.J. Cole. 2001. Human influence on nitrogen export: a comparison of mesic and xeric catchments. *Marine Freshwater Research* 52: 119-125

Çelik, M. 2002. Water quality assessment and the investigation of the relationship between the River Delice and the aquifer systems in the vicinity of Yerköy (Yozgat, Turkey) *Environmental Geology* 42:690–700

Center for International Earth Science Information Network (CIESIN), Columbia University; United Nations Food and Agriculture Programme (FAO); and Centro Internacional de Agricultura Tropical (CIAT). 2005. Gridded Population of the World: Future Estimates (GPWFE). Palisades, NY: Socioeconomic Data and Applications Center (SEDAC), Columbia University. Available at http://sedac.ciesin.columbia.edu/gpw. Retrieved May 7, 2012.

Chakrapani, G.J., R.K. Saini, and S.K. Yadav. 2009. Chemical weathering rates in the Alaknanda–Bhagirathi river basins in Himalayas, India. *Journal of Asian Earth Sciences* 34: 347–362

Clair, T.A., I.F. Dennis, and S. Bélanger. 2012. Riverine nitrogen and carbon exports from the Canadian landmass to estuaries. Biogeochemistry. *Biogeochemistry*, *115*(1-3), 195-211

Clark, G.M., D.K. Mueller, and M.A. Mast. 2000. Nutrient concentrations and yields in undeveloped stream basins of the United States. *Journal of the American Water Resources Association* 36(4): 849-860.

Cox, M.E., A. Moss, and G.K. Smyth. 2005. Water quality condition and trend in North Queensland waterways. *Marine Pollution Bulletin* 51: 89–98

Cozzi, S., and M. Giani. 2011. River water and nutrient discharges in the Northern Adriatic Sea: Current importance and long term changes. *Continental Shelf Research* 31: 1881–1893

Crenshaw, C.L., N.B. Grimm, L.H. Zeglin, R.W. Sheibley, C.N. Dahm, and A.D. Pershall. 2010. Dissolved inorganic nitrogen dynamics in the hyporheic zone of

reference and human-altered southwestern U. S. streams. *Fundam. Appl. Limnol., Arch. Hydrobiol.* 176(4): 391–405

Dawson, R.N., and K.L. McMurphy. 1972. The temperature dependency of biological denitrification. *Water* Research 6: 71-83

de Viliers, S., and C. Thiart. 2007. The nutrient status of South African rivers: concentrations, trends and fluxes from the 1970s to 2005. *South African Journal of Science* 103: 343-349.

Demirel, Z., Z. Özer, and O. Özer. 2011. Investigation and modeling of water quality of Göksu River (Cleadnos) in an international protected area by using GIS. *J. Geogr. Sci.* 21(3): 429-440

Depetris, P.J., D. M. Gaiero, J. L. Probst, J. Hartmann and S. Kempe. 2005. Biogeochemical output and typology of rivers draining Patagonia's Atlantic seaboard. *Journal of Coastal Research* 21(4): 835-844

Dlamini, V., Z. Hoko, A. Murwira, and C. Magagula. 2010. Response of aquatic macro-invertebrate diversity to environmental factors along the Lower Komati River in Swaziland. *Physics and Chemistry of the Earth* 35: 665–671

Dumitrache, F., and E. Diacu. 2010. Study on the seasonal variation of nitrogen nutrients content from Ialomita River Hydrographic Basin. *Rev. Chim. (Bucureşti)* 61(3): 328-332

Duran, M., M. Tüzen, and M. Kayımet. 2003. Exploration of biological richness and water quality of stream Kelkit, Tokat-Turkey. *Fresenius Environmental Bulletin* 12(4): 368-375

Duran, M., and M. Suicmez. 2007. Utilization of both benthic macroinvertebrates and physicochemical parameters for evaluating water quality of the stream Cekerek (Tokat, Turkey). *Journal of Environmental Biology* 28(2): 231-236

Ejaz, N., H.N. Hashmi, and A.R. Ghumman. 2010. Water quality assessment of effluent receiving streams in Pakistan: A case study of Ravi River. *Mehran University Research Journal of Engineering & Technology* 30(3), 383-396

Ertel, A.-M., A. Lupo, N. Scheifhacken, T. Bodnarchuk, O. Manturova, T. U. Berendonk, and T. Petzoldt. 2012. Heavy load and high potential: anthropogenic pressures and their impacts on the water quality along a lowland river (Western Bug, Ukraine). *Environ Earth Sci* 65:1459–1473

Fan, A.M., C.C. Willhite, and S.A. Book. 1987. Evaluation of the nitrate drinking water standard with reference to infant methemoglobinemia and potential reproductive toxicity. *Regulatory Toxicology and Pharmacology* 7: 135-148

Fekete, B.M. 2001. Global Annual River Discharge (km3/yr). Water Systems Analysis Group, University of New Hampshire, NH. Available at http://wwdrii.sr.unh.edu/download.html

Florescu, D., R.E. Ionete, C. Sandru, A. Iordache, and M. Culea. 2011. The influence of pollution monitoring parameters in characterizing the surface water quality from Romania southern area. *Rom. Journ. Phys.* 56(7-8): 1001–1010

Furnas, M. 2003. Catchments and corals: terrestrial runoff to the Great Barrier Reef. Australian Institute of Marine Science and CRC Reef Research Centre, Townsville, Australia. Cited in Brody & Mitchell 2005.

Gabche, C.E., and V.S. Smith. 2002. Water, salt, and nutrients budgets of two estuaries in the coastal zone of Cameroon. *West African Journal of Applied Ecology* 3: 69-89

Galloway, J.N., and E.B. Cowling. 2002. Reactive nitrogen and the world: 200 years of change. *Ambio* 31(2): 64-71

Galloway, J.N., A.R. Townsend, J.W. Erisman, M. Bekunda, Z. Cai, J.R. Freney, L.A. Martinelli, S.P. Seitzinger, and M.A. Sutton. 2008. Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. *Science* 320: 889-892

Geoscience Australia. 2013. Australia's River Basins 1997. Available at http://koordinates.com/layer/741-australias-river-basins-1997/

Goolsby, D.A., W.A. Battaglin, B.T. Aulenbach, and R.P. Hooper. 2000. Nitrogen flux and sources in the Mississippi River Basin. *The Science of the Total Environment* 248 : 75-86

Gordeev, V.V., J.M. Martin, I.S. Sidorov, and M.V. Sidorova. 1996. A reassessment of the Eurasian river input of water, sediment, major elements, and nutrients to the Arctic Ocean. *American Journal of Science* 296: 664-691.

Green, P.A., C.J. Vörösmarty, M. Meybeck, J.N. Galloway, B.J. Peterson, and E.W. Boyer. 2004. Pre-industrial and contemporary fluxes of nitrogen through rivers: a global assessment based on typology. *Biogeochemistry* 68: 71-105

Growns, I., I. Reinfelds, S. WIlliams, and G. Coade. 2009. Longitudinal effects of a water supply reservoir (Tallowa Dam) on downstream water quality, substrate and riffle macroinvertebrate assemblages in the Shoalhaven River, Australia. *Marine and Freshwater Research* 60: 594–606

Ha, K., M.-H. Jang, and G.-J. Joo. 2002. Spatial and temporal dynamics of phytoplankton communities along a regulated river system, the Nakdong River, Korea. *Hydrobiologia* 470: 235–245

Hancock, P.J., and A.J. Boulton. 2005. The effects of an environmental flow release on water quality in the hyporheic zone of the Hunter River, Australia. *Hydrobiologia* 552:75–85

Helland, A., G. Holtan and P. Jørgensen. 2003. Riverine Inputs of Organic Carbon and Nitrogen to Norwegian Coastal Areas. *Ambio* 32(6): 412-417

Hijmans, R.J., S.E. Cameron, J.L. Parra, P.G. Jones and A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology 25: 1965-1978.

Holmes, R.M., J.B. Jones, Jr., S.G. Fisher, and N.B. Grimm. 1996. Denitrification in a nitrogen-limited stream ecosystem. *Biogeochemistry* 33(2): 125-146

Holmes, R.M., J.W. McClelland, B.J. Peterson, S.E. Tank, E. Bulygina, T.I. Eglinton, V.V. Gordeev, T.Y. Gurtovaya, P.A. Raymond, D.J. Repeta, R. Staples, R.G. Striegl, A.V. Shulidov, and S.A. Zimov. 2012. Seasonal and annual fluxes of nutrients and organic matter from large rivers to the Arctic Ocean and surrounding seas. *Estuaries and Coasts* 35: 369-382

Howarth, R.W., G. Billen, D. Swaney, A. Townsend, N. Jaworski, K. Lajtha, J.A. Downing, R. Elmgren, N. Caraco, T. Jordan, F. Berendse, J. Freney, V. Kudeyarov, P. Murdoch, and Z. Zhao-Liang. 1996. Regional nitrogen budgets and riverine N & P fluxes for the drainages to the North Atlantic Ocean: Natural and human influences. *Biogeochemistry* 35(1): 75-139

Howarth, R.W., A. Sharpley, and D. Walker. 2002. Sources of nutrient pollution to coastal waters in the United States: Implications for achieving coastal water quality goals. *Estuaries* 25(4): 656-676

Howarth, R.W., D. Swaney, G. Billen, J. Garnier, B. Hong, C. Humborg, P. Johnes, C.-M. Mörth, and R. Marino. 2012. Nitrogen fluxes from the landscape are controlled by net anthropogenic nitrogen inputs and by climate. *Front. Ecol. Environ.* 10(1): 37-43

Huang, X., M. Sillanpää, E.T. Gjessing, and R.D. Vogt. 2009. Water quality in the Tibetan Plateau: Major ions and trace elements in the headwaters of four major Asian rivers. *Science of the Total Environment* 407: 6242–6254

Hudon, C., and R. Carignan. 2008. Cumulative impacts of hydrology and human activities on water quality in the St. Lawrence River (Lake Saint-Pierre, Quebec, Canada). *Can. J. Fish. Aquat. Sci.* 65: 1165–1180

Hunter, H.M., and R.S. Walton. 2008. Land-use effects on fluxes of suspended sediment, nitrogen and phosphorus from a river catchment of the Great Barrier Reef, Australia. *Journal of Hydrology* 356: 131– 146

Hur, J., S.-J. Hwang, and J.-K. Shin. 2008. Using synchronous fluorescence technique as a water quality monitoring tool for an urban river. *Water Air Soil Pollut.* 191: 231–243

Igwilo, I.O., O.J. Afonne, U.J.-M. Maduabuchi, and O.E. Orisakwe. 2010. Toxicological study of the Anam River in Otuocha, Anambra State, Nigeria. *Archives of Environmental & Occupational Health* 61(5): 205-208

Jaji, M.O., O. Bamgbose, O.O. Odukoya, and T. A. Arowolo. 2007. Water quality assessment of Ogun river, South West Nigeria. *Environ. Monit. Assess.* 133: 473–482

Jarvie, H.P., C. Neal, A.D. Tappin, J.D. Burton, M. Harrow, L. Hill, and M. Neal. 2000. The water quality of the River Trent: from the lower non-tidal reaches to the freshwater tidal zone. *The Science of the Total Environment* 251/252: 335-367

Jarvis, A., H.I. Reuter, A. Nelson, and E. Guevara. 2008. Hole-filled SRTM for the globe Version 4, available from the CGIAR-CSI SRTM 90m Database: http://srtm.csi.cgiar.org.

[JMP] WHO/UNICEF Joint Monitoring Programme for Water Supply and Sanitation. 2014. Available at http://www.wssinfo.org/data-estimates/tables/

Karikari, A.Y., J. K. Bernasko, and E. K. A. Bosque-Hamilton. 2009. An Assessment of Water Quality of Angaw River in Southeastern Coastal Plains of Ghana. *West African Journal of Applied Ecology* 11(1)

Kenyanya, M.M. (1999) *Water quality and nutrient dynamics - Lake Victoria, Kenya.* In: *Report on Fourth FIDAWOG Workshop held at Kisumu, 16 to 20 August 1999*. Jinja, Uganda, Lake Victoria Fisheries Research Project , pp. 118-126. (LVFRP Technical Document, 7)

Khan, I., H. Ullah, and M. Imran. 2007. Nitrate and phosphate pollution in surface and ground water in western Malaysia. *Jour. Chem. Soc. Pak.* 29(4): 315-320

Kitamura, K., K. Saeki, and N. Funamizu. 2013. Semi-decentralized water recycling in megacities: the example of Tokyo Shinjuku Area. In: *Milestones in Water Reuse: The Best Success Stories*. Eds.: V. Lazarova, T. Asano, A. Bahri, and J. Anderson. IWA Publishing, London, UK.

Kulekana, J.J. 2004. Levels of nitrate and phosphate in some satellite lakes within the Lake Victoria basin, Tanzania. *Tanzania Journal of Science* 30(1): 1-10

Kumar, R.N., R. Solanki, and N. Kumar. 2011. An assessment of seasonal variation and water quality index of Sabarmati river and Kharicut Canal at Ahmedabad, Gujarat. *Electronic Journal of Environmental, Agricultural and Food Chemistry* 10(5): 2248-2261

Langhammer, J. 2001. Water quality in the Berounka river basin. *Acta Universitatis Carolinae* 2: 111-131

Lassaletta, L., H. García-Gómez, B.S. Gimeno, and J.V. Rovira. 2010. Headwater streams: neglected ecosystems in the EU Water Framework Directive. Implications for nitrogen pollution control. *Environmental Science & Policy* 13: 423–433

Laznik, G., and G. Matisone. 1994. Variations in the loadings and riverine transport of nitrate and nitrite in Latvia. *Marine Pollution Bulletin,* 29(6-12): 484-490

Le, T.P.Q., G. Billen, J. Garnier, S. Théry, D. Ruelland, X.A. Nghiem, and V.M. Chau. 2010. Nutrient (N, P, Si) transfers in the subtropical Red River system (China and Vietnam): Modelling and budget of nutrient sources and sinks. *Journal of Asian Earth Sciences* 37: 259–274

Lewis, W.M. Jr., J.M. Melack, W.H. McDowell, M. McClain, and J.E. Richey. 1999. Nitrogen yields from undisturbed watersheds in the Americas. *Biogeochemistry* 46: 149-162.

