PROJECT DESCRIPTION

I. BACKGROUND

A. Nitrate uptake and retention in streams

Human activities are flooding the biosphere with fixed nitrogen (N), changing the chemistry of the atmosphere, soils, and waters by adding biologically available N, primarily from combustion of fossil fuels, agricultural fertilizers and legume cultivation (Vitousek et al. 1997). We are now matching each gram of naturally fixed N with a gram of biologically available N generated by human activities. Anthropogenic N pollution is especially pronounced in urban areas, where deliberate (legume fixation, food and fuel import) and inadvertent (e.g., conversion of N_2 to NO_3 during fossil fuel combustion) anthropogenic sources may account for >90% of N inputs (Grimm et al. 2001, Baker et al. in revision). Whatever its source, this N addition should lead to a greening of the biosphere as N is a major building block of life on Earth and is frequently a limiting factor for plant production in both terrestrial and aquatic environments. However, this anthropogenic N is distributed in amounts and locations where it is not merely stimulating primary production but actually disrupting the balance of life in a variety of ecosystems. Forests in the US, Europe, and China are in decline as trees die in response to the syndrome called N saturation (Aber et al. 1998). Landscapes that historically assimilated and retained most available N are now leaking substantial amounts to surface and groundwaters. The fate of much of the anthropogenic N load to the biosphere is uncertain because mass balance studies for major watersheds of the nation show that most (on the order of 60 to 80%) of the N added to the catchments is not exported to the oceans but is missing, having disappeared from our accounting somewhere within these watersheds (Howarth et al. 1996). Even so, the residual 20-40% produces a N loading to estuaries and coastal oceans that is unacceptable when we have large areas of oxygen depletion in places such as Chesapeake Bay and the Mississippi Plume (Kemp, et al. 1992, Turner and Rabalais 1994). To judge the full impact of humans on the biosphere we must discover the fate of this vast quantity of unaccounted N that is stored or processed within watersheds. We have to understand this aspect of the N cycle if we are to successfully manage and protect sensitive ecosystems and ecosystem services, such as drinking water supply and coastal marine fisheries.

The several possible fates of the missing N include plant uptake, immobilization and denitrification in soils, loss during passage through wetlands and riparian zones, and uptake and denitrification in streams, lakes, and large rivers. We propose an intensive isotopic tracer study of the fate of N in stream networks. Most of the inorganic N transported by streams and rivers is in the form of nitrate (NO_3) and traditionally NO_3 has been thought to flow, for the most part, freely downstream to lakes and coastal ecosystems once it enters streams. Recently this view has been vigorously challenged because mass balance analyses for the Mississippi River drainage have shown that large quantities of total N and NO_3 are evidently lost as water travels through its tributary streams and rivers (Alexander et al. 2000). Loss rates within stream channels were as high as 50% per day in streams of 50-cm depth but declined rapidly to only 0.5 % per day in rivers several meters in depth. Thus, N traveled readily to the mouth of the Mississippi if it reached a large river channel but very little N was delivered from sites drained by series of smaller channels. This synthesis of many years of water quality monitoring shows quite clearly that stream channel networks themselves are major sinks for anthropogenic N, although the mechanisms of N loss are not known.

The results of the Alexander et al. study are of great practical significance because they suggest a strategy for controlling coastal zone pollution - routing N through a series of small streams and rivers where maximal N loss will occur. This conclusion is consistent with results from our recently completed study of ammonium dynamics in streams throughout North America (the Lotic Inter-site Nitrogen Experiment, LINX; Peterson et al., in review). This inter-site ^{15}N-tracer study demonstrated experimentally that smaller streams are most retentive of ammonium, with shortest ammonium uptake distances (i.e., greatest retention efficiencies) in the smallest streams and uptake distances increasing

logarithmically with stream discharge and depth. Experimental tracer studies have an advantage over mass balance studies because the rates of individual processes such as nitrification, nitrate uptake and denitrification can be measured in-situ. In the LINX study the focus was on ammonium uptake and transformation and food web transfers of N in pristine streams. In this proposal we focus on the fate of stream water NO_3 by directly adding a $^{15}NO_3$ tracer to the stream channel. The power of this approach is that it facilitates a specific analysis of the assimilation and denitrification processes that control the fate of NO_3 in streams. In this proposal we also expand our focus to consider streams in human-altered landscapes (agriculture, urban).

We propose to evaluate the factors controlling NO_3 uptake and retention in relatively pristine streams as well as in streams that carry elevated NO_3 as a result of their agricultural or urban settings using ^{15}N-tracer experiments. At each of eight sites distributed in diverse biomes throughout the U.S., we will trace the fate of NO_3 in nine streams - 3 reference, 3 agricultural and 3 urban - for a total across all sites of 72 streams. Our experience suggests that small streams with high surface to volume ratios will be most retentive of NO_3. We expect that the impact of humans on the structure of stream channels will affect the uptake efficiency of streams, often decreasing uptake as streams are channelized. Elevated NO_3 concentrations will affect the rates of both NO_3 assimilation and denitrification in streams, but which one will be affected most? Will the high NO_3 levels stimulate the biota to be more retentive or will the biotic retention processes be overwhelmed? Will carbon limitation of denitrification occur in NO_3-enriched systems? We will answer these questions and use the new information to develop a process-based model of N retention in stream reaches. Finally, we will extend our results to large spatial scales by using our stream model with watershed-scale information on hydrography and land use to predict N retention in large river basins at each of our eight study sites, and will test these predictions with synoptic field measurements.

Our proposed research is broadly integrative in several important respects. It integrates across a large range of spatial scales, from the micro-scale (microelectrode surveys of anoxia and sediment denitrification enzyme assays) to stream reaches (assimilatory NO_3 uptake and denitrification rates) to landscapes in diverse biomes (nitrate retention throughout the fluvial systems of 5^{th} or 6^{th} order river basins). It also integrates across land use categories ranging from relatively undisturbed lands with native vegetation to those dominated by human activities. Finally, our research will integrate experimental results using a relatively new experimental technique (^{15}N tracer additions) with modeling at the stream reach and landscape scales.

B. Conceptual Model of N dynamics in streams

Our conceptual model of nitrate processing in the stream network of watersheds is based upon process-based understanding of NO_3 dynamics at the stream reach (km) scale (Fig. 1) combined with analysis of land-use and stream network geometry at the landscape scale (Fig. 2). There are numerous data sets on the statistical relationships between land-use and nitrate loading to streams. What is new in this proposal is the linkage between the watershed level data on land-use and nutrient loading with the field studies of NO_3 uptake and retention in streams in relatively undisturbed, agricultural and urban catchments in 8 regions distributed throughout the U.S.

Our reach model (Fig. 1) is derived from our prior research on the LINX project (see Results from Prior NSF Support) but emphasizes the fate of NO_3 rather than NH_4. The stream channel segment (or reach) receives NO_3 inputs from upstream and from lateral seepage plus any tributary inputs. Nitrate concentration within the reach is maintained by the dynamic equilibrium between these upstream and lateral inputs plus nitrate produced from in-situ nitrification balanced against NO_3 losses due to assimilation by biota, denitrification to gaseous N and export downstream. In the field tracer studies, $^{15}NO_3$ will be added to measure *in-situ* the rates of nitrification, nitrate assimilation and denitrification. The net retention of NO_3 within the reach is calculated from the mass balance of NO_3 and $^{15}NO_3$ within the stream reach. The specific fate of nitrate retained is determined directly by measuring both the assimilatory uptake of NO_3 on the stream bottom and the denitrification rate. These mass balances will be

determined for reaches of 72 streams with different NO_3 concentrations and in catchments with different land uses distributed in 8 regions across the U.S. These data will be synthesized for use in calibration of both empirical mass balance models and simulation models of NO_3 dynamics in stream reaches and at the large watershed scale.

