Nitrogen Uptake, Retention and Cycling in Stream Ecosystems: An Intersite N-15 Tracer Experiement
A Proposal Submitted to the Ecosystems Studies Progran of NSF (ABRIDGED VERSION)
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II. BACKGROUND, RESEARCH OBJECTIVES AND SIGNIFICANCE A. Nitrogen cycling in streams Nitrogen is an element of considerable interest in lotic ecosystems. It limits productivity in some flowing waters (e. g. Grimm and Fisher 1986), while in others it is present to such excess that legislators have called for a 50% reduction by the year 2000 (Haycock et al. 1993). In the U. S. nitrogen loading has increased 20-fold in the last 50 years, and loading varies by an order of magnitude in different parts of the country (Puckett 1995). Knowledge of factors regulating nitrogen cycling in streams is integral to understanding lotic ecosystem structure and function (Meyer et al. 1988). There is a rich literature of process-level studies in which temporal and spatial variation in rates of key nitrogen transformations have been documented (Stream Solute Workshop 1990). Rates of nitrogen transport in some rivers are well documented (e. g. Triska et al. 1993). However, a good understanding of the controls of nitrogen cycling in lotic ecosystems is lacking. Although the theory of nutrient spiraling in streams is well-developed and is being widely applied (Stream Solute Workshop 1990), it treats the processes responsible for nitrogen uptake and release as a black box. A robust model linking hydrodynamics, nitrogen transformations, and food web dynamics in streams is needed. Because streams serve as key hydrologic and biogeochemical links between uplands and downstream ecosystems, understanding how changes in climate, atmospheric deposition, or land use will affect entire landscapes requires working models of the structure and function of streams. Such models are needed to address important large-scale issues such as water quality, biotic diversity, and coastal eutrophication. The role of lotic ecosystems in the response of the biosphere to anthropogenic change has been largely ignored by the scientific community. In a recent assessment of the sources of water pollution, no consideration was given to in-stream transformations of nitrogen that could alter nitrogen transported by rivers (Puckett 1995). The reason for this is not that in-stream processes are unimportant, but rather that stream ecosystem functions have not been synthesized in robust models that are general and can speak to entire watershed, regional and continental-scale changes (Naiman et al. 1995). Such models are available for oceans and for terrestrial ecosystems but not for the networks of rivers that link land and ocean. Comparisons of streams in different biomes with respect to hydrologic and nitrogen transport processes are important and necessary steps in the development of general

quantitative models of lotic systems. An NSF-sponsored workshop on solute dynamics in streams was held at the Coweeta Hydrologic Laboratory in July 1995. The workshop focused on the use of field tracer addition experiments and stream hydrodynamic and nutrient mass balance models to study nutrient cycling in stream ecosystems. It became clear from the workshop proceedings that the combination of field tracer experiments (conservative and reactive tracers) and ecosystem simulation modeling used in a multisite comparative study would greatly increase our understanding of nutrient dynamics as well as trophic interactions in stream ecosystems. This proposal is an outgrowth of that workshop. We propose to refine and test predictions derived from a model of stream nitrogen dynamics in a wide range of streams that differ in their hydrodynamic (size of transient storage zones), chemical (streamwater N concentrations and N: P ratios, detritus C: N ratios), and metabolic (GPP, P: R ratios) characteristics. In each stream, we will use conservative tracer injections to determine hydrodynamic properties, chemical measurements to determine nutrient concentrations and ratios, whole-stream measures of GPP and respiration to determine metabolic characteristics, and additions of 15N tracers to determine N cycling rates and pathways. Results of these empirical studies will be used to test site-specific model predictions as well as to develop a generalized conceptual model of the factors controlling N cycling in lotic ecosystems.

B. 15N Tracer Experiments The cycle of nitrogen in ecosystems is receiving increasing attention due to the magnitude of the human perturbation of the cycle through fossil fuel combustion, fertilizer production and waste disposal (Galloway et al. 1994). New, more powerful tools are needed for more secure understanding of the uptake, storage and transformation of N in ecosystems. One of the more exciting new approaches is the use of 15N-enriched nitrogen compounds as tracers of nitrogen in whole ecosystem experiments (Fry et al. 1995). 15N enriched fertilizer has long been used in agricultural research to investigate nitrogen use efficiency of crops. Recently, ecologists have begun experimenting with the addition of tracer amounts of enriched N to study how ecosystems process nitrogen. For example, aircraft application of 15N-enriched nitrogen to small catchments in Maine aided the study of the effects of nitric acid deposition (Nadelhoffer et al., in press). The 15N tracer allowed the determination of the allocation of added N to leaves, wood, forest floor, mineral soil and to stream export. Because of the large standing stock of N in the forest, this would have been impossible to measure without the tracer, yet the information is critical to testing the forest nitrogen saturation hypothesis (Aber et al. 1989). Experiments in aquatic systems have shown that 15N tracer addition allows much improved definition of N flow and storage in lakes and streams (Kling 1994, Fry et al. 1995, Peterson et al. in review). Researchers at the Arctic Toolik Lake LTER site performed the first stream experiment on an unfertilized reach of the Kuparuk River. The tracer addition allowed an integrated assessment of ammonium uptake rate and uptake distance in the river over a continuous 6 week addition period. This information is similar to that obtained with the solute injection (slight enrichment) technique but, being a tracer, it requires no detectable increase in nutrient level. The big difference between the two approaches, however, is the additional information that is derived from the 15N tracer study. Nitrification rate can be determined by analysis of 15N in nitrate downstream of the addition site. Uptake of N by primary producers and grazing of primary producer N by insects and fish

