LINX LOTIC INTERSITE NITROGEN EXPERIMENT

LOTIC INTERSITE NITROGEN EXPERIMENT (LINX) PROTOCOLS (January 1997 Revision) CHOICE OF STREAM STUDY REACH

General. The choice of the study reach should be based on matching, as closely as possible, the characteristics provided in Table 1 in the proposal (e. g., discharge, As: A, water chemistry, metabolism) for the time of year the experiment will be conducted. The streams were "chosen" to provide a gradient of values across each of these characteristic axes, so it is important that in choosing the experimental reach you try to match Table 1. Select a reach that is typical of your stream but try to: (1) avoid tributaries entering the reach, (2) avoid large springs or seeps entering the reach (unless you are prepared to monitor these closely), (3) avoid a reach with unusually large pools or unusually long riffles, as these features may make it impossible to obtain an adequate distribution of samples for travel distance calculations. Length. The length of the study reach will depend on flow and expected NH4 uptake length (see also 15N experiment rationale). In general, for small streams (< 10 L/ s) a length of about 100-150 m should be sufficient, whereas for larger streams (> 25 L/ s, e. g., Upper Ball Cr., Mack Ck, Smith Ck) a length of at least 300 m is probably needed. Note for the conservative tracer and nutrient injections it might be necessary to use only the upper one-half to two-thirds of the reach so as to reach tracer steady state within a reasonable length of time. Care should also be taken in selecting an injection site for the conservative tracer and N-15 additions. The injection site should be a meter or two above a constriction or other natural or artificially-enhanced mixing zone to produce complete mixing of the added tracer (a preliminary dye or conservative tracer release may be needed for this). The upstream sampling station in the study reach should be just a meter or two below the zone of complete mixing so as to minimize the distance between it and the injection site (but it is important to have complete mixing prior to the most upstream station). This may need to be tested with a preliminary dye or salt injection. Groundwater inflow. It would be helpful if the study reach had relatively little groundwater inflow (< 10% increase in discharge from upper to lower station). This is particularly important for the metabolism measurements. **MEASUREMENTS AND EXPERIMENTS I. Pre-15N Addition Measurements/ Experiments A. Benthic Organic Matter and Invertebrate Biomass** Rationale. Biomass and N content of each of the 15 compartments in the model are needed for the period of study in order to compute the amount of N going into these compartments. For compartments that are sampled in bulk (e. g., epilithon, CBOM, FBOM), we only need to know the biomass and N content of the bulk material. For compartments that have several different species that are important contributors of biomass (e. g., grazers, collectors, shredders), we need to know the biomass and N content of each species that contributes significantly to the total (> 5%).

Sampling. Because sampling for benthic organic matter and invertebrates may be somewhat disruptive, it should be done approximately 2 weeks prior to the 15N addition (hopefully long enough prior to the 15N addition to allow recovery from any disturbance during sampling). It may be necessary to sample macroinvertebrates and benthic organic matter again at the end of the 15N addition if it appears that there have been substantial changes in invertebrate densities and organic matter standing stocks. Sampling of each of the bulk-type compartments (e. g., CBOM, FBOM, epilithon, filamentous algae, bryophytes, etc.) should use standard methods appropriate for each stream (e. g., transect sampling -material collected from standard areas along several transects; or stratified random sampling -material collected from each of several types of stream habitat according

to the relative importance of each in the study reach). For CBOM, if both woody and leaf material is distinguishable and important, then it might be best to get the biomass of each type separately. For FBOM, determine total biomass using the most appropriate method for the site (e. g., removal of all material within a cylinder placed into the sediments). If sediments are deep, it would probably be best to sample to a standard depth of 10 cm (since it is unlikely that the 15N would move farther into the sediments than that depth). In the case of epilithon, samples should be scraped from rock surfaces of known area and the scraped material collected on a pre-combusted and tared glass fiber filter (preferably Whatman GF/ F). Replicate samples for chlorophyll analysis of epilithon should also be taken at the time of epilithon biomass sampling. Chlorophyll should be determined using a 95% acetone extraction (24 hours in refrigerator in the dark) unless a more rigorous method is needed. For bryophytes, see the sampling protocol developed by Breck Bowden in Appendix C. This protocol may also be appropriate for filamentous algae if they are rather patchily distributed. For the organism-type compartments (e. g., grazers, shredders, invertebrate predators, vertebrate predators) use sampling methods appropriate for the site. If several species within a compartment are important contributors to the total biomass of that compartment and will be sampled separately for 15N analysis, then their biomass within the reach and their average N content should be determined separately. All samples should be dried (60 o C) and ground. One portion of the sample is sent to Judy Meyer at UGA for %N analysis and another portion is weighed, combusted at 500 o C for > 4 hours, and reweighed to determine ash free dry mass (AFDM) and AFDM/ dry mass ratio.

B. Microbial Biomass Rationale. Microbial assimilation of inorganic nutrients may be a significant term in many of the streams. Estimates of bacterial and fungal biomass will be made for surficial BOM and hyporheic sediments for each stream. These data should provide some predictive power as independent variables in multiple regression models of N cycling, as well as provide interesting intersite comparisons of microbial abundance. Sampling. Approximately 1-2 weeks prior to the 15N addition, samples for microbial biomass should be collected from the stream. For each stream Stuart Findlay is expecting 5 samples each of CBOM, FBOM, and hyporheic sediments, and 5 extra samples at the discretion of the site PI. Thus each stream site will provide 20 samples for bacteria analysis and a paired set of 20 samples for ergosterol. However, it is advisable to take more samples and preserve them for later analysis in case they are needed. Samples for microbial biomass should be collected so as to be representative of reach-scale standing stocks. For example, FBOM should be collected from several depositional areas along the reach. Each sample could represent a single microsite (e. g., a single debris dam or backwater) or samples from several microsites of the same type could be bulked (e. g., several debris dams or several backwaters). The decision as to bulking should be based on how many types of microsites need to be sampled and the limitation on number of samples to be analyzed mentioned above. For example, if FBOM in a reach occurs primarily in backwaters and to a lesser degree in the thalweg, then it might be best to collect individual samples from about 4-5 representative backwater sites and about 3 representative thalweg sites (this would then use up 2-3 of the optional samples). Alternatively, if FBOM is distributed equally among backwater, debris dam, and thalweg sites, it would be best to bulk several sites of each type together and then submit 2-3 bulked samples from type site. For CBOM, if both woody and leaf material is important, it would be best to take about 3 samples of each type for analysis. Remember, take extra samples and preserve them for possible future analysis if more funding becomes available. For hyporheic sediments, sample from 10-20 cm depth in areas known (or at least expected) to have active exchange with surface water. This may be done by carefully removing surface materials to the desired sampling depth and then collecting the necessary amount of sediments for bacterial and fungal (ergosterol) analysis.

Sample volumes and preservation. For bacterial count samples, Stuart needs 10-25 cc of sediment (surficial FBOM or hyporheic sediments) or about 10 cc of CBOM preserved in 15 mL of 5% buffered Formalin. Samples should be kept cold, but not frozen. For ergosterol (fungi), Stuart needs about 25 cc of sediment (surficial FBOM or hyporheic sediments) or about 10 cc of CBOM preserved in 15 mL of

HPLC-grade methanol. Samples should be kept cold but not frozen. Site investigators will need to provide data on wet: dry wt ratios for each sample and the organic content. Thus, for each sample, it would probably be best to collect enough material to split it three ways: (1) a sample for bacterial counts, (2) a sample for ergosterol (fungi), and (3) a sample for wet: dry wt ratio and organic content to be performed on site. The wet weight of each sample and wet: dry weight ratios must be recorded on the container for normalization of the data.

C. Conservative Tracer and Nutrient Injections Rationale. The general design of this task is that a known concentration of solute is released at a constant rate into the stream for one to several hours and measurements are made downstream to determine the concentration and timing of the passage of the solute pulse. There are two components of this task. The first involves injection of a conservative solute tracer (Cl or Br) to determine the hydraulic properties of the stream, which are then used as independent variables in the intersite analysis of N cycling. Chloride has been widely used a conservative tracer in many studies because it is cheap and easily measured. Plan to use Cl unless your background Cl concentrations are high (> 5 mg/ L) or unless discharge is so high that you cannot release Cl fast enough to achieve measurable concentrations (approx. 10 mg/ L above background concentration). In these cases, plan to use bromide as an alternative. Bromide can be measured at levels about 0.1 below the detection limit of Cl. Both Cl and Br can be measured on site with ion specific electrodes (Webster and Meyer have 4 Cl-specific probes that can be borrowed if you don't have them or can't get them). Chloride can also be measured with a high quality conductivity meter. The injection should be performed using a peristaltic pump (we will try to provide a pump for the site if one is not available). While a Mariotte Bottle would also work, we feel that a peristaltic pump is a little more reliable for giving a constant injection. The length of the injection time will vary depending on size and discharge of the stream. In general, the injection should continue until the concentration at the downstream station has reached a constant level (plateau) for at least 0.5 hour. In most streams this will require 1-3 hours. The downstream station should be located 50-300 m downstream from the injection site, again depending on the size and discharge of the stream (this should be the same reach used for the 15N addition, or at least the upper part of the 15N addition reach). Data from the conservative tracer injection will be analyzed using an advection-dispersion model with transient storage. Webster is currently using a Fortran version that runs on most computer platforms, and he plans to do all the simulations for this study. The second part of this task is conducted along with the conservative tracer injection -the stream is slightly enriched with an inorganic nutrient and uptake of the nutrient is measured assuming 1st-order uptake kinetics and the nutrient uptake length is calculated. Because nutrients are added at levels above background, this method may overestimate uptake length, however, it will give us relative numbers for comparisons. Injections of NO_3 , NH_4 , and PO_4 should be done on consecutive days (a conservative tracer must be added with each nutrient injection to correct for dilution, but intensive sampling of the conservative tracer for determination of hydraulic properties need only be done with one of the nutrient injections). Rather than specify one standard addition at all sites, each site should **aim for additions that are about 2x to 5x the background levels of the nutrient being added.** In choosing the concentration addition level, use the following criteria: 1) the addition should be a small as possible so as to remain below the saturation level (if at all possible) for uptake if it is the limiting nutrient; 2) the concentration increase must be able to be accurately measured even after about 2/ 3 of it has been removed from solution. Set up lots of stations within the expected uptake length distance below the injection site at which to collect samples (6-8 stations at a minimum -the more measurements of the nutrient increase relative to conservative tracer over distance, the better the estimate of uptake length given the limits on analytical precision), and collect numerous background samples (at least 4-5) and steady state samples (5-10) at each station. Because there is less carryover effect of NO_3 , its injection should be done on the first day, then NH_4 , then PO_4 . For streams with low background NO_3 concentrations (< 10 ugN/ L), NO3 additions of about 25-50 ugN/ L would be best. However, for streams with high NO_3 concentrations (> 200 ugN/ L), NO_3 additions of at least 200-400 should probably be used. For

streams with low background NH 4 concentrations (< 5 ugN/ L), NH4 additions of about 20-30 ugN/ L would be best (although NH 4 measurement accuracy might necessitate a slightly higher addition level). For low SRP streams (< 5 ugP/ L), PO 4 additions of about 10-25 ugP/ L would be best. Also, at sites at which N and P are likely to be co-limiting you may want to do a combined NH 4 and PO 4 after each of the individual injections are complete.