The landscape-scale model of nitrate processing in stream channels draws on information from the stream reach model developed from results of our field experiments and from catchment land use and hydrography data organized by a geographic information system (GIS) (Fig. 2). N inputs to stream channels are calculated based upon land use and riparian characteristics of the subcatchment contributing water and solutes to each stream or river segment. The stream reach model first computes NO_3 retention and export downstream for the most upstream reach. This exported NO_3 along with additional seepage and tributary NO_3 loading is input to the next downstream reach and so on. Thus, the landscape-level model computations are performed sequentially from the primary (1st order) streams to higher order reaches for each of the contributing subcatchments downstream until the mouth of the catchment of interest is reached. The data stored from the simulation will provide a GIS-based map of NO_3 loading, NO_3 assimilation, denitrification and export from each stream or river reach. The summary statistics will be the location and magnitude of nitrate sinks plus total NO_3 export as a percent of inputs at each node in the stream network scaled up to a 5th to 6th order river basin.

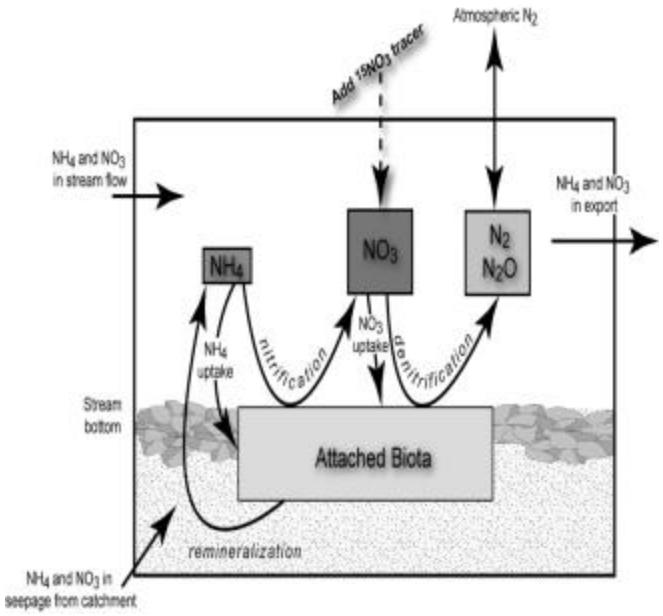


Figure 1. Stream reach-scale model of nitrate dynamics.

Figure 2. Landscape-scale conceptual model (large watersheds).

The LINX project data set has allowed us to obtain preliminary estimates of NO_3 retention and transformation in pristine headwater streams. We have found that in headwater streams throughout North America less than 50% of the nitrate entering the stream will remain in the stream water for 1 km of travel downstream (Peterson et al. in review). This proposal extends this analysis in two ways by 1) measuring denitrification directly *in-situ* and 2) focussing on land-use factors that perturb the N cycle. These additions are the critical ones for developing our understanding of stream channel N cycling and for the practical management application of these N cycle simulation models.

C. Significance of research

We have learned a great deal from several decades of research on various aspects of N dynamics in stream channels, but most of that research has been carried out in piecemeal fashion and a comprehensive synthesis is still unavailable. For example, we know that denitrification occurs at relatively high rates in riparian wetlands (reviewed by Hill 1996) and in many kinds of stream sediments (Christensen et al. 1990, Triska et al. 1993, Pinay et al. 1994, Hill et al. 1998), but we have little idea of the relative importance of these two environments in regulating the export of N from watersheds. Most research has dealt with the riparian wetlands as key points of N interception, leaving the impression that most stream water originates in riparian wetlands and that the recipient stream channels are simply conduits for N. In fact, substantial amounts of groundwater discharge to streams can bypass riparian wetlands. Furthermore, our previous LINX project results showed how dynamic inorganic N can be in stream channels under the simultaneous influences of production and consumption processes (Peterson et al., in review). The N mass balance study of Alexander et al. (2000) for the Mississippi basin suggests that this dynamic behavior extends to larger stream channels as well.

Collectively, our LINX studies cited throughout this proposal have perhaps come closest to attaining an integrated understanding of within-channel N cycling processes, but they focused on ammonium dynamics in relatively undisturbed stream channels. The proposed study will yield a much more comprehensive picture of nitrate dynamics in streams, and thus will advance our conceptual and theoretical understanding of N cycling in streams. Furthermore, and in contrast to the LINX studies and much of the other published research on streams, the proposed study emphasizes N dynamics in disturbed streams, with consideration of the effects of stream channel modifications and N enrichment resulting from urbanization and agriculture. Such disturbances are ubiquitous in North America and impact the majority of streams found in urban and agricultural landscapes. Understanding the roles of disturbance in controlling N cycling and transport is fundamental to the management and restoration of stream ecosystems. Finally, in taking a more integrative approach that spans broad spatial scales and disciplines, we will provide the most comprehensive and synthetic view of N retention in stream channels to date.

The research outlined in this proposal is particularly timely because it addresses a major water quality issue that has attracted increasing attention in recent years - the increasing incidence of undesirable algal blooms and oxygen depletion in estuaries and nearshore ocean waters (Paerl and Whitall 1999). For example, the growing hypoxic zone in the Gulf of Mexico is believed to result from eutrophication stimulated by enhanced N delivery by the Mississippi River (Turner and Rabalais 1994). The recognition that nitrate loads in the Mississippi may be causing environmental harm offshore has spawned intensive efforts to evaluate sources of N pollution in the river's watershed as well as the factors that regulate N transport through the fluvial system (Goolsby 2000). A recent agreement reached by the Mississippi River/Gulf of Mexico Task Force calls for a 30% reduction in N discharge from the river system to the Gulf by 2015 (Showstack 2000). One potential way to achieve this goal is to restore smaller stream channels to their natural morphology and riparian vegetation, but we do not know the extent and degree of restoration that would be required to significantly reduce N export from watersheds. The present study speaks to precisely this question, with an eye toward how anthropogenic disturbances have affected these N retention processes (and therefore how restoration of natural stream channels might ameliorate the problem). Furthermore, NO_3^- contamination is a significant drinking water problem, causing methemoglobinemia and perhaps acting as a carcinogen (Hartman 1983).

The integrated experimental and modeling design that we propose here represents a novel approach to the study of nitrate dynamics in streams. Whole-stream isotope addition experiments are infrequently performed because of logistical and analytical challenges, as well as high costs. The extensive measurements organized around the isotopic addition experiments that we propose can only be accomplished with the contributions of multidisciplinary team members with diverse scientific training and experience, including isotope geochemistry, nutrient biogeochemistry, microbial ecology, spatial data analysis, and simulation modeling. Our success with the LINX project has demonstrated our capabilities

as a team and we are poised to succeed in the new research proposed here. As in the LINX project, we will provide numerous opportunities for undergraduate student participation and graduate research projects within the proposed project, and we will invite outside researchers to attend our planning and synthesis meetings. Students will have the opportunity to participate in synthesis activities as well as site-specific work, which will provide these individuals with a valuable perspective on how research projects involving multiple sites, disciplines, and institutions are conducted. Female scientists will be well represented on our team, as they were in our previous LINX project (records of attendance at our data synthesis meetings show 10-11 women out of 23-25 participants). The scientific results will be broadly disseminated in the form of peer-reviewed scientific publications and conference presentations as well as outreach efforts, such as presentations to local audiences composed of citizens and resource managers and posting of results on the internet.

II. PROPOSED RESEARCH

A. Objectives and Hypotheses

This multi-site, inter-biome study of nitrate dynamics in streams is designed to achieve four **objectives**:

1. Quantify whole-stream rates and mechanisms for nitrate removal and retention in streams within and across biomes (assimilatory uptake pathways, denitrification).
2. Quantify the effects of two dominant types of human disturbance (urbanization, agriculture) on processes of nitrate removal and retention in streams, and determine whether these effects are consistent across biomes.
3. Develop a biome-independent, stream-reach model of nitrate uptake and retention based on stream physical, chemical, and biological characteristics and riparian and catchment land use information.
4. Determine the role of nitrate retention in headwater streams at the landscape level by evaluating (a) whether nitrate retention in large, mixed land-use river basins can be predicted from retention in small streams using a GIS-based model incorporating hydrography and land use information (i.e., whether nitrate retention in higher-order rivers is low relative to retention in small, headwater streams).