can be followed by sequential sampling of the biota. Production of 15N-labeled sestonic and benthic detrital N can be determined and the dynamics (settling, transport, and resuspension) of FPON studied in detail. After 6 weeks of continuous tracer addition to the Kuparuk the addition was stopped. At this point many compartments had reached their maximum 15N content and all compartments contained measurable label. Subsequent sampling measured the rate of 15N loss from each component and also showed surprisingly long N storage (up to 2 years) in certain insect species and the epilithon. The experiment provided sufficient information to test many facets of our nitrogen model of the Kuparuk. Specifically, 15N distributions over space and time were used to estimate ammonium uptake length and uptake rate, transport distance for fine particles, rate of nitrification, rate of N turnover in detritus, algae, mosses, insects and fish, and flow pathways from ammonium to each consumer. The 15N tracer addition experiment with a similar design has been repeated in three other Alaskan streams (Blueberry Creek, Imnavait Creek and the fertilized reach of the Kuparuk River) for the purpose of validating a whole watershed biogeochemical model. Hugh White Creek at the Coweeta LTER site also received 15N tracer in June 1995 as part of an LTER intersite workshop on methods and models for the study of solute dynamics in streams. A major objective of the workshop was to develop the rationale and approach for this intersite proposal to compare N cycling in different stream types. The whole-ecosystem 15N addition experiment is ideal for an interbiome comparison because it provides in situ, simultaneous information on several of the most important N cycle processes in streams and because it can be effectively and safely applied with similar protocol and without significant perturbation to most small or medium-sized streams. With careful attention to experimental design, it should provide a powerful way to test hypotheses about how N cycling varies across stream types.

C. The Stream Nitrogen Model Models can help to synthesize current understanding of N dynamics, allowing investigators to test hypotheses by generating specific predictions based on differences in parameter values among streams. Our model consists of a tracer box model of the stream N cycle. The model computes how 15N introduced into a stream will become distributed among system compartments over time and distance from the point of introduction. We chose a simple model structure to facilitate the initial intersite comparison. This structure consists of 15 compartments within each stream segment (Fig. 1). The downstream flux of nitrogen is represented by inputs of NH4, NO3, DON and PON. Nitrogen spiraling during transport is calculated by linking a series of the stream segment models such that the results of the N cycle transformations in segment 1 are passed to segment 2 and so on. In our intersite comparisons we used a series of 50 segments to represent a stream reach, which should encompass several N spiraling lengths. The model will be expanded beyond this simple structure as necessary to accommodate intersite differences.

Figure 1. Fifteen compartment stream N model used to simulate uptake, storage, andregeneration of 15N tracer. For simplicity, downstream transport of the lower four compartments as well as transfers among compartments are not shown.

The model tracks the flow of total N and tracer 15N according to the nitrogen stocks and fluxes provided by the investigator. The most useful model output is the predicted 15N content (del

15N) of the 15 different compartments over time and distance because these predictions can be directly compared with the field samples (of organisms, dissolved nitrogen, and detritus) which will be analyzed for 15N content (Fig. 2).

Figure 2. Comparisons of model simulations with field data showing spatial distributions of 15N in compartments representing primary uptake pathways and primary consumers in two streams in which 15N additions have been conducted.

There is no stream where we have enough information to achieve good agreement between the model predictions of tracer distributions and the field tracer data on the first try. However, developing the model calibration forces us to document what we know and do not know for each site. Direct comparisons between field data and model predictions help focus attention on areas of uncertainty. Revisions of model structure and changes in fluxes permit exploration of possible reasons for lack of agreement. Finally, the field data and model calculations provide a solid basis for comparing the N cycle in streams with contrasting characteristics.

D. Hypothesis and Objective Results of previous 15NH4 addition experiments in the Kuparuk R., Blueberry Ck., and Imnavait Ck., Alaska, and in Hugh White Ck., North Carolina, and comparison of N model simulations for several other streams during the July 1995 Coweeta Workshop lead us to the central hypothesis for the proposed research:

The considerable variability among streams in uptake, retention, and cycling of nitrogen is controlled by key hydrodynamic, chemical, and metabolic characteristics that determine water retention, degree of N deficiency, and energy flow through food webs in stream ecosystems.

