LINX II

STREAM ^{15}N EXPERIMENT PROTOCOLS
Revision 5 – August 10, 2004
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1. INTRODUCTION

The following are the protocols for the LINX II project. They are the minimum requirements needed to determine nitrate uptake rates and lengths, nitrification rates, and denitrification rates as outlined in the proposal. Section 2 describes the rationale for selection of streams to be used in the ^{15}N addition experiments and the protocol for streamside well installation. Section 3 describes the field procedures for the preliminary nitrate injection and the ^{15}N addition experiment. Section 4 describes procedures for a variety of ancillary measurements that will provide data to interpret and extrapolate the ^{15}N experiment results and develop the stream N cycling model. Section 5 is a summary of the various measurements and suggested schedule for completing them. Section 6 discusses data management. Section 7 describes in detail the procedures for processing the various ^{15}N samples (water, biomass). Finally, the procedures and equations for determining uptake lengths and rates are given in Appendix A.

Each regional site group is also encouraged to undertake additional studies and measurements as they see fit to answer additional region-specific questions. In addition, regional groups may wish to coordinate additional studies and measurements across several regions to answer inter-site questions involving a subset of regions. Please notify Pat Mulholland of additional studies and measurements or inter-site analyses as they are planned and so that we can keep a record of these and distribute this information to the entire project team.

2. STREAM SELECTION FOR ^{15}N EXPERIMENTS

2A. Stream selection

Site selection may be the most important thing we do. Reconnaissance for site selection should be done during the same time of year the ^{15}N experiments are expected to be conducted. This will probably require a good deal of map work, field scouting, and some preliminary measurements of discharge and nitrate concentration. Selection of streams for the experimental ^{15}N additions is scheduled for 2002. Site selection and data needs for the large basin studies are provided in the Landscape Analysis protocols.

For the ^{15}N addition experiments at each site we need 9 streams in the same general biome and physiographic province (e.g., similar slope, etc.), although they don't necessarily need to be near one another. Three streams will be references (native vegetation, relatively minimal human impact), three in urban/suburban areas, and three in agricultural areas (but probably not areas with heavy N-fertilizer use). Land use immediately adjacent to and within 1 km upstream of the study reach will define the stream category. Streams within each of the 3 land use categories are not considered to be strict replicates. In fact it is best to select 3 streams within a land use category that include a typical range of specific types within that land use in each region/biome (or typical within the large river basin selected for the modeling work, see Landscape Analysis protocols). For example, 3 urban streams in a particular region might include residential, commercial, and golf course. If a particular type of land use dominates a particular category within a region (e.g., pasture, rangeland within the agriculture category) then 2 or even 3 streams of this type could be selected.

Discharge should be approximately 5 to 50 L/s (but probably could go as high as 100-200 L/s or so for some streams if nitrate concentrations aren't too high), and a study reach chosen of approximately 500 m to 1000 m or longer without large surface tributaries if at all possible (reach length depends on discharge, longer reaches needed for larger discharges). Study reaches of < 500 m may be acceptable for small streams (< 10 L/s) with low nitrate concentrations and/or high uptake rates, but given that nitrate uptake lengths are likely to be long in many streams, reach lengths of 500 m or more are probably needed for many streams. The nitrate concentrations of the study streams will vary with category. We have calculated the isotope budget for each region assuming a discharge of 20 L/s in all streams, nitrate concentrations of 50 ugN/L in reference streams and 500 ugN/L in agriculture and urban streams, and a target ^{15}N enrichment of the nitrate to 20,000 per mil. In other words, each site has an isotope budget for a total nitrate flux of 63,000 ugN/s to achieve an ^{15}N enrichment of 20,000 per mil. Streams should be chosen so as to stay within this constraint, whenever possible. However, for regions with streams with relatively high nitrate concentrations two options are possible: (1) reduce the target ^{15}N enrichment, and (2) transfer some isotope from a site where stream nitrate concentrations are lower. See section 3C for the formula for calculating the isotope requirement per unit nitrate flux to achieve a 20,000 per mil enrichment.