The research we propose will be guided by three primary hypotheses. Our work is founded on the idea that nitrate uptake and retention in streams can be predicted from physical, chemical, and biological characteristics of stream ecosystems. We propose a comprehensive plan of experimental research and predictive model development to identify and quantify the broad-scale controls on stream nitrate retention, which will allow us to test the first of our three **hypotheses**:

H_1: The relative importance of assimilatory uptake of nitrate and denitrification in streams changes within and across biomes due to differences in physical characteristics (geomorphology and hydraulics) and rates of biological activity (primary production and heterotrophic microbial metabolism).

We are concerned with the effects of human disturbance on stream nitrate retention processes. We predict that the nitrate retention capacity of headwater streams is significantly reduced by human disturbances that alter the physical, chemical, and biological structure of these ecosystems. Foremost among the human disturbances to headwater streams are the effects of land conversion to agricultural and urban/suburban uses. We believe that changes in nitrate retention resulting from human disturbances are predictable from knowledge of which physical, chemical, and biological characteristics control nitrate dynamics and how these characteristics are affected by disturbance. This then brings us to the second of our hypotheses guiding the research:

H_2: The fractional retention of nitrate in streams (percent of nitrate in transport) is significantly reduced by human disturbances to the landscape (agriculture, urbanization), and disturbance effects on in-stream nitrate retention can be predicted within and across biomes from the effects of disturbance on key physical, chemical, and biological characteristics.

Finally, we are interested in testing the hypothesis raised by the study of Alexander et al. (2000) that low-order streams and rivers ($\leq 4^{th}$ order) are the critical sinks for N retention in river basins. We believe that it is the smallest streams (1^{st} up to 3^{rd} order) that are the most N-retentive portions of the stream/river network, as stated by our third hypothesis:

H_3: Small, headwater streams are the most important sites within large river basins for retention of nitrate transported from land to water because they have relatively high surface to volume ratios and large organic matter inputs that promote intense biological uptake and transformation of nitrate.

These hypotheses lead to a number of specific testable predictions listed below together with a brief overview of the work designed to test each of them.

B. Predictions and tests

P1: Nitrate uptake rates and the relative importance of assimilatory uptake and denitrification processes can be predicted from rates of ecosystem metabolism, nitrate concentrations, and stream geomorphology and hydraulic characteristics.
Test: Regression analyses will be used to investigate the degree to which nitrate uptake rates, nitrification rates, denitrification rates, and assimilatory uptake:denitrification ratios in reference streams can be predicted across biomes from the following independent variables: whole stream metabolism rates (gross primary production, ecosystem respiration), nitrate concentration (and N limitation status), and several geomorphologcal and hydraulic characteristics (e.g., organic matter accumulations, relative size and water exchange rates of transient storage zones within the stream channel).

P2: Elevated nitrate concentrations resulting from anthropogenic N sources in disturbed landscapes result in *increased rates* of assimilatory nitrate uptake and denitrification relative to undisturbed conditions, but *reduce* the *efficiencies (% retention)* of uptake and denitrification (i.e., increase uptake lengths, reduce transfer velocities) at within-biome and across-biome scales.
Test: Multivariate regression analyses will be used to identify predictive relationships between stream water nitrate concentrations and nitrate uptake and denitrification rates and efficiencies (i.e., nitrate removal as a percent of concentration or flux). In the across-biome analyses we will normalize uptake rates and efficiencies and nitrate concentrations to the reference condition by calculating the fractional changes (dividing values for disturbed streams by the reference stream mean values for each site). This data normalization procedure will allow us to minimize the effect of inter-biome differences in reference stream conditions that could obscure the effects of disturbances related to nitrate concentrations across biomes.

P3: Reductions in the physical complexity of stream channels as a result of human *disturbances reduce rates and efficiencies* of both assimilatory nitrate uptake and denitrification within and across biomes.
Test: Analysis of variance (ANOVA) using land use categories, and multivariate regression analyses using GIS-based land use data for riparian corridors and entire catchments will be employed to determine the effects of disturbance on various geomorphological (e.g., debris accumulations, hydraulic radius, substrate size variability, riparian canopy density) and hydraulic characteristics (e.g., transient storage

zone size and water exchange rates, dispersion coefficients, average water velocities per unit channel gradient). Then, multivariate regression analyses will be employed to determine if the rates and efficiencies of nitrate uptake and denitrification can be predicted from measures shown in the first set of analyses to be most affected by disturbance. In the across-biome analyses values of the dependent (uptake rates and efficiencies) and independent variables (disturbance-related geomorphological and hydraulic characteristics) will be normalized to the reference condition by calculating fractional changes as in the test of P2.

P4: Nitrification rates and the importance of nitrification as an in-stream source of nitrate can be predicted from the concentration of ammonium and the ecosystem demand for N within and across biomes.
Test: Regression analyses will be used to determine whether nitrification rates can be predicted from ammonium concentrations and ecosystem rates of metabolism used as surrogate measures of autotrophic (gross primary production) and heterotrophic (ecosystem respiration) N demand.

P5: The retention of nitrate inputs within large river basins can be predicted from a general model of nitrate uptake rates in small streams scaled up to the landscape level using GIS-based data on hydrography and land use.
Test: We will develop and validate a biome-independent model of stream nitrate dynamics based on the experimental ^{15}N results, apply it to a 5^{th} to 6^{th} order river basin in each biome using land use and hydrography data, and compare the model results with longitudinal measures of NO_3 concentration throughout each large river basin.

C. General Approach

Our research involves two elements: (1) a large set of field ^{15}N-tracer experiments in small streams at multiple sites (diverse biomes) to quantify the mechanisms, rates and controls on stream nitrate dynamics, and (2) extension of our results to the landscape scale by combining reach-scale experimental results with watershed-scale spatial data on hydrography and land use to predict nitrate retention in a 5^{th} or 6^{th} order river basin at each site. The second research element will also include tests of the river basin predictions using synoptic measurements of nitrate throughout each river basin.

Field ^{15}N-tracer experiments in streams. We will perform one major field experiment and an extensive set of measurements in an identical manner in reference and disturbed streams at each of 8 sites representing diverse biomes and physiographic regions of the United States. At each site we will select 3 reference streams that have minimal direct human disturbances and drain catchments with vegetation typical for that biome. The disturbed streams at each site will include 3 streams in watersheds heavily influenced by agriculture of a type typical for the region represented by that site, and 3 streams in watersheds heavily influenced by urban/suburban development typical for that region. Thus, the study will include 9 streams at each of 8 sites, for a total of 72 streams. The field experiments will be spread out over 3 years at each site, with one stream from each land use category (reference, agricultural, urban/suburban) studied each year.

The field experiment will consist of a tracer-level addition of ^{15}N-nitrate released continuously over a 24-hour period. We will measure ^{15}N in water (NO_3 and N gases) along a longitudinal transect several times during the ^{15}N addition to determine whole-stream rates of assimilatory nitrate uptake and denitrification in each stream. We also will measure ^{15}N in all major biomass compartments after the tracer addition to partition assimilatory uptake by mechanism and to determine N turnover and long-term retention rates associated with different biomass compartments. Finally, we will measure a number of physical, chemical, and biological characteristics 1-2 days prior to, during, or 1-2 days after the ^{15}N addition to identify the most important factors controlling N uptake and retention, both within and across biomes. These measurements range from the micro-habitat to landscape scales. They include surveys of microscale anoxic zones and microbial denitrification enzymes, inputs, concentrations, and downstream

fluxes of different forms of N, biomass standing stocks, whole-stream rates of metabolism, N limitation indices, channel morphological and hydraulic characteristics (including transient storage zone characteristics), and watershed and riparian land use information organized in a GIS.