From this hypothesis we derive several specific predictions (presented in section III. C) that will be explicitly tested by the empirical research. Thus, our objective is to examine the N cycle in a variety of streams and identify the role of hydrodynamic, chemical, and metabolic characteristics in controlling the uptake, retention, and transfer of N through the stream food web. These objectives will be met by conducting 15NH4 additions and solute injections to streams of contrasting characteristics across several biomes and comparing results of these experiments across streams and with simulations of the N model calibrated for each stream. E. Significance of Proposed Research Comparisons of streams in different biomes, using the same set of experiments, and analysis of experimental results using a single, standardized mass balance model will allow the development of a generalized model of N cycling in streams. This combined empirical and modeling study will provide the increased understanding necessary to address the effects of large-scale anthropogenic and climate changes on water quality, certain aspects of biotic diversity, and the structure and function of stream ecosystems.

III. PROPOSED RESEARCH A. General Approach We will combine data synthesis (previous 15N experiments), modeling, and comparative empirical studies to gain a better understanding of N cycling and its determinants in stream ecosystems. The stream N model was run for most of the proposed study streams as part of the July 1995 Solute Dynamics

Workshop. Based on these preliminary model runs and our current understanding of stream N cycling, we developed a number of predictions (listed in III. C below) that expand on our central hypothesis. During the early stages of the project, the model will be updated and re-run for each stream to generate site-specific predictions concerning how added 15N will be incorporated into various compartments over space and time. Site-specific and overall predictions will be tested using two types of experiments: (1) short-term (several hours) solute injections (conservative tracer, NH4, NO3, PO4) to define hydrodynamic characteristics (e. g., average velocity, size and water exchange rates of transient storage zones) and to determine nutrient uptake lengths (i. e., inverse of distance-normalized uptake rates, see Newbold et al. 1981) based on the commonly-used enrichment approach (Solute Dynamics Workshop 1990), and (2) longer-term (6 wk) additions of 15NH4 at tracer levels (i. e., negligible increase in NH4 concentrations) to measure N uptake, retention, and cycling rates through the ecosystem under ambient nutrient concentrations. During the 15N experiments, we will also measure streamwater and detrital chemistry, microbial biomass, and whole-stream GPP and respiration rates to define the overall chemical and metabolic characteristics of each stream. Results of the 15N experiments will be compared with the model simulations to identify areas of agreement and disagreement. Results of the 15N experiments across all sites will be analyzed statistically (primarily by regression analysis) to test predictions concerning relationships between N dynamics (as quantified by measures of 15N uptake, transfer, and turnover) and hydrodynamic (relative size of transient storage zones), chemical (streamwater N concentrations and N: P ratios, detritus C: N ratios), and metabolic (GPP, P: R ratios) factors (independent variables in the regression analysis) in streams.

B. Site Selection The study uses an intersite comparative approach by conducting the same set of experiments across a broad range of streams, encompassing large gradients in the hydrodynamic, chemical, and metabolic factors hypothesized to regulate N uptake, retention and cycling in streams. Streams selected for study range in latitude from the tropics to the arctic (Table 1). Together, they provide broad gradients in hydrodynamic characteristics, as defined by discharge and the importance of transient storage zones (quantified as As: A, Bencala and Walters 1983), in potential N deficiency, as defined by streamwater DIN concentrations and N: P ratios and by the C: N ratios and microbial biomass of detritus, and in metabolic status, as defined by gross primary production and P: R ratios. Thus, conducting the 15N addition experiment in this set of streams will provide robust tests of the influence of these potential determinants on N uptake, retention and cycling. Streams were also selected based on previous research; considerable amounts of data on nutrient cycling and food web characteristics are available for all streams selected. This information will be used to construct the preliminary N cycling models for each stream prior to the 15N experiment.

Table 1. Selected study streams and hydrodynamic, chemical, and metabolic characteristics estimated from previous measurements. 15N addition experiments have already been conducted in Hugh White Creek, NC and in several Alaska streams (denoted by *) and the results of these studies will also be used in the intersite comparative analyses.

Season Hydro-dynamics Water Chemistry Detritus Chemistry Metabolism Stream Biome of Study Q As: A DIN N: P C: N GPP P: R Quebrada Bisley, PR Trop/ Forest winter 10 < 0.2 250 220 high < 200 < 0.5

Sycamore Ck., AZ Temp/ Arid spring 10 > 1 33 1.9 low 8000 1.4

Gallina Ck., NM Temp/ Semi-arid autumn 5 > 1 26 9 low 400 0.5

S. Kings Ck., KA Temp/ Prairie spring 5 0.2 20 < 20 low 600 4.9

Ball Ck., NC Temp/ Forest autumn 60 0.4 8 9.5 high < 50 < 0.2

HughWhite Ck., NC* Temp/ Forest summer 10 1.5 12 12 high < 10 < 0.1

Walker Br., TN Temp/ Forest spring 10 0.1 30 20 high 300 0.4

Mack Ck., OR Temp/ Forest summer 70 <0.2 12 2 low 600 1.4

Smith Ck., MI Temp/ Forest summer 40 > 1 550 22 high 100 0.2

Bear Br., NH Temp/ Forest summer 5 < 0.2 420 940 high < 10 < 0.2

Kuparuk R. Trib., AK Tundra summer 25 ? 10 5 high ? >1

Kuparuk R., AK* (control)

Tundra summer 1000 0.2 20 14 high ? >1

Kuparuk R., AK* (P fert.)