Liljeström, I. 2007. Nitrogen and phosphorus dynamics in the Mekong basin: Nutrient balance assessment in a catchment scale. M.S. Thesis, Helsinki University of Technology

Ludwig, W., E. Dumont, M. Meybeck, and S. Heussner. 2009. River discharges of water and nutrients to the Mediterranean and Black Sea: Major drivers for ecosystem changes during past and future decades? *Progress in Oceanography* 80: 199–217

Maillard, P., and N.A. Santos. 2008. A spatial-statistical approach for modeling the effect of non-point source pollution on different water quality parameters in the Velhas river watershed – Brazil. *Journal of Environmental Management* 86: 158–170

Markich, S.J., and P.L. Brown. 1998. Relative importance of natural and anthropogenic influences on the fresh surface water chemistry of the Hawkesbury-Nepean River, south-eastern Australia. *The Science of the Total Environment* 217: 201-230

McKee, L. B. Eyre, and S. Hossain. 2000. Intra- and interannual export of nitrogen and phosphorus in the subtropical Richmond River catchment, Australia. *Hydrol. Processes* 14: 1787-1809

Melaku, S., T. Wondimu, R. Dams, and L. Moens. 2007. Pollution status of Tinishu Akaki River and its tributaries (Ethiopia) evaluated using physico-chemical parameters, major ions, and nutrients. *Bull. Chem. Soc. Ethiop.* 21(1): 13-22

Meybeck, M. 1982. Carbon, nitrogen, and phosphorus transport by world rivers. *American Journal of Science* 282: 401-450

Meyer, J.H., and R. van Antwerpen. 1995. Trends in water quality of selected rivers in the South African sugar industry. *Proceedings of the South African Sugar Technologists' Association* (June 1995): 60-68

Mikhailik, T.A., P. Ya. Tishchenko, A. M. Koltunov, P. P. Tishchenko, and M. G. Shvetsova. 2011. The effect of Razdol'naya River on the environmental state of Amur Bay (The Sea of Japan) *Water Resources* 38(4): 512–521

Mishra, R.R., B. Rath, and H. Thatoi. 2008. Water quality assessment of aquaculture ponds located in Bhitarkanika Mangrove Ecosystem, Orissa, India. *Turkish Journal of Fisheries and Aquatic Sciences* 8: 71-77

Mitamura, O., N. Nakamoto, M. do Socorro Rodrigues Ibañez, P.R.S. Calvacante, J.P.C. Neto, and R. Barbieri. 2012. Biogeochemical cycling of urea in the aquatic systems of Pindaré and Turiaçu River basins, a pre-Amazonian floodplain, Baixada Maranhense, Brazil. *Acta Limnologica Brasiliensia* 24(2): 167-180

Mithani, I., N.R. Dahegaonkar, J.S. Shinde, and S.D. Tummawar. 2012. Preliminary studies on physico-chemical parameters of river Wardha, District Chandrapur, Maharashtra. *Journal of Research in Ecology* 1: 14-18

Mokaya, S.K., J.M. Marhooko, and M. Leichtfried. 2004. Influence of anthropogenic activities on water quality of a tropical stream ecosystem. *African Journal of Ecology* 42(4): 281–288

Neal, C., A.J. Robson, P. Wass, A.J. Wade, G.P. Ryland, D.V. Leach, and G.J.L. Leeks. 1998. Major, minor, trace element and suspended sediment variations in the River Derwent. *The Science of the Total Environment* 210/211: 163-172

Neal, C., H.P. Jarvie, R.J. Williams, L.C.V. Pinder, G.D. Collett, M. Neal, and L. Bhardwaj. 2000. The water quality of the Great Ouse. *The Science of the Total Environment* 251/252: 423-440

Ólafsdóttir, S.R., and J. Ólafsson. 1999. Input of dissolved constituents from River Þjórsá to S-Iceland coastal waters. *Rit Fiskideildar* 16: 79-88

Pagano, M., R. Arfi, and D. Defaye. 2011. Zooplankton communities of the Sélingué reservoir (Mali, West Africa). The role of environmental factors. IN *Studies on Freshwater Copepoda: a Volume in Honour of Bernard Dussart*. Eds.: D. Defaye, E. Suarez-Morales, and J.C. von Vaupel Klein, pp. 385-401. Koninklijke Brill, Leiden, The Netherlands

Patazova, P., and P. Simeonova. 2012. Long-term statistical assessment of the water quality of Tundja River. *Ecol Chem Eng S.* 19(2): 213-226

Potter, P., N. Ramankutty, E.M. Bennett, and S.D. Donner. 2010. Characterizing the spatial patterns of global fertilizer application and manure production. *Earth Interactions*, 14(2): 1-22

Peierls, B.L., N.F. Caraco, M.L. Pace, and J.J. Cole. 1991. Human influence on river nitrogen. *Nature* 350: 386-387

Pekka, L., J. Ingri, A. Widerlund, O. Mokrotovarova, M. Riabtseva, and B. Öhlander. 2004. Geochemistry of the Kola River, northwestern Russia. *Applied Geochemistry* 19: 1975–1995

Peterson, B.J., R.M. Holmes, J.W. McClelland, C.J. Vörösmarty, R.B. Lammers, A.I. Shiklomanov, I.A. Shiklomanov, and S. Rahmstorf. 2002. Increasing river discharge to the Arctic Ocean. *Science* 298: 2171-2173.

Pfennig, K.S., and P.B. McMahon. 1996. Effect of nitrate, organic carbon, and temperature on potential denitrification rates in nitrate-rich riverbed sediments. *Journal of Hydrology* 187: 283-295

Pizarro, J., P.M. Vergara, J.A. Rodríguez, P.A. Sanhueza, and S.A. Castro. 2010. Nutrients dynamics in the main river basins of the centre-southern region of Chile. *Journal of Hazardous Materials* 175 : 608–613

Prabhahar, C., K. Saleshrani, and K. Tharmaraj. 2012. Seasonal variation in physico-chemical parameters of Palar River in and around Vaniyambadi Segment, Vellore District, Tamil Nadu, India. *International Journal of Pharmaceutical & Biological Archives* 3(1): 99-104

Räike, A., O.-P. Pietiläinen, S. Rekolainen, P. Kauppila, H. Pitkänen, J. Niemi, A. Raateland, and J. Vuorenmaa. 2003. Trends of phosphorus, nitrogen and chlorophyll *a* concentrations in Finnish rivers and lakes in 1975–2000. *The Science of the Total Environment* 310: 47–59

Rani, N., R.K. Sinha, K. Prasad, and D.K. Kedia. 2011. Assessment of temporal variation in water quality of some important rivers in middle Gangetic plains, India. *Environ. Monit. Assess.* 174: 401–415

Rasmussen, J.B., and V. Trudeau. 2007. Influence of velocity and chlorophyll standing stock on periphyton $\delta^{13}C$ and $\delta^{15}N$ in the Ste. Marguerite River system, Quebec. *Can. J. Fish. Aquat. Sci.* 64: 1370–1381

Ravichandran, S., R. Ramanibai, and N.V. Pundarikanthan. 1996. Ecoregions of describing water quality patterns in Tamiraparani basin, South India. *Journal of Hydrology* 178: 257-276

Razumov, V.A., and F. I. Tyutyunova. 2001. Nitrite contamination of the Moskva River: causes and effects. *Water Resources 28(3): 324–334*

Rim-Rukeh, A., G.O. Ikhifa, and P.A. Okokoyo. 2007. Physico-chemical characteristics of some waters used for drinking and domestic purposes in the Niger Delta, Nigeria. *Environ. Monit. Assess.* 128:475–482

Rosales Hoz, L., A. Carranza Edwards, P. Carvajal Romero, C. Mendez Jaime, and M. E. Ruiz Santoyo. 2003. Physico-chemical seasonal variability of a tropical estuary: major and minor elements in water and air. *Environmental Geology* 44:790–798

Ryder, D., R. Veal, C. Sbrocchi, and J. Schmidt. 2011. *Bellinger-Kalang Rivers Ecohealth Project Assessment of River and Estuarine Condition 2009-2010*. Final Technical Report to the Bellingen Shire Council. University of New England, Armidale, NSW, Australia.

Şahan, A., T. Altun, F. Çevik, İ. Cengizler, E. Nevşat, and E. Genç. 2007. Comparative study of some haematological parameters in European Eel (*Anguilla anguilla* L., 1758) caught from different regions of Ceyhan River (Adana, Turkey). *E.U. Journal of Fisheries & Aquatic Sciences* 24(1-2): 167-171

Schaefer, S.C., and M. Alber. 2007. Temperature controls a latitudinal gradient in the proportion of watershed nitrogen exported to coastal ecosystems. *Biogeochemistry* 85: 333-246

Segal-Rozenhaimer, M., U. Shavit, A. Vengosh, I. Gavrieli, E. Farber, R. Holtzman, B. Mayer, and A. Shaviv. 2004. Sources and transformations of nitrogen compounds along the lower Jordan River. *J. Environ. Qual.* 33:1440–1451

Seitzinger, S.P., R.V. Styles, E.W. Boyer, R.B. Alexander, G. Billen, R.W. Howarth, B. Mayer, and N. van Breemen. 2002. Nitrogen retention in rivers: model development and application to watersheds in the northeastern USA. *Biogeochemistry* 57/58: 199-237Turner, R.E., N.N. Rabalais, D. Justic, and Q. Dortch. 2003. Global patterns of dissolved N, P and Si in large rivers. *Biogeochemistry* 64: 297-317

Serdal Sakcali, M., R. Yilmaz, S. Gucel, C. Yarci, and M. Ozturk. 2009. Water pollution studies in the rivers of the Edirne Region–Turkey. *Aquatic Ecosystem Health & Management*, 12(3): 313–319

Silva-Benavides, A.-M. 1996. The use of water chemistry and benthic diatom communities for qualification of a polluted tropical river in Costa Rica. *Rev. BioI. Trop.* 44(2): 395-416

Singh, A.K., G.C. Mondal, S. Kumar, T.B. Singh, B.K. Tewary, and A. Sinha. 2008. Major ion chemistry, weathering processes and water quality assessment in upper catchment of Damodar River basin, India. *Environ. Geol.* 54:745–758

Singh, K.P., A. Malik, D. Mohan, and S. Sinha. 2004. Multivariate statistical techniques for the evaluation of spatial and temporal variations in water quality of Gomti River (India)—a case study. *Water Research* 38: 3980–3992

Slabbert, N. 2007. The potential impact of an inter-basin water transfer on the Modder and Caledon River systems. Ph.D. Thesis, University of the Free State, Bloemfontein, South Africa.

Stalnåcke, P., A. Grimvall, K. Sundblad, and A. Wilander. 1998. Trends in nitrogen transport in Swedish rivers. *Environmental Monitoring and Assessment* 59: 47–72

Stalnåcke, P., A. Grimvall, K. Sundblad, and A. Tonderski. 1999. Estimation of riverine loads of nitrogen and phosphorus to the Baltic Sea, 1970–1993. *Environmental Monitoring and Assessment* 58: 173–200

Szalińska, A., and J. Dominik. 2006. Water quality changes in the Upper Dunajec Watershed, southern Poland. *Polish J. Environ. Stud.* 15(2): 327-334

Tafangenyasha, C., and L.T. Dube. 2008. An investigation of the impacts of agricultural runoff on the water quality and aquatic organisms in a lowveld sand river system in southeast Zimbabwe. *Water Resour. Manage.* 22:119–130

Taleb, A., N. Belaidi, and J. Gagneur. 2004. Water quality before and after dam building on a heavily polluted river in semi-arid Algeria. *River Res. Applic.* 20: 943–956

Thani, I., and C. Phalarksh. 2008. A preliminary study of aquatic insect diversity and water quality of Mekong River, Thailand. *KKU Sci. J.* 36(Supplement): 95-106

Thongdonphum, B., S. Meksumpun, and C. Meksumpun. 2011. Nutrient loads and their impacts on chlorophyll a in the Mae Klong River and estuarine ecosystem: an approach for nutrient criteria development. *Water Science & Technology* 64(1): 178-187

[USGS EROS] United States Geologic Survey Earth Resources Observation Center. 1996. HYDRO1k Elevation Derivative Database. Sioux Falls, South Dakota.

van Vliet, M.T.H., and J.J.G. Zwolsman. 2008. Impact of summer droughts on the water quality of the Meuse River. *Journal of Hydrology* 353: 1–17

Vattenfall Power Consultant. 2009. *Lao People's Democratic Republic: Preparing the Cumulative Impact Assessment for the Nam Ngum 3 Hydropower Project. Final Report from the Monitoring Programme*. Project Number 40514.

Vega, M., R. Pardo, E. Barrado, and L. Deban. 1998. Assessment of seasonal and polluting effects on the quality of river water by exploratory data analysis. *Wat. Res.* 32(12): 3581-3592

Vitale, K., M.M. Rajčić, and A. Senta. 2002. Waters in Croatia between practice and needs: Public health challenge. *Croatian Medical Journal* 43(4): 485-492

Vörösmarty, C.J., B.M. Fekete, M. Meybeck, and R.B. Lammers. 2000. Global system of rivers: Its role in organizing continental land mass and defining land-to-ocean linkages. *Global Biogeochemical Cycles* 14(2): 599-621

Wollheim, W.M., B.A. Pellerin, C.J. Vörösmarty, and C.S. Hopkinson. 2005. N retention in urbanizing headwater catchments. *Ecosystems* 8(8): 871-884

Zeb, B.S., A.H. Malik, A. Waseem, and Q. Mahmood. 2011. Water quality assessment of Siran River, Pakistan. *International Journal of the Physical Sciences* 6(34): 7789 – 7798

Table 4.1. Watersheds included in this study, including sampling frequency and method of NO_3^- analysis. Blank spaces denote that sampling frequency or method was unknown. APHA = American Public Health Association; GEMS=Global Environment Monitoring System (United Nations).

Continent	River	Reference	Sampling frequency	Method	Notes
Africa	Akaki	Melaku et al. 2007	One-time	Colorimetry	Assumed mg NO3, not mg NO3-N
	Anambra	Igwilo et al. 2010	One-time	Colorimetry (brucine)	Assumed mg NO3, not mg NO3-N
	Angaw	Karikari et al. 2009	Monthly, 1 year	Colorimetry (hydrazine red)	
	Awash	Kenyanya 1999	Monthly, 2 years	GEMS	Assumed mg NO3, not mg NO3-N
	Berg	deViliers & Thiart 2007	Long-term median, government monitoring		
	Breede	deViliers & Thiart 2007	Long-term median, government monitoring		
	Caledon	Slabbert 2007	Monthly	Sulfuric acid digestion	
	Chiredzi	Tafangenyasha & Dube 2008	Winter season	Colorimetry (sulfanilamide)	digitized
	Gouritz	deViliers & Thiart 2007	Long-term median, government monitoring		
	Great Fish	deViliers & Thiart 2007	Long-term median, government monitoring		
	Groot	deViliers & Thiart 2007	Long-term median, government monitoring		
	Harts	deViliers & Thiart 2007	Long-term median, government monitoring		
	Imo	Rim-Rukeh et al. 2007	Rainy season	Colorimetry (brucine)	Assumed mg NO3, not mg NO3-N
	Incomati (Komati)	Dlamini et al. 2010	5x (Feb.-April)	APHA	
	Kei	deViliers & Thiart 2007	Long-term median, government monitoring		
	Keiskamme	deViliers & Thiart 2007	Long-term median, government monitoring		
	Keurbooms	deViliers & Thiart 2007	Long-term median, government monitoring		
	Letobo	Barker 2006	2x (high & low flow)	Merck cell test kit	
	Mara	Kulekana 2004	2x (dry & wet seasons)	APHA	
	Medjerda	Bouraoui et al. 2005	N/A	SWAT model	Digitized
	Mkuze	Meyer & Antwerpen 1995	Multi-year mean, government monitoring		Assumed mg NO3, not mg NO3-N