Nutrient uptake lengths computed from solute injections will then be compared at each site among each other (e. g., NH 4 vs. PO 4 uptake lengths) to determine relative nutrient deficiency (nutrient with shortest uptake length is most limiting) and with the uptake length determined from the 15N addition (NH 4 only). Nutrient uptake lengths will also be compared between sites to determine relative nutrient deficiency among sites. Uptake lengths are determined by computing the slope of the regression between ln((N sx -N ix)/(C sx -C ix)) (as the y variable) and distance below the injection site in meters (as the x variable), where N sx = steady state nutrient concentration at station x, N ix = pre-injection nutrient concentration at station x, C sx = steady state conservative tracer concentration at station x, and C ix = pre-injection conservative tracer concentration at station x. The uptake length is just the inverse of this slope. Jack Webster will check the calculations of nutrient uptake length for all sites. It would probably be best to complete the conservative tracer and nutrient injections before the 15N addition begins, although they could be done during the 15N addition (short-term addition of nutrients -even NH 4 -should not affect the longer-term 15N dynamics). If conditions change substantially during the 6-week 15N addition, it would be best to repeat the short-term conservative tracer and nutrient injections during the latter part of the 15N addition.

C. 1. Conservative Tracer Injection Methods The methods below have been modified from: J. R. Webster and T. P. Ehrman, 1996, Solute dynamics, pages 145-160 in F. R. Hauer and G. A. Lamberti (eds.), Methods in Stream Ecology, Academic Press, San Diego. They are for a 100-m reach and using a Mariotte bottle and Cl specific probe. However, we should have a peristaltic pump at all sites to do the injections, so you can replace the Mariotte bottle instructions with those for a peristaltic pump (the critical thing is to get a constant injection rate right from the start of the injection). Laboratory Preparations: 1. Mix stock solution of sodium chloride by dissolving 238 g salt per 1 L distilled water. Total volume needed will depend the duration of releases and release rate. Heating the mixture in a water bath aides in dissolution. Mix vigorously and repeatedly for the solution is close to saturation. Make certain the salt is completely dissolved. 2. Prepare a series of chloride standards (1-20 mg/ L) for calibrating the probes. We use 0.5, 1, 2, 3, 4, 5, 10, and 20 mg/ L.

Field Pre-Injection: 1. Calculate stream flow and necessary release rate to raise stream concentration appropriately (by about 10-15 mg/ L at the upstream station). 2. Use a tape measure to delimit the extent of the experimental reach. Mark every 5 m within the reach with labeled flagging tape. 3. At each 5-m cross-section, measure wetted channel width, depth across the stream (every 10-20 cm depending on width, minimum 10 depth measurements per cross-section), and thalweg velocity (optional). It may be better to do these measurements after the releases to avoid unnecessary stream stomping. Stream temperature and gradient should also be measured. 4. Calibrate the Cl probes with the standards. The standards should be placed in the stream until they equilibrate with ambient stream temperature.

Field Injection: 1. Collect a series of background (immediately pre-injection) water samples in mid-stream at 10-m intervals over the reach. Work from downstream up and avoid unnecessary stomping in the stream. 2. Position chloride probes at 20, 50, and 100 m sites. Place probes securely in a well-mixed areas. 3. Add solute solution to the Mariotte bottle and seal with rubber stopper. Position the Mariotte bottle on a stand directly in the stream (if shallow and stable enough) or on bank (with sufficiently long tubing to reach stream) such that the solution will enter a turbulent, well-mixed zone. Do not attach tip to Mariotte bottle at this time. 4. With a bucket under the spigot, open to full and

allow Mariotte bottle to equilibrate; you will hear a glug-glug-glug sound as air comes down through the tube. Turn off spigot. Do not break the seal at the rubber stopper or this step will have to repeated. 5. Connect appropriate tip to end of spigot or tubing. Place bucket under tip and open to flush out any air bubbles. Measure the release rate with a graduated cylinder and stop watch (keep the bucket under the tip to avoid any premature addition to the stream). If rate is unacceptably higher or lower than expected, a new tip with a larger or smaller opening should be used. During the release, periodically recheck the release rate, emptying solute in the graduated cylinder into the stream (but do not do this prior to the release --empty the graduated cylinder into the bucket). 6. Synchronize stop watches and open spigot to commence release. 7. Frequency of chloride readings at downstream site depends upon rate at which the concentration changes in the stream. Record probe readings every 1-5 min (flow dependent) until pulse arrives and then measure every 15-30 seconds as chloride concentration increases rapidly. 8. At plateau (10 min to several h after commencing release), working from downstream to upstream, take 1 sample in a clean bottle from mid-stream at 10-m intervals for Cl analysis. Again, avoid unnecessary stomping in the stream. Shut off the Mariotte bottle once samples have been collected from all sites. Record the total time of release. 9. Continue recording chloride concentration until stream levels return to pre-release levels. Once measurement in the stream has been terminated, use the probe to measure chloride concentrations of background and plateau samples collected. These samples can then be discarded. Recalibrate the probes, for they may experience electronic drift during the release.

Personnel: These releases can be done with 2 experienced people, but with more people, the chances of getting good data are much better. It works well with 5 people: one to do the release, 3 reading Cl probes (downstream and 2 intermediate sites), and one person to "coordinate", i. e., make sure everything is done correctly, cover while someone else takes a break, pass out coffee and donuts, and generally keep everyone else happy. The post-doc, technician, and/ or Webster should be available for most releases, so we'll need about 3 additional site people. No experience necessary.

Materials and supplies: Lab -conservative solute (we use non-iodized table salt) distilled water containers for standards --8 carboys for stock solution of solutes graduated cylinders (100 mL and 1000 mL) Field -Peristaltic pump (supplied by MBL if site doesn't have one) or Mariotte bottle supplied by Virginia Tech velocity meter (optional) meter stick stop watches flagging tape permanent marking pen tape measure (50-100 m) squirt bottles with distilled water --2 or 3 thermometer water resistant paper or notebooks, pencils --for each Cl probe site and release site stand for Mariotte bottle --VT bucket graduated cylinder (100 mL) sample bottles --20 clean Cl probes --4, VT C. 2. Nutrient Injection Methods. The first nutrient injection (NO_3) can be done in conjunction with the conservative tracer injection above. The other nutrient injections (NH_4 and PO_4) should be done on the next two days, but conservative tracers (Cl or Br) must be added with the nutrients (but the intensive measurement of the conservative tracer concentrations need not be done). On a fourth day do a combined NH_4 and PO_4 injection if N and P are likely co-limiting.

Laboratory Preparations: 1. Mix stock solution of sodium chloride by dissolving 238 g salt per 1 L distilled water. Total volume needed will depend the duration of releases and release rate. Heating the mixture in a water bath aides in dissolution. Mix vigorously and repeatedly for the solution is close to saturation. Make certain the salt is completely dissolved. 2. Prepare stock solutions of nutrient solutes (sodium or potassium salts). Concentration should be such to produce the desired increase in concentration when mixed in the stream at the upstream station. Field Pre-Release: 1. Calculate stream flow and necessary release rate to raise stream concentrations appropriately. Field Injection: 1. Collect a series of background (immediately pre-injection) water samples in mid-stream at several stations over the reach (these are for background nutrient and Cl concentrations). The stations should consist of the upstream reach station (located just downstream from complete mixing of the solute) and several other stations downstream that coincide with stations for the 15N addition (e. g., for a small stream at

about 10 m, 25 m, 50 m, 100 m downstream from the injection site). Take 3 replicate background water samples at each station, filtering each through washed (50 mL of distilled water or stream water through the filter), 0.45-um pore size membrane filters (we use plastic syringes and pre-packaged Syrfil-MF syringe filters made by Costar Corp. for this). Place these samples in clean, acid-washed bottles (probably will need about 50 mL of volume for each sample) and place on ice. Work from downstream up and avoid unnecessary stomping in the stream. 2. Position the Cl probe at the most downstream station to determine when steady state has arrived. 3. Add nutrient/ Cl solution to the Mariotte bottle and commence injection as described for the conservative tracer injection above. 4. After plateau (steady state Cl concentrations) has been reached at the downstream site, take at least 5 samples at mid-stream from each station for nutrient and Cl analysis in the same way that the background samples were collected. It is best to do this in 5 or more separate rounds of sampling, each round consisting of 1 sample from each station from downstream to upstream (being careful not to step in the stream if at all possible, or to minimize disturbance if it is necessary to step in the stream). This sampling scheme will average over short-term variation in concentrations. The samples should be placed on ice immediately. 5. After steady state sampling has been completed, the injection can be shut off. Laboratory analysis (post field injection): 1. Within 2-3 days, NO_3, NH_4, and PO_4 (soluble reactive P) analysis should be done in the laboratory. Samples should be kept cold until analysis. If samples cannot be analyzed within 3 days, they can be frozen until analysis. It is a good idea to do analysis of NO_3, NH_4, and PO_4 on each sample for each injection (to determine if adding one nutrient affected concentrations of another, and in the case of the NH_4 addition, to determine nitrification). Measurement of the conservative tracer (e. g., Cl) in each sample must also be made, either by wet chemical methods or by ion-specific electrode. We would like to have a measurement precision of + 1 ugN or P/ L for these analyses, particularly at sites with low concentrations. For the PO_4 analysis, the standard molybdenum blue/ ascorbic acid method will provide this accuracy is a 10 cm long cell is used in the spectrophotometer.

D. Periphyton Nutrient Limitation Assay Rationale. To evaluate whether N or P is limiting periphyton in each stream, a set of nutrient-diffusing substrates (N, P, N+ P, control) will be set out at the downstream end of each study reach (so as to preclude any enrichment effects in the study reach) at the start of the N15 addition. This limitation will be expressed as an increase in chlorophyll content with N, P, or N+ P amendment relative to the controls. Six replicate substrata from each treatment will be collected at 6 weeks and analyzed for chlorophyll. The determination of nutrient limitation will be used as an independent variable in the intersite comparison analysis of N cycling characteristics. Bioassays for wood degradation by fungi as a function of nutrient amendment will also be done by the project post-doc, in part to test if the heterotrophic community shows similar patterns of nutrient limitation.