Tundra summer 4000 0.2 9 3.5 high ? >1

Blueberry Ck, AK* Tundra summer 300 0.3 9 3.4 high ? >1

Imnavait Ck, AK* Tundra summer 100 ? 8 7.6 high ? >1

Units: Q (L/ s), As: A (m2/ m2), DIN (ugN/ L), N: P (atomic, inorganic), GPP (mgO2/ m2/ d), P: R (atomic). Estimates of detritus C: N ratios are based on importance of algae (e. g., high GPP= low C: N) and dominant type of allochthonous input (e. g., tree leaves= high, peat= high, grasses= low). The low estimate for Lookout Creek reflects the dominance of a riparian N-fixing species (alder).

C. Predictions and Tests Ammonium uptake and retention. Ammonium uptake and retention should be related to geomorphic/ hydraulic characteristics that control solute retention and residence time, and to water chemistry and metabolic characteristics that control the demand for N relative to its supply. Transient storage zones, particularly those produced by surface

water exchange with hyporheic zones, should increase N uptake and retention by increasing water retention and by increasing the surface area available for microbial colonization, thereby increasing microbial N demand. Low streamwater DIN concentrations should result in greater potential for N deficiency and hence greater fractional rates of NH4 uptake (shorter uptake lengths) and tight recycling of assimilated N. Because algae may be more strongly dependent on streamwater nutrients than microbes growing on terrestrial detritus, streams in which algal uptake is an important component of total N uptake (higher GPP and P: R) may retain and recycle streamwater N more efficiently than streams in which algae are not important. Transient storage zones (e. g., hyporheic zones) provide habitat primarily for heterotrophic microbes and their consumers, and their importance in streams is one determinant of the overall importance of heterotrophy as a pathway for nutrient uptake (Findlay 1995). The demand for nutrients by autotrophs should be proportional to primary production rate, and the demand for nutrients by microbial heterotrophs should be related to ecosystem respiration rate and the C: nutrient ratio of allochthonous organic matter inputs. Thus, P: R ratios and the C: N ratios in organic matter inputs should determine the relative demand for N by autotrophs and heterotrophs. Nitrification rates should also be lower with greater N deficiency as competition between nitrifiers and other organisms for available ammonium increases. Therefore we predict the following:

P1: Ammonium uptake lengths determined from the 15N experiment will be shorter and/ or ammonium uptake rates will be greater in streams with higher As: A ratios, in streams with lower streamwater DIN concentrations and N: P ratios, and in streams with higher GPP rates and P: R ratios.

P2: Among streams with low P: R ratios (< 0.5), ammonium uptake lengths will be shorter and/ or ammonium uptake rates greater in streams with higher R and higher detritus C: N ratios than in streams with lower R and lower detritus C: N ratios.

P3: The relative importance of autotrophs (epilithon, filamentous algae, mosses) in total 15NH4 uptake will be greater in streams with lower As: A and higher GPP rates and P: R ratios.

P4: Nitrification rates will be lower in streams with lower DIN concentrations and higher detrital C: N ratios.

P5: Retention time of assimilated N will be longer in streams with higher As: A ratios, lower DIN concentrations, lower N: P ratios, and higher detrital C: N ratios.

P1 will be tested by computing uptake lengths and uptake rates of NH4 for each stream using data from the 15N experiment, and determining relationships between uptake lengths and rates (dependent variables) and As: A ratio, DIN concentration, N: P ratio, GPP rate, and P: R ratio (independent variables) in an intersite regression analysis. Also, bacterial abundance in hyporheic sediments will be measured to determine if there are biological differences that influence N uptake in hyporheic zones. We use both ammonium uptake length and uptake rate

per unit time as dependent variables in this analysis because neither by itself presents a complete picture of the uptake of streamwater N (uptake length being a measure of uptake efficiency and uptake rate a measure of mass uptake, Newbold et al. 1981). Further, because of the strong dependence of uptake length on discharge, large differences in discharge among some of our sites may obscure the effect of other independent variables on uptake length (but not on uptake rate). P2 will be tested by determining relationships between NH4 uptake lengths and rates and ecosystem R and detritus C: N ratios in an intersite regression analysis using results only from sites with P: R ratios < 0.5. P3 will be tested by calculating 15N uptake by epilithon, filamentous algae, and mosses (specific 15N content times total N in pool) as a fraction of total 15N uptake from stream water (calculated from uptake lengths and streamwater NH4 flux) for each stream, and using an intersite regression analysis with As: A ratios, GPP rates, and P: R ratios as independent variables. P4 will be tested by determining nitrification rate from the rate at which 15N appears in the streamwater nitrate pool and the total nitrate pool size in each stream, and comparing across the ranges in streamwater DIN and detrital C: N ratios. P5 will be tested by measuring the rate of 15N loss in streamwater (streamwater 15N flux/ 15N standing stock) and the rate of decline in specific 15N content (15N: 14N ratio, hereafter referred to as del 15N) in the various ecosystem compartments during the first week following termination of 15N addition in all streams. These rates of loss or decline will be analyzed using an intersite regression with the independent variables being those used in tests of P1 and P2. **Food Web Transfer of N.** We believe that the effectiveness with which streamwater N is transferred to consumers in stream food webs is dependent on the pathways of N uptake and the relative N deficiency of the ecosystem. In general, we assume that herbivory is a more efficient mechanism of food web N transfer than is detritivory. N transfer to consumers from detrital pools is inefficient because microbes and meiofauna do not appear to be effectively harvested by macroconsumers in streams (Findlay et al. 1986, Borchardt and Bott 1995). We also assume that autotrophs obtain a greater fraction of their N requirements from the stream water compared with microbial heterotrophs which meet some of their N needs by uptake of detrital N. Thus, we predict that the relative importance of autotrophy and heterotrophy will strongly influence the amount of 15N that is acquired by consumers in each stream (relative to total uptake). Further, we predict that N deficiency will enhance the efficiency of trophic transfer of 15N because consumers are more likely to be N-limited or co-limited by N and C in highly N-deficient streams. Therefore, we predict the following:

P6: The del 15N of herbivores (scrapers) at the end of the addition experiment (integrated over the study segment) will be greater than that of detritivores (shredders, collector/ gatherers) in all streams.

P7: The fraction of the 15N removed from stream water that eventually is acquired by herbivores and detritivores will be greater in streams with lower streamwater DIN concentrations than in streams with higher DIN concentrations.

P8: Among streams with low P: R ratios (< 0.5), the 15N acquired by detritivores will be greater in streams with higher detritus C: N ratios than in streams with lower detritus C:

N ratios.

P9: The fraction of the 15N removed from stream water that eventually is acquired by all consumers will be greater in streams with higher GPP rates and higher P: R ratios than in streams with lower GPP rates and lower P: R ratios.

P6 will be tested by comparing distance-integrated del 15N values for the most common herbivore (scraper) taxa with del 15N values for the most common detritivore (shredder, collector/ gatherer) taxa in each stream at the end of the 15N addition. P7 will be tested by calculating the ratio of herbivore plus detritivore 15N uptake (del 15N times total biomass N in dominant taxa at end of experiment) to total 15N uptake from stream water in each stream and comparing this ratio (transfer efficiency) across the range in streamwater DIN concentrations using an intersite regression. P8 will be tested by comparing total detritivore 15N uptake (normalized to average streamwater ammonium del 15N during the experiment) across the range in detritus C: N ratios using regression analysis only among streams with P: R ratios < 0.5. Fungal biomass on benthic organic matter (BOM) will also be measured to help explain variability in detrital C: N ratio and in the trophic transfer of detrital 15N. To test P9, the ratio of consumer 15N uptake (all important consumers) to total 15N uptake from stream water will be compared across the range of GPP rates and P: R ratios using intersite regressions. **N cycling and turnover.** We believe that the cycling rate of N differs within the various organic pools in streams (e. g., epilithon, CBON, FBON, DON), and differs within the same pool between streams because of differences in trophic status and in N deficiency. We believe that N in organic matter pools derived from algae will turn over more rapidly than N in pools associated with allochthonous organic matter, because, as noted above, the latter may be less efficiently consumed and transferred up the food web. We also assume that N cycles more rapidly in all organic pools in streams that are more N deficient. Therefore, we predict the following:

P10: The maximum del 15N and turnover rate of 15N will be greater in the epilithon than in the CBON, FBON, or DON pools in all streams.

P11: The maximum del 15N and turnover rate of 15N in epilithon, CBOM, and FBOM pools will be greater in streams with lower streamwater DIN concentrations and N: P ratios and higher detritus C: N ratios.

P10 will be tested by comparing maximum del 15N values in epilithon with maximum del 15 N values in CBON, FBON and DON pools in each stream. P11 will be tested using intersite regression analyses with maximum del 15N in epilithon, CBOM, and FBOM (normalized to average streamwater ammonium del 15N during the experiment) and turnover rates of 15N in each of these pools as dependent variables, and streamwater DIN concentrations and N: P ratios and detritus C: N ratios as independent variables. **N deficiency.** N deficiency should be an important factor in determining N uptake, retention and cycling. In addition to streamwater DIN concentrations and N: P ratios, N uptake lengths provide relative measures of N deficiency. The spiraling concept holds that nutrients that are in the lowest supply relative to

demand (most deficient) should have the shortest uptake lengths (Newbold et al. 1982); however, this hypothesis has never been rigorously tested. An alternative approach to nutrient tracer additions for determining nutrient uptake lengths involves small, short-term nutrient additions to streams (Webster and Ehrman, in press). Although this small enrichment approach may result in somewhat longer uptake lengths than using nutrient tracers (Mulholland et al. 1990), it can be a useful approach for comparing among different nutrients if nutrient additions are small and similar in their relative magnitude. Under greater N deficiency we expect the difference in uptake lengths between NH4 and PO4 to be greater, whereas the difference between NH4 and NO3 uptake lengths should be smaller. Comparison of uptake lengths measured using small enrichments and using tracer additions also provides an indication of nutrient deficiency. The difference in NH4 uptake lengths measured using the enrichment and 15N-tracer approaches should be smaller under greater N deficiency, because enrichments more strongly stimulate nutrient uptake rate under high nutrient deficiency. Therefore, we predict the following:

P12: The relative magnitude of NH4 and PO4 uptake lengths will be related to measures of N deficiency, with NH4 uptake length being shorter than PO4 uptake length in streams with lower DIN concentrations and N: P ratios.