	Mphongolo/Shingwedzi	Barker 2006	2x (high & low flow)	Merck cell test kit	Assumed mg NO3, not mg NO3-N
	Mutirikiwi	Tafangenyasha & Dube 2008	Winter season	Colorimetry (sulfanilamide)	
	Njoro	Mokaya et al. 2004	6x (May-August)	APHA	Station near wastewater ponds
	Nzoia	Kenyanya 1999	Monthly (2 years)	GEMS	Assumed mg NO3, not mg NO3-N
	Ogun	Jaji et al. 2007	Bimonthly (1 year)	APHA	
	Olifants (Gouritz)	deViliers & Thiart 2007	Long-term median, government monitoring		
	Pongola	deViliers & Thiart 2007	Long-term median, government monitoring		
	Riet	deViliers & Thiart 2007	Long-term median, government monitoring		
	Runde	Tafangenyasha & Dube 2008	Winter season	Colorimetry (sulfanilamide)	
	Sabie	Barker 2006	2x (high & low flow)	Merck cell test kit	Assumed mg NO3, not mg NO3-N
	Sand	Ashton et al. 2001	Government monitoring		
	Sankarani	Pagano et al. 2011	One-time, high water	Strickland & Parsons 1972	
	Sundays	deViliers & Thiart 2007	Long-term median, government monitoring		
	Swartkops	deViliers & Thiart 2007	Long-term median, government monitoring		
	Tafna	Taleb et al. 2004	Monthly	HACH	Assumed mg NO3, not mg NO3-N
	Tokwe	Tafangenyasha & Dube 2008	Winter season	Colorimetry (sulfanilamide)	Site 18
	Tugela	deViliers & Thiart 2007	Long-term median, government monitoring		
	Umfolozi	deViliers & Thiart 2007	Long-term median, government monitoring		
	Umzimvubu	deViliers & Thiart 2007	Long-term median, government monitoring		
	Wilge	deViliers & Thiart 2007	Long-term median, government monitoring		
	Zio	Boukari et al. 1999		French standard methods	Midpoint of range
Asia	Alaknanda	Chakrapani et al. 2009	Daily & seasonal	Ion chromatography	
	Amguema	Gordeev et al. 1996	Weekly		Assumed mg NO3, not mg NO3-N
	Betwa	Avtar et al. 2011	One-time	UV spectrophotometer screening	
	Bhagirathi	Chakrapani et al. 2009	Daily & seasonal	Ion chromatography	Average

Brahamani	Mishra et al. 2008	Monthly (6 months)	APHA	Assumed mg NO3, not mg NO3-N
Cekerek	Duran & Suicmez 2007	Monthly (1 year)		Average of all seasons
Ceyhan	Şahan et al. 2007	Summer	Cd reduction	Station II
Damodar	Singh et al. 2008	Pre-monsoon	Ion chromatography	Assumed mg NO3, not mg NO3-N
Delice	Çelik 2002	November 1998 & May 1999		
Dzachu	Huang et al. 2009	One-time	Ion chromatography	Site 46
Gandak	Rani et al. 2011	March 2004-February 2006	APHA	
Geum	Hur et al. 2008	3x (low flow)	APHA	
Gomati	Singh et al. 2004	Monthly (5 years)	APHA	
Jordan	Segal-Rozenhaimer et al. 2004	2x (wet & dry seasons)	Cd reduction	
Kelkit	Duran et al. 2003	Monthly (1 year)	Turkish government standards	Average of second 5 stations; assumed mg NO3, not mg NO3-N
Ken	Avtar et al. 2011	One-time	UV spectrophotometer screening	
Lengchu	Huang et al. 2009	One-time	Ion chromatography	
Lhasa	Huang et al. 2009	One-time	Ion chromatography	
Litangchu	Huang et al. 2009	One-time	Ion chromatography	
Lo (Red)	Le et al. 2010	Monthly (2 years)	Nitrite reduction	
Mae Klong	Thongdonphum et al. 2011	3x (early & middle loading periods)	Segmented flow analyzer	Digitized
Muar	Khan et al. 2007			Assumed mg NO3, not mg NO3-N
Mun/Moon	Thani & Phalarksh 2008	One-time?	Cd reduction	
Nakdong	Ha et al. 2002	Biweekly (1 year)	Quikchem ion analyzer	
Nam Ngum/Lik	Vattenfall Power Consultant 2009	Monthly		Assumed mg NO3, not mg NO3-N
Ngomchu	Huang et al. 2009	One-time	Ion chromatography	
Nyangchu	Huang et al. 2009	One-time	Ion chromatography	
Pahang	Khan et al. 2007			Assumed mg NO3, not mg NO3-N
Palar	Prabhahar et al. 2012		Cd reduction	Assumed mg NO3, not mg NO3-N
Parlung Tsangpo	Huang et al. 2009	One-time	Ion chromatography	
Penganga/Wardha	Mithani et al. 2012	Monthly (1 year)	APHA	Assumed mg NO3
Rapti	Agrahari & Kushwaha 2012	Monthly (1 year)	APHA, test kits	Station 3 average
Ravi	Ejaz et al. 2010	3 years, low flow season	Nitrate electrode	Station 14
Razdol'naya	Mikhailik et al. 2011	February-October 2008	Cd reduction	Station 1; digitized
Ruak (Golden Triangle)	Thani & Phalarksh 2008	One-time?	Cd reduction	

	Sabarmati	Kumar et al. 2011	Monthly (1 year)	APHA	Assumed mg NO3, not mg NO3-N
	Siran	Zeb et al. 2011	3x in winter and summer	APHA	
	Swat	Alam et al. 2009			Assumed mg NO3, not mg NO3-N
	Yarmouk	Segal-Rozenhaimer et al. 2004	4x (wet & dry seasons)	Cd reduction	Average
	Tamiraparani	Ravichandran et al. 1996	4 months (pre & post monsoon)	APHA	
	Yuchu	Huang et al. 2009	One-time	Ion chromatography	
Australia	Barron	Cox et al. 2005	Monthly (long-term)	APHA	
	Bellinger	Ryder et al. 2011	Monthly (1 year)	Cd reduction	Station BR3
	Burrum	Cox et al. 2005	Monthly (long-term)	APHA	
	Haughton	Cox et al. 2005	Monthly (long-term)	APHA	
	Hawkesbury	Markich & Brown 1998	Monthly (1 year)	Cd reduction	Assumed mg NO3, not mg NO3-N
	Hunter	Hancock & Boulton 2005	September & November	Cd reduction	
	Johnstone	Hunter & Walton 2008	1991-1996	Segmented flow colorimetry	
	Kolan	Cox et al. 2005	Monthly (long-term)	APHA	
	Normanby	Furnas 2003 (cited in Brody & Mitchell)			
	O'Connell	Cox et al. 2005	Monthly (long-term)	APHA	
	Proserpine	Cox et al. 2005	Monthly (long-term)	APHA	
	Richmond	McKee et al. 2000	Monthly 1994-1996	Colorimetric (Lachat)	Average of baseflow and stormflow
	Shoalhaven	Growns et al. 2009	2x (spring & autumn)	APHA	
Europe	Adige	Cozzi & Giani 2011	Monthly 1995-2007	Colorimetry	
	Ahtavanjoki	Räike et al. 2003	Long-term monitoring	Cd reduction	
	Aire (England)	Neal et al. 1998	Multi-year		
	Aliakmon	Ludwig et al. 2009	European Environmental Agency monitoring		
	Arda	Serdal Sakcali et al. 2009	Yearly (6 years)	Colorimetry	Assumed mg NO3, not mg NO3-N
	Arges	Florescu et al. 2011	Spring 2010	Probe	Digitized; Assumed mg NO3, not mg NO3-N
	Arno	Ludwig et al. 2009	European Environmental Agency monitoring		
	Aude	Ludwig et al. 2009	European Environmental Agency monitoring		
	Azuer	Alvarez-Cobelas et al. 2010	Spring 2003		Assumed mg NO3, not mg NO3-N
	Ätran	Stalnåcke et al. 1998	Monthly (long-term)	Cd reduction	

Ångermanälven	Stalnåcke et al. 1998	Monthly (long-term)	Cd reduction	
Barta	Laznik & Matisone 1994	Long-term monitoring	Colorimetry	Assumed mg NO3, not mg NO3-N
Berounka	Langhammer 2001	Long-term monitoring		
Bug	Ertel et al. 2012	September 2009 & April 2010	Spectrophotometry	
Dalälven	Stalnåcke et al. 1998	Monthly (long-term)	Cd reduction	
Delangersan	Stalnåcke et al. 1998	Monthly (long-term)	Cd reduction	
Derwent	Neal et al. 1998	Multi-year		
Drava	Vitale et al. 2002	6-12x/year	APHA	
Dunajec	Szalińska & Dominik 2006	4-24x/year	Polish government standards	
Ega	Lassaletta et al. 2010	~Monthly	Cd reduction	Assumed mg NO3, not mg NO3-N
Eman	Stalnåcke et al. 1998	Monthly (long-term)	Cd reduction	
Ergene	Serdal Sakcali et al. 2009	Yearly (6 years)	Colorimetry	Assumed mg NO3, not mg NO3-N
Gauja	Laznik & Matisone 1994	Long-term monitoring	Colorimetry	
Gideälven	Stalnåcke et al. 1998	Monthly (long-term)	Cd reduction	
Glomma	Helland et al. 2009	Monthly (8 years)	Norwegian government standards	
Great Ouse	Neal et al. 2000	Weekly (15 months)	Colorimetry	Assumed mg NO3, not mg NO3-N
Göksu	Demirel et al. 2011	4x, 2006-2008	Ion chromatography	Assumed mg NO3, not mg NO3-N
Helgeå	Stalnåcke et al. 1998	Monthly (long-term)	Cd reduction	
Herault	Ludwig et al. 2009	European Environmental Agency monitoring		
Ialomita	Dumitrache & Diacu 2010	Monthly (5 years)	“Standard methods”	Tandarei station
Iijoki	Räike et al. 2003	Long-term monitoring	Cd reduction	
Indalsälven	Stalnåcke et al. 1998	Monthly (long-term)	Cd reduction	
Jucar	Ludwig et al. 2009	European Environmental Agency monitoring		
Kalixälven	Stalnåcke et al. 1998	Monthly (long-term)	Cd reduction	
Kasari	Piriisoo et al. 2007	Low discharge	Grasshoff et al. 1999	
Kemijoki	Stalnåcke et al. 1999	Monthly (long-term)	Cd reduction	
Kokemienjoki	Räike et al. 2003	Long-term monitoring	Cd reduction	
Kola	Pekka et al. 2004	5x (1 year)	Ion chromatography	Site K13
Kyronjoki	Räike et al. 2003	Long-term monitoring	Cd reduction	
Lagan	Stalnåcke et al. 1998	Monthly (long-term)	Cd reduction	
Lielupe	Laznik & Matisone 1994	Long-term monitoring	Colorimetry	Assumed mg NO_3, not mg NO_3-N

	Ljungan	Stalnåcke et al. 1998	Monthly (long-term)	Cd reduction	
	Ljusnan	Stalnåcke et al. 1998	Monthly (long-term)	Cd reduction	
	Luleälven	Stalnåcke et al. 1998	Monthly (long-term)	Cd reduction	
	Meuse	van Vliet & Zwolsman 2008	National monitoring program	Autoanalyzer	Keizersveer station, reference average
	Mezen	Gordeev et al. 1996	Weekly		Assumed mg NO_3, not mg NO_3-N
	Moskva	Razumov & Tyutyunova 2001	25 years, summer & winter		Downstream of Moscow
	Motala strom	Stalnåcke et al. 1998	Monthly (long-term)	Cd reduction	
	Mörrumsån	Stalnåcke et al. 1998	Monthly (long-term)	Cd reduction	
	Neretva	Ludwig et al. 2009	European Environmental Agency monitoring		
	Neva	Stalnåcke et al. 1999	Monthly (long-term)	Cd reduction	
	Nissan	Stalnåcke et al. 1998	Monthly (long-term)	Cd reduction	
	Olt	Florescu et al. 2011	Spring 2010	Probe	Digitized; Assumed mg NO_3, not mg NO_3-N
	Onega	Gordeev et al. 1996	Weekly		Assumed mg NO_3, not mg NO_3-N
	Oreälven	Stalnåcke et al. 1998	Monthly (long-term)	Cd reduction	
	Oulujoki	Räike et al. 2003	Long-term monitoring	Cd reduction	
	Ouse	Neal et al. 1998			
	Pineios	Ludwig et al. 2009	European Environmental Agency monitoring		
	Pirita	Piriisoo et al. 2007	Low discharge	Grasshoff et al. 1999	
	Pisuerga	Vega et al. 1998	Every 3 months (2.5 years)	Spectrophotometry	Cabezon station; assumed mg NO_3, not mg NO_3-N
	Piteälven	Stalnåcke et al. 1998	Monthly (long-term)	Cd reduction	
	Po	Ludwig et al. 2009	European Environmental Agency monitoring		
	Pyhajoki	Räike et al. 2003	Long-term monitoring	Cd reduction	
	Rhone	Ludwig et al. 2009	European Environmental Agency monitoring		
	Severnaya (Northern) Dvina	Gordeev et al. 1996	Weekly		
	Siikajoki	Räike et al. 2003	Long-term monitoring	Cd reduction	
	Skellefteälven	Stalnåcke et al. 1998	Monthly (long-term)	Cd reduction	
	Strimonas	Ludwig et al. 2009	European Environmental Agency monitoring		
	Thjorsa (Þjórsá)	Ólafsdóttir & Ólafsson 1999	14x (1 year)	Automated colorimetry	

				(Grasshoff)	
	Torneälven	Stalnåcke et al. 1998	Monthly (long-term)	Cd reduction	
	Trent	Jarvie et al. 2000	Weekly (3.5 years)	Colorimetry	
	Tundja	Patazova & Simeonova 2012	2004-2009	"Local and international standard methods"	
	Tweed	Neal et al. 1998	Multi-year		
	Umeälven	Stalnåcke et al. 1998	Monthly (long-term)	Cd reduction	
	Vefsna	Helland et al. 2003	Monthly (8 years)	Norwegian government standards	
	Venta	Laznik & Matisone 1994	Long-term monitoring	Colorimetry	Assumed mg NO_3, not mg NO_3-N
	Viskan	Stalnåcke et al. 1998	Monthly (long-term)	Cd reduction	
North America	Agua Fria	Crenshaw et al. 2010	3-year experiment	Ion chromatography	Assumed mg NO_3, not mg NO_3-N
	Allegheny	Goolsby et al. 2000	Long-term data		Digitized
	Altamaha	Schaefer & Alber 2007	National monitoring system		
	Androscoggin	Boyer et al. 2002	National monitoring system		
	Big Black	Goolsby et al. 2000	Long-term data		Digitized
	Black	Schaefer & Alber 2007	National monitoring system		
	Blackstone	Boyer et al. 2002	National monitoring system		
	Cape Fear	Schaefer & Alber 2007	National monitoring system		
	Cheyenne	Goolsby et al. 2000	Long-term data		Digitized
	Chippewa	Goolsby et al. 2000	Long-term data		Digitized
	Coatzacoalcos	Rosales Hoz et al. 2003	4x (rainy & dry seasons)	Portable HACH spectrometer	
	Connecticut	Boyer et al. 2002	National monitoring system		
	Cumberland	Goolsby et al. 2000	Long-term data		Digitized
	Delaware	Boyer et al. 2002	National monitoring system		
	Des Moines	Goolsby et al. 2000	Long-term data		Digitized
	Deschutes	Schaefer et al. 2009	National monitoring system		
	Eastmain	Clair et al. 2012	National monitoring system		
	Edisto	Schaefer & Alber 2007	National monitoring system		
	Eel	Schaefer et al. 2009	National monitoring system		
	G. Miami	Goolsby et al. 2000	Long-term data		Digitized
	Gatineau	Millot et al. 2002	2x (medium & high flow)	Ion chromatography	
	Grand	Goolsby et al. 2000	Long-term data		Digitized
	Hudson	Boyer et al. 2002	National monitoring system		
	Iowa	Goolsby et al. 2000	Long-term data		Digitized
	James	Boyer et al. 2002	National monitoring system		
	Kanawha	Goolsby et al. 2000	Long-term data		Digitized