Most of the methods that we propose here have been used successfully in our previous LINX project. In particular, the stream ^{15}N addition technique, measurements of ^{15}N in streamwater ammonium, nitrate and stream biomass compartments, and application of process models to longitudinal ^{15}N data to determine uptake rates and lengths were developed and/or refined for use in streams during LINX. We have built on the experience gained in the previous inter-site study in designing this more comprehensive study of nitrate dynamics in streams.

Data analyses will address three fundamental questions: (1) are nitrate dynamics in agricultural and urban streams significantly different from those in reference (undisturbed) streams, (2) what physical, chemical, and biological factors are most important as controls on nitrate dynamics (in particular, rates of assimilatory uptake, nitrification, and denitrification), and (3) how do human disturbances alter the controls and predictability of nitrate dynamics in streams? We will focus our analyses at two levels: within site (n=9), to identify disturbance effects and controls within particular biomes, and across sites (n=72), to identify disturbance effects and controls across all biomes and thus gain a more fundamental understanding of factors governing nitrate dynamics in streams. Initially, ANOVA will be used to determine effects of land use and, with post hoc comparisons, effects of disturbance type within and across sites. To address questions concerning controls on nitrate dynamics and how disturbance affects those controls, we will apply univariate and multivariate regression and multivariate ordination techniques using data from all streams, irrespective of land use category. These analyses also will be conducted within each site, to determine predictive relationships within biomes, and across sites, to determine broader relationships across biomes. The predictive relationships that we develop for various nitrate uptake and retention mechanisms will be used with our GIS-based land use data to scale up our predictions and simulate the role of streams and land use changes on nitrate flux at the landscape scale.

This study will not address temporal variation in nitrate dynamics in streams because we will conduct only one ^{15}N experiment in each stream. We recognize that nitrate uptake and retention may be highly variable over an annual period. However, seasonality in nitrate uptake in streams is the subject of an ongoing NSF-supported research project (NPARS, M. Valett lead PI; see section G) and we believe that our focus should be on broad spatial patterns and controls of nitrate dynamics. All experiments at each site will be performed during the same season to enable effective within-site comparisons. The period chosen for the experiments at each site will be that during which nitrate uptake is expected to be moderately high. Although experiments at different sites will be conducted at different times of the year, this is of less concern in the across-site comparisons because the seasonality of many factors relating to stream nutrient dynamics (e.g., organic matter inputs) differs across biomes and it would not be possible to choose one season with similar characteristics at all sites.

Scaling up to landscapes: nitrate retention in large river basins. The recent analysis of N flux in the Mississippi basin by Alexander et al. (2000) suggested that nitrate retention in headwater streams accounts for the majority of N removal during transit through the river system (Alexander et al. 2000). This result was derived indirectly by integrating monitoring data from a range of stream sizes into a mass-balance model. We intend to expand on and test this hypothesis by using a simple NO_3 dynamics model to scale our experimental results from study reaches of small streams up to entire stream networks draining 5^{th} or 6^{th} order watersheds (Fig. 3).

In their analysis, Alexander et al. included all streams of approximately 1^{st} to 4^{th} order as headwater streams. We hypothesize that it is the smaller streams in this category (1^{st} to 3^{rd} order) that account for the majority of Alexander's headwater nitrate storage. We further hypothesize that the structural complexity of 4^{th} through 6^{th} order streams is an important determinant of nitrate retention in these larger systems, although at lower rates than in smaller streams. To test these hypotheses, we will develop a GIS-based water and N routing model for application to larger watersheds and apply it to a 5^{th} or 6^{th} order river basin at each of our 8 study sites. Because widely available hydrography data (USGS/EPA National Hydrography Dataset) are compiled at scales too coarse for studying headwater

streams, watershed hydrography within each large basin will be derived from USGS 30m digital elevation models (DEMs) (Tarboton et al 1991). The DEM/hydrography model will then allow delineation of sub-watersheds for each stream segment using ARCINFO algorithms (Djokic and Ye 2000).

The model will simulate N delivery to the stream network, assimilatory NO_3 uptake and denitrification within stream segments, and N and water transport between stream segments. N delivery to stream segments via groundwater will be estimated from sampling streamside wells installed in each of our experimental study reaches, and extrapolated to the rest of the stream network based on adjacent land use determined from cover classes derived from Landsat Thematic Mapper (TM) data (Khan 1993, Vorosmarty et al. 1997). Surface, point-source inputs of N will be estimated from environmental permit records available from state regulatory agencies and other available records.

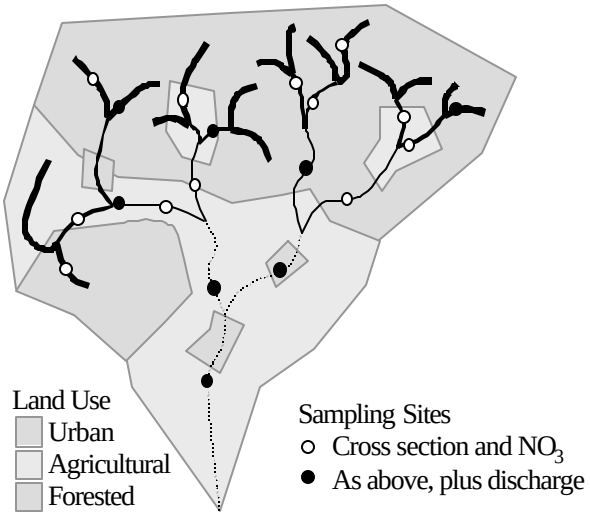


Figure 3. Schematic of a 5th order watershed, associated stream network, and sampling design for channel cross sections, NO_3 concentration, and stream discharge. Thickness of stream denotes hypothesized in-stream NO_3 retention calculated by an associated model (a function of NO_3 concentration, stream discharge, and adjacent land use). As denoted by dotted lines, 4th and greater order streams will be assumed to retain no NO_3 in order to test hypotheses described in the text. NO_3 delivery to the stream channel will be estimated based on adjacent land use.

Throughout headwater streams (1st to 3rd order) in the stream network, NO_3 uptake and denitrification will be modeled as a function of stream discharge, NO_3 concentration, and adjacent land-use type using relationships derived from our field ^{15}N-tracer experiments. The model will assume that NO_3 uptake and retention in the remaining streams (4th order and greater) does not occur. If our extension of Alexander's hypothesis holds true (i.e., 1st to 3rd order streams account for the majority of N retention in stream networks), we would expect this model to overpredict NO_3 concentrations in 4th to 6th order streams, but that the overprediction will be small relative to the predicted retention in the smaller streams. However, we may find substantial overprediction of NO_3 concentrations in 4th to 6th order streams at some of our study sites and only modest or little overprediction in others. This would suggest that NO_3 processing in large streams varies across biomes. If this is the case, we will use regression analyses to test whether stream channel complexity is an important determinant of NO_3 retention/processing in large streams. Indices of channel complexity in 4th order and larger streams will be compiled using measures such as sinuosity, variation in scour-zone width, variation in floodplain width, number of side channels, etc. derived from USGS digital ortho quads (see Gurnell et al. 2000 as an example of this type of approach). Strong correlations between channel complexity indices and residuals from regressions of modeled vs. measured NO_3 concentrations would indicate that human activities that alter channel complexity within the stream corridor contribute to the low N retention observed in large streams.

In order to model surface water flow within the stream network of the large basins, channel cross-sections will be sampled at 20 to 40 locations per basin (Fig. 3). Stream discharge will be sampled at a subset of these cross sections each year to establish area/discharge curves to estimate discharge at any point in the basin. For comparison against model predictions, the pattern of NO_3 concentrations within

each basin will be documented using synoptic sampling of NO_3 concentration at the cross section locations. Specifically, NO_3 concentrations will be measured during each of years 2 to 4 of the study. To the extent practical, the synoptic NO_3 measurements will be timed to correspond with the headwater stream ^{15}N-tracer experiments and during periods when there has not been substantial recent rainfall. Thus, sampling will coincide with the period in which longitudinal processing of N has the greatest influence on N transport and the magnitude of lateral N inputs is minimal.