P13: NH4 uptake lengths will be shorter than NO3 uptake lengths in all streams, but the difference in these uptake lengths will be lower in streams with lower DIN concentrations and lower N: P ratios.

P14: Uptake lengths of NH4 will be shorter using 15N tracer additions than using NH4 additions in all streams, but the discrepancy between methods will be smaller in streams with lower DIN concentrations and lower N: P ratios.

P12 and P13 will be tested by intersite regression analyses comparing differences in NH4 , NO3, and PO4 uptake lengths computed from short-term injections of NH4, NO3, and PO4 with streamwater DIN concentrations and N: P ratios. P14 will be tested by an intersite regression comparing differences in uptake lengths computed from the short-term NH4 injections and from the initial 4 hours of the 15NH4 additions with streamwater DIN concentrations and N: P ratios.

D. Workplan, methods, and data analysis **Task 1. N Model Simulations.** The 15-compartment, stream N model will be run for a simulated 6-wk 15NH4 addition to each stream using the best available estimates for all compartment standing stocks and fluxes. The model simulations will be performed prior to the start of 15NH4 addition experiments in each stream in order to provide an initial conceptualization and predictions of N cycling (based on our current assumptions) to compare with the 15N experiment results. All sites except Bear Brook have already run the N model for a 15NH4 addition, but the model will be rerun for each stream using updated information from the solute injection experiments (task 2) and sampling of standing stocks (task 4). **Task 2. Solute injection experiments.** There are two components of this task. The first involves measuring hydrodynamic properties of each stream using an

injection of a conservative hydraulic tracer and analysis of the data with a transient storage model. Use of conservative tracer injections has recently become a standard technique in stream studies and provides important data on the dynamics of water relevant to the uptake and cycling of nutrients (Triska et al. 1989, Solute Dynamics Workshop 1990, Ehrman and Lamberti 1992, DeAngelo et al. 1993, Mulholland et al. 1994). The second part involves an addition of inorganic nutrients (NH4, NO3, and PO4) and is conducted simultaneously with the conservative tracer injection. It will allow us to calculate uptake lengths for these nutrients and will provide information on the relative importance of N and P to organisms in each stream. Methods for both components of this task are described in detail by Webster and Ehrman (in press). Briefly, the conservative tracer injection will consist of either Cl or Br, depending on background concentrations and discharge. Both of these solutes can be measured on site in real time with ion-specific electrodes or a high quality conductivity meter (Cl). The injection will be accomplished using either a Mariott bottle or a peristaltic pump. The injection will continue until the tracer concentration at a downstream site (50-300 m downstream from the injection site, depending on stream size) has reached a constant level for at least 0.5 h (probably 1-3 h at most sites). At the downstream site the tracer concentration will be recorded every 0.25 to 5 min, depending on the rate of change. Tracer concentration data will be analyzed using an advection-dispersion model with transient storage (Bencala and Walters 1983). Several computer simulators of this model are available (e. g., Runkel and Broshears 1991). We will use a Fortran version developed by J. R. Webster that runs on a PC. This model analysis of the data will allow us to calculate discharge, nominal transport time, average water velocity, dispersion rate, size of the transient storage zone (As), the ratio of transient storage area to surface cross section area (As: A), the rate of exchange between surface and transient storage zones, and the average uptake length of water (distance traveled by water molecule before uptake into a transient storage zone). Measurements of stream width and depth will be made within 1-2 days of the conservative tracer injections. Wetted width will be measured at 2 to 5 m intervals (depending on study reach length) and water depth will be measured at 10 to 20 cm intervals across the stream at each place where width is measured. These measurements will allow a direct calculation of surface water cross section area (A) to compare with estimates of effective cross section area determined from the conservative tracer injections. Uptake (immobilization) of non-conservative solutes (nutrients) will be measured simultaneously with the conservative tracer injection. Nitrate, ammonium, and phosphate will be added to the injection solution (as soluble salts) at concentrations necessary to raise stream levels to about 3-5x background levels. Injections will be performed on two consecutive days, with NO3, PO4 and conservative tracer injections on day 1 and NH4 and conservative tracer injections on day 2. For each injection, samples will be taken from the stream at 5-10 sites located geometrically (increasing distance downstream) along the reach both before the release (to measure background levels) and after the conservative tracer has reached plateau levels. These samples will be filtered and placed on ice for analysis of nutrients at the site laboratory using standard procedures (APHA 1995). If samples cannot be analyzed within 24 h, they will be frozen for storage. Nutrient uptake lengths will be determined from the ratio of background-corrected concentration of the nutrient to that of the conservative tracer concentration, assuming first-order kinetics (e. g., Webster et al. 1991, Newbold 1992). Although this method may