	Kaskaskia	Goolsby et al. 2000	Long-term data		Digitized
	Kennebec	Boyer et al. 2002	National monitoring system		
	Kentucky	Goolsby et al. 2000	Long-term data		Digitized
	Klamath	Schaefer et al. 2009	National monitoring system		
	Merced	Schaefer et al. 2009	National monitoring system		
	Merrimack	Boyer et al. 2002	National monitoring system		
	Milk	Goolsby et al. 2000	Long-term data		Digitized
	Minnesota	Goolsby et al. 2000	Long-term data		Digitized
	Mississippi headwaters	Goolsby et al. 2000	Long-term data		Digitized
	Missouri headwaters	Goolsby et al. 2000	Long-term data		Digitized
	Mohawk	Boyer et al. 2002	National monitoring system		
	Monongahela	Goolsby et al. 2000	Long-term data		Digitized
	Muskingum	Goolsby et al. 2000	Long-term data		Digitized
	Natashquan	Clair et al. 2012	National monitoring system		
	Nehalem	Schaefer et al. 2009	National monitoring system		
	Neuse	Schaefer & Alber 2007	National monitoring system		
	Ogeechee	Schaefer & Alber 2007	National monitoring system		
	Osage	Goolsby et al. 2000	Long-term data		Digitized
	Ouachita	Goolsby et al. 2000	Long-term data		Digitized
	Pajaro	Schaefer et al. 2009	National monitoring system		
	Pamlico	Schaefer & Alber 2007	National monitoring system		
	Pee Dee	Schaefer & Alber 2007	National monitoring system		
	Penobscot	Boyer et al. 2002	National monitoring system		
	Potomac	Boyer et al. 2002	National monitoring system		
	Puerco (Colorado basin)	Crenshaw et al. 2010	3-year experiment	Ion chromatography	Assumed mg NO_3, not mg NO_3-N
	Raccoon	Goolsby et al. 2000	Long-term data		Digitized
	Rappahannock	Boyer et al. 2002	National monitoring system		
	Roanoke	Schaefer & Alber 2007	National monitoring system		
	Rock	Goolsby et al. 2000	Long-term data		Digitized
	Rogue	Schaefer et al. 2009	National monitoring system		
	Rupert	Clair et al. 2012	National monitoring system		
	Russian	Schaefer et al. 2009	National monitoring system		
	Saco	Boyer et al. 2002	National monitoring system		
	Saint Francois	Hudon & Carignan 2008	Monthly (April-November)	Lachat autoanalyzer	
	Saint Maurice	Millot et al. 2002	2x (medium & high flow)	Ion chromatography	
	Salinas	Schaefer et al. 2009	National monitoring system		
	San Pedro (Colorado basin)	Crenshaw et al. 2010	3-year experiment	Ion chromatography	Assumed mg NO_3, not mg NO_3-N

	Santa Ana	Schaefer et al. 2009	National monitoring system		
	Santee	Schaefer & Alber 2007	National monitoring system		
	Satilla	Schaefer & Alber 2007	National monitoring system		
	Savannah	Schaefer & Alber 2007	National monitoring system		
	Schuylkill	Boyer et al. 2002	National monitoring system		
	Scioto	Goolsby et al. 2000	Long-term data		Digitized
	Siuslaw	Schaefer et al. 2009	National monitoring system		
	Skeena	Clair et al. 2012	National monitoring system		
	Skunk	Goolsby et al. 2000	Long-term data		Digitized
	Spokane	Schaefer et al. 2009	National monitoring system		
	St. Croix	Goolsby et al. 2000	Long-term data		Digitized
	St. Francis	Goolsby et al. 2000	Long-term data		Digitized
	Stanislaus	Schaefer et al. 2009	National monitoring system		
	Ste. Marguerite	Rasmussen & Trudeau 2007	One-time	?	
	Tarcoles	Silva-Benavides 1996	Monthly (1 year)	APHA	
	Tuolumne	Schaefer et al. 2009	National monitoring system		
	Upper Illinois	Goolsby et al. 2000	Long-term data		Digitized
	Willamette	Schaefer et al. 2009	National monitoring system		
	Wisconsin	Goolsby et al. 2000	Long-term data		Digitized
	Yakima	Schaefer et al. 2009	National monitoring system		
	Yazoo	Goolsby et al. 2000	Long-term data		Digitized
South America	Aconcagua	Ribbe et al. 2008	National monitoring network		Digitized
	Bio-Bio	Pizarro et al. 2010	National monitoring network	Molecular absorption spectroscopy	
	Caura	Lewis et al. 1999			
	Chico	Depetris et al. 2005	8x, 3 years, range of discharges	Cd reduction	
	Coig	Depetris et al. 2005	8x, 3 years, range of discharges	Cd reduction	
	Deseado	Depetris et al. 2005	8x, 3 years, range of discharges	Cd reduction	
	Gallegos	Depetris et al. 2005	8x, 3 years, range of discharges	Cd reduction	
	Huasco	Ribbe et al. 2008	National monitoring network		Digitized
	Imperial	Pizarro et al. 2010	National monitoring network	Molecular absorption spectroscopy	
	Itata	Pizarro et al. 2010	National monitoring network	Molecular absorption spectroscopy	
	Limari	Ribbe et al. 2008	National monitoring network		Digitized
	Negro (Argentina)	Depetris et al. 2005	8x, 3 years, range of discharges	Cd reduction	
	Pindare	Mitamura et al. 2012	Rainy season (low water levels)	Bendschneider & Robinson 1952	
	Rapel	Pizarro et al. 2010	National monitoring network	Molecular absorption	

				spectroscopy	
	Santa Cruz (Argentina)	Depetris et al. 2005	8x, 3 years, range of discharges	Cd reduction	
	Velhas	Maillard & Santos 2008	Quarterly (long-term); government monitoring		Average; Assumed mg NO_3, not mg NO_3-N

Table 4.2. Watershed characteristics of rivers included in this study. Latitudinal zone classifications: P=polar T=temperate R=tropical

Continent	River	Size (km^2)	[NOx] (µM)	Disch. (mm/yr)	NOx load (kmol/ km^2/yr)	Pop. density (pers./ km^2)	Pop. density unimp. sanit. (pers./ km^2)	Mean temp. (°C)	Mean precip. (mm/ month)	Mean slope (°)	Manure use (kg/ha)	Fert. use (kg/ha)	Lat. zone	Precip./ disch. flag	Units flag	Sampling flag
Africa	Akaki	8,442	92	241	22.284	532	421.6	17	1,065	2.1	487	21	R		X	X
	Anambra	14,376	4	5,377	19.087	485	336.6	26	1,620	0.9	127	33	R	X	X	X
	Angaw	16,211	22	151	3.277	83	71.5	26	1,353	1.0	40	5	R			
	Berg	12,057	27	32	0.857	47	12.3	16	527	4.1	90	58	T			
	Breede	6,669	10	69	0.664	16	4.1	17	487	3.5	127	50	T			
	Caledon	19,375	23	116	2.657	59	26.9	14	694	2.4	214	19	T			
	Chiredzi	3,404	69	121	8.389	39	23.2	21	719	1.1	-	-	R			X
	Gouritz	45,584	2	19	0.048	4	1.0	16	415	3.6	98	35	T			
	Great Fish	30,423	31	2	0.073	7	1.9	16	483	2.7	177	7	T			X
	Groot	28,780	2	-	-	1	0.3	24	337	2.3	64	7	T	X		
	Harts	30,270	15	4	0.056	14	3.6	17	496	0.4	110	70	T			
	Imo	9,073	342	6,674	2,282.13	665	461.3	26	2,595	0.3	308	25	R	X	X	X
	Incomati	46,085	4	14	0.061	41	22.4	20	785	1.3	103	12	T			X
	Kei	18,642	11	0	0.001	32	8.3	16	660	3.1	281	8	T			
	Keiskamme	8,144	24	0	0.007	111	29.0	18	696	2.1	137	8	T			
	Keurbooms/ Krom(me)	7,527	1	2	0.002	71	18.6	16	780	3.0	-	-	T			
	Letobo	13,954	32	0	0.008	68	17.6	21	685	1.5	53	4	T			X
	Mara	13,223	35	46	1.587	73	55.8	19	1,051	1.6	173	7	R			X
	Medjerda	23,149	120	39	4.645	118	10.0	16	537	2.3	248	96	T			
	Mkuze	9,320	8	2	0.012	41	10.8	21	850	1.7	120	23	T		X	
	Mphongolo/ Shingwedzi	11,590	38	8	0.312	49	22.1	22	561	0.8	93	2	R		X	X

	Mutirikiwi	8,146	146	24	3.463	41	24.6	20	716	1.2	136	47	R			X
	Njoro	5,605	27	89	2.405	183	129.4	15	955	2.8	359	11	R			X
	Nzoia	28,826	0	44	0.014	362	255.2	19	1,381	1.7	477	18	R		X	
	Ogun	22,057	671	117	78.296	351	244.0	27	1,192	0.7	131	18	R			
	Olifants (Gouritz)	11,134	7	1	0.004	10	2.5	15	475	3.8	56	5	T			
	Pongola	10,273	36	3	0.112	48	13.1	19	771	2.7	122	28	T			
	Riet	35,408	8	1	0.008	22	5.6	16	477	0.7	136	40	T			
	Runde	41,666	215	62	13.308	27	16.4	20	601	0.9	77	35	R			X
	Sabie	10,858	60	23	1.394	44	11.5	19	866	3.6	137	20	T		X	X
	Sand	13,839	1	12	0.018	50	13.1	19	602	1.1	97	9	R			
	Sankarani	32,901	10	157	1.582	29	23.1	26	1,381	0.6	56	9	R			X
	Sundays	21,263	8	-	-	4	1.0	17	348	2.4	64	5	T	X		X
	Swartkops	2,675	30	-	-	366	95.3	17	509	2.4	-	-	T	X		X
	Tafna	30,110	451	1	0.319	173	12.2	16	410	2.2	137	12	T		X	X
	Tokwe	7,194	142	69	9.802	36	21.3	19	663	1.0	169	26	R			
	Tugela	30,053	14	18	0.250	63	16.7	16	838	3.5	221	84	T			X
	Umfolozi	10,091	5	4	0.019	70	18.3	19	869	2.6	220	18	T			X
	Umzimvubu	19,925	13	7	0.095	53	13.9	15	805	3.8	356	12	T			X
	Wilge	13,440	16	35	0.544	32	8.6	14	731	1.7	197	64	T			X
	Zio	2,537	41	677	27.857	240	212.6	26	1,214	1.0	295	17	R			X
Asia	Alaknanda	11,223	15	161	2.414	88	57.1	7	1,201	15.1	197	37	T			
	Amguema	30,927	0	107	0.003	0	0.0	-12	356	-	0	-	P		X	
	Betwa	34,020	6	238	1.382	282	182.8	26	1,019	0.4	406	217	T			X
	Bhagirathi	7,618	6	168	1.005	87	56.0	6	1,179	13.6	213	36	T			
	Brahamani	9,936	56	379	21.298	220	142.8	25	1,399	1.4	434	187	R		X	X
	Cekerek	12,027	56	11	0.621	54	4.8	10	444	3.6	207	240	T			
	Ceyhan	8,470	90	607	54.629	133	12.0	17	763	3.3	396	437	T			X
	Damodar	42,374	5	97	0.472	688	446.4	26	1,231	0.6	814	281	T		X	X

Delice	18,769	230	31	7.135	53	4.7	10	429	2.1	105	318	T			X
Dzachu	15,556	16	288	4.558	5	1.6	0	510	6.6	37	1	T			X
Gandak	46,223	14	536	7.651	329	201.7	14	1,309	10.2	408	162	T			
Geum	9,766	81	426	34.343	367	0.0	11	1,284	2.9	463	364	T			X
Gomati	12,431	29	145	4.143	950	616.9	26	962	0.1	575	461	T			
Jordan	28,802	555	158	87.938	314	0.0	17	459	3.6	312	187	T			
Kelkit	13,034	203	37	7.568	49	4.4	8	543	6.2	216	148	T			
Ken	26,674	5	715	3.649	206	133.6	25	996	0.6	420	172	T			X
Lengchu	2,688	4	3,004	13.080	4	1.2	0	568	10.9	1	0	T	X		X
Lhasa	32,970	4	43	0.187	18	6.4	-1	313	7.9	58	11	T			X
Litangchu	19,033	10	388	3.947	24	8.3	7	862	8.3	210	38	T			X
Lo (Red)	35,183	20	193	3.890	140	41.0	20	1,526	5.2	234	144	R			
Mae Klong	27,643	17	11,745	198.296	254	16.6	25	1,128	0.6	155	125	R	X		X
Muar	6,282	122	816	99.244	90	3.9	27	2,065	1.4	152	281	R		X	X
Mun/Moon	49,562	114	34	3.871	154	10.2	27	1,238	0.5	159	225	R			X
Nakdong	23,345	200	114	22.854	269	0.0	11	1,244	3.8	486	314	T			
Nam Ngum/ Lik	17,694	7	996	6.905	44	16.9	23	2,089	4.6	35	6	R		X	
Ngomchu	19,317	10	164	1.720	2	0.8	-2	487	6.0	22	1	T			X
Nyangchu	17,719	7	382	2.587	3	1.2	0	390	11.9	20	2	T			X
Pahang	28,306	126	311	39.186	32	1.4	25	2,446	3.1	64	130	R		X	X
Palar	17,934	163	3	0.427	424	275.3	27	867	1.3	485	136	R		X	X
Parlung Tsangpo	12,096	11	521	5.542	2	0.6	2	597	14.6	29	1	T			X
Penganga/ Wardha	47,883	62	6,380	396.725	220	142.5	27	1,245	0.6	340	86	R	X	X	
Rapti	28,044	8	321	2.614	627	406.1	23	1,375	2.2	597	492	T			
Ravi	36,086	216	235	50.919	814	470.0	21	732	3.1	389	685	T			
Razdol'naya	16,826	66	68	4.519	30	9.8	3	630	2.4	61	22	T			
Ruak (Golden Triangle)	1,928	79	3,860	303.173	58	18.7	21	1,500	4.8	91	36	R	X		X

	Sabarmati	23,554	6	65	0.361	540	350.2	27	774	0.7	295	340	R		X	
	Siran	2,832	271	879	238.442	549	288.6	17	1,174	6.3	416	238	T			
	Swat	14,178	90	114	10.341	282	148.7	12	806	10.8	596	207	T		X	X
	Tamiraparani	5,488	7	175	1.292	462	299.6	28	985	1.9	307	51	R			
	Yarmouk	16,319	341	21	7.168	159	7.2	16	271	1.6	206	102	T			
	Yuchu	8,995	7	1,429	10.605	3	1.2	1	564	8.5	30	1	T	X		X
Australia	Barron	2,145	2	904	2.034	23	0.0	22	1,948	2.3	10	0	R			
	Bellinger	3,459	12	69	0.849	16	0.0	18	1,543	3.1	56	1	T			
	Burrum	3,347	0	-	-	12	0.0	21	1,077	0.5	753	15	T	X		
	Haughton	4,350	0	18	0.001	3	0.0	24	932	1.5	49	34	R			
	Hawkesbury	21,964	7	0	0.002	72	0.0	14	973	2.6	147	3	T		X	
	Hunter	21,446	7	-	-	18	0.0	16	817	2.8	174	16	T	X		X
	Johnstone	2,322	6	1,087	6.846	13	0.0	22	2,899	2.5	140	1	R			
	Kolan	2,911	0	-	-	3	0.0	21	1,022	1.6	464	48	T	X		
	Normanby	24,317	0	57	0.009	0	0.0	26	1,162	0.8	5	12	R			
	O'Connell	2,372	0	225	0.034	21	0.0	22	1,506	2.7	44	11	R			
	Proserpine	2,601	3	186	0.469	7	0.0	23	1,238	2.0	28	8	R			
	Richmond	7,031	6	10	0.063	19	0.0	19	1,351	2.0	368	30	T			
	Shoalhaven	7,205	2	30	0.063	9	0.0	13	1,006	2.5	167	1	T			X
Europe	Ätran	2,698	27	338	9.296	23	0.0	6	860	0.8	70	38	T			
	Ångeman-älven	32,429	4	105	0.380	1	0.0	2	690	-	3	-	T			
	Adige	5,001	79	1,536	121.368	354	0.0	12	876	3.5	677	465	T	X		
	Ahtavanjoki	4,040	32	131	4.224	16	0.0	3	555	-	51	96	T			
	Aire (England)	2,447	192	-	-	659	0.0	9	-	0.9	521	460	T			
	Aliakmon	12,364	73	1,802	131.252	44	0.6	11	640	4.6	210	375	T	X		
	Arda	5,624	20	178	3.558	41	0.0	11	631	3.9	108	157	T		X	X
	Arges	13,084	286	2,734	781.721	264	0.0	9	654	2.0	212	123	T	X	X	X
	Arno	11,042	171	49	8.426	210	0.0	13	844	3.9	181	407	T			