D. Site Selection

Eight widely separated sites representing different biomes and/or physiographic regions have been selected (Table 1). The general locations of reference and disturbed streams at each site have also been chosen, but final selection of streams will be based on more extensive field investigations and land use data analysis during the first year of the study. We will select small streams (1^{st} to 3^{rd} order), ranging in discharge from about 5 to 50 L/s at baseflow. Streams of this size form the headwaters of drainage networks and the initial terrestrial/aquatic interfaces within most watersheds. Reference streams will have areas of human disturbance comprising < 5% of the watershed, whereas disturbed streams will be selected from watersheds with > 25% of the land use in either agriculture or urban categories.

Table 1. Study sites

Site Location	Biome, Physiographic Province	Reference streams	Agricultural streams	Urbanized streams
Coastal MA	Cool temperate deciduous forest, New England Coastal Plain	Parker River watershed (Plum Island Ecosystem LTER)	Ipswich River watershed (improved pasture)	Ipswich R. watershed (commercial, suburban)
Western NC	Warm temperate deciduous forest, Southern Appalachians	Coweeta LTER	Little Tennessee R. drainage near Coweeta (primarily pasture)	Franklin/Asheville/ Silva, NC areas (commercial, suburban)
Southern MI & Northern IN	Cool temperate deciduous forest, North Central tillplain	Yankee Springs State Recreation Area, Barry State Game Area, Fort Custer Training Center	Near Kellogg LTER and South Bend, IN (intensive row-crop agric., mostly corn and soybean)	Kalamazoo/Battle Creek, MI and/or South Bend, IN areas (suburban, golf course)
Central KS	Grassland, Great Plains	Konza Prairie LTER (1 with grazing bison, 2 without)	Flint Hills near Konza (cattle rotation-grazing)	Manhattan, KS (suburban, golf course)
Western WY	Semi-arid coniferous, Rocky Mountains	Grand Teton Nat'l Pk. & adjoining nat'l forest/BLM lands	Thomas Fork drainage (heavily grazed pasture)	Jackson, WY area (suburban, ski resort areas)
Western OR	Humid coniferous, Cascade Mountains	Tributaries of Clackamas & Tualatin R	Tributaries of Clackamas, Pudding, Mollala R (grass seed, produce agr.)	Portland area (Johnson & Fanno Cks., commercial, suburban)
AZ, NM	Arid grassland, Southwestern Basin and Range	Reference reaches within San Pedro, upper Gila, Mimbres, and lower Salt River basins in AZ and NM	Reaches of San Pedro, upper Gila, Mimbres, lower Salt River basins in agr. areas	Reaches of San Pedro, upper Gila, Mimbres, lower Salt River basins in urban areas

Puerto Rico	Moist evergreen tropical forest, Montane to Coastal Plain	Luquillo LTER (Espiritu Santo and Mameyes basins)	Rio Canovanos basin (pasture and banana plantation)	Rio Piedras basin (commercial, dense suburban)

E. Workplan, methods, and data analysis

^{15}N Experiments. The central experiment performed in all streams is a 24-h addition of ^{15}N-nitate (99 atom% $K^{15}NO_3$) to increase the δ^{15}N of streamwater nitrate to 20,000 per mil. This level of ^{15}N addition will result in an increase in dissolved nitrate concentration of 7.3% in all streams, a relatively small increase and one not expected to have substantial stimulatory effects due to enrichment. Thus, the experiment is a nitrate tracer experiment. The 24-h period for the experiment is sufficiently long to ensure approximately steady state conditions in surface and subsurface water $^{15}NO_3$ pools over several hundred meters of stream length. A conservative tracer (either NaCl or NaBr) will also be added with $^{15}NO_3$ to determine discharge rate and other hydraulic characteristics as well as to provide an indication when hydrologic steady state is achieved (Stream Solute Workshop 1990). The 24-h experiment duration will also allow us to evaluate diel fluctuations in nitrate dynamics resulting from autotrophic uptake processes.

The sampling strategy during and after the ^{15}N addition is based on using the longitudinal variation in tracer ^{15}N in water and biomass compartments to determine whole ecosystem rates of nitrate uptake (removal from water) and partition uptake by process (Mulholland et al. 2000). Samples of all compartments also will be collected immediately prior to the ^{15}N addition to allow correction for background levels of ^{15}N so that only variations in ^{15}N tracer are considered in determining uptake rates. In each stream, approximately 8 stations along a 200- to 500-m longitudinal transect immediately below the ^{15}N addition point and one station upstream (for background ^{15}N) will be established for sample collection

To determine whole-stream rates of nitrate uptake, samples of water for analysis of ^{15}N in nitrate and dissolved N gases (N_2 and N_2O) will be collected at each station at 5 times (prior to ^{15}N addition, 3 times during daylight, and once during darkness). To determine regeneration rates of ^{15}N taken up by biota and entrainment and downstream transport of particulate ^{15}N, we will collect one additional set of longitudinal samples approximately 24 hours after the end of the ^{15}N experiment for analysis of ^{15}N in nitrate, ammonium, dissolved organic N (DON), and suspended particulate organic N (SPON). Samples for ammonium, nitrate, DON, and SPON concentration will be collected from each station during each sampling period to calculate mass flux rates of N from the ^{15}N tracer data. Sample processing and analytical methods are presented in table 2.

To determine uptake rates of nitrate by specific biotic processes involving assimilation of N into living organisms (assimilatory uptake rate), samples of the major biomass compartments will be collected along the longitudinal transect approximately 24 hours after the ^{15}N addition is terminated. Benthic biomass compartments to be sampled include epilithon (biofilms scraped from surfaces of rocks), bryophytes, filamentous algae (if their distribution is patchy and they are not included in epilithon sampling), decomposing leaves, wood, and benthic fine particulate organic matter. Each sample will consist of composited material from five locations within 5 m of each of the 8 longitudinally distributed stations. The longitudinal distributions of ^{15}N in these compartments will be used in a mass balance analysis of the fate of ^{15}N removed from water within the study reach. Samples of these biomass compartments also will be collected 1, 6, and 12 months after the end of the experiment to determine long-term retention of ^{15}N removed from water.

Table 2. Sample processing and analysis.