Methods. The general method will be similar to that employed by Corkum (Arch. Hydrobiol. 136: 391-411) who has modified past assays used by Winterbourn, Pringle, and Bower. The nutrient diffusing substrates will be constructed of 50mm diameter by 40mm high plastic capped cups. Each top will have a hole cut in it thereby exposing an ashed 47mm glass fiber filter (GFF) that lies over the nutrient amended agar (nitrate, phosphate, both, or neither (control)). Nitrate amended agar will consist of 0.5M N as NaNO 3 in 2% agar. Phosphate amended agar will consist of 0.5M P as KH 2 PO 4 in 2% agar. Nitrate and phosphate amended agar (N+ P) will consist of 0.5M N and 0.5M P using NaNO 3 and KH 2 PO 4 in 4% agar (otherwise agar will not solidify). There will be 6 replicates of each treatment. The bioassay cups will be attached to plastic

holders (holding 12 cups each) in a random fashion and secured to the stream bottom with metal stakes downstream of the experimental reach just before the tracer experiment begins. Cups will be removed when there is visible response (growth of algae) or 6 weeks, whichever occurs first. The filters are to be carefully removed from each cup and analyzed for chlorophyll using the spectrophotometric

method for determination of phaeophytin-corrected chlorophyll a (Standard Methods, 1995 APHA, method 10200H. 2). For the analysis, the entire filter should be extracted with 20 ml of 90% acetone in the dark at 4 %C for 12-20 h. In cases where there is a significant cyanobacterial component, the samples should be ground. After this point all samples should be kept in the dark and on ice. After extraction, pour extractant into a clean vial and centrifuge until solution is optically clear. Place 3 ml of solution in a 1cm pathlength cuvette, read absorption at 750 nm and 664 nm. Acidify solution with 0.1ml of) 1N HCl, mix gently, and after 90 sec read absorption at 750 nm and 665 nm. Chlorophyll is calculate as:

mg/ m 2 chla = 26.7 * ((E664-E750)-(E665-E750) * 0.02 L extract)/ (filter area in m 2)

II. 15 N Addition and Companion Measurements A. Whole-stream Metabolism Rationale. Measures of Gross Primary Production (GPP) and Community Respiration (CR) at the scale of the entire stream reach will be made using the upstream-downstream diurnal dissolved oxygen change technique with direct determination of the air-water exchange coefficient from propane injections. GPP and CR will be used as independent variables in intersite comparisons of N cycling. If possible, these measurements should be made at several times during the 15 N addition period on relatively clear days (but at least one set of measurements must be made at each site during the 15 N experiment). The measurements can be made by site investigators if they have the necessary equipment; if not they will be made by a central investigator (either Jen Tank or Pat Mulholland). It would be best to use the entire study reach for these measurements (upstream and downstream stations corresponding to the ends of the study reach), but if groundwater inflow is > 10% over the reach, then a shorter reach length can be used to minimize groundwater inflow. If a shorter reach is used, then it should be representative of the entire reach and have an average water travel time of at least 15-20 min. Methods. The methods described in Marzolf et al. (1994) (Can. J. Fish. Aquat. Sci. 51: 1591-1599) will be used. This work will be performed by central project personnel, unless site investigators have the necessary equipment and want to do the work themselves. Essentially, the technique involves doing an oxygen mass balance across the study reach every 5 minutes using the difference in D. O. concentrations between upstream and downstream stations, the discharge, and the calculated flux of oxygen into or out of the reach due to air/ water exchange (determined from the fractional loss of injected propane across the reach corrected for dilution, the water temperature, and the barometric pressure -the latter two measurements used to calculate the D. O. saturation level in stream water). Mulholland has an Excel spreadsheet that he will give you that helps process the data which consists of D. O. concentration and water temperature measurements at 5 min. intervals at upstream and downstream stations over a 40-hour period. It is very important to have an accurate determination D. O. saturation levels every 5 min., so accurate measurements of water temperature (at each station at each 5 min. sampling interval) and elevation-corrected barometric pressures (hourly readings at a nearby weather station corrected to the stream elevation are ideal) are very important in this regard.

B. 15 N Addition Isotope addition. The 15 N will be added at the same site as the conservative tracer and nutrient injections. Rapid mixing of the isotope is needed with the 15 N addition also. The more rapidly the isotope becomes dispersed, the easier it will be to select stations downstream and determine uptake distances. For some streams it may be necessary to use a diffuser or multiple dripper tubes to speed mixing (or some re-engineering of the channel just below the dripper). Test mixing with a conservative tracer. Sampling Sites. It is necessary to know if there is any preexisting gradient in isotope values along your study reach. Such gradients may result from non-uniform seepage inputs especially if originating from fertilizer or sewage sources (probably not important for most of our streams). Before the addition starts, collect a preliminary sample set at all stations. One or two stations should be upstream of the addition site and 6 or 7 stations should be downstream (giving a total of 7-9 stations). Depending on your site, you may want to have both riffle or run and pool stations to increase your sample diversity. It is likely that your chosen stations will not turn out to be ideally distributed for

describing the tracer movement, so analyze a set of epilithon or grazer samples very soon after the dripper starts (day 2-3) and readjust accordingly. Notify the isotope lab manager in advance that you need rapid turnaround. Use a geometric progression of stations with 3-4 within the NH 4 uptake length (calculated from the nutrient injection data) and 3-4 more widely separated downstream (e. g., for a small stream: at about 5m, 10m, 25m, 50m, 75m, 100m, 150m downstream from injection site).

But remember that travel distances will change as discharge or nutrient level changes. What to sample. Samples representing each of the 15 compartments in the model (NH 4 , NO 3 , DON, suspended PON, FBON, CBON, epilithon, filamentous algae, bryophytes, grazers, collectors, filterers, shredders, invertebrate predators, vertebrate predators -the latter two being representatives of trophic level > 3) will be collected. Additional samples may be collected on some dates or at some stations to elaborate some compartments (e. g., CBON split into wood and leaf subcompartments, FBON or suspended PON separated into different size fractions, individuals of other invertebrates from particular functional groups, epilithon on introduced tiles along a transect). Bias your samples toward taxa that are most abundant and can be obtained most reliably. For the standard 15 compartment samples, select one species of grazer, collector, filter feeder, and shredder that can be collected at as many stations and times as possible (you may wish to collect individuals of other species of a particular functional group on some sampling dates/ stations if they are also important). Be opportunistic and take many more samples than you can afford to analyze (about 500 per site) because it is impossible to go back and you never know what may turn up in the data later. For example, think of terrestrial critters that depend on your reach and sample them. You can always try to get more sample analysis money later if something exciting appears. Also sample analysis costs are decreasing over time and samples can be stored indefinitely but the overall costs (mainly personnel) of these experiments is not. When to sample. To optimize effort and avoid depleting any compartment during the study, there will be four primary levels of sampling intensity: (1) full sampling of all 15 compartments at all 7-9 stations (1-2 upstream, 6-7 downstream), (2) abbreviated sampling consisting of only the abundant compartments (not in danger of depletion -e. g., epilithon, FBON, CBON, bryophytes, filamentous algae, abundant invertebrates) or rapidly cycling compartments (e. g. epilithon, small grazers, filamentous algae) and only at 3-4 stations (1 upstream, 3 downstream), (3) water compartments only, and (4) expanded sampling consisting of all 15 compartments at all of the full sampling stations plus sampling of the abundant compartments at 4- 5 additional stations that both fill in within the original segment (2-3 additional stations) as well as expand the segment length (2-3 additional stations) by about 50% to 100%.

The sampling schedule should be as follows: Pre-N15 Addition -approx. 1-2 weeks pre-N15 addition (full sampling) -to determine any background longitudinal gradients.

During N15 Addition -day 0/~ 4-6 hr (water compartments only/ filtered -NH4, NO3, DON). -day 3 (abbreviated sampling -only rapidly cycling compartments, ~4 stations). -day 7 (full sampling). -day 14 (abbreviated sampling). -day 20 (water compartments only/ filtered -NH4, NO3, DON). -day 21 (full sampling). -day 28 (abbreviated sampling). -day 35 (abbreviated sampling). -day 40 (water compartments only/ filtered -NH4, NO3, DON). -day 42 (expanded sampling) -DO NOT SHUT OFF N15 DRIPPER UNTIL COMPLETED. -Also, sampling of one rapid-turnover compartment every other day at one station (see below).

Post-N15 Addition -day 1 post/ am (water compartments only/ filtered -NH4, NO3, DON). -day 7 post (full sampling). -day 14 post (abbreviated sampling). -day 28 post (full sampling). -3 month post (full sampling). -6 month post (full sampling). -9 month post (full sampling). -12 month post (full sampling).

If organism/ material depletion or disturbance is still a problem with this schedule, it can be adjusted

somewhat (but should consult with Jen Tank, Bruce Peterson, Pat Mulholland). The most important samplings are day 7 and day 42. The 7d post drip measurements are also important for determining turnover of N in the different compartments (at 3 days post N15 you may want to collect samples from the most rapid turnover compartments at the most upstream station below the dripper). Given the limitation on the total number of samples we can analyze it is best to collect pooled samples of material or organisms from each station and thus allow samples for more stations and dates to be analyzed. Send only a portion of the pooled sample to be analyzed for 15N, retaining some in case reanalysis is needed. The only samples to perhaps avoid pooling are the larger organisms like fish if there are few available and/ or we want to dissect parts for analysis. In addition to the above sampling schedule, we would like to sample one or two rapid turnover compartments at approximately 2 d intervals at one station (the most upstream station below the 15N dripper where mixing is complete under all flow regimes) during the 15N addition. This will help us determine if there are large changes in specific activity of 15N in the dissolved ammonium pool in water due to changes in ammonium concentration or changes in flow rate during the experiment. The best compartment(s) to choose for this sampling is one that is abundant, easily sampled, and that turns over rapidly. Epilithon and/ or a small, fast-turnover grazer (e. g., chironomid, small Baetis) is probably the best compartment to select for this purpose. To be most useful, epilithon should be free of large detritus accumulation (we need epilithon consisting mostly of algal cells). One suggestion is to place unglazed ceramic tiles out in a riffle area (to minimize sedimentation of detritus on the tiles) at one station far enough in advance to allow good algal colonization (probably about 3 months for most sites). A couple of tiles can be collected every 2 days, scraped, and the epilithon collected on small GFF filters, dried, placed in scintillation vials, and sent off for 15N analysis (remember, need to collect enough tiles to provide enough N for the 15N measurement -assuming 1% N content, need at least 3 mg of dry mass of epilithon). Finally, at a couple of times, we would like to separate and analyze microbial N in FBON and CBON samples (separated from the non-cycling N in the detrital matrix). This is optional, but we would like to encourage all sites to try this if possible. The procedures and sampling schedule for microbial N and N15 analysis are provided in Appendix B. The method for final analysis of the K 2 SO 4 extracts for N15 is still under development to some degree and may be modified. However, these extracts can be held indefinitely until analysis so this should not prevent any site from going ahead with this technique. Sample processing. Detritus, epilithon, primary producer, insects, fish parts, etc can be held by freezing, by preserving in alcohol, or by drying. The most expeditious approach for preparation is to dry the samples and grind samples to a fine powder with a teflon mortar and pestle. In the case of small insects, mashing with a spatula in the bottom of a scintillation vial will suffice. **To minimize the possibility of cross-contamination of samples, process your samples in order from reference (least labeled) to highest del values just below the dripper. Clean your grinding implements thoroughly so that no dust carries over from sample to sample.** But remember that a dried bug is difficult to identify. It is important to have reference samples preserved for identification if there is any doubt as to the species. Samples held in alcohol are best for identification and are easily dried and ground for subsequent analysis if you don't have a duplicate dried sample. We often freeze larger fish if it is not convenient to do immediate dissection. Large organisms can be dissected to assess isotope distributions inside the organism, for example, within moss tissues (holdfast, stem, leaf base, leaf tip) or fish bodies (scales, muscle, liver, gonad, blood). These large species may have a measurable signal only in the most metabolically active tissues if the experiment is short relative to their N turnover. Ultimately all samples must be dried and homogenized for analysis. Tougher samples are ground with mortars or wiggle bugged (a small shaker with impact). The procedures for dissolved N species (NO 3 , NH 4 , and DON) and the sampling schedule for these samples are provided as Appendix A.