Aude	5,887	108	129	13.911	53	0.0	12	779	3.1	314	565	T			
Azuer	3,196	602	12	6.978	21	0.0	14	447	0.6	135	519	T		X	X
Barta	2,615	24	280	6.598	19	0.0	7	734	0.4	85	44	T		X	
Berounka	8,794	417	221	92.230	85	0.0	7	668	1.8	197	324	T			
Bug	36,464	171	3	0.568	78	2.6	7	571	0.4	246	159	T			X
Dalälven	29,020	9	569	5.154	9	0.0	2	638	0.5	79	4	T			
Delangersan	2,221	9	991	9.153	10	0.0	3	649	-	9	-	T	X		
Derwent	1,118	423	1,183	499.964	401	0.0	9	924	1.9	-	-	T	X		
Drava	24,980	100	282	28.281	81	0.3	7	1,017	5.8	248	138	T			
Dunajec	6,760	77	220	16.873	152	0.1	6	836	3.5	175	126	T			
Ega	1,382	273	3,250	885.845	28	0.0	11	770	3.6	-	-	T	X	X	
Eman	4,390	25	109	2.715	14	0.0	6	605	0.8	36	56	T			
Ergene	11,082	158	79	12.487	98	8.8	13	626	1.0	132	401	T		X	X
Gauja	11,972	70	96	6.705	34	0.1	5	658	0.4	25	23	T			
Gideälven	3,681	4	359	1.306	4	0.0	1	606	-	4	-	T			
Glomma	32,811	27	139	3.771	22	0.0	2	688	1.4	65	66	T			
Göksu	10,466	171	193	33.020	85	7.6	12	667	5.8	252	100	T		X	X
Great Ouse	9,897	361	166	60.089	258	0.0	10	588	0.5	439	341	T		X	
Helgeå	10,989	69	52	3.593	26	0.0	6	723	0.6	48	49	T			
Herault	2,535	41	238	9.854	56	0.0	13	735	3.4	247	375	T			
Ialomita	9,226	223	916	204.206	111	0.0	10	549	0.9	209	147	T	X		
Iijoki	16,274	3	97	0.299	2	0.0	0	546	-	18	0	T			
Indalsälven	29,149	6	74	0.445	4	0.0	2	727	-	9	-	T			
Jucar	21,841	286	62	17.789	34	0.0	13	455	1.8	191	374	T			
Kalixälven	10,780	4	238	0.968	1	0.0	-1	483	-	1	-	P			
Kasari	3,030	16	427	6.764	10	0.2	5	669	0.2	37	47	T			X
Kemijoki	43,057	4	101	0.418	2	0.1	-1	528	-	14	-	P			
Kokemien-joki	26,689	68	59	3.989	27	0.0	3	608	-	58	116	T			

Kola	26,239	7	117	0.834	8	2.2	-1	510	-	4	-	P			
Kyronjoki	4,358	43	120	5.172	19	0.0	3	566	-	81	155	T			
Lagan	4,252	23	375	8.456	76	0.0	7	762	0.7	125	330	T			
Lielupe	14,429	40	127	5.012	27	0.0	6	644	0.2	74	83	T		X	
Ljungan	9,566	7	241	1.770	6	0.0	2	618	-	4	-	T			
Ljusnan	20,315	5	116	0.624	3	0.0	2	654	-	7	0	T			
Luleälven	24,157	2	184	0.422	1	0.0	-1	636	-	1	-	P			
Mörrumsån	3,929	19	519	9.652	27	0.0	6	676	0.5	37	79	T			
Meuse	17,099	239	374	89.490	313	0.0	9	909	1.1	1,167	495	T			
Mezen	35,947	1	111	0.087	0	0.1	-1	583	-	2	-	T		X	
Moskva	2,024	193	842	162.318	48	14.3	5	638	0.5	53	13	T	X		
Motala strom	12,813	28	28	0.783	33	0.0	6	645	0.7	76	158	T			
Neretva	10,794	47	183	8.632	90	3.8	9	1,191	5.9	64	90	T			
Nissan	2,251	25	299	7.555	32	0.0	7	902	0.6	46	17	T			
Olt	11,194	253	1,944	491.178	71	0.0	9	647	2.7	229	101	T	X	X	X
Onega	24,671	1	178	0.140	1	0.4	1	611	-	7	1	T		X	
Oreälven	2,564	4	1,951	7.331	2	0.0	1	598	-	3	-	T	X		
Oulujoki	5,048	3	581	1.992	23	0.0	1	524	-	25	74	T	X		
Ouse	8,086	217	656	142.454	361	0.0	9	846	1.6	708	302	T			
Pineios	11,145	149	58	8.604	41	0.6	14	644	4.1	203	213	T			
Pirita	2,264	42	154	6.471	29	0.6	5	668	0.2	66	49	T			X
Pisuerga	15,272	181	84	15.242	35	0.0	10	578	1.7	160	455	T		X	
Piteälven	12,116	2	224	0.481	2	0.0	-1	600	-	1	-	T			
Po	34,952	136	379	51.681	278	0.0	11	921	4.3	654	422	T			
Pyhajoki	5,353	20	89	1.780	12	0.0	2	525	-	22	33	T			
Rhône	20,282	103	859	88.265	116	0.0	11	824	3.6	214	366	T	X		
Severnaya (Northern) Dvina	37,373	0	917	0.393	7	2.0	1	568	-	7	2	T	X	X	

	Siikajoki	24,957	14	79	1.134	3	0.0	1	574	-	27	22	T				
	Skellefte-älven	5,353	3	583	1.554	4	0.0	0	563	-	2	-	T	X			
	Strimonas	16,870	99	10	0.946	57	0.9	10	541	5.3	185	179	T				
	Thjorsa	7,603	1	354	0.439	0	0.0	1	1,390	-	0	-	T				
	Torneälven	30,094	3	90	0.260	1	0.0	-2	502	-	1	-	P				
	Trent	7,933	500	143	71.397	592	0.0	9	699	0.7	673	311	T				
	Tundja	8,610	196	90	17.657	50	0.3	11	634	2.7	97	3	T				X
	Tweed	4,802	59	426	24.930	25	0.0	7	945	2.3	639	552	T				
	Umeälven	12,316	3	317	0.864	1	0.0	0	724	-	2	-	T				
	Vefsna	4,303	7	645	4.418	1	0.0	2	1,426	-	5	-	T				
	Venta	9,569	28	54	1.512	38	0.0	6	699	0.3	81	52	T		X		
	Viskan	2,485	51	409	20.830	55	0.0	7	891	1.1	98	18	T				
North America	Agua Fria	6,519	0	1	0.000	142	0.6	17	393	2.5	43	23	T		X		
	Allegheny	30,271	47	66	3.091	49	0.2	8	1,104	2.2	76	31	T				
	Altamaha	35,112	57	30	1.703	51	0.2	18	1,207	0.4	155	56	T				
	Androscoggin	8,451	45	555	25.028	17	0.1	5	1,128	3.1	121	1	T				
	Big Black	8,770	17	208	3.612	19	0.1	17	1,440	0.4	97	96	T				
	Black	3,274	39	296	11.660	32	0.1	17	1,211	0.2	99	198	T				
	Blackstone	1,115	125	1,032	129.059	276	1.1	9	1,199	0.9	-	-	T				
	Cape Fear	13,599	50	93	4.651	82	0.3	16	1,235	0.3	257	53	T				
	Cheyenne	44,094	72	-	-	3	0.0	8	349	0.9	76	13	T	X			
	Chippewa	24,722	39	93	3.614	13	0.1	5	829	0.4	131	97	T				
	Coatza-coalcos	19,418	104	230	23.946	33	5.1	25	2,435	2.6	264	65	R				
	Connecticut	25,019	60	258	15.415	65	0.3	6	1,139	2.5	30	2	T				
	Cumberland	46,428	27	111	2.967	49	0.2	13	1,304	2.9	159	51	T				
	Delaware	17,560	125	228	28.611	85	0.3	10	1,135	1.3	55	26	T				
	Des Moines	37,496	359	1	0.313	23	0.1	9	688	0.2	440	594	T				
	Deschutes	27,787	9	60	0.556	4	0.0	7	548	2.8	54	16	T				

Eastmain	48,978	1	205	0.293	0	0.0	-3	810	0.8	5	-	T			
Edisto	6,944	48	43	2.076	39	0.2	18	1,241	0.3	149	76	T			
Eel	8,058	20	483	9.558	4	0.0	11	1,423	6.1	46	2	T			
G Miami	13,915	262	65	16.924	111	0.5	10	948	0.3	557	519	T			
Gatineau	25,950	5	155	0.753	4	0.0	3	988	1.3	21	5	T			X
Grand	47,428	60	-	-	1	0.0	6	403	0.7	84	80	T	X		
Hudson	11,942	58	549	31.661	32	0.1	8	1,135	2.1	28	4	T			
Illinois	28,242	301	88	26.485	307	1.3	9	970	0.2	149	510	T			
Iowa	32,796	348	36	12.556	26	0.1	8	826	0.2	420	690	T			
James	16,206	55	196	10.815	24	0.1	13	1,094	1.9	131	13	T			
Kanawha	31,692	41	81	3.329	29	0.1	11	1,150	2.2	53	3	T			
Kaskaskia	15,023	51	193	9.863	32	0.1	12	989	0.2	168	605	T			
Kennebec	13,994	42	285	11.985	9	0.0	5	1,065	2.0	50	1	T			
Kentucky	18,026	65	106	6.815	46	0.2	12	1,190	1.9	213	33	T			
Klamath	40,356	10	53	0.556	3	0.0	8	771	5.0	43	6	T			
Merced	2,876	10	316	3.209	4	0.0	9	973	6.8	112	1	T			
Merrimack	12,005	60	274	16.593	143	0.6	7	1,122	2.0	16	0	T			
Milk	38,852	22	74	1.633	1	0.0	5	460	1.5	43	117	T			
Minnesota	43,715	274	1	0.177	21	0.1	7	562	0.5	268	550	T			
Mississippi	30,175	21		#VALUE!	10	0.0	4	-	-	87	49	T			
Mohawk	8,935	104	467	48.337	54	0.2	7	1,144	1.7	55	11	T			
Monongahela	19,114	67	153	10.269	79	0.3	10	1,290	2.2	67	14	T			
Muskingum	20,819	111	90	9.994	74	0.3	10	965	0.8	262	184	T			
Natashquan	17,034	1	202	0.289	0	0.0	-2	1,035	1.7	3	-	T			
Nehalem	1,747	64	13,164	842.903	5	0.0	9	2,228	3.6	-	-	T	X		
Neuse	7,033	93	136	12.693	103	0.4	16	1,235	0.3	980	209	T			
Ogeechee	8,415	61	163	9.999	29	0.1	18	1,212	0.3	52	57	T			
Osage	39,023	31	55	1.726	15	0.1	13	953	0.4	285	98	T			

Ouachita	41,669	17	23	0.409	17	0.1	17	1,391	1.5	133	20	T				
Pajaro	3,063	81	238	19.262	33	0.1	14	494	3.7	508	42	T				
Pamlico	5,748	95	490	46.691	35	0.1	16	1,272	0.2	188	78	T				
Pee Dee	21,448	60	138	8.244	62	0.3	16	1,191	0.6	285	68	T				
Penobscot	20,109	38	209	8.061	8	0.0	4	1,047	1.4	12	3	T				
Peribonka	21,641	5	153	0.773	0	0.0	-2	941	1.3	3	0	T				X
Potomac	29,940	195	87	16.931	63	0.3	11	1,011	2.0	294	62	T				
Povungnituk	25,214	2	90	0.182	0	0.0	-8	401	0.4	0	-	T				X
Puerco (Colorado basin)	7,664	0	-	-	9	0.0	10	299	1.5	15	-	T	X	X		
Raccoon	3,779	441	-	-	39	0.2	12	-	-	97	19	T				
Rappa-hannock	4,134	93	178	16.595	24	0.1	13	1,071	1.1	204	32	T				
Roanoke	21,984	40	128	5.128	40	0.2	14	1,126	1.0	138	19	T				
Rock	28,348	248	25	6.217	64	0.3	8	825	0.4	394	519	T				
Rogue	10,188	8	185	1.571	20	0.1	9	979	5.9	27	2	T				
Rupert	28,842	6	470	2.684	0	0.0	-2	810	0.6	5	0	T				
Russian	3,470	25	294	7.415	86	0.4	13	1,108	4.0	93	12	T				
Saco	3,349	41	810	33.457	16	0.1	7	1,181	1.7	81	2	T				
Saint Francois	11,887	23	884	20.578	35	0.1	4	1,062	1.4	177	52	T				
Saint-Maurice	32,975	7	559	3.787	5	0.0	2	1,016	1.6	9	3	T				X
Salinas	10,568	13	41	0.541	10	0.0	14	434	3.4	122	87	T				
San Pedro (Colorado basin)	11,850	4	-	-	12	0.3	16	420	2.7	22	4	T	X	X		
Santa Ana	3,881	68	44	3.018	432	1.8	16	447	4.1	69	2	T				
Santee	32,017	51	22	1.122	71	0.3	16	1,243	0.9	142	13	T				
Satilla	7,348	95	97	9.159	14	0.1	19	1,253	0.2	175	75	T				
Savannah	25,488	46	196	9.087	25	0.1	16	1,288	0.7	240	29	T				
Schuylkill	4,903	257	404	103.676	293	1.2	10	1,149	1.5	325	115	T				

	Scioto	16,882	222	171	37.813	105	0.4	11	952	0.3	206	416	T			
	Siuslaw	1,531	49	2,749	134.575	4	0.0	10	1,759	3.1	25	0	T	X		
	Skeena	43,141	8	307	2.410	1	0.0	1	778	9.6	1	-	T			
	Skunk	11,246	348	182	63.152	20	0.1	9	861	0.4	443	621	T			
	Spokane	9,932	7	393	2.895	7	0.0	7	734	4.3	9	3	T			
	St. Croix	20,030	13	4	0.048	18	0.1	5	762	0.4	90	56	T			
	St. Francis	23,635	17	16,382	278.105	21	0.1	15	1,233	0.1	88	422	T	X		
	Stanislaus	2,485	9	366	3.372	5	0.0	9	850	6.8	40	-	T			
	Ste. Marguerite	6,107	3	1,138	3.574	0	0.0	-2	1,054	2.7	1	-	T	X		X
	Tarcoles	2,147	443	2,112	934.894	882	55.5	21	2,424	4.9	235	63	R			
	Tuolumne	4,307	8	210	1.704	15	0.1	9	879	5.9	3	-	T			
	Willamette	28,992	51	539	27.351	11	0.0	9	1,609	4.3	92	12	T			
	Wisconsin	30,889	39	41	1.577	20	0.1	6	803	0.3	183	110	T			
	Yakima	14,542	21	997	21.146	15	0.1	7	740	4.6	86	21	T	X		
	Yazoo	35,008	23	34	0.792	18	0.1	17	1,429	0.5	97	265	T			
South America	Aconcagua	15,223	228	131	29.936	452	5.8	9.535 832	496	9.0	111	28	T			
	Bio-Bio	41,519	15	186	2.796	33	0.4	8	1,472	4.8	88	32	T			
	Caura	47,190	4	977	4.330	1	0.0	25	2,868	2.5	21	0	R			X
	Chico	43,713	9	10	0.086	0	0.0	7	187	1.9	11	0	T			
	Coig (aka Coyle)	30,468	1	74	0.058	2	0.1	6	267	1.9	26	2	T			
	Deseado	42,547	7	55	0.377	1	0.0	9	206	1.8	10	-	T			
	Gallegos	8,897	47	44	2.056	3	0.1	6	254	1.0	46	6	T			
	Huasco	9,887	26	6	0.162	2	0.0	6	81	10.4	10	5	T			
	Imperial	12,129	29	402	11.491	47	0.6	11	1,479	2.5	193	126	T			
	Itata	14,975	71	239	17.079	64	0.8	12	1,133	3.3	149	82	T			
	Limari	2,239	17	150	2.506	19	0.2	15	148	3.6	48	157	T	X		
	Pindare	38,942	2	222	0.413	15	3.0	26	1,492	1.1	116	12	R			X
	Rapel	14,259	112	1,811	202.947	119	1.5	10	557	5.4	342	216	T	X		

Santa Cruz (Argentina)	24,748	1	450	0.514	0	0.0	6	421	4.3	10	0	T	X	
Velhas	26,605	10	181	1.726	142	27.2	21	1,287	2.0	120	5	R		X

Table 4.3. Means, medians, and number of watersheds in each geographic area. Statistical group was determined through a Wilcoxon rank sum test.