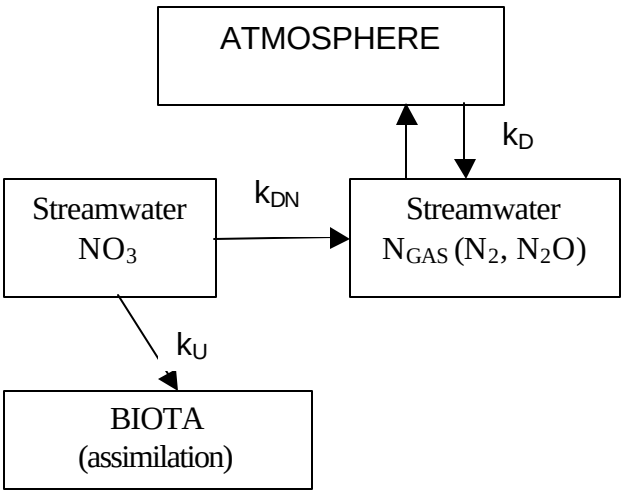
Analysis	Brief description	Reference
^{15}N-nitrate	Removal of ammonium by alkaline diffusion, reduction (Devarda's alloy), headspace diffusion, sorption onto acidified filter, ^{15}N analysis of sorbed N by mass spectrometry	Sorensen and Jensen 1991 Sigman et al. 1997
^{15}N-ammonium	Same as for ^{15}N-nitrate except w/o reduction	Holmes et al. 1998
^{15}N-DON	Initial removal of ammonium and nitrate as above, DON conversion to nitrate by alkaline persulfate digestion, ^{15}N-nitrate processed and analyzed as above.	Sigman et al. 1997 Holmes et al. 2000
^{15}N-SPON	Filtration of water through glass fiber filter, dry, in-line high temperature combustion and analysis of ^{15}N by mass spctrometry	Mulholland et al. 2000
^{15}N-N_2, N_2O	Headspace equilibration of water with helium, liquid N_2 cryotrapping of headspace gas to remove N_2O and other gases, isolation via molecular sieve GC column, and ^{15}N analysis of N_2 by mass spectrometry. N_2O released from cryotraps and separated by GSQ capillary GC followed by ^{15}N analysis by mass spectrometry.	Stevens et al. 1993
^{15}N-benthic biomass	Separation by biomass type, drying, in-line high temperature combustion and analysis of ^{15}N by mass spectrometry.	Mulholland et al. 2000
Streamwater NO_3	Automated Cu-Cd reduction, azo dye colorimetry.	APHA 1995
Streamwater NH_4	Automated phenate colorimetry.	APHA 1995
Streamwater DON	Alkaline persulfate digestion, NO_3 analysis as above.	APHA 1995
Streamwater SPON	Filtration of water through precombusted glass fiber filter, gravimetric analysis of dry mass, % N content by high temperature combustion using Carlo Erba CN analyzer.	APHA 1995
Streamwater N_2O	Headspace equilibration of stream water with helium, headspace gas analysis by gas chromatography (electron capture detector).	Ioffe and Vitenberg 1984 Robertson and Tiedje 1984

Analysis of ^{15}N data. We will compute three measures of nitrate uptake from the longitudinal streamwater $^{15}NO_3$ data: uptake length (Sw), uptake rate (U), and uptake velocity (V_f). Each of these measures provides a different perspective on the importance of in-stream nitrate uptake. Sw (units of m^{-1}) is a measure of how effectively in-stream uptake can alter the downstream flux of a nutrient, and is calculated by taking the inverse of the 1st order rate coefficient for $^{15}NO_3$ decline in water over distance (Newbold et al. 1983, Stream Solute Workshop 1990). U (units of $mg/m^2/s$) is a measure of the mass flux of nutrient removed from the water per unit area and is the most common measure for reporting nutrient uptake. V_f (units of cm/s) is a measure of how rapidly uptake can deplete the nutrients in a given depth of water. Both U and V_f can be calculated from Sw and measurements of stream discharge, the downstream flux of nutrient in water, and average stream width as described in detail by Mulholland et al. (2000).

We will determine nitrate uptake rates by individual biomass compartments using the data on ^{15}N in biomass measured one day after the ^{15}N addition and the ratio of ^{15}N:^{14}N in streamwater nitrate during the ^{15}N addition as described by Mulholland et al. (2000). These biomass compartment uptake rates provide a measure of the relative importance of different biomass types in nitrate uptake. The sum of all biomass compartment uptake rates is equivalent to the total assimilatory nitrate uptake rate.

Nitrification rate is the internal supply of nitrate via oxidation of streamwater ammonium and coupled mineralization/ammonium oxidation of organic forms of N. We will determine total nitrification rate from the rate of isotopic dilution of streamwater $^{15}NO_3$ with distance (decline in $\delta^{15}N$-nitrate), corrected for groundwater inputs of nitrate (this is similar to the approach used to determine gross nitrification rates in forest soils, Stark and Hart 1997). Groundwater inputs of nitrate will be determined from the increase in discharge over the study reach (determined from dilution of the conservative tracer added with ^{15}N) and measured nitrate concentrations and ^{15}N:^{14}N ratios in groundwater samples collected from approximately 8 streamside wells during the experiment.

Denitrification rate will be determined from measurements of ^{15}N-tracer in streamwater N_2 and N_2O pools over distance downstream from the $^{15}NO_3$ addition, and application of a simple two box model to the longitudinal $^{15}N_2$ and $^{15}N_2O$ data. This approach is a modification of the N isotope pairing method developed for use in sediment cores (Nielson 1992, Seitzinger et al. 1993). The model we will fit to the longitudinal ^{15}N data to determine denitrification rate (Fig. 4) is a modification of the model used to determine nitrification rate and nitrate uptake rate in our previous LINX study (Mulholland et al. 2000). The model includes assimilatory uptake (k_U), denitrification (k_{DN}), and atmospheric exchange (k_D). Equations describing the rate of change with distance of ^{15}N-tracer in each reservoir are listed in Fig. 4, as are the steady state solutions for ^{15}N in each reservoir over distance. We then use a Marquardt-Levenbuerg least squares technique to determine values for the two unknowns in the solution (k_{DN} and k_D) that best fit the observed longitudinal profile in ^{15}Ngas for each stream. We will also estimate k_D directly for each stream from propane injections (Marzolf et al. 1994), and values of propane k_D will be converted to Ngas k_D using conversion factors based on differences in aqueous diffusion coefficients for these gases and surface renewal models of gas transfer in turbulent waters (Rathbun et al. 1978, Duran and Hemond 1984, Jahne et al. 1987). Values of k_{DN} will then be determined by refitting the model to the longitudinal ^{15}Ngas profiles and compared to those determined from model fitting with both k_{DN} and k_D as unknowns.



Tracer ^{15}N over distance, x:

$d^{15}NO_3/dx = -(k_{DN}+k_U)^{15}NO_3$

$d^{15}N_{GAS}/dx = k_{DN}{}^{15}NO_3 - k_D{}^{15}N_{GAS}$

Steady state solutions:

$^{15}NO_3 = (^{15}NO_3)_0 * e^{-(kDN+kU)x}$

$^{15}N_{GAS} = [k_D(NO_3)_0/(k_D-k_{DN}-k_U)]$
$* [e^{-(kDN+kU)x} - e^{-kDx}]$

where:

$^{15}NO_3$ and $^{15}N_{GAS}$ are ^{15}N tracer concentrations in each pool

k_U+k_{DN} is the measured rate of $^{15}NO_3$ decline with distance

$(^{15}NO_3)_0 = {}^{15}NO_3$ at x=0

$(^{15}N_{GAS})_0 = 0$

Figure 4. Model of nitrate dynamics that will be fitted to the longitudinal $^{15}NO_3$ data to determine denitrification rate

This approach will provide a whole-stream measure of denitrification rate under ambient nitrate concentrations. Most previous attempts to determine stream denitrification rates have used the acetylene block technique on sediment core samples and suffer from problems associated with reaeration during sampling, incomplete penetration of acetylene into sediments, and inadequate representation of different habitat types (Hill 1983, Christensen et al. 1990, Holmes et al. 1996). For these reasons, most previous studies have determined potential rather than actual denitrification rates. Our proposed ^{15}N-based approach will provide an actual *in situ* measure of denitrification at the whole-stream scale. It is dependent on observing a measurable level of ^{15}N-tracer in streamwater N_2 and N_2O pools. For example (Fig. 5), for a low nitrate stream (case 1) if denitrification is 20% of total nitrate uptake from water, then we should be able to observe the tracer ^{15}N signal in the N_2 pool ($\delta^{15}N$ peak of 0.9 per mil), relative to the precision of the analysis (approximately 0.1 per mil). For a high nitrate stream (case 2), a denitrification rate of 20% of nitrate uptake should produce a peak ^{15}N signal in the N_2 pool of about 5.5 per mil, a value that should be easily detected.