overestimate uptake length, as demonstrated by Mulholland et al. (1990) for phosphorus, it will allow us to compare the tracer and enrichment approaches for determining NH4 uptake length. If elevated nutrient concentrations cause saturation of nutrient uptake, we should observe an increase in the slope of a plot of ln(NH4/ Cl ratio) versus distance from upstream to downstream (i. e., an increase in the first-order uptake rate coefficient with decline in NH4 concentration downstream), as did Mulholland et al. (1990) for PO4. **Task 3. Metabolism, sediment bacteria, and detritus chemistry measurements.** Measurements of GPP and ecosystem R will be made in each stream during the first week of the 15N addition experiment (see Task 4 below). GPP and R will be measured using the upstream-downstream diurnal dissolved oxygen change technique, as modified by Marzolf et al. (1994). Briefly, this technique involves high frequency (min) measurements of dissolved oxygen concentrations at two stations over a 40-h period. GPP and R rates will be determined by performing an oxygen mass balance over the stream segment using measured changes in dissolved oxygen concentration from upstream to downstream, discharge rate, and air-water oxygen flux determined by measuring evasion rates of experimentally-injected propane (determined using gas chromatography) and scaled to oxygen. The high precision dissolved oxygen meters (Orbisphere Model 2607) and dataloggers (Campbell Scientific Model CR10) needed for these measurements are available at ORNL for use in this project. Measurements of BOM standing stock, detritus C: N ratio, and microbial biomass will be made during the week prior to the 15N experiment. BOM, separated into coarse and fine fractions, will be determined from standard transect collections. Bacteria associated with fine BOM will be determined by epifluorescent direct counts (Sinsabaugh and Findlay 1995). Fungal biomass on coarse BOM will be measured as ergosterol (Sinsabaugh and Findlay 1995) to quantify the major microbial component of decomposing litter (Findlay and Arsuffi 1989). Subsamples of coarse and fine BOM will be analyzed for C and N content using a CN analyzer (Carlo Erba Model NA1500). The bacterial counts and ergosterol analysis on samples from all sites will be performed centrally at the Institute of Ecosystem Studies (S. Findlay), and the C/ N analysis will be performed centrally at the University of Georgia (J. L. Meyer). **Task 4. 15NH4 addition experiments.** The experiment will consist of a 6-wk continuous addition of 15NH4 with co-injection of a conservative tracer (e. g., Br or Cl) during the first 4 hours. The co-injection of conservative tracer will allow computation of NH4 uptake lengths based on calculated streamwater 15N/ conservative tracer ratios and their rate of decline with distance (Newbold et al. 1981). Sampling for 15N in each of the 15 model compartments (see section II. C) will be conducted at several locations within each stream prior to, during, and for approximately 12 months after 15N addition. Streamwater samples will also be collected at each sampling location weekly, filtered, and analyzed for NH4, NO3, DON, PON, and PO4 using standard procedures (APHA 1995). Each stream will be sampled prior to the 15N experiment in order to determine biomass and community composition within each of the 15 model compartments and to define the specific samples to be collected for 15N analysis. Although we will attempt to collect samples for all 15 model compartments in all streams, all compartments may not be represented in every stream and some compartments are likely to be represented by 2 or more important species. Whenever possible, taxa will be sorted to the species level prior to analysis for 15N content (i. e., del 15N, defined as the 15N: 14N ratio in the material being analyzed).

Sampling is designed to monitor an upstream control reach for any variations in natural abundance del 15N and to describe the distribution of the 15N tracer over time along a downstream transect starting at the site of the addition. Two control stations and 6 stations below the addition site will be sampled on a routine basis. On one occasion near the end of the tracer addition, selected samples will be collected along a longer downstream transect of 10 stations (the normal 6 plus 4 more) to define the downstream extent of tracer movement. Stations will be spaced in a geometric progression within the constraints of the study reach but the first two will be within the estimated uptake length of dissolved ammonium. Sampling frequency will vary to some extent according to system characteristics. In general, one transect will be sampled one week prior to starting the addition, and then sampled at 3 d, 7 d, and weekly until 2 weeks after the addition has been terminated. Follow up samples of selected compartments will be collected at 1, 3, 6 and 12 months after the addition is terminated. However, if a stream has a dominant organism with a N turnover time of < 3d (e. g., black fly larvae), that organism will be sampled daily during the first week of tracer addition and daily the first week after the tracer addition is stopped. We estimate that the standard sampling will produce 1560 samples (15 components* 8 stations* 13 sampling dates), or 1620 samples when the long transect is included, for each stream. Experience has shown that it pays to collect more samples than ultimately will be processed. An initial screen of samples will allow prioritization and selection of a subset of these samples for 15N analysis. We will analyze about one-third of the samples collected from each stream at a cost of $7.50/ sample using the stable isotope laboratory at MBL. The del 15N of streamwater NH4+ and NO3-will be determined after concentration on ion exchange resins (Dowex 50X-8 and Dowex 1X-8, respectively), elution with KCl, addition of MgO and DeVarda's alloy (for NO3 only), and sorption of concentrated NH4+ onto a Whatman GF/ F filter permeated with H2SO4 (Sorensen and Steen Jensen 1991). The filters are dried and assayed for 15N by mass spectrometry using an automated sample combustion system and a Finnigan Delta S isotope ratio mass spectrometer. DON will be concentrated by lyophilization to dryness of stream water following removal of NH4+ and NO3-by ion exchange, and the lyophilized material assayed for del 15N as above. Particulate organic matter samples (e. g., detritus, whole organisms) will be dried, homogenized, and assayed for del 15N by mass spectrometry as above. The del 15N of samples collected just prior to the 15N addition and from the upstream control stations in each stream will be used to define natural abundance values and will be subtracted from del 15N values of samples collected after the 15N addition has begun to determine the tracer del 15N of each sample.