Sample selection for analysis. Inventory your available samples completely and formulate a strategy for obtaining the most information from the fewest analyses. It would be best to pick one or two sample types (e. g., grazer and filter feeder) and running each time at one station (say station #2

downstream of the dripper because station #1 may be not completely mixed) and also running each station at one time (e. g., day 21 when there should be a large signal). This will show you the time and distance dimensions of the best signal and prevent wasting analyses on samples unlikely to add much information. The next step is to select the samples that relate most closely to the hypotheses of greatest interest at your site. In general, the entire suite of samples from day 7, 21, and 42 will likely need to be analyzed to test the model predictions. We proposed in the original proposal to run 15N on approximately 1/ 3 of the total samples collected by carefully prioritizing our analyses to get the most information. This number of **500 to 600 samples per site** still seems like a good target (although we had to cut the budget for 15N analyses, we have used grant funds to get the Europa combustion furnace demonstrated at the Coweeta workshop that will lower the per sample cost). Samples for the dissolved N species will require much more time for processing than ground tissue samples. These types of samples will likely be run on a reduced schedule (e. g., 5 stations on 4 dates), but this will depend on ongoing methods work and ambient concentrations at each site (see Appendix A). If NH4 or NO3 concentrations are over 1 uM, the analysis becomes much more feasible. If concentrations are near detection limits (0.1 uM), the analyses can be nearly impossible because getting quantitative recovery from 5-10 liters of water is tough and many fewer samples can be processed. Thus, for these samples we will have to do what sample characteristics and technician time will allow rather than proposing a fixed number as with ground tissue and detritus samples. In summary, it will take a good deal of thought to select the best samples to run, but remember that dried, preserved, or frozen samples can be held indefinitely for future analysis. Thus, collect as many samples as you can and yet still avoid damaging or depleting your study reach.

Sample labeling for analysis After drying and grinding all samples, place samples into new scintillation vials labeled with Nalgene Poly-Paper 7/ 8" by 7/ 8" labels (cat. No. 6309). These labels are extra high quality and reduce risk of falling off glass vials. On each label use a 2-3 letter code for your stream name on first line. On second we will use a 3 part code recognized by staff at MBL. So a sample label would look like this: UBC 28 SH 8

The first part is the sample day (e. g. 28 is day 28, P7 is post day 7 etc.). The middle part of the code is the organism or sample type, usually two letters. And the last part is the station number sample was taken from. Sample codes used thus far. SH = shredder 1 = station 1 at -10m (i. e. 10m above dripper) SC = scraper 2 = station 2 at farthest site from dripper FL = filterer 3 = station 3 at next farthest site from dripper IP = invertebrate predator blah blah etc. VP = vertebrate predator to 8 = station closest below dripper (highest 15N) CH = chironomidae SL = salamander Each stream will of course have different representatives for these groups but consistency of labeling from site to site will help Kris Tholke at MBL to easily deal with our samples for 15N analysis. Analysis. Compare the distributions of 15N with your model predictions to see if your preliminary predictions were any good. Do the comparisons support or falsify your working hypotheses? Set up a new model calibration that incorporates measured discharge and nutrient levels. Does this calibration improve the data vs predicted del comparisons or not? Finally set up a model calibration (s) incorporating measured biomass, production, growth, stomach content, etc from your intensively studied reach. What aspects of the N cycle still appear to be poorly understood? Do you have stored samples that can shed light in this area? Detailed methods. The following detailed methods for the 15N addition and sampling are taken from the chapter by Hershey and Peterson in the book on methods in stream ecology edited by Hauer and Lamberti (reference given above). Remember, the post-doc will be on-site to help coordinate this work.

N-15 Addition Details: 1. Materials: carboy narrow PVC pipe (with ID just big enough to thread tygon tubing through) NH4Cl with 10% as 15N (acidify to pH 3 with HCl, using a pH meter to monitor the acidification) Peristaltic pump drive tubing silicone tubing tygon tubing pipet tips (several sizes) and/ or tubing connectors to connect drive and tygon tubing funnel 12-V battery solar panel (or swap

batteries daily) graduated cylinder stopwatch 2. The carboy with the 15N solution should be set on the stream bank with the pump and battery. Add a week's worth of solution at a time. Place setup out of reach of potential flooding. Hook up the peristaltic pump to the 12-V battery. Connect the posts of the battery to the solar panel (battery and panel in parallel). These should be checked regularly to ensure good connections and a charged battery. For heavily shaded sites, the solar panel will probably not keep the batteries charged and several batteries will need to be rotated (maybe two batteries coupled together to run the pump and two batteries being charged to replace them). The pump should have a short length of drive tubing threaded through the pump head (see pump Operator's manual if you need help). Drive tubing wears out gradually. Use a long enough piece of drive tubing to be able to move the tubing once a week so that the force of the pump heads is placed on fresh tubing (if tubing wears out, the pump won't work). If wildlife is a problem, consider setting up an electric fence. 3. Run a length of tygon (or silicon) tubing from the funnel to the outlet end of the drive tubing. Connect the tygon to the drive tubing with a pipette tip. Thread the tygon (or silicon) tubing through the PVC pipe so that when the pipe is placed in the carboy, the bottom of the tubing sits above the carboy bottom. Cut an angle into the bottom of the PVC pipe to allow the carboy solution to pass freely into the tygon tubing. Run the tygon tubing/ PVC pipe into the carboy, all the way to the bottom. Attach a pipet tip to the end of the tygon tubing by the funnel. Insert tip into the funnel. Funnel should be attached to tygon which is extended to the middle of the stream where the 15N will be dripped in. The tubing from the carboy to the pump, and from the pump to the funnel, should be of small enough inner diameter to allow the pump to easily draw the solution. 4. When the pump is engaged, the 15N should be pumped into the funnel end of the dripper tubing and thus be dripped into the stream (it will take several minutes for the 15N to reach the funnel; the drip rate may be low; so to get it through the tubes faster turn the pump up to maximum speed (99) until the solution is actually dripping into the river, and then slow it down). Bubbles may develop in the tubing due to degassing of the solution (or temperature changes) in the carboy. Work these out at the beginning and regularly thereafter to prevent any potential blockage caused by their accumulation. 5. Check the rate of dripping with a graduated cylinder and a stop watch at the point where the tube enters the funnel to be certain that the 15N label is being dripped at the appropriate rate. With fresh tubing, the drip rate may be slightly off until it reequilibrates, though this has not been a big problem.

6. The volume of 15 NH 4 Cl in the carboy should be checked regularly to ensure that the pump has been pumping at the proper rate. Check the dripper as often as possible. Make sure the solution in the carboy is free of all debris to prevent blockage of the tubing. If you have a cold spell, freezing in the line may occur, depending on the concentration of the solution. If this appears to be a problem, you should consult with Bruce Peterson and the MBL people to see what can be done to minimize it. At Coweeta, salt was added to the carboy to prevent freezing and this worked well. Sampling of compartments: **NH 4 , NO 3 , DON** -The methods are quite detailed for these analyses and are included as an appendix to this set of protocols (see appendix A).

Suspended PON -Filter approximately 1 L of stream water from each station onto a 25 mm diameter GF/ F filter (need at least 1 mg of ash-free dry mass, so may need to filter larger volumes in streams with low seston concentrations). Place filter in scintillation vial. Include one blank filter per sampling period. Dry at 60 o C to complete dryness.

Epilithon -Using a wire brush (or something comparable), scrub enough rock surfaces to provide at least 2 mg (dry mass) of material from each station. Sample from several rocks at each station and pool scrubbate to account for potential variation. Pour scrubbate into 50 mL centrifuge tube. Filter the material onto pre-combusted GF/ F filter (25 mm diameter), place filter in scintillation vial and dry (60 o C). Include one blank filter per sampling period. It is important to sample the same types of rocks throughout the study reach (to ensure sampling the same type of epilithic community). This will simplify comparison of the 15 N content in the epilithic compartment. For example, do not sample

"clean" rocks at some stations, and rocks with large amounts of detritus at other stations. If several types of epilithic communities exist (e. g., thin communities in fast flowing sections, thicker communities or detritus-dominated communities in pools or slowly flowing areas), treat them as separate types of samples (epilithon A, epilithon B). Also, consider that certain rocks may turn over or get scoured during high discharge and will have a less mature algal community. Try to sample rocks that appear relatively stable. Finally, be very thorough about cleaning the scrub brush between stations to reduce potential cross-contamination. A separate brush, etc. should be used for the reference station upstream from the N15 dripper. Sample stations in the following order: reference, then downstream to upstream (lowest N15 to highest N15) in the labeled reach. **Bryophytes** -Pick clumps of submerged bryophyte species of interest. For bryophytes (as well as other compartments such as filamentous algae below) it is often helpful to have a viewscope to see what you are sampling (a sheet of plexiglass attached to a clear plastic tube with a handle works well). Place the collected bryophytes in 50 mL centrifuge tubes with some stream water to keep moist. Back at the lab, pick off the green tips and place in scintillation vial and dry (60 o C). Because bryophytes grow from the tip outward, we would like to sample the actively growing portion of the thallus as consistently at possible. Therefore, be very careful and consistent in cutting off the end portions to be analyzed, choosing only green material of the same length from the tip. Need at least 2 mg of dry material. **Filamentous algae** -Pick from stream bottom and place in 50 mL centrifuge tube w/ some stream water to keep moist. Back in the lab, make sure to remove as much detritus as possible and place in scintillation vial and dry at 60 o C (it might be necessary to collect this material on a GF/ F filter first). Need at least 2 mg of dry algal material.

FBOM (< 1 mm) -Collect material from FBOM accumulations that are most available to collector/ gatherers by suctioning off the stream bottom (a turkey baster works well) and filtering the slurry onto pre-combusted GF/ F filters, and place in scintillation vial and dry at 60 o C (one blank filter included on each sampling date). Try to avoid collecting inorganic material (e. g., sand) as much as possible (we want the organic rich sediment deposits). On some sampling dates or at some stations you may want to stratify the sampling by habitat type (e. g., pool, riffle). During the last collection of the 15N addition period (42d) we need to do a more quantitative sampling of FBOM so that we can do a 15N mass balance. This will likely require a more intensive sampling effort. For streams with deep sediments, the same depth cutoff (e. g., 10 cm) that was used in sampling of FBOM mass should be used here, except that for sampling 15N it is best to obtain a depth profile of 15N by collecting individual samples from several depths (e. g., top mm, top cm minus top mm, 1-5 cm, 5-10 cm, etc.). Each sample should consist of about 10 mg of dry material.