2B. Installation of streamside wells (contact: Maury Valett)

We would like to sample groundwater along the experimental stream reach in order to estimate subsurface inputs of nitrate and the ^{15}N content of these inputs to use in the calculation of total nitrification rate. Ideally, this would involve installing wells that access groundwater entering the stream. Below is a description of how wells could be constructed and installed. However, the calculation of nitrification rate (see Appendix A-3) is not particularly sensitive to the value of $\delta^{15}N$-nitrate in groundwater (within the expected range for groundwater), and if it will be very difficult to install wells to get representative groundwater samples of sufficient volume for $\delta^{15}N$-nitrate analysis, then these could be eliminated. The nitrification rate calculations are somewhat more sensitive to groundwater nitrate concentration, however. Thus, some method of estimating groundwater nitrate concentration entering the experimental reach is desirable.

Structure: We can use 2" PVC wells with cases constructed of Schedule 40 PVC, slip-slip couplers, and caps (or well points). We suggest use of 0.04" (ca. 1 mm) screen (although 0.02" or 0.01" will work). Screen lengths will vary depending on the nature of the near-stream groundwater system and annual variation in water table height.
Construct all wells according to the depth to water (see below) while being sure to leave approximately 30 cm of casing above ground. For all wells, record the lengths of each component (i.e. case, coupler, screen, cap) before installation. Wells bonded with PVC cement should be leached in water for at least one week while replacing water frequently.

Construction and Installation: Once locations are identified, wells should be installed during low flow conditions, but need to be in place at least one month before the ^{15}N experiments (ideally > 3 months before) to allow re-equilibration of groundwater in the vicinity of the well after soil disturbance. Approximately 8 wells should be installed along each stream appropriately spaced to accommodate variation in inflow.

Bore-hole method of installation - Once the location of the well is determined, a 4" bucket auger is used to generate a bore hole through the unsaturated sediments to the water table. Native fill withdrawn from the bore-hole should be kept for later use. Measure the depth to the water table and construct the well with the coupler to be located approximately 15-20 cm beneath ground level and a screen length appropriate to reach 30 cm beneath the water table. Try to extend the bore hole to ca. 30-50 cm beneath the water table. Place the well down the bore hole as far as possible by hand. Drive the well further into the saturated zone by covering the top of the case with a board and striking the board with a sledge hammer until the coupler has reached the appropriate depth (i.e.15-20 cm beneath the ground surface, Figure 2). Fill the annular space around the well with silica sand (#3 size available in 50lb bags) and ensure that the sand is well packed around the screen. Fill to just above the coupler then cap with native fill to within 10 cm of the ground surface. Provide a shallow (i.e. 2-3 cm deep) layer of bentonite (typically purchased as medium size Kwikplug pellets). Apply a few cups of water to the Kwikplug and pack the remaining hole with native sediments. Number the well and cap when done.

Punch and chase method of installation - Insert a 2" metal punch to depth then remove it quickly and place the constructed well into the pilot hole. Punch removal may require use of

pipe wrenches since it may become wedged during insertion. Depending on the stability of the pilot hole, rapid punch removal and well insertion may be critical to ensure proper depth. Wells should be constructed with drive points and installed to the appropriate depth using a board and a sledge hammer. An alternative to the pilot hole approach is to equip the well with a steel drive point and pound it directly into the saturated zone.

Working wells: Wells should be 'worked with brushes and/or bailers. The brush is run up and down the length of the screen to clear fine sediments. Brushing is typically combined with bailing to clean the well. In addition, bailers may be placed into the well and used to create pressure waves by steadily elevating and dropping the bailer within the well. Waves generated in this manner will help clear fine material from the annular space. Wells should be worked on a regular basis to maintain yield quality. Cap the wells after installation to prevent rainwater from entering.