Geographic area	# of watersheds	Average NO_3^- concentration (μM)	Standard deviation	Median	Statistical group
Africa	41	69	133	23	a
Asia	41	81	115	20	ab
Australia	12	4	4	2	c
Europe	76	97	128	40	a
North America	85	77	103	46	b
South America	15	39	61	15	ab
Tropical	39	82	136	27	A
Temperate	225	78	112	31	A
Polar	7	3	2	3	B
Total	271	77	114	28	

Table 4.4. Results of regressions between NO_3^- concentrations, loads, and potential explanatory factors. NO_3^- concentrations and loads were log transformed. Other transformations as indicated in table. NS=not significant.

Explanatory factor	Relationship with NO_3^- concentrations			Relationship with NO_3^- loads		
	Equation	R^2	p	Equation	R^2	p
Population density	y = 0.52*log(x) + 0.66	0.32	2e-24	y = 0.46*log(x) + 0.84	0.14	5e-9
Precipitation	NS			y = 1.70*log(x) - 3.45	0.10	8e-7
Temperature	y = 0.01*x + 1.23	0.02	0.04	NS		
Population density without access to improved sanitation	NS			NS		
Watershed slope	NS			NS		
River discharge	NS			Co-correlated		
Watershed size	NS			y = -1.9e-5*x + 1.84	0.05	0.0007
Fertilizer use	y = 0.0024*x + 1.11	0.22	4e-16	y = 0.0024*x + 1.23	0.12	6e-8
Manure use	y = 0.0015*x + 1.11	0.12	1e-8	y = 0.0018*x + 1.19	0.09	6e-6

Table 4.5. Results of regressions between nitrate concentrations and watershed characteristics on different continents. NS=not significant.

Explanatory factor	Africa	Asia	Australia	Europe	North America	South America
Population density	y=0.27*log(x)+1.36 R^2=0.11 p=0.04	y=0.60*log(x)+1.02 R^2=0.28 p=0.0004	y=0.43*log(x)+0.93 R^2=0.31 p=0.048	y=0.88*log(x)+0.02 R^2=0.75 p=5e-24	y=0.76*log(x)+0.03 R^2=0.32 p=1e-8	y=0.91*log(x)-0.07 R^2=0.39 p=0.01
Precipitation	NS	NS	NS	NS	y=0.10*log(x)+2.82 R $R^2$2=0.17 p=0.0001	NS
Temperature	NS	y=5.20x+7.19 R^2=0.14 p=0.01	NS	y=4.83x-1.32 R^2=0.71 p=1e-21	y=2.82x+5.48 R^2=0.11 p=0.002	NS
Population density w/o access to impr. sanit.	NS	NS	NS	NS	NS	NS
Watershed slope	NS	NS	y=0.57x+2.04 R^2=0.44 p=0.01	NS	y=-0.69x+3.00 R^2=0.07 p=0.02	NS
River discharge	NS	NS	NS	NS	NS	NS
Watershed size	NS	NS	NS	NS	NS	NS
Fertilizer use	NS	y=79.81x+54.70 R^2=0.15 p=0.01	y=-10.08x+14.21 R^2=0.36 p=0.03	y=147.6x-72.50 R^2=0.44 p=1e-10	y=111.41x-70.59 R^2=0.21 p=1e-5	NS
Manure use	NS	NS	NS	y=162.72x-88.24 R^2=0.34 p=4e-8	y=113.56x-34.44 R^2=0.27 p=4e-7	y=68.91x+9.39 R^2=0.29 p=0.04

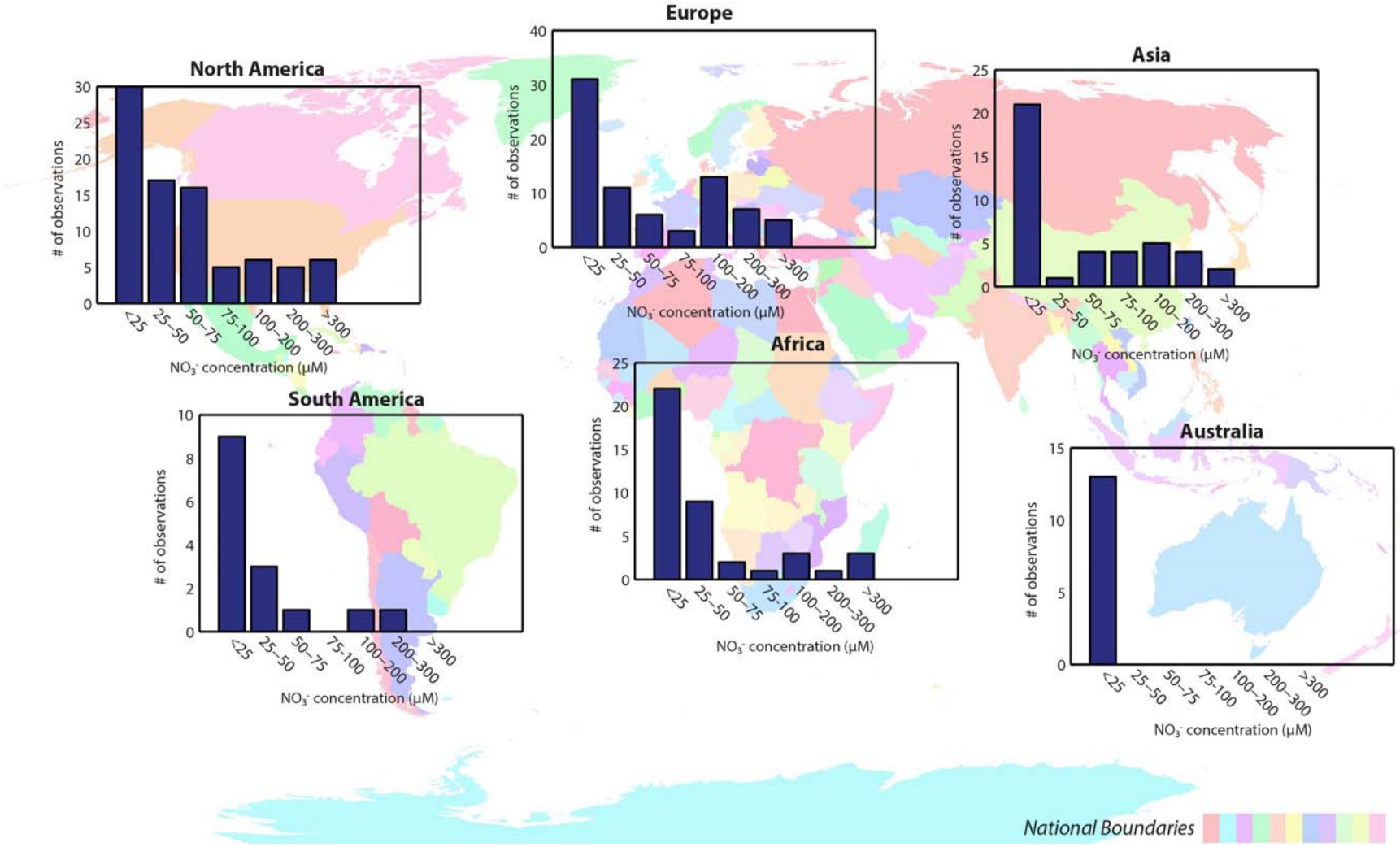


Figure 4.1. Histograms of nitrate concentrations on each continent. Background map shows political boundaries.

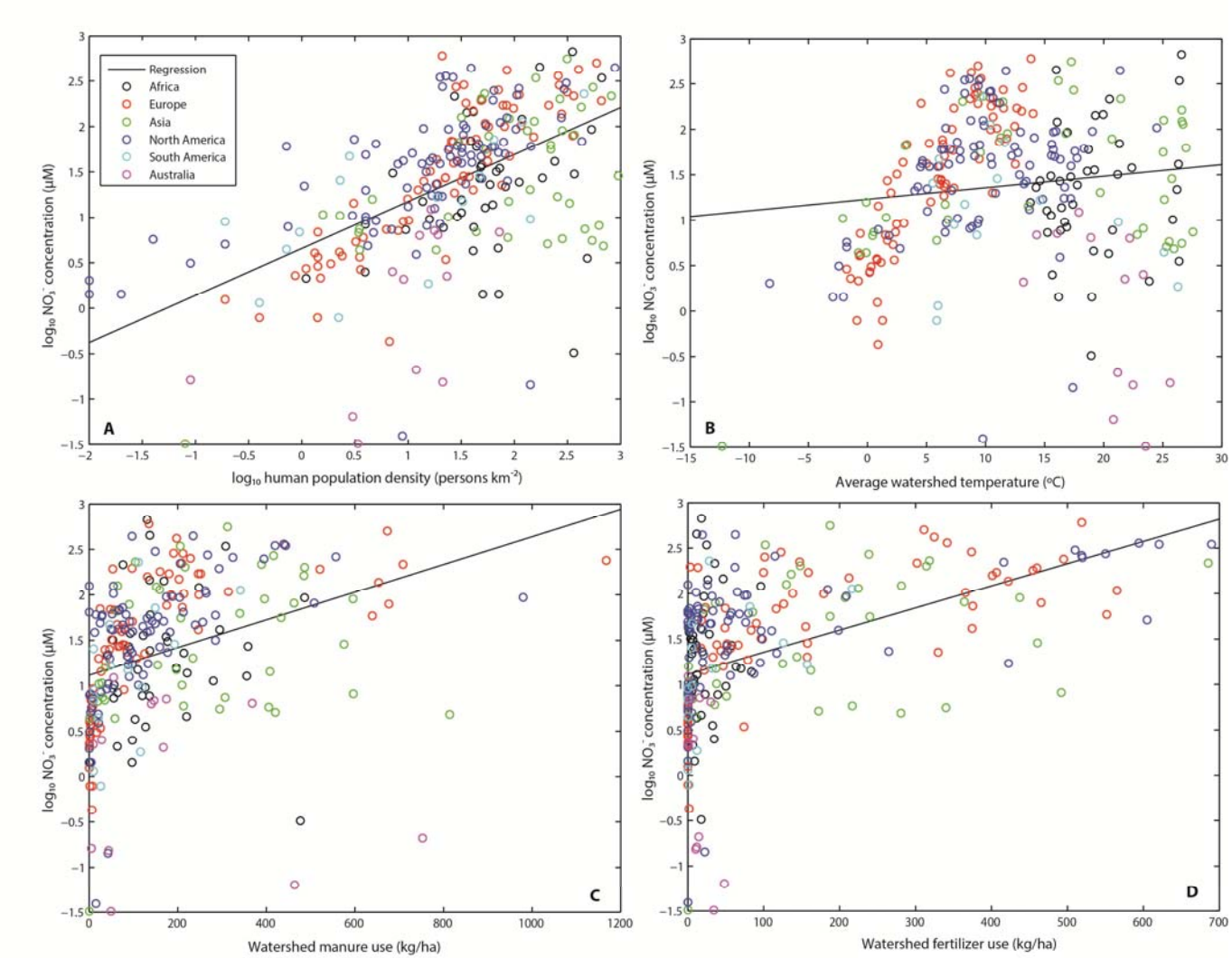


Figure 4.2. Population density and temperature vs. nitrate concentration in 271 world watersheds. (a) population density, (b) average watershed temperature, (c) manure use, (d) fertilizer use.

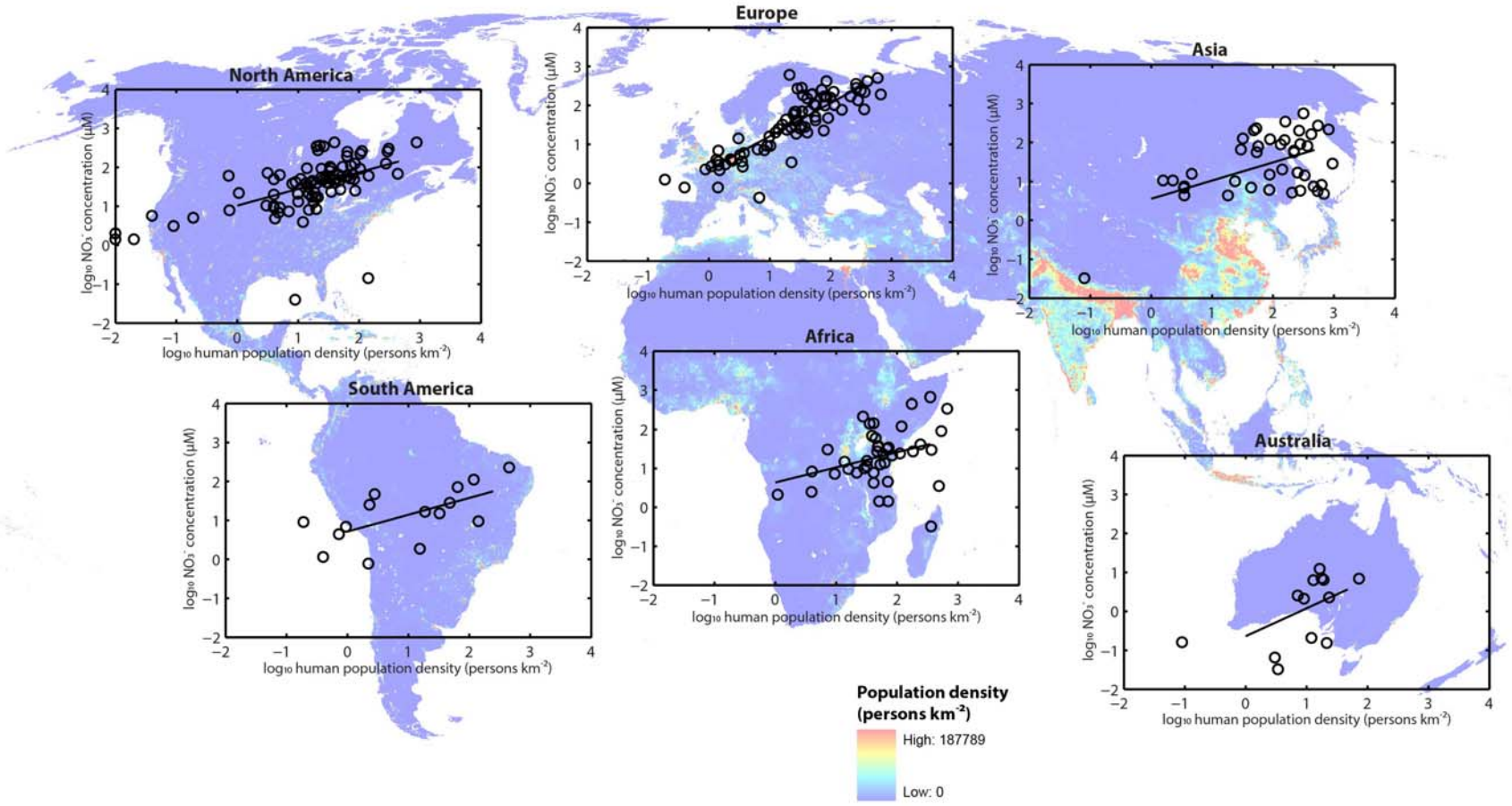


Figure 4.3. Population density vs. nitrate concentration for each continent (excluding Antarctica). Background map represents population density. Equations are given in Table 4.5.