CBOM (> 1 mm) -Collect by removing appropriate size material from the bottom, dry at 60 o C, grind, and place ground material into a scintillation vial. On some sampling dates it would be best to collect separate samples of different types of material if they are important contributors of CBOM (e. g., woody material, leaf detritus). Need about 10 mg of dry material.

Invertebrates -Sample all common taxa and place at least 5 individuals of each taxa in separate scintillation vials. Concentrate on most common taxon from each functional feeding group which occur throughout the reach in order to get a complete transect (you may wish to collect representatives of a more than one taxon from a particular feeding group on days 7, 21, and 42 if more than one taxon is an important contributor to that feeding group). Also, try to get a complete transect of the dominant organism in each compartment (e. g., grazer, shredder, filterer, predators). Back in the lab, remove the organisms from the vials, rinse the vials, and add filtered stream water. Place the organisms back into their vials and allow to sit overnight for gut clearance so that the measured 15N will not reflect unassimilated food particles in the gut. Then dry (60 o C), grind and place in a scintillation vial or place in alcohol for identification. Need about 1 mg of dry material per sample. **Vertebrates** -It is probably best to focus on the youngest age class of salamanders or fish because it is more likely that they will pick up measurable 15N during the study than adults. Depletion may be a problem in many

streams, so selection of sampling dates and stations will be important. It might be best to choose one central station to sample from on most dates, and then only do the complete longitudinal survey on the last date (and even then maybe not at all stations). Also, it might be a good idea to discuss with Peterson whether its best to send dissected parts or whole organisms for analysis, given the particular situation at your site. **Microbial N in FBOM and CBOM (optional: not part of 15 compartment sampling)** -It is the microbial N pool (living biomass and extracellular material produced by microbes) associated with detritus that will likely be cycling most rapidly and most available to detritivores. A portion of the N associated with detritus is likely to be refractory (not cycling with the water pools and not readily available to detritivores) and thus will not become labeled by the 15N addition. Thus, our measures of 15N in bulk FBOM and CBOM material will probably be lower than that in the detrital N pool actively cycling and being transferred through the food web. To separate and measure the 15N content of microbial N pools associated with FBOM and CBOM we will try the chloroform fumigation technique to extract microbial N and then analyze the extracted N for 15N and total N. This will be a rather time consuming process, so it would be best to do it on FBOM and/ or CBOM from only some stations and only on some dates. The following schedule is suggested: -day 7: collect samples of FBOM and CBOM (pooling material from several locations) at one station above dripper (background) and 1-2 of the upper stations below dripper (2 replicates from each station and each material type). -day 42 (or slightly before): collect samples of FBOM or CBOM (whichever is most important at the site) at one station above dripper and about 6-7 of the stations below the dripper (one rep. per station, except 2 reps. at station above and upper station below dripper). For the other material type (FBOM or CBOM) collect samples at one station above dripper and 1-2 stations below dripper (2 reps.). -day 3 post N15 drip: collect samples of CBOM and FBOM at one station above dripper and 1-2 of the upper stations below dripper (2 reps. per station per material type).

Detailed methods for sample processing are given in Appendix B. We are generally using the chloroform fumigation method for soils (we should use the fumigation and extraction after pre-extraction method). Basically, it involves doing a 30-min pre-extraction of adsorbed pools of NH 4 using 0.05 M K 2 SO 4 , then fumigating with chloroform in the dark at 25 o C for 24 hours to kill and lyse microbes, and finally extracting the microbial N using a somewhat more concentrated K 2 SO 4 solution (0.5 M). For each fumigated sample there is a replicate non-fumigated sample that is also extracted with the 0.5 M K 2 SO 4 to correct for any extraction of non-microbial pools by the K 2 SO 4 solution. The final K 2 SO 4 extracts are then be split in two portions with one aliquot analyzed for total soluble N using either UV or persulfate digestion followed by DIN analysis (to obtain total N in this pool). The other aliquot will be used for N15 analysis (we're not sure yet whether this will be done at Woods Hole or at Univ. of Georgia). About 25 g of soil (dry wt) normally used for this, but we can probably get by with about 2-5 g (dry wt) of FBOM or 1-2 g of CBOM for this procedure. We probably need to do this only on the surface pool of FBOM.

Additional notes: For samples being collected on glass fiber filters, **use pre-combusted Whatman GFF filters of the 25 mm diameter size.** This size fits easily into a scintillation vial and is small enough to fit into the boats used in the automated 15N analysis instrumentation. Use glass scintillation vials to hold samples (not plastic which can mess up 13C analyses). All samples in scintillation vials should be dried completely (24-48 hours may be long enough for most samples) at 60 o C. Once dry, cap the vials for storage and shipment to MBL. **Make sure all vials are labeled with stream name, station, compartment, and date.**

Bruce Peterson has also written a little more guidance on sampling strategies that is attached at the end of this set of protocols as Appendix D.

Ancillary Measurements During 15N Addition: Water temperature, nutrient concentrations, light -Water temperature is a potential determinant of many biological processes. Therefore, it is

important to have frequent measures of water temperature throughout the N-15 addition experiment. Ideally each site should try to have a high-frequency (15-min to 1 hour frequency) record of water temperature at a middle site in the study reach. It is also critical to have at least weekly measurements of nutrient concentrations at the 15N dripper site, and perhaps several other stations down the study reach during the 15N experiment. These measurements should include NH_4, NO_3, DON, and SRP at a minimum (also dissolved organic P if possible), using standard methods (use UV oxidation with peroxide for DON, or if you want to use an alternative method do some intercomparisons with a site that uses the UV method or make arrangements for another site to do your DON analysis). Each site should also try to get continuous light measurements at the stream surface to help interpret differences in algal uptake. Light will be measured during the whole-stream metabolism measurements, but a record throughout the 6-week 15N addition would be very helpful.

III. Data Handling Each site has primary responsibility for data entry on electronic files and storage. There should be separate data files for the different experiments or measurement types. For uniformity it would probably be best for files (where this is appropriate) to have as the first three columns: date, experimental time (pre, _d, _d-PostDrip), station (UP, or m downstream from injection site). In addition to storage of data at each site, data should also be sent to a central storage location (Webster). However, this data will not be released to anyone outside of the project until we discuss this issue at one of our final project meetings.

APPENDIX A. Protocol for Collecting/ Processing DIN and DON Samples for 15 N Overview: The following document describes the field procedure for collecting and preparing samples for measurement of 15N in NH4, NO3, and DON as part of the Stream Intersite Comparison Study. The field procedures are designed to be as simple as possible. Much of the preparatory work will be done in Woods Hole before and after the samples are collected. The basic method to be used is the ammonium diffusion procedure (Sorensen and Jensen 1991, Sigman et al. in review). 15N-NH4 diffusions will be started immediately after samples are collected, whereas NO3 and DON samples will be shipped to Woods Hole for further processing before starting diffusions. Each site will be provided with materials to collect samples at 7 stations (2 upstream and 5 downstream of the dripper). Samples will be shipped to Woods Hole after collection, and then sampling materials will then be recycled (coolers, water bottles, diffusion bottles) or prepared fresh (filter packs, reagents) and shipped back to the field site. It is important to realize that sample processing for 15N-DIN and DON takes at least 3 weeks, so there will be no feedback during the course of the experiment.

Sampling Design and Schedule: Samples for 15N-DIN and 15N-DON should be collected at 7 stations (2 upstream and 5 downstream of the dripper) on 4 dates (within 12 hours after dripper is started, midway through isotope addition, near end of isotope addition, and within 6 hours after turning off dripper). The first set of samples will be best for measuring NH4 uptake distance (because recycling of 15N will be insignificant in the first few hours) and may show enrichment in the nitrate pool. DON will probably not be enriched on the first sampling date. The second and third sets of samples should show high enrichments in all pools. The primary reason for taking the fourth set of samples soon after the dripper is turned off is to have our best chance of measuring 15N-DON. We are still not sure how well our DON method will work when the ammonium pool is highly enriched. Therefore, by sampling a few hours after the dripper is turned off, the ammonium pool should be minimally enriched whereas the DON pool should still have near maximum signal.

Sample processing for 15N-NH4, NO3, and DON is an expensive and time-consuming process, so we will try to select the most important samples for processing (Table 1). Note, however, that sample collection in the field is identical on all dates. 15N-NH4 will be analyzed at all stations on all dates, but 15N-NO3 and 15N-DON will initially be analyzed at 3 stations. We expect that isotopic signals will be highest in nitrate and DON at the downstream stations. Therefore, we will first look for 15N at

stations 6 and 7 (as well as an upstream control station). If samples from these stations are not enriched, additional stations will not be analyzed. Conversely, if stations 6 and 7 do show strong enrichment in nitrate or DON, additional samples will be run.

Table 1: Sample processing schedule for 15N-NH4, 15N-NO3, and 15N-DON. Numbers refer to stations, with 1 and 2 being controls (upstream of the dripper) and 3-7 being downstream of the dripper, with station 7 farthest downstream.

I. Within 12 hours after initiation of drip stations 1-7 15N-NH4 stations 1, 6, 7 15N-NO3 stations 1, 6, 7 15N-DON

II. Midway through addition stations 1-7 15N-NH4 stations 1, 6, 7 15N-NO3 stations 1, 6, 7 15N-DON

III. Near end of addition stations 1-7 15N-NH4 stations 1, 6, 7 15N-NO3 stations 1, 6, 7 15N-DON

IV. Within 6 hours after ending drip stations 1-7 15N-NH4 stations 1, 6, 7 15N-NO3 stations 1, 6, 7 15N-DON

Sampling Procedure: Materials: Ammonium Diffusion Bottles: 4.3-liter Nalgene bottles Filter Packs: For NH4 diffusions MgO Vials: For NH4 diffusions NaCl and scoop for 200 g aliquots Bottles for NO3/ DON samples Geopump peristaltic pump 14.2 cm diameter filter holder 14.2 cm diameter ashed GFF's batteries for Geopump Procedures: . Using Geopump and 14.2 cm ashed GFF's, filter 4 liters water into appropriately labeled Ammonium Diffusion Bottle . Add 200 g ashed NaCl to Diffusion Bottle (use scoop provided to get approx. 200g) . Cap sample and shake until all salt is dissolved . Add filter pack to bottle. Note: The filter packs contain acidified GFD's. They trap NH3. Therefore, to avoid contamination, it is important to keep contact with air to a minimum. . Immediately after adding filter pack, add pre-measured vial of MgO. . TIGHTLY close diffusion bottle and gently shake to distribute MgO throughout container. . Place bottle in cooler (no ice needed). . Collect an additional 4-liter, filtered sample and place on ice immediately. This sample will be subsampled in Woods Hole for 15NO3 and 15N-DON. Also collect a separate sample for analysis of NH4, NO3, and DON (see note below). . Move to next sampling station and repeat above steps. . NH4 Standard: We will run one standard with each set of ammonium diffusions. The diffusion standard should be started at the same time the ammonium samples are collected. A 4-liter Nalgene bottle containing nanopure water will be shipped with the other supplies. Add 4 mL of the NH4 stock solution (provided) to the 4-liter diffusion bottle then add salt, filter pack, and MgO as detailed above. Important: A separate sample should be collected at each location for nutrient analysis (NH4, NO3, and DON). These analyses should be done on site as soon as possible. Precise ammonium values are needed for final 15N-NH4 calculation (but not required before starting NH4 diffusions in the field). Approximate nitrate concentration (if > 1 &M) is required before processing 15NO3 samples in Woods Hole. If nitrate concentration is < 1 &M (14 &g/ L), precise concentrations are needed before processing samples.