Questions on well construction and installation can be addressed to Maury Valett (mvalett@vt.edu).

Alternative: Please note that there is an alternative to installing wells to collect representative groundwater samples. See second subsection of section 3B below.

3. FIELD NITRATE AND ^{15}N ADDITION EXPERIMENTS

3A. Preliminary nitrate addition to establish sampling locations

Equipment and materials:
Injection pump (battery operated, capable of constant delivery during injection) and tubing
Nitrate and conservative tracer solution (concentrations based on discharge, pump rate, and targets of 100 μgN/L increase for nitrate, 10-15 mgCl/L increase for NaCl or 40 μg/L increase for Br)
Sample bottles (see below)
Field conductivity/temperature meter (or Br-specific electrode if using Br as conservative tracer)

Approximately 1-2 weeks prior to the ^{15}N experiment, a short-term (e.g., 2-6 hours) nitrate and conservative tracer addition is conducted to locate sampling locations and to determine responses to nitrate spikes. **The nitrate concentration increase should be 100 μgN/L for all streams.** Use of the same nitrate concentration increase will enable cross-site comparisons of these data (cross-site comparisons using different concentration increases are problematic because we have shown in the LINX uptake length comparison paper that the overestimation of uptake length using nutrient additions is related to the level of concentration increase, JNABS 21:544-560). However, if the precision of nitrate analysis is too low for streams with high ambient nitrate concentrations (e.g., streams with nitrate concentrations of 1 mgN/L or greater where declines in nitrate of several μgN/L would not be detectable), then use a nitrate increase of 500 μgN/L. But only use this higher concentration increase if you are sure you cannot measure declines using the lower concentration increase.

The nitrate samples collected must be analyzed quickly to be of use in sample location selection. Choose a location for the injection within a good mixing zone; a turbulent restriction is ideal (you may need to do a little channel engineering to achieve this). This will also become the ^{15}N addition point so the choice of injection site that provides good lateral and vertical mixing is critical. Choose the 1st station as close to the injection as possible and yet still be assured of complete mixing (probably at least 20 m downstream from the injection point, maybe longer for larger streams). **It is very important that there be complete mixing of the injection by the 1st sampling station (engineer the stream immediately above this station if necessary).** Choose the other stations based on the expected nitrate decline with distance. Ideally the stations should span a distance over which the nitrate concentration increase (relative to the conservative tracer) has declined to $< 1/2$ of its value at the 1st station (although this may not be possible in many instances and a reach spanning a distance over which the nitrate concentration increase has declined to 3/4 to 2/3 of its value at the 1st station should be sufficient). Collect samples for nitrate and measure conservative tracer concentrations at each station just prior to the nitrate injection (background values) and again after the reach has come to steady state. The conservative tracer concentrations can be determined using a conductivity meter or Br electrode, depending on whether Cl or Br is chosen as the conservative tracer. Calculate the steady state nitrate:conservative tracer concentration ratio at each station after correcting the nitrate and conservative tracer concentrations for the background values (prior to the injection).

3B. Collection of $^{15}NO_3$ samples from wells

To determine groundwater nitrate concentrations and the ^{15}N content of groundwater nitrate, samples for $^{15}NO_3$ and nitrate concentration are collected from each well. These samples can be collected the day before the ^{15}N addition begins, or at the time the PRE stream water samples are collected (if they can be collected rapidly and won't delay the start of the ^{15}N addition). These data will be used to determine nitrification rates (direct nitrification of ammonium in water and coupled mineralization/nitrification). Nitrification rates will be calculated from the isotopic dilution in streamwater $^{15}NO_3$ over the study reach (decline in del$^{15}NO_3$ values with distance downstream from the ^{15}N addition), corrected for groundwater inputs of nitrate and the ^{15}N content of those inputs. Groundwater inputs of nitrate are determined from the increase in discharge over the study reach (determined from the conservative tracer data) multiplied by the average groundwater nitrate concentration. Each well should be pumped (at least 3 well volumes) about 1 hour prior to sampling. A sample of water from each well should then be pumped and filtered. Two filtered samples are needed from each well: (1) a 1 L sample for $^{15}NO_3$ analysis and a small sample (e.g., 50 mL) for nitrate and ammonium analysis (you may also want to do additional chemistry on this sample).