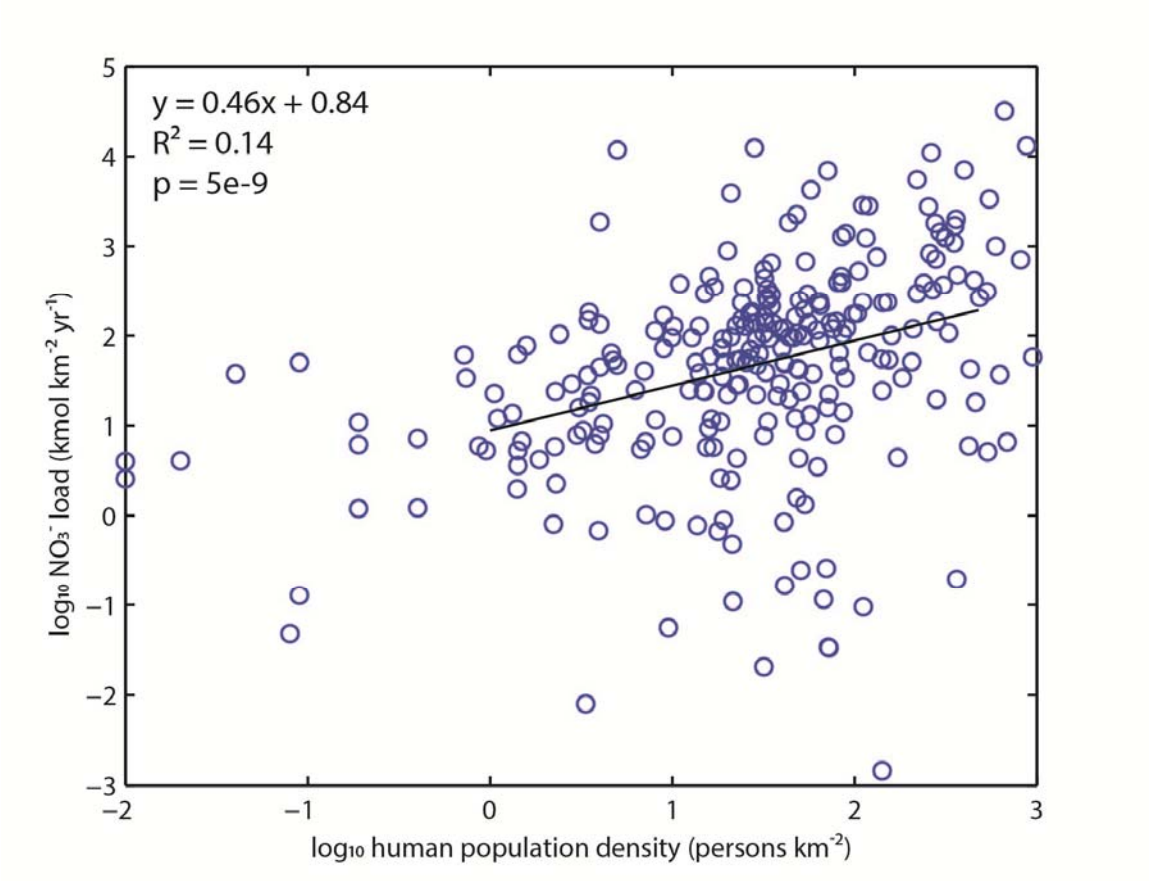


Figure 4.4. Population density vs. nitrate loads in world watersheds.

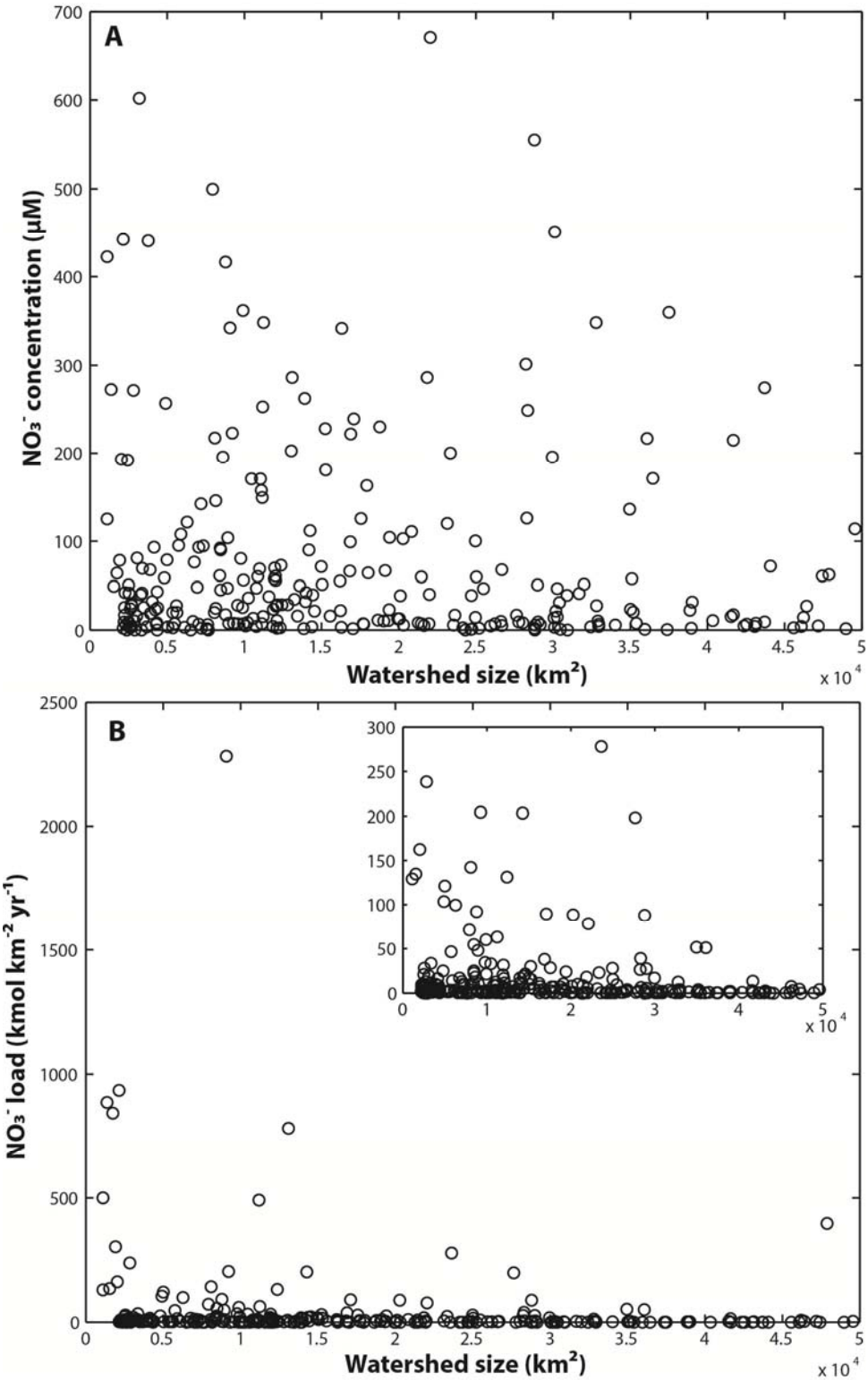


Figure 4.5. Watershed size versus nitrate in watersheds between 2,000 and 50,000 km^2. (a) Nitrate concentrations; (b) nitrate loads. Inset in (b) shows a reduced scale.

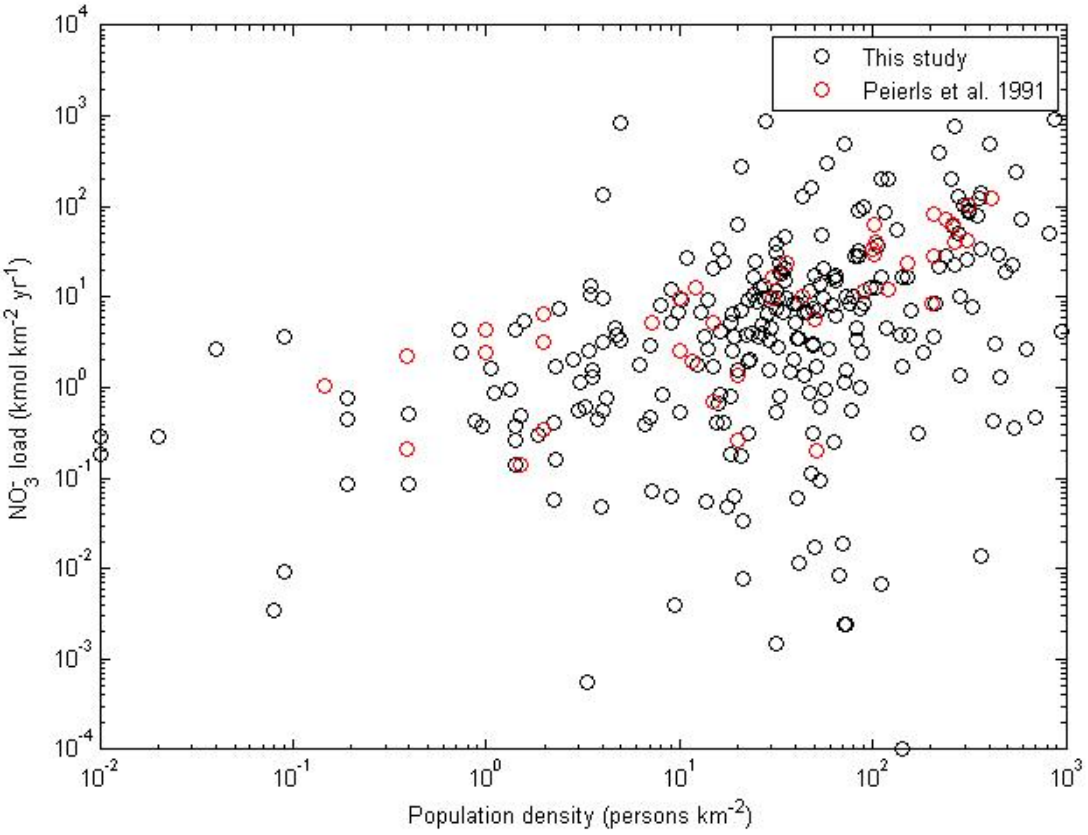


Figure 4.6. Log of population density versus log of nitrate loads. Red points, Peierls et al. 1991; black points, this study.

CHAPTER 5

CONCLUSIONS

Nitrogen is an element of great importance to living organisms, and the increased availability of fixed, biologically available N since the Industrial Revolution and the invention of the Haber-Bosch process has resulted in detrimental changes to aquatic ecosystems worldwide (Vitousek et al. 1997). Rivers deliver nitrogen to the coast, where the adverse effects of nutrient-induced eutrophication are particularly felt, but even fluvial systems are susceptible to the effects of increased nutrient input. Because of the complexity of the nitrogen cycle and the spatial variability of the processes comprising it, however, the controls on nitrogen behavior on a landscape scale remain less than completely understood.

Previous research has suggested that N loading from rivers is controlled primarily by the anthropogenic nitrogen inputs to the watershed surface (Howarth et al. 1996). Chapter 2 shows that this is not always the case. Nitrogen budgets were constructed for 18 watersheds on the west coast of the United States. Here, streamflow was found to be the primary factor controlling N exports, although the addition of anthropogenic inputs to the regression improved the relationship. Streamflow was also the primary control on the percentage of N inputs that are exported from these systems. This is potentially due to the large dynamic range of streamflow in the region studied, which ranges from temperate rainforest to desert ecosystems.

An in-depth examination of the Altamaha River watershed in Georgia is presented in Chapter 3. Monthly measurements of the concentration of various species of nitrogen in the Altamaha River and its tributaries were undertaken and compared with inputs to the land surface. Concentrations of the various species of N did not exhibit any seasonal patterns, but load was highest in the late winter and spring, when streamflow was also highest. Concentrations of inorganic N were highest in the upper portion of the watershed, corresponding to higher human and animal populations, while the lower watershed, which was dominated by agricultural activity, had higher organic N concentrations. The two blackwater tributaries, the Little Ocmulgee and Ohoopee Rivers, were particularly dominated by organic matter and exhibited other characteristics common to blackwater streams.

The best predictor of nitrate as well as total nitrogen concentrations was human population density, rather than any of the calculated anthropogenic N inputs. This was true for loads as well, and relationships between population density and nitrogen loads were even better than for concentrations. This was somewhat surprising, as anthropogenic N inputs have generally been found to be excellent predictors of riverine N delivery.

The composition of in-stream nitrate in each of the subwatersheds was analyzed for isotopes of both nitrogen and oxygen. This dual isotope technique can give insight into the sources of NO_3^-. Nitrate isotopes in rivers in the Altamaha watershed generally exhibited a signature similar to that of nitrate derived from sewage or manure. Excepted were samples with a very low NO_3^- concentration. These samples tended to exhibit a nitrate similar to that of atmospheric deposition. This suggested

that background NO_3^- in this system is of atmospheric origin, while at higher concentrations, nitrate of manure and sewage origin overwhelms the background signal.

To put the measurements taken in the Altamaha River into a global context, a metadata analysis of nitrate measurements in rivers of comparable size was undertaken in Chapter 4. While these types of studies have been undertaken before, they have primarily focused on large rivers, which deliver the majority of freshwater and of nitrogen to the coasts through their sheer size. However, most of the runoff from the world's land surface flows into small and medium-sized rivers (which may then later drain into a larger stream), making an understanding of these smaller streams important. Additionally, studies focusing on large rivers have included estuarine measurements in their compilations, which may contain marine-derived nitrogen as well as that coming from terrestrial runoff.

The compilation of nitrate measurements in 271 medium-sized rivers, which had basins ranging from 2,000 to 50,000 km^2 in size, covered all continents except Antarctica. Concentrations ranged from close to 0 to 671 μm. However, concentrations were generally quite low, with measurements of less than 25 μm reported in more than half of the watersheds in the dataset. Both mean and median concentrations were highest in Europe and lowest in Australia.