When all samples have been collected, ship coolers to Woods Hole (Bob Holmes, Ecosystems Center, Marine Biological Laboratory, 7 MBL Street, Woods Hole, MA 02543). FEDEX may be the way to go. There will probably be 1 cooler for ammonium samples and 1 cooler for nitrate/ DON samples. In addition, we will probably also provide a few extra bottles/ filter packs/ etc to allow extra samples to be taken if desired. Again, ice nitrate/ DON samples but not ammonium diffusions. Cooler/ bottles/ supplies will be shipped back within a few days and we'll start the process again.

APPENDIX B Microbial 15 N Determination on CBOM and FBOM Rationale: This procedure is intended to extract microbial N pools associated with FBOM and CBOM (maybe leaves and wood

separately) for measurement of 15 N and total N. The protocol uses the chloroform fumigation method (a standard method for soil microbial N analysis) to kill and lyse microbial cells followed by a potassium sulfate extraction of the cytoplasm N released upon cell lysis. Before performing the fumigation step, any adsorbed ammonium is removed by a pre-fumigation extraction with potassium sulfate so that only microbial N is obtained with the post-fumigation extraction.

Detailed method: Collect FBOM and CBOM (leaves and wood separately if both are present consistently) samples from each station by pooling material collected from several locations. Collect only surface FBOM material because this is likely to have the greatest microbial colonization. The sampling schedule and stations were given in the subsection on sampling of compartments (p. 13). For each replicate sample, split into two portions and add material to 120-mL flasks for pre-fumigation extraction (one portion will be fumigated and the other will be a non-fumigated control). Add enough moist FBOM or CBOM to the flasks to ensure that you have at least 1-3 g AFDM in each flask (probably at least 50 g moist weight of FBOM and 10-20 g moist weight CBOM should do it, but be sure to record wet mass added to each flask). Add 60 mL of 0.05 M K 2 SO 4 to each flask for pre-fumigation extraction of any adsorbed pools of ammonium. Place on shaker for 30 min. Remove extract by filtering the extracted sample. It is not necessary to transfer all of the material out of the flask onto the filter because the solid material must be placed back into the flask for the chloroform fumigation. Place filter containing the FBOM or CBOM material back into the same flask from which it came (place the filter on its edge in the flask so that it doesn't cover other material in the flask). To the flasks containing the sample portions to be fumigated, add 3 drops of ethanol-free chloroform (CHCl 3) to the flask. Place the fumigated sample flasks into a desiccator (with top valve) lined with wet tissue paper and a vial of soda lime. After the desiccator is filled with sample flasks, place a beaker containing about 25 mL of ethanol-free chloroform and a few boiling chips in the center of the desiccator. Close the desiccator lid and draw a vacuum on the desiccator until the chloroform has boiled vigorously for 2 min. The desiccator is then incubated in the dark at 25 o C for 24 h. After fumigation, the chloroform is removed by repeated (six-fold) evacuations of the desiccator. The fumigated samples are removed from the desiccator, 60 mL of 0.5 M K 2 SO 4 added to each flask, and the flasks placed on a shaker table for 30 min. to extract the released N. The flasks containing the non-fumigated controls are also placed in a desiccator (but without chloroform) and incubated in the dark at 25 o C for 24 h. The non-fumigated samples are also extracted in 60 mL of 0.5 M K 2 SO 4 , identically to the fumigated samples. After extraction of the fumigated and non-fumigated samples, filter the extract through pre-combusted glass fiber filters and divide the filtrate into two subsamples - one subsample (about 25 mL) for analysis of total N (following either persulfate or UV oxidation) and the other subsample (25 mL) for 15 N analysis. Refrigerate these subsamples until they can be digested.

For the extract subsamples for 15N analysis, we will try to pipet extract aliquots into the small cups used in the mass spec, letting them dry, and then repeating this procedure until we have enough N to get an N15 measurement. This processing will be done at the site of the mass spec (either MBL or Georgia). We are still working out details of this technique. Just make sure to refrigerate the extract samples for N15 analysis and hold them until they can be processed. For each sample, AFDM/ wet mass ratios must be determined on a portion of the wet material in order to be able to compute the AFDM of each sample fumigated (and non-fumigated) and thus compute the microbial N per unit AFDM in each sample, using the difference between total N in fumigated and non-fumigated samples. For more information on this technique I have enclosed a description of the basic fumigation extraction method copied from a text on soil methods (Methods in Applied Soil Microbiology and Biochemistry, edited by Kassem Alef and Paolo Nannipieri, Academic Press, 1995).

APPENDIX C Protocol for quantification of bryophyte cover at stream sites

Purpose: To provide additional quantitative information that will assist in interpretation of results from the NSF Stream N-cycling Comparison Project. In addition, relatively few data on stream bryophyte community characteristics and cover exist, so these data will be valuable, in and of themselves. These procedures will not be applicable at all sites.

Objectives: to provide estimates of bryophyte cover (done by site team) to identify the bryophyte species present in each stream (done by Bowden at UNH) to provide estimates of bryophyte biomass (done by site team) Procedures: Moss "cover" or "abundance" will be estimated by a "point-transect" method (see for example Bowden et al. 1994. Freshwater Biology 32: 445). The only items you will need are:

* a field notebook and pencil, a tape measure and, optionally, stakes to fix the ends of the tape measure
* waders, if the water is deep, a viewscope

* a quadrat, frame or hoop of known area (0.01 to 0.5 m 2), single-edge razor blades, ziplocks or other storage bags

The viewscope can be constructed from any appropriate materials. For example, you can glue a piece of Plexiglas sheet on an 8-10" long piece of 8" OD PVC or Plexiglas tube. You may find the viewscope easier to work with if you put one or more handles on it. A simple hand hole near the top edge of the tube will do. Draw a target point in the center with an indelible marker to mark "the spot". Identify a set of stations that are representative of the experimental stream reach. For the purposes of this project, we are most interested in the bryophytes that are actually in, or would normally be exposed to, water at the time of the 15 N/ whole stream metabolism experiments. Typically mosses are not abundant in pool environments. One strategy might be to simply examine pool environments to confirm that bryophytes are absent, estimate the area of stream that is in pools, as allocate your station assignments to the riffle environments in which the bryophytes are likely to be most abundant. The number of stations you establish will depend on your experimental reach length. Five stations in an reach of 200-250 m ought to suffice; more is better. For each station location chosen, fill out a "Station Environment Data Sheet" (see attached). This sheet is designed to provide correlative information about the general environment of the bryophytes at a given station. Note special conditions. At each station you should do five transects. Begin your transects at the most downstream location at each station. It is most convenient if two persons work the transects, especially if the stream is wide. If the stream is narrow, one person can do the work. If two workers are available, one should sit on the bank with the tape measure and notebook, while the other person wades with the viewscope. Fix or tie the end of the tape to the view scope. The person on the bank then plays out the tape at fixed intervals. The wader keeps the tape taut and identifies *the single dominant cover class* under the target spot in the viewscope at each interval. The wader calls out the cover type for the person on the bank to record. If only one person is available, run a tape across the steam and tie or stake it out so that it can be stretched taut. This is easy to do on narrow streams, but more difficult on wider streams. Wade across the stream and at set intervals identify *the single dominant cover class* under the target spot. Because it is awkward to juggle the viewscope, the field notebook, and the pencil simultaneously, you may want to use an inexpensive pocket tape recorder to record the cover classes "on the fly", then transcribe these notes later. The sampling interval you should use should be about 5 cm for streams less than 3 m wide and 10 cm for streams wider than 3 m. Thus for a stream that is 2 m wide, at a 5 cm sampling interval, you will get 40 points per transect and so for five transects, you will get 200 points per station. If your stream is less the 1 m wide, you will obtain fewer than 100 points for a 5- transect station. In this case, increase the number of transects, to insure at least 100 sample points per station. On the Kuparuk River (15-20 m wide) we get up to 1000 points per station and find that we can do a station in about 30-40 mins. It is critical that you assign only one cover category to a point. With practice you can identify bryophytes and even macroalgae to genera. It is convenient to assign one or

two letter codes to each cover class (e. g. "-" for "bare" rocks, "F" for "Fissidens", "S" or "Scorpidium" , etc.). Then, field notes can be very efficiently recorded by noting the station and transect location, the sampling interval, followed by the cover codes in a simple serial listing (see example Station Field Data Sheet). Note that for this project, "bare" rocks are ones that don't have bryophytes on them. They may, however, have macro-or microalgae. Include both true mosses and liverworts (leafy and thalloid). In most cases, the field personnel will probably *not* be able to identify the mosses. Reference to a simple, popular field guide (e. g. Conard and Redfern, Vitt et el.) or plant text will provide you with some indication of what to look for. If there is no one available who can identify species, then identify what you see as "morphotypes" based on whatever criteria seem to be most distinctive (e. g. color, leaf or stem shape, branching pattern, presentation). These morphotypes can be identified as "unknowns" in the field book with codes such as "U1", "U2", etc. for each different morphotype. It is critical to take a voucher specimen for each new morphotype defined. Take voucher specimens liberally. Voucher specimens should include as many taxonomic clues as possible (i. e. leaves and stems intact, capsules, fruiting bodies, etc.). Be sure to include the entire plant, but you needn't save more than will fit easily in a business envelope. You can either use business envelopes or make a cheap specimen envelop as follows: Take an ordinary sheet of 8.5" x 11" bond paper and fold it three times, as you would for a letter. Open the top fold and fold the left and right edges in 0.5-1". Write a specimen description on the outside of the top fold (the back of the "lid" of the envelope). Your specimen identification should include the stream reach name, the station location information, and the *unique specimen code* (e. g. U1, U2, &#65533;etc.) for each *new* specimen you collect. Insert your wet specimen and close the lid. As soon as possible, set the voucher specimen out to air-dry (oven drying is not necessary or recommended). Once dry, send the specimens and data sheets to: Breck Bowden **or after mid-August 1997** Department of Natural Resources Manaaki Whenua Landcare Research James Hall -UNH Canterbury Agri & Sci Centre Durham, NH 03824 Gerald Street PO Box 69 ATTN: Moss specimens Lincoln, NEW ZEALAND

ATTN: Moss specimens
Take voucher specimens liberally. Even if they end up all being the same species, it is better to have multiple examples of a species than none. Describe the morphotype in your field notes and on the envelope used to save the voucher specimen. Be sure the identify the location from which the voucher specimen was taken.