If wells are not installed or if it is not possible to obtain a large enough sample for $\delta^{15}N$-nitrate analysis of groundwater, then try to obtain some samples for determining nitrate concentration of groundwater inputs to the study reach. Alternative approaches for obtaining water samples for nitrate concentration analysis are use of well points driven into riparian areas and sampling of sediment interstitial water in areas of known upwelling (Steve Hamilton has information on this latter approach).

Alternative approach to groundwater sampling (contact: Steve Hamilton):

As mentioned in section 2B, we need to sample groundwater along the experimental stream reach to estimate subsurface inputs of nitrate and, ideally, the ^{15}N content of these inputs (although ^{15}N content is not as critical as NO_3 concentration – see Appendix A-3 bold text for explanation). Installation of PVC wells to collect groundwater lateral to the stream is the standard approach to obtain large samples of groundwater, but it is costly, and the water that is sampled may not reflect that which actually enters the stream due to riparian-zone processes that take place between the wells and the sediment-water interface. This protocol is proposed as a simpler alternative to yield samples of smaller volume that would be adequate for analysis of nitrate and ammonium concentrations, but may not be sufficient for ^{15}N analysis unless the NO_3 concentration is relatively high (we need a minimum of about 25 μg of N in sample to be processed for ^{15}N – note that these samples are NOT spiked with nitrate, see second part of table 2 in section 7B for volume needed). With this alternative approach, many more samples can be obtained in less time, thereby increasing our confidence in the estimation of inflow concentrations.

Steve Hamilton has designed an economical groundwater "sipper" and can make one and send it to you at no cost; **let him know if you would like a sampler** (hamilton@kbs.msu.edu). The device consists of a hollow 3/8" metal tube with an opening cut in the lower 5 cm. This tube is inserted into the streambed to bury the intake about 5-20 cm below the surface. Inside the tube is a stainless-steel fine-mesh fuel filter connected to 1/8" O.D. TFE tubing that leads through the

tube to a 60-mL syringe equipped with a 4-way plastic stopcock. Sediment porewater is slowly extracted via the syringe, discarding the first ~10 mL via the side port of the stopcock, and then withdrawing a sample of ~40 mL. A syringe filter can be attached to the stopcock so the sample can be filtered upon collection. It should be easy to obtain enough sample (e.g., 60 mL) to measure nitrate and ammonium concentrations, and those concentrations should reflect the water entering the stream. Do not withdraw more volume than necessary to avoid pulling streamwater into the sediments.

It is critical that such samples be collected only from areas where groundwater from adjacent uplands is known to be emerging into the stream. Points of high groundwater inflow would best represent the overall groundwater entering the stream, and also such points can more likely be sampled close to the sediment-water interface without drawing in water from above the interface. There are two easy ways to locate such points. One is to look for seeps along the stream edge where inflow is obvious from sloping water surface profiles, but seeps could represent shallow subsurface runoff, their inflow rates can be small, and they are not always present. Another way, which Steve recommends, is to measure the temperature of the sediments to locate points of relatively high inflow. At most times of the year there will be a temperature differential between inflowing groundwater and streamwater; during the summer, the groundwater is generally cooler, reflecting the mean annual temperature of infiltrating waters. Insert the probe deep into an area where you are sure groundwater is discharging to find the expected temperature, then walk along the stream and look for similar temperatures close to the surface of the bed. Be careful not to step close to potential sampling points.