Denitrification will also be estimated from the mass balance of tracer ^{15}N. The difference between measured loss of ^{15}N-nitrate from stream water during the experiment and the sum of ^{15}N recovered from all benthic biomass compartments after the experiment (determined from the tracer ^{15}N in benthic biomass, total benthic biomass, and %N content of biomass measured within 1-2 d after the experiment) will provide an estimate of denitrification rate. This approach will provide an upper estimate of denitrification rate because it is unlikely that we will be unable to account for the tracer ^{15}N in all benthic biomass compartments that take up nitrate from water. Nonetheless, it will serve to constrain denitrification rate in streams where results from the direct ^{15}N-gas method described above are poor.

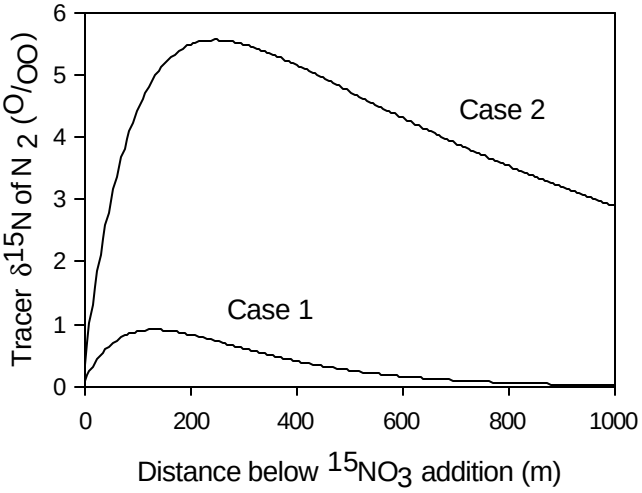


Figure 5. Longitudinal variation in tracer ^{15}N in streamwater N_2 downstream from $^{15}NO_3$ addition for a stream with discharge of 20 L/s, average width of 4 m, average water velocity of 5 m/min and atmosphere-water N_2 exchange rate of 0.05 min^{-1} (values typical for streams based on our LINX work). Case 1 is a relatively low nitrate stream (nitrate concentration of 50 μgN/L) with a relatively short nitrate uptake length (200 m), as expected for many of our reference streams. Case 2 is a relatively high nitrate stream (nitrate concentration of 1 mgN/L) with a much longer nitrate uptake length (1000 m) as expected for many of our disturbed streams. In both cases denitrification rate is assumed to be 20% of total nitrate removal from water.

Additional measurements. In conjunction with the ^{15}N experiments in each stream, we will measure a number of physical, chemical, and biological characteristics that provide information on mechanisms of nitrate uptake, allow us to do a mass balance of tracer ^{15}N, and may serve as predictors in a general, landscape-scale stream model of nitrate uptake and retention. Standing stocks of all important biomass compartments (dry mass per unit area and %N content of coarse and fine benthic organic matter, bryophytes, epilithon, and filamentous algae) will be determined within 2 days of the ^{15}N addition in each stream using conventional sampling methods previously used as part of the LINX project (Mulholland et al. 2000). We will use these data with the longitudinal ^{15}N profiles to calculate tracer ^{15}N incorporated by each biomass compartment (equivalent to assimilatory ^{15}N uptake).

A conservative tracer (Cl or Br) also will be added continuously with the $^{15}NO_3$ in order to determine groundwater input along the study reach (via dilution of the tracer) and channel hydraulic characteristics. The tracer concentration will be monitored at a downstream station and the size and water exchange rates between flowing water and transient storage zones (e.g., interstitial water within sediment and hyporheic zones) will be quantified by applying a stream transient storage zone model (Bencala and Walters 1983, Hart 1995).

Whole-stream rates of metabolism will be determined during the ^{15}N experiments in each stream. Gross primary production and ecosystem respiration will be determined using the upstream-downstream diurnal dissolved oxygen change method with direct measures of air-water oxygen exchange rate using propane injections (Marzolf et al. 1994, Young and Huryn 1998).

Denitrification enzyme activity (DEA) assays will be performed on sediments collected from approximately 10-15 locations in each stream using standard procedures (Teidje 1982,

Knowles 1990). The DEA assays will be used to assess the spatial heterogeneity of denitrification and to provide a comparative measure of potential denitrification activity across sites. DEA assays are increasingly being used to infer the denitrification potential in soils and sediments because DEA reflects recent enzyme synthesis by denitrifiers, and because direct rate measurement by acetylene inhibition has proven problematic (Groffman et al. 1999). Relationships that we will develop between actual denitrification rates (from our ^{15}N experiments) and DEA assays will allow the latter to be used in future stream surveys to estimate denitrification rates across broad spatial scales. All DEA assays will be performed at a central laboratory at the Institute of Ecosystem Studies (Dr. Stuart Findlay) for consistency. Sediments will be shipped overnight on ice and the assays begun within 24 hours of sample collection.

Surveys of sediment dissolved oxygen profiles will be conducted in each stream using O_2 microelectrodes to identify the spatial distribution and variability of anaerobic zones, an important requirement for denitrification (Dodds and Jones 1987, Dodds 1989, 1991, Dodds et al. 1999). We will measure sediment O_2 profiles along five cross-channel transects in each stream. Electrodes will be constructed in Walter Dodds' laboratory for use at all sites (cathode-type electrodes with 20-µm diameter sensing tips embedded in hypodermic needles, Revsbeck and Jorgensen 1986). The results of these surveys will be correlated with DEA rates to help provide a mechanistic model for factors that control denitrification.

Nutrient limitation assays of biofilm communities will be conducted using inorganic and organic nutrient releasing substrata deployed in each stream for 3 weeks (Winterbourn 1990, Tank and Webster 1998, Tank and Winterbourn 1995, 1996). Response of autotrophs (on inorganic substrata) and heterotrophs (on organic substrata) will be determined from analysis of chlorophyll a, bacterial counts, or ergosterol (for fungi) performed at a central laboratory (Univ. of Notre Dame, Dr. Jennifer Tank).

Channel and riparian characteristics that are potentially related to nitrate uptake and retention will be determined in each stream. Average channel slope will be determined and width, depth, hydraulic radius, and sediment size structure (Wolman 1954) will be measured along 10 transects in each stream to assess both mean and variation. Large woody debris accumulations will be mapped and volumes estimated per unit stream length (Wallace and Benke 1984). Riparian canopy cover along each study reach will be estimated using hemispherical canopy photography (Davies-Colley and Payne 1998). These photos will be digitized and analyzed at a central location (to minimize analytical variation) using GLA software (Frazer et al. 2000). These data will be used to estimate daily photon flux density to the stream reach. In addition, we will measure photon flux in an open area near the stream during the metabolism measurements to correct for cloud cover.

Land use data at two spatial scales will be acquired for each stream. First, we will determine the proportions of various land uses within the watershed drained by each stream using available GIS databases or generated from existing aerial photographs of each site. Second, riparian land use (within 25m of the channel) in the experimental reach of each stream and for about 1 km upstream will be determined by manually mapping the near-stream environment, because we expect that existing land-use classifications will not have adequate spatial resolution. We will use consistent land-use categories to facilitate inter-site comparisons.

F. Inter-site data synthesis

Inter-site synthesis of data is a critical component of the project. This synthesis will seek to identify the dominant mechanisms and best predictors of nitrate uptake and retention rates across biomes. This synthesis also will seek to determine how individual controls (predictors) may differ when compared across biomes. Finally, inter-site synthesis at the landscape scale will focus on comparing the predictions of nitrate retention in large river basins derived from the model of nitrate retention in small streams. Thus, the inter-site synthesis work will build on the

data synthesis at each individual site, which should be nearing completion near the end of the final year of experimental work (year 4).

We will hold a series of three workshops in the final year of the project (year 5) for the inter-site data analysis and synthesis. The first workshop will be devoted to presentation of each site's most important findings on the mechanisms and controls on nitrate uptake, and discussion of similarities and differences. Specific tasks to identify inter-site controls and predictors will be developed and small groups assigned to each task. In addition, at this workshop the first round of results from the large river basin analyses will be presented. The second workshop will be devoted to presentation of results from the specific inter-site synthesis tasks and to develop a set of common predictors for nitrate uptake and retention across biomes. Additional inter-site synthesis tasks will be developed and new groups formed to address them. The second iteration of the large river basin simulations will be presented as well. The third workshop will be focused on finalizing the inter-site synthesis activities and evaluating the tests of the large river basin analyses using the synoptic nitrate measurements.