"Cover" is defined as number of "hits" for a species divided by the total number of points on a transect. The *mean* of the 5 transects is used as the smallest replicable unit for purposes of ANOVA testing. However, the standard deviation *among* the individual transects at a station is also a useful indicator of the variability of cover at a location. It would be convenient if personnel from the individual sites would summarize the data for each cover class, on each transect, for each station (see for example Station Data Summary Sheet). Both the station "Field" and "Summary" data sheets are easy to set up as Excel or QuattroPro spreadsheets. If you do this, please send copies of these files, on disk. In addition, please send a photocopy of your field notes as a backup, just in case. Estimate bryophyte biomass by harvesting all of the biomass from small quadrants (0.01 to 0.5 m 2) in areas with different bryophyte densities (estimated by eye-balled). The harvested biomass should be dried at 65 o C to a constant weight and weighed. A simple regression of dry weight on cover then provides a tool to convert the point quadrat cover data to biomass (adjusted for the portion of the *total* stream area that is actually habitable by bryophytes, e. g. riffles versus pools). This method works reasonable well, but is clearly destructive. It should be sufficient in this project to modify this protocol as follows: Identify an area with "100%" cover (or the maximum cover you ever encounter) and harvest an appropriate area (0.01 to 0.5 m 2). Dry the harvested material to 65 o C, weigh it and use the ratio of the dry weight to the area harvested (in m 2) as the cover-to-biomass conversion factor. If your harvested area was not 100% covered with bryophyte, include a "cover factor", i. e. the *portion*" of the m 2 that was actually covered by bryophytes. It would be good to get 3-5 replicate estimates; more if small

quadrants are used, fewer if large quadrants are used. The cover to biomass conversion factor would then be: CBf = (g dry biomass) / [(quadrat area) * (percent cover in quadrat)] Expected effort: The field work (recording the point transect data and collecting specimens) should take a day or so for 5 stations. It will take more time at first, as to become familiar with the species or morphotypes that are present. The biomass collections could be done at the same time and might take another day to process. The specimen identification work is the really time-consuming part. Unless you are keen to do so, don't bother. Just send you voucher specimens, field data sheets, data summary sheets, files (if you have them), and photocopied field notes to Breck Bowden at UNH or Landcare, as noted above. You may reach Breck at: () 862-4523 (603) 862-4976 breck. bowden@ unh. edu or after mid-August 1997 at +64 3 325 6701 (voice) +64 3 325 2418 (FAX) bowdenb@ landcare. cri. nz

APPENDIX D SAMPLING STRATEGIES FOR PRODUCERS, PREDATORS AND RARE CONSUMERS

There will be taxa that can not be sampled repeatedly at several sites without depletion. In these cases it is wise to consider carefully how many individuals are available and when they should best be sampled. The best strategy for collecting the optimum isotope tracer data will vary depending on the species. In the case of primary producers, rocks with moss tuft can be moved into strategic locations in your reach, assuring sufficient material for successive sampling from each site. For epilithon, artificial or natural but precolonized substrates can be situated to give an optimum sample number and spacing. We have used small unglazed ceramic tiles. Sampling predators that are relatively rare puts a premium on each individual taken. For these taxa, consider collecting your reference samples from a similar reach of the same stream well away from the study site. It may be too disruptive to increase predator abundance by stocking the reach but this might be considered especially in cases where the reason for scarcity is unrelated to food supply. Assuming that you can't import sufficient specimens, consult your model to see when this taxa would be expected to reach its maximum del value. This will probably be near the end of the isotope addition. If stocks permit, sample at times of half the expected maximum and again at the maximum at two sites. This should give the most information from the fewest samples. If only one collection is possible, collect at the end of the 15N addition period. If only a very few individuals can be taken, consider running each specimen separately rather than pooling tissue from several individuals. For example, on the Kuparuk we pool several YOY grayling but dissect each adult fish to assay muscle, liver, gonad , etc. separately. Liver and gonad tissue reach near equilibration with grayling food within a few weeks whereas muscle does not. Blood also equilibrates rapidly and some organisms can be bled or otherwise sampled without sacrificing the organism. If we were doing natural abundances, we could clip fish fins but this won't work for most species for our tracer study. Perhaps someone can develop a muscle tissue biopsy that is non-destructive and would have the great advantage of following isotope changes in the same individual over time. The smallest sample feasible but expensive to run should contain 100 nM N (0.1 uM or about 2 ug). One trick is to pump fish stomachs to assess trophic relationships both visually and isotopically while putting the fish back for resampling later. One bonus is that fish stomachs often contain organisms that are difficult to collect and these can be run for isotopic content to fill out your food web diagram. Because predators and large omnivores are mobile, interpreting their isotopic tracer signals is difficult. In Alaska, we place mesh weirs across the stream to prevent migration between reference and labelled reaches. Knowledge of life history is important because many species have periods of movement and relatively stationary periods. Marking individuals just prior to the study is one way of determining the likelihood of movement during the experiment. Our working assumption has been that adult grayling tagged and released in a pool and later recaptured in the same pool, have been resident during the interval. This tagging approach is time consuming but can yield a lot of additional information. For example, it can provide estimates of abundance and growth helpful for calibrating the box model.

LINX LOTIC INTERSITE NITROGEN EXPERIMENT

Addendum # 2 to LINX Project Protocols (January 1998) Addition of Two Sites for Collection of Water Samples for 15NO3 Analysis

Based on results from the completed N15 addition experiments, it appears that collection of two additional downstream water samples for analysis of N15 in NO3 pools will greatly aid in determining nitrification rate and nitrate uptake length. Preliminary calculations indicate that nitrification rate accounts for nearly 25% of the total ammonium uptake rate in Walker Branch, and estimates of nitrate uptake length using the N15 data are considerably shorter than estimates calculated from the NO3 additions prior to the start of the N15 experiment. Therefore, please collect two additional 4L water samples for 15NO3 at distances 1.5 times and 2 times the distance of the most downstream station on each of the 4 water collection dates (day 0, day 20, day 41, and 12-16 hours post N15 drip). In other words, if your most downstream routine collection station for organisms, etc. is at 200 m below the N15 dripper, then collect these extra water samples at 300 m and 400 m.

LINX LOTIC INTERSITE NITROGEN EXPERIMENT

Addendum to LINX Project Protocols (December 1997) Calculations of NH4 Uptake Length and Uptake Rates

Based on the d15N data and streamwater NH4 concentrations we can calculate uptake length and uptake rates of NH4 according to the following methods.

A. NH4 Uptake Length There are three methods for calculating NH4 uptake length using the 15N data: (1) using the water (d15N= delta 15N) d15N-NH4 data corrected for downstream dilution (increases in flow), (2) using the water 15N-NH4 mass flux data (determined from d15N-NH4 data, NH4 concentrations, and flow), and (3) using the organism (or biomass compartment) d15N data corrected for downstream dilution. Each of these methods involves the same calculation: **a regression of the natural log of the 15N value (corrected for background, and corrected for dilution if d15N data are used) against distance below the 15N dripper (in meters).** The slope of this regression is the distance-normalized NH4 uptake rate and the inverse of the slope is the NH4 uptake length. It is also a good idea to compute the 95% confidence interval for the uptake length using the regression statistics for the slope. See below for details for each method.

The uptake length obtained using the water 15N data (methods 1 and 2) is an instantaneous measure for the time the water samples were collected. The water 15N mass flux method (method 2) should be the most accurate approach; however, it requires good measures of NH4-N concentration at each station where water samples for 15N are collected. This may be difficult in streams with very low (or undetectable) concentrations of NH4. The water d15N-NH4 data can be used directly to calculate uptake length (method 1) assuming that there are minimal changes in streamwater NH4 concentration over distance within the experimental reach (i. e., assumes that regeneration of NH4 plus lateral inputs approximately balance uptake). This may be the situation in many N limited streams (e. g., streams with very low or undetectable NH4-N concentrations; it was the case in Walker Branch and Upper Ball Creek). If so, then the two water 15N data methods should give similar uptake length values. Finally, the two water 15N data methods may overestimate NH4 uptake length on days 20 and 41 slightly due to regeneration of some 15N back to water within the study reach (the uptake length calculation assumes that the tracer flux is changed only by uptake as a parcel of water moves downstream). Likely, there will be some regeneration of 15N back to water at least by day 20. We can correct for this on day 41 by subtracting the background-corrected 15N flux or d15N values determined from the post 12 hour water samples at each station from the day 41 values prior to doing the regressions of ln(15N flux or d15N) vs. distance. We can also apply this correction to the day 20 water data, although this is likely to be an over-correction because the amount of 15N in the reach will be lower than at post 12 hours.

The uptake length calculated using the organism or biomass compartment 15N data (method 3) is a cumulative uptake length over the period from the beginning of the 15N addition to the time when samples were collected (it is not a time-linear average but rather is weighted somewhat toward more recent periods). The organism/ biomass compartment method also may be an overestimate of NH4 uptake length if there is substantial regeneration back to water of 15N taken up for the same reason the water methods overestimate in this situation (i. e., the water d15N values at the downstream stations are somewhat greater than they would be if only NH4 uptake were occurring). This may be a problem in the latter stages of the 15N addition period; thus, we expect this method to give good results

probably only for day 7. The organism/ biomass compartment method may also give poor results if there is considerable movement of organisms/ materials within the stream such that a sample from any one location may not be representative of materials that have remained at that location. This would probably be evident in a poor r2 for the regression. It is likely to be more of a problem for highly mobile organisms or highly transportable materials (e. g., FBOM, leaves). Further, the organism/ biomass compartment method may give poor results for organisms that are feeding on several different food sources that take up 15N at different rates. We would expect more accurate results for primary consumers with a narrow feeding strategy than for omnivores or organisms at higher trophic levels. In summary, we expect that the organism/ biomass method for calculating uptake length will give results similar to the water 15N data methods only early in the 15N addition period (within the first week or so) as long as the stream flow and NH4 concentrations remain roughly constant within that period. In such cases, we expect uptake lengths computed using water 15N data on day 0 and using organism/ biomass 15N data on day 7 to be similar.

Method 1 -Uptake length calculation using streamwater d15N-NH4 data. To calculate uptake length using the streamwater d15N-NH4 data, the d15N-NH4 values at each station below the dripper are corrected for background (by subtracting the water d15N-NH4 value at the station above the dripper) and corrected for dilution (by multiplying the background-corrected d15N-NH4 values by the ratio of flow at that station to flow at the uppermost station below the 15N dripper). Uptake length is then computed as the inverse of the slope of the regression of Ln (background-and dilution-corrected d15N-NH4 values vs. distance downstream from the dripper).