Human population density was the single best predictor of nitrate concentrations worldwide. Average watershed temperature was also a significant predictor of nitrate concentrations, but the relationship was not as strong. A relationship between population density and nitrate concentration was present on all continents when

considered individually, but varied in strength. When latitudinal zones were considered, tropical rivers tended to have higher nitrate concentrations than temperate streams, and the relationship between population density and NO_3^- concentration was steeper in tropical rivers. Polar streams exhibited an even steeper relationship, but this may have been due to the low number of observations in these latitudes. Other watershed characteristics were important in predicting nitrate concentrations only on certain continents. Overall, a combination of population density, precipitation, and discharge in combination was the best predictor of nitrate concentrations. Nitrate load was best predicted by a combination of population density, precipitation, and temperature.

The relationships between nitrate concentrations and human population density in smaller rivers have far more scatter than in larger rivers. A weak but significant negative relationship between watershed size and riverine nitrate concentration suggested that smaller watersheds may indeed be more susceptible to variation in nitrate retention and processing.

Nitrate concentrations in the Altamaha River subwatersheds (2.3-54.1 μM) were similar to the average North American concentration (37.1 μM) and somewhat lower than the worldwide average (64 μM). However, nitrate concentrations in these watersheds are lower than would otherwise be expected based on population density. One potential explanation for this is that, unlike many other watersheds, the major population centers in the Altamaha (in particular, portions of the city of Atlanta) are located in the headwaters rather than near the coast. This could permit more time for processing of in-stream

nitrate, particularly as headwater streams account for a greater proportion of in-stream denitrification compared to higher-order streams (Seitzinger et al. 2002).

The effect of humans on concentrations, particularly in medium-sized watersheds such as those examined in this dissertation, has not been particularly well-studied. This dissertation adds additional evidence that anthropogenic activities are indeed affecting riverine systems. There is far more variability in the relationship between population density and nitrate concentrations or loads in medium-sized watersheds as compared to large rivers, which have been the subject of most previous studies. This suggests that other watershed characteristics can come into play when considering the controls of N in medium-sized watersheds.

This dissertation suggests that the controls of nitrogen export from watersheds can vary from region to region. Previous studies have shown that watershed nitrogen export is controlled primarily by human N inputs to the watershed surface (Howarth et al. 1996). This was the case on the east coast of the United States, although the precise nature of the relationship differed between the northeast and the southeast regions (Boyer et al. 2002, Schaefer and Alber 2007). On the U.S. west coast, nitrate export was primarily related to streamflow rather than anthropogenic nitrogen inputs, although when added to the relationship inputs held some additional explanatory power (Chapter 2). When only the Altamaha River watershed was considered, in-stream N was very strongly related to population density (Chapter 3). On a global scale (Chapter 4), population density is the best predictor of in-stream N in medium-sized rivers; however, the relationship contains a great deal of scatter and can be improved by the inclusion of additional factors such as fertilizer and manure use. These results suggest that the dynamics of which inputs to a

watershed reach the stream are complex and not uniform, particularly when considered at a global scale.

This work raises several avenues for future study. In this study, the blackwater tributaries to the Altamaha River displayed somewhat different chemical characteristics from the other rivers. In particular, the isotopic composition of nitrate in the Little Ocmulgee River was dramatically different from other sampling stations. Nitrate isotopes in blackwater rivers as a class have not been investigated, and this would be an interesting question to pursue further. Unlike in previous work, this study also found that background nitrate appeared to be of atmospheric origin, rather than associated with increased human activity. This could be explored further with broader-scale investigations of nitrate isotopes in undisturbed watersheds. Finally, the lack of information on in-stream nitrate concentrations in medium-sized watersheds (I only found data for 271 out of 4,024 watersheds queried) suggests that these could be further studied, particularly given the potential for increased variability in nitrogen processing in smaller watersheds. Although Europe and North America were well represented in this compilation, there was less data available for other continents. Given the potential effects of climate change, polar regions in particular should not be neglected in such work.

REFERENCES

Boyer, E.W., C.L. Goodale, N.A. Jaworski, and R.W. Howarth. 2002. Anthropogenic nitrogen sources and relationships to riverine nitrogen export in the northeastern U.S.A. *Biogeochemistry* 57/58: 137–169

Howarth, R. W., G. Billen, D. Swaney, A. Townsend, N. Jaworski, K. Lajtha, J. A. Downing, R. Elmgren, N. Caraco, T. Jordan, F. Berendse, J. Freney, V. Kudeyarov, P. Murdoch, and Z. L. Zhu. 1996. Regional nitrogen budgets and riverine N&P fluxes for the drainages to the North Atlantic Ocean: Natural and human influences. *Biogeochemistry* 35: 75-139.

Schaefer, S.C., and M. Alber. 2007. Temperature controls a latitudinal gradient in the proportion of watershed nitrogen exported to coastal ecosystems. *Biogeochemistry* 85: 333-246.Seitzinger, S.P., R.V. Styles, E.W. Boyer, R.B. Alexander, G. Billen, R.W. Howarth, B. Mayer, and N. van Breemen. 2002. Nitrogen retention in rivers: model development and application to watersheds in the northeastern U.S.A. *Biogeochemistry* 57/58: 199-237.

Vitousek, P.M., J.D. Aber, R.W. Howarth, G.E. Likens, P.A. Matson, D.W. Schindler, W.H. Schlesinger, and D.G. Tilman. 1997. Technical Report: Human alteration of the global nitrogen cycle: Sources and consequences. *Ecological Applications* 7(3): 737-750.

APPENDIX A

NUTRIENT BUDGETER: A MATLAB-BASED PROGRAM TO SIMPLIFY THE CALCULATION OF WATERSHED NITROGEN AND PHOSPHORUS BUDGETS

ABSTRACT

Identifying the sources of excess nitrogen (N) and phosphorus (P) entering the landscape is the first step in controlling down-stream eutrophication of coastal waters. Nutrient budgets that sum total inputs to a watershed are extremely useful in this regard, but can be time-consuming and complicated to construct. Data from the U.S. Census of Agriculture present a particular challenge, as information is frequently withheld and the missing values must be estimated before these data can be used. We have developed a MATLAB-based program (Nutrient Budgeter) designed to simplify the calculation of nitrogen and phosphorus inputs to watersheds.

INTRODUCTION

Having an understanding of nitrogen and phosphorus inputs to watersheds is an important tool in managing coastal eutrophication. Budgets based on the methodology used in the SCOPE Nitrogen Project (e.g. Boyer et al. 2002, Schaefer and Alber 2007) consider four types of watershed nitrogen inputs (atmospheric deposition, fertilizer use, net food and feed import, and nitrogen fixation) and two types of phosphorus inputs (fertilizer use and net food and feed import). However, performing these calculations can be challenging and time-consuming. In particular, data from the U.S. Department of

Agriculture's Census of Agriculture are difficult to handle because values are sometimes withheld to protect the privacy of farmers.

This program was designed to simplify the construction of nutrient input budgets for watersheds (or other geographical areas of interest), and to automate the processing of Agricultural Census data. Although the program was developed for data sources available in the U.S., it is flexibly designed and could also accommodate other data types.

SOFTWARE DESIGN AND INPUT REQUIREMENTS

The program features a graphical user interface that walks the user through the process of calculating N and/or P inputs to a watershed (Figure 1). It is based on county-level data, and the user must provide information on the fractions of each county's area located inside the watershed as well as total watershed area. (These are relatively easy to calculate in a GIS.) Input data are read from Microsoft Excel spreadsheets and/or comma-delimited text files. There are currently 6 modules, which can be run independently.

- Population module: Requires per-capita N and P consumption rates (program provides default option). Module output: watershed population and human N and P consumption.
- Livestock module: Requires per-capita consumption, excretion, and manure N volatilization rates for each type of livestock (suggested values provided), and fraction of total N volatilization to be considered a long-range transport (default option provided). Module output: livestock consumption, excretion, and N volatilization from manure.

- Crop module: Requires N and P conversion rates (suggested values provided); N fixation rate (suggested values provided); whether the crop is a forage/silage crop (non-forage/silage crop harvests are assumed to spoil at a rate of 10%); whether the crop is a non-food crop (considered an export). Missing values are estimated by running a linear regression between area and harvested quantities for counties in each state. Module output: crop production, non-food crop production, and biological N fixation by crops.
- Fertilizer module: calculates watershed fertilizer use and N volatilization from county-level fertilizer use data (Alexander and Smith 1990, Battaglin and Goolsby 1994, Ruddy et al. 2006, or derived from data available from The Fertilizer Institute). Requires volatilization rate for each type of N fertilizer (suggested values provided), fraction of N volatilization to be considered an export (default option provided). Module output: fertilizer N and P use and N volatilization.
- Forest module: calculates forest N fixation from county-level areas of forest types (available from the U.S. Forest Service Forest Inventory program) and per-area forest N fixation rates. Requires per-unit area N fixation rate and the fraction of area to which it is to be applied. Module output: biological N fixation in forest lands.
- Atmospheric deposition module: calculates atmospheric N deposition from county-level atmospheric N deposition values (Ruddy et al. 2006). Module output: watershed atmospheric N deposition.

OUTPUTS

Per-unit-area watershed nutrient inputs (or exports) are calculated by multiplying county-level data by the fraction of each county located within the watershed, multiplying by any applicable rates, summing all the counties, and dividing by the watershed area. A set of bar graphs are produced that depict inputs of each type for each watershed, and output is written to Excel and comma-delimited files.

CONCLUSIONS

This program automates the tedious and potentially error-prone process of performing the calculations necessary to create a nutrient budget. The graphical interface further aids users who may not be not well-versed in MATLAB. The different modules of the program can be run independently, so that even if a complete nutrient budget is not of interest specific components could easily be calculated. Although this program is geared specifically towards N and P, it could be easily adapted to allow for calculation of other inputs (e.g. pollutants) to a watershed.

ACKNOWLEDGMENTS

We thank Adrian Burd and Joan Sheldon for assistance with MATLAB programming. Support was provided by the GCE-LTER (NSF Award OCE-0620959).

REFERENCES

Alexander, R.B., Smith, R.A., 1990. County-level estimates of nitrogen and phosphorus fertilizer use in the United States, 1945 to 1985, USGS Open-File Report 90-130. Available online at http://pubs.usgs.gov/of/1990/ofr90130/

Battaglin, W.A., Goolsby, D.A., 1994. Spatial data in geographic information system format on agricultural chemical use, land use, and cropping practices in the United States. USGS Water Resources Investigations Report 94-4176. Available online at http://pubs.er.usgs.gov/usgspubs/wri/wri944176

Boyer, E.W., Goodale, C.L., Jaworski, N.A., Howarth, R.W., 2002. Anthropogenic nitrogen sources and relationships to riverine nitrogen export in the northeastern USA. Biogeochemistry 57, 137-169.

Howarth, R.W., Billen, G., Swaney, D., Townsend, A., Jaworski, N., Lajtha, K., Downing, J.A., Elmgren, R., Caraco, N., Jordan, T., Berendse, F., Freney, J., Kudeyarov, V., Murdoch, P., Zhao-Liang, Z., 1996. Regional nitrogen budgets and riverine N & P fluxes for the drainages to the North Atlantic Ocean: natural and human influences. Biogeochemistry 35, 75-139.

Ruddy, B.C., Lorenz, D.L., Mueller, D.K., 2006. County-level estimates of nutrient inputs to the land surface of the conterminous United States, 1982-2001. USGS Scientific Investigations Report 2006-5012, Reston, VA. Available online at http://pubs.er.usgs.gov/usgspubs/sir/sir20065012

Schaefer, S.C., Alber, M., 2007. Temporal and spatial trends in nitrogen and phosphorus inputs to the watershed of the Altamaha River, Georgia, USA. Biogeochemistry 86, 231-249.

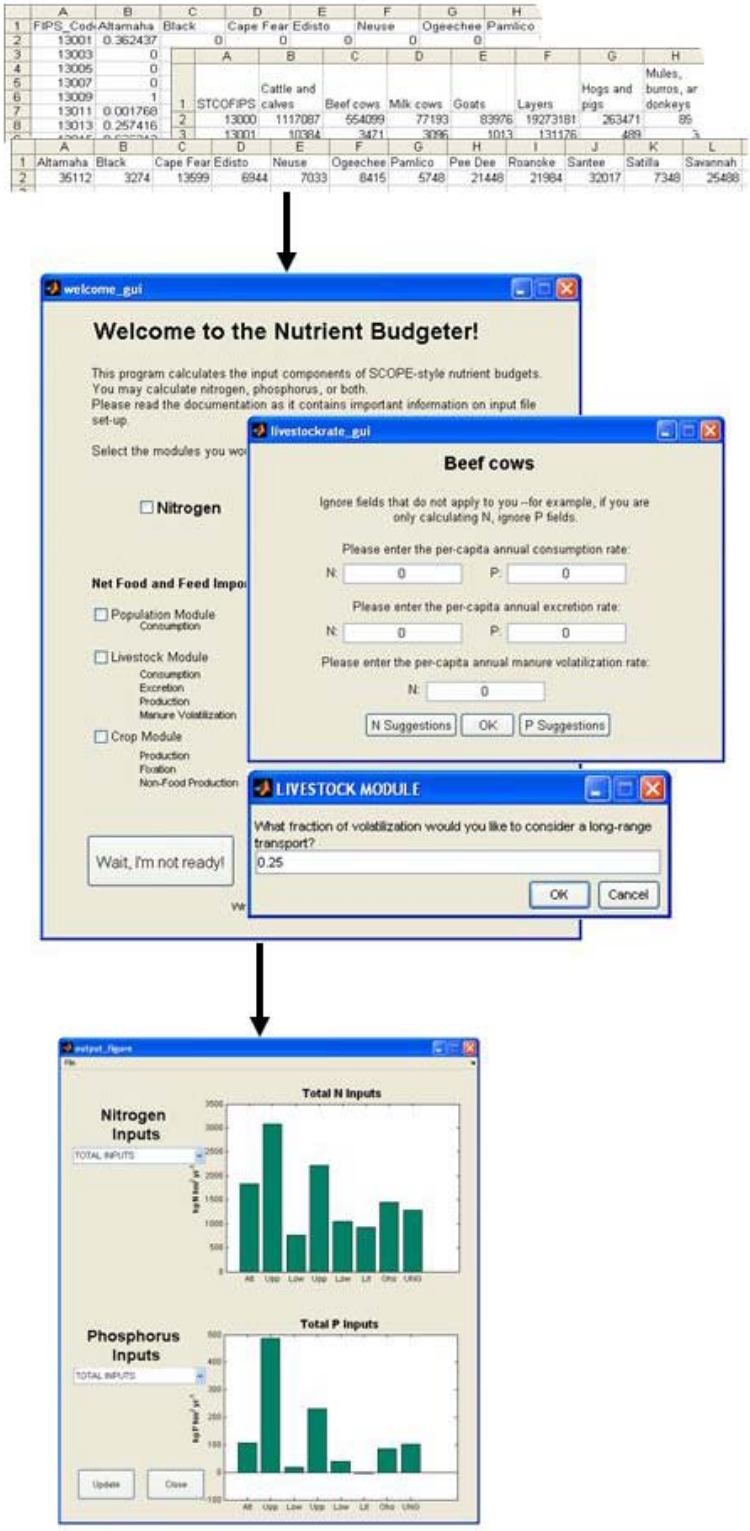


Figure A1. Structure of the Nutrient Budgeter program. The first section lists input data, the second section shows modules and the components each module calculates, and the final section shows the output figure generated by the program.