We recognize that we will not complete all synthesis activities in the fifth year of the study. Additional insights will undoubtedly arise as synthesis topics are discussed and papers written over the years following the formal end of the project. All PI's will make a concerted effort to attend one common national scientific meeting each year (annual meeting of either the North American Benthological Society or the Ecological Society of America) and hold a 1-day workshop to continue the project synthesis activities after the formal end of the project. This approach was successful in the LINX project, with all sites having several representatives at all synthesis meetings.

G. Relationships to ongoing research

Many of the sites used in the proposed research have substantial ongoing research, particularly the LTER sites (e.g., Coweeta Hydrologic Laboratory, Luquillo, Plum Island, Konza Prairie, Kellogg Biological Station). The proposed research will benefit from and be a benefit to those efforts. In addition, our proposed study relates very closely to another ongoing NSF-funded project entitled "Nitrate retention in headwater streams: influences of riparian vegetation, metabolism, and subsurface processes" (NPARS, DEB-98-15868, H. M. Valett, lead PI). Valett's study is examining controls on nitrate uptake and retention using ^{15}N-nitrate addition experiments in two small streams at each of three sites (Coweeta Hydrologic Laboratory, Oak Ridge National Laboratory, and the mountains of northern New Mexico). The ^{15}N experiments are being performed during different seasons and in paired streams with contrasting surface/subsurface water exchange patterns at each site. Valett's study differs from the study proposed here in its much more limited extent (2 of the 3 sites are in the same biome) and the inclusion of only relatively pristine streams. The research we propose here will benefit greatly from Valett's study by using many of the methods they have developed or refined (e.g., processing and analysis of ^{15}N-nitrate and ^{15}N-N_2 samples), and possibly co-locating 1 or 2 of our undisturbed streams (Coweeta and/or New Mexico). The proposed study will go well beyond Valett's study by focusing on streams across many diverse biomes, by developing a more robust, predictive model of nitrate dynamics that includes the effects of disturbance from land use changes, and by extending the results to large spatial scales via the river basin analysis of nitrate retention in each biome.

III. MANAGEMENT STRATEGY

We recognize that significant management problems may arise in multi-site, multi-investigator research projects. These include issues such as coordination of logistical support, establishment of clear policies for publication and authorship, and maintaining uniform experimental methods across numerous study sites operated by different groups of scientists. We have successfully addressed these and other issues in our previous collaborative project (LINX), and will build on this experience here. The proposed project management is designed to ensure consistency of all site work and facilitate inter-site synthesis and modeling (Table 3).

Table 3. Project responsibilities and personnel.

Task	**Individual**	**Institution**
Project Leader	Patrick Mulholland	Univ. of Tennessee
Project Steering Committee	Patrick Mulholland	Univ. of Tennessee
	Bruce Peterson	MBL, Woods Hole
	Steve Hamilton	Michigan State Univ.
	Jennifer Tank	Notre Dame Univ.
	Robert Hall	Univ. of Wyoming
Central Activity Leaders:		
$^{15}N_{GAS}$ analysis	Lee Cooper	Univ. of Tennessee
^{15}N-DON analysis	Bruce Peterson	MBL, Woods Hole
River basin modeling	Judy Meyer, Geoffrey Poole	Univ. of Georgia
DEA assays	Stuart Findlay	Institute for Ecosystem Studies
Anoxic microzone surveys	Walter Dodds	Kansas State Univ.
N limitation assays	Jennifer Tank	Univ. of Notre Dame
Site Leaders:		
Coastal MA	Bruce Peterson	Marine Biological Lab.
Western NC	Jack Webster, Maury Valett Judy Meyer	VPI Univ. of Georgia
Southern MI/ Northern IN	Steve Hamilton Jennifer Tank	Michigan State Univ. Univ. of Notre Dame
Central KA	Walter Dodds	Kansas State Univ.
Western WY	Robert Hall Jennifer Tank	Univ. of Wyoming Univ. of Notre Dame
Southern AZ/ Northern NM	Nancy Grimm Clifford Dahm	Arizona State Univ. Univ. of New Mexico
Western OR	Stanley Gregory Sherri Johnson	Oregon State University Oregon State University
Puerto Rico	William McDowell	Univ. of New Hampshire

Dr. Patrick Mulholland (aquatic ecologist/biogeochemist) will be responsible for overall project leadership and coordination. He served this role in the LINX project. Mulholland and 4 other individuals will form a project steering committee to organize synthesis activities and ensure overall project coordination. This committee will communicate frequently by email and teleconference. Several tasks will be performed centrally to ensure consistency and efficiency and to provide particular expertise. Dr. Lee Cooper (aquatic ecologist with extensive experience

with ^{15}N techniques) will oversee the ^{15}Ngas analysis for the denitrification work. Dr. Bruce Peterson (aquatic ecologist/biogeochemist with experience in ^{15}N analysis of DON) will lead the DO^{15}N analysis work. Dr. Judy Meyer (aquatic ecologist with experience in landscape analyses) will lead the river basin modeling work and supervise a postdoctoral associate (Dr. Geoffrey Poole) who has considerable experience in landscape-level stream ecosystem modeling. Dr. Stuart Findlay (microbial ecologist) will oversee the dentrification enzyme activity task. Dr. Walter Dodds (aquatic ecologist with experience with microelectrode techniques) will lead the anaerobic microzone survey work. Dr. Jennifer Tank (aquatic ecologist with experience with N limitation assays) will lead the N limitation assay work. At each site, a lead PI or co-lead PIs will plan and coordinate the experimental work at that site to ensure that it is carried out on schedule and according to protocols. The site leaders also will ensure that all site data are available for inter-site synthesis efforts and, together with the central task leaders, will comprise the core of the inter-site synthesis group.

The project schedule (Table 4) includes a one year start-up phase, 3 years of field experiments, sample processing, and data compilation, and a final year focused primarily on inter-site synthesis and scaling up of results. Although we have identified the geographic areas from which we will select the study streams at each site, final selection of stream reaches for the experiments will involve additional reconnaissance (e.g., watershed land use, riparian conditions) and preliminary measurements (e.g., NO_3 concentrations, discharge) during year 1. It is critical that we select streams that provide a range of characteristics typical for each site and disturbance category - a task that will involve considerable effort and was beyond our resources available during proposal preparation. Also, we have tentatively identified a 5^{th} or 6^{th} order river basin at each site to be used in the landscape level analysis, but the choice can not be finalized until we are able to more completely evaluate the availability of land use data for each basin during the first year of the study. For the inter-site synthesis, it is imperative that experimental methods and data compilation be identical at all sites and ensuring this will be an important aspect of project management. A 3-day workshop will be held during the first year of the project to ensure that key project personnel understand all field, laboratory, and data handling protocols. This approach of using a common field experiment at all sites followed by an extensive inter-site synthesis of the results is the strength of the project. Our planned schedule will provide the time needed for the organization, experimental work, and synthesis to ensure project success.

Table 4. Project schedule.

Year	**Task**
Year 1	Final selection of streams and experimental reaches Recruit students and postdocs Methods coordination workshop Develop GIS-based land use information (maps, aerial photos TM images) Install streamside wells in experimental reach in each stream ^{15}N purchase
Year 2	^{15}N experiments at each site (1 reference, 1 agriculture, 1 urban stream)
Year 3	^{15}N experiments at each site (1 reference, 1 agriculture, 1 urban stream)
Year 4	^{15}N experiments at each site (1 reference, 1 agriculture, 1 urban stream)
Year 5	Inter-site synthesis of data: All-investigator meetings (3) Develop general stream nitrate model River basin scale-up at each site