Method 2 -Calculation of uptake length using streamwater 15N mass flux data. To calculate NH4 uptake length using the 15N mass flux data a few more computations are involved. The mass concentration of 15N-NH4 tracer in water at each station is calculated from the water d15N-NH4 values and NH4-N concentrations ([NH4], in ⍰gN/ L) at station I as follows:

([NH4] i * 0.003663) * (d15Nb-c/ I /1000) (1)

where d15Nb-c/ I is the background-corrected value in water at station I (note that the dilution correction to d15Ni is not necessary here). The 15N mass flux at each site I is then computed by multiplying by the streamflow at site I (Qi) as follows:

([NH4] i * 0.003663) * (d15Nb-c/ I /1000) * Qi (2) Uptake length is then calculated as the inverse of the slope of the regression of Ln (mass 15N flux at station I, as given by equation 2) vs. distance downstream from the dripper.

Method 3 -Calculation of uptake length using organism/ biomass d15N values. Calculation of NH4 uptake length using the organism/ biomass d15N values at each station below the dripper is essentially the same as using the water d15N values. It involves first correcting for the background 15N by subtracting the d15N values for that compartment at the station upstream from the dripper and then correcting for dilution by multiplying by the ratio of flow at that station to flow at the uppermost stations below the dripper. Then, uptake length is calculated as the inverse of the slope of the regression of Ln (background-and dilution-corrected d15N value at station I) vs. distance downstream from the dripper.

B. NH4 Uptake Rates We can compute two types of NH4 uptake rates from the 15N data: (1) whole-stream uptake rate, and (2) compartment-specific uptake rate (for each of the primary uptake compartments, i. e., epilithon, filamentous algae, bryophytes, CBOM-leaves, CBOM-wood, FBOM, etc.). Both the whole-stream and compartment-specific uptake rates are computed in terms of N mass area-1 time-1 (e. g., ⍰gN m-2 s-1).

Whole-stream NH4 uptake rate. The whole-stream uptake rate is computed from the NH4 uptake length, streamwater NH4 flux (F, computed as streamflow in L/ s * stream NH4 concentration in ‰gN/ L), and average stream width (w, wetted width in m) according the following equation from Newbold et al. (1981, Can. J. Fish. Aquat. Sci. 38: 860-863):

NH4 Uptake Rate (‰gN m-2 s-1) = (F)/(Uptake Length* w) (3) This is the total NH4 uptake rate (rate of removal of NH4 from stream water) by all stream compartments.

Compartment-specific NH4 uptake rate. Uptake rates of NH4 by individual stream compartments can be computed using the biomass d15N values and the streamwater d15N-NH4 values at one of the stations near the dripper (but one that you‰re sure is well-mixed, we used the 10m station in Walker Branch) from the early period of the release (e. g., day 7 biomass samples and day 0 water samples), and

the data on total N standing stock in that compartment per unit streambed area (TN/ m2, in gN/ m2). The compartment TN standing stock values can be computed from the biomass standing stock measurements that were made at the beginning of the experiment (converted to dry mass per m2 using AFDM/ dry mass conversions) and the %N content (% of dry mass) obtained from the C/ N analyses done on subsamples of each compartment from this initial sampling (in most cases done at Univ. of Georgia). The compartment-specific NH4 uptake rates can be computed as the mass rate of 15N uptake (background-corrected) per unit area at site I times 1/(15N/ 14N) ratio in water (also background-corrected) at site I. Using the compartment d15N values measured on day 7 (background-corrected d15Nbiomass values) and the streamwater d15N-NH4 values (also background-corrected) measured on day 0 (and assuming this is a good average for the period from day 0 to day 7), we can estimate NH4 uptake rates (gN m-2 d-1) into each compartment as (pay close attention to parentheses and brackets):

NH4 Uptake Rate (‰gN m-2 d-1) = [(((d15Nbiomass/ 1000)* 0.003663)*(TN/ m2))/ 7] * [1/((d15Nwater/ 1000)* 0.003663)] (4)

Actually, equation 4 gives only the 14NH4 uptake rate, but this is approximately equal to the total NH4 uptake rate (14N+ 15N) into that compartment because 15N is less than 1% of 14N+ 15N. Also, equation 4 will generally underestimate NH4 uptake rate by each compartment because we must assume that there has been no recycling of 15N (release back to the water) up to day 7 from that compartment. We know that this is not really true, but this is the best we can do. The underestimation will be greater for biomass compartments with rapid N turnover rates (e. g., epilithon), as evidenced by d15N values that approach isotopic steady state (asymptote values) earlier in the experiment, than for biomass compartments with slower N turnover rates (e. g., bryophytes in the Walker Branch study). This is why we can only use this method to estimate compartment-specific NH4 uptake rates early in the experiment.

The compartment-specific mass uptake rates for all major primary uptake compartments can then be summed to give an approximate whole-stream uptake rate that can be compared with the whole-stream uptake rate computed from the uptake length data (method 1, equation 3). Almost certainly the summed compartment-specific uptake rates will be lower than the whole-stream rate from the uptake lengths for two reasons: (1) there may be compartments involved in uptake that were not sampled, and (2) the underestimation of the true compartment-specific uptake rates because of the assumption of no 15N recycling up to day 7 (see above). For some compartments with relatively slow N turnover rates this might be a reasonable approximation in the first few days or so of the 15N addition. This can be verified for different compartments by plots of their d15N values over time. If a particular compartment is near d15N steady state at the time of sampling (flattening of its d15N vs time curve),

its NH4 uptake rate is likely

highly underestimated. In Walker Branch, the sum of all compartment-specific NH4 uptake rates was only a little over 1/ 3 the whole-stream NH4 uptake rate computed using the uptake length and NH4 flux (see above). Nonetheless, the biomass-specific calculations give a rough indication of relative importance of different compartments in NH4 uptake (although remember that uptake rates for compartments closer to isotopic steady state are underestimated more than compartments farther from steady state).

There is a second approach for calculating compartment-specific uptake rates that involves determining the N-specific NH4 uptake rate (k, in units of d-1) from the rate of change in background-corrected d15Nbiomass values, assuming first-order tracer kinetics that result in d15Nbiomass values approaching a known asymptote value at a particular station x. Therefore, this approach can only be used when the asymptote d15Nbiomass value for a particular compartment can at a particular station x be determined during the experiment (i. e., the compartment approaches steady state with respect to the 15N tracer). The stream water d15N-NH4 value at a particular station might be used as the estimate of the asymptote d15Nbiomass value for that station if we knew that all the biomass N was actively cycling with the water NH4 pool and if NH4 was the only form of N that was taken up from the water (i. e., no uptake of NO3). But we know that these assumptions are likely not true. Therefore, the asymptote d15Nbiomass value for a particular primary uptake compartment is likely to be considerably lower than the water d15N-NH4 value at that station. However, if we can determine the asymptote for a particular primary uptake compartment from the d15Nbiomass data over time during the experiment at a particular station x (call it (d15Nbiomass) asymptote x), then we can determine the N-specific uptake rate, k (units of d-1), by solving the following equation:

(d15Nbiomass) x, t = (d15Nbiomass) asymptote x * (1 -e-kt) (5) Rearranging, and using the d15Nbiomass data from day 7 gives: k = -(Ln(1-((d15Nbiomass) x /(d15Nbiomass) asymptote x)))/ 7 (6)

Then, once we have the N-specific NH4 uptake rate, k, for each compartment we can compute the the compartment specific NH4 uptake rate (in units of gN m-2 d-1) by multiplying k (in d-1) by the fraction of the compartment that is actively cycling with the water NH4 pool (which is equal to the compartment TN/ m2 value times the ratio (d15Nbiomass) asymptote x / (d15Nwater) x):

k * (TN/ m2)*((d15Nbiomass) asymptote x / (d15Nwater) x)) (7) Finally, it should be emphasized that we are calculating only NH4 uptake rate rates, both on a whole-stream basis and for each compartment. If there is appreciable uptake of NO3 as well, then the total N uptake rate will be greater than the uptake rate of NH4.

C. Caveat Concerning d15N values (15NH4 Blank effect) The purpose of this section is to discuss some potential problems with the measurement of d15N-NH4+ and caution against over-interpretation of this data. We intend to run some tests this winter in order to increase our confidence in the d15N-NH4+ values.

The general d15N-NH4+ protocol we follow is as outlined in Holmes et al (in press, Marine Chemistry). In brief, NH4 is converted to NH3 under basic conditions, and the NH3 is collected on an acid trap which is later run on the Mass Spec. One complication with this method is fractionation which we currently correct for with the standard we run with each transect. Another problem with this technique is blanks, and the magnitude of the problem increases with the level of isotopic enrichment, especially at low NH4 concentrations. Therefore, the "true" del value of ammonium is particularly difficult to estimate near the dripper site where the del values are highest. We currently are not able to

correct for the blank because the exact amount and del value of the blank are not well known.

In each 4L diffusion, the reagents we add introduce extra nitrogen that is not from stream water. Based on very limited data, the reagents (salt and MgO) introduce something like 0.25 ?mol N onto the filter disks for the 4L diffusion sample, even after being ashed (Holmes, pers comm). This is a fairly large amount relative to the total mass of N we recover on the filter in most of these diffusions (range of 0.7 to 2 ?mol/ 4L). The d we get from the mass spectrometer is therefore a combination of the blank d and the sample d. If we assume the d of the blank is near 0 (which is likely), then the sample d?s we get back from the mass spec are lower than the actual d. The problem is more severe for highly enriched samples because the spread between blank and d15N-NH4+ values will be much greater; samples close to the dripper will be relatively more underestimated (Fig. 1). Therefore, any comparisons between the d15N-NH4+ at a given distance downstream and the biota at that same station will have to be made with this potential problem in mind. Obviously, the greater the percent of mass that is derived from a blank the greater the underestimate will be (Fig. 1). We intend to get a better estimate of the blank mass this winter.

As we have shown, the effect the reagent blank might have on absolute d values can be large. However, the effect on the Sw calculation is relatively minor. Based on calculations using hypothetical conditions, the effect of the blank on Sw is likely to be less than 10% (Table 1). In Table 1, we set up a hypothetical transect with a maximum measured d of 500, an uptake length of 50m, and an N-recovery of 0.7 ?mol/ filter (all reasonable estimates from the various experiments run so far). Of this 0.7 ?mol, we varied the blank component equal to 0.1, 0.3 and 0.6 ?mol/ filter (roughly 17%, 50%, and 86% of the N-recovered). We also varied the d15N of the blank equal to 5, 0, and -5 o/ oo. If the blank is 0, there is no effect on the estimate of

Sw because the slope of the natural log transformed data is changed proportionally along the entire transect. If blank d?s are 5 or -5 (a likely range), the error in the Sw calculation is less than 10% if the mass of the blank is less than 50% of the total mass (Table 1). Even if most of the mass on the filter is derived from the blank (which is unlikely), the effect is relatively small. In short, the blank effect on Sw will be minor.

In summary, (1) interpret d15N-NH4+ data cautiously, being aware that the d values of the highly enriched samples may be significantly underestimated. This could significantly affect compartment specific uptake rates, as discussed in this addendum. After running some experiments this winter, we may revise the d15N-NH4+ values reported thus far.

(2) Problem #1 is only a minor annoyance with regard to calculating Sw.