IV. PROJECT ADMINISTRATION, ORGANIZATION AND SCHEDULE The project will be administered from one institution (VPI) with subcontracts let to nine other institutions to conduct most of the central and site activities. Project organization and personnel are summarized in Table 2. Four individuals will serve central project roles. P. J. Mulholland will serve as overall project leader and will coordinate intersite synthesis activities. B. J. Peterson will oversee 15N analyses and interpretation of the 15N data. The 15N analyses for the entire project will be performed in the stable isotope laboratory at MBL and will be costed on a per sample basis. J. R. Webster will oversee subcontracting at Virginia Tech and will oversee application of the transient storage model to the conservative tracer injection data for

estimation of hydrodynamic characteristics at each site. One post-doctoral fellow will be hired through Virginia Tech to serve as field coordinator for the 15N experiments and help with 15N data analysis and interpretation. This individual will travel to each site approximately 2 weeks prior to the start of 15N additions and remain onsite for approximately one month to ensure adherence to standard experimental and sampling procedures and aid in the resolution of any site-specific problems. One part-time technician (6 months per year) will also be hired through Virginia Tech to conduct the metabolism measurements in each stream during the first week of the 15N addition experiment. One individual will serve as PI for each site (Table 2). In addition, each site may support one other senior person to assist with site activities and will support one student or technician to assist with field and laboratory work and modeling. Responsibilities of each site are: (1) to conduct the short-term solute injection experiments, including chemical analysis of water samples, and apply the stream transient storage model to the conservative tracer data; (2) to conduct the 15N addition experiment, including streamwater chemistry sampling and analysis and sampling and sample preparation for 15N analysis; and (3) to run the N simulation model and compare model output with empirical results. Experimental activities will be focused during primarily one year at each site, with five sites scheduled each of the first two years. Because it is essential that the project post-doctoral fellow be present at each site for approximately one month at the beginning of experimental work, initiation of experiments will be staggered among the five sites scheduled in each year. Sites scheduled for initiation of experimental studies in year 1 are: Mack Ck. (July), Gallina Ck. (September), Upper Ball Ck. (December), Walker Br. (April), and Sycamore Ck. (May). Sites scheduled for year 2 are: Bear Br. (June), Kuparuk R. tributary (July), Smith Ck. (August), Q. Bisley (February), and South Br. Kings Ck. (April). The scheduling of experiments during different seasons will also enable us to determine N uptake and cycling over a greater range in hypothesized controlling factors (e. g., DIN, GPP, P: R). An all-investigator meeting will be held during the final year of the project for intersite comparisons and synthesis. Site results and intersite comparisons will be presented as talks and posters at national meetings by PI's and students.

Table 2. Project Personnel and Responsibilities Position/ Site Name Institution Central Project: Project Leader P. J. Mulholland ORNL 15 N Task Coordinator B. J. Peterson MBL Hydrodynamics Task Coordinator J. R. Webster VPI Field Leader Jennifer Tank VPI Site Leaders: Q. Bisley, Luquillo LTER, PR W. H. McDowell, S. L. Johnson UNH Sycamore Ck. N. B. Grimm, S. G. Fisher, E. Marti ASU Gallina Ck., Sevilleta LTER, NM H. M. Valett UNM S. Kings Ck., Konza LTER, KA W. K. Dodds KSU Ball Ck., Coweeta LTER, NC J. L. Meyer, J. R. Webster UGA and VPI Walker Br. P. J. Mulholland ORNL Mack Ck., Andrews LTER, OR S. V. Gregory, L. R. Ashkenas OSU Smith Ck., Kelogg LTER, MI S. K. Hamilton MSU/ KBS Bear Br., Hubbard Brook LTER, NH W. B. Bowden, S. Findlay UNH and IES Kuparuk R. Trib., Arctic Tundra LTER, AK B. J. Peterson, A. E. Hershey MBL and UMD

Most of the scientists that will be involved in the proposed study participated in the July 1995 Coweeta workshop (see Results from Prior NSF Support). At the workshop we demonstrated the feasibility of the techniques that we are proposing, a necessary criteria for this type of intersite comparative study. Also, the workshop participants demonstrated their willingness and

commitment to participate in a group effort.

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