Temperature can easily be measured in subsurface sediments with a penetrating probe and a readout unit. A relatively cheap option is available from Omega Corporation:

Digital Thermometer:
Splash proof, dust proof, Type K thermocouple connection.
PN: HH501AK - $79

Penetration Temperature Probe:
Utility Handle Thermocouple Probe with Conical Tip
PN: KHSS-14G-RSC-24-NP - $53

3C. ^{15}N Addition experiment and water sampling

Equipment and materials (water sampling):
Injection pump (battery operated, capable of constant 20 mL/min delivery for 24 h) and tubing
Injection carboy (30 L capacity)
Note: Rinse thoroughly if using same carboy, pump, and tubing as used for nitrate addition (to avoid any isotopic dilution of ^{15}N solution)
^{15}N injection solution (appropriate $K^{15}NO_3$ and conservative tracer concentration – Cl or Br, depending on streamflow and background Cl conccentration)
Field filtration apparatus (e.g., battery operated Geopump II with standard pumphead and 142 mm polycarbonate filter holder, cost approx. $1000, vendor: Geotech Geopump @ 800-833-7958, www.geotechenv.com).
Filters for filtration apparatus – 140 mm pre-ashed Whatman GFF.
Field conductivity/temperature meter (or Br-specific electrode if using Br)
Sampling bottles (1 L or 0.5 L) and cubitainers (1/2 gal) for $^{15}NO_3$ and DO^{15}N samples (see text)
Sampling bottles (4 L) for $^{15}NH_4$ samples (see text) (suggested vendor: www.usplastic.com, industrial square or round 1 gallon jugs, item #66224 or #66152, $1.12 each)
Small sample bottles (60 mL for TDN, 125 mL or 250 mL for other solutes, HDPE) for chemistry samples (see text)

Note: Equipment and supplies for ^{15}N-gas sampling are listed separately under this subsection below.

Overview. The ^{15}N addition experiment consists of a 24-hour addition of ^{15}N ($K^{15}NO_3$) together with a conservative tracer and sampling of water at various stations and times during the addition. The experiment should **begin at 1 p.m. and continue for 24 hours**. The day the experiment begins is designated as day 1. Based on a pump rate of 20 mL/min, 28.8 L of injection solution is needed for a 24-hour injection (add between 29 and 30 L to injection jug to ensure enough solution to complete 24-h injection – measure total volume accurately). The injection solution should consist of deionized water and a conservative tracer (e.g., NaCl or NaBr) at a concentration needed to achieve the necessary increase for the ambient stream discharge (e.g., Cl increase of about 10-15 mg/L, Br increase of about 100 µg/L or high enough given the precision of the measurement). Because it is difficult to get more than about 300 gNaCl/L into solution, for streams with discharge rates > 10 L/s NaBr should probably be used as the conservative tracer if a pump rate of 20 mL/min and an injection solution volume of 28.8 L is to be used.

Amount of ^{15}N to add. The $K^{15}NO_3$ should be carefully weighed out **(choose a location not in the same room/building in which the water and biomass samples will be processed)**, placed in a vial/bottle, added to the injection solution in the field just prior to the start of the experiment and mixed thoroughly (to make sure the $K^{15}NO_3$ is completely dissolved, you may want to add to the injection solution and shake one day prior to the experiment). The amount of ^{15}N to weigh out for each experiment to achieve a 20,000 per mil enrichment is calculated as:

45.3 g $K^{15}NO_3$ salt per 1000 ugN/s of stream nitrate flux (1)

In other words, if you anticipate a discharge of 10 L/s and a nitrate concentration of 1 mgN/L, then you would weigh out 453 g $K^{15}NO_3$ for that stream (or ½ of this amount to achieve a 10,000 per mil enrichment). Add deionized water to the vial/bottle containing the ^{15}N salt to dissolve it prior to adding it to the injection solution to ensure that it is all transferred. Mix the injection solution very well after adding the ^{15}N solution. It is important to record the mass of $K^{15}NO_3$ salt added to the injection carboy, the initial volume of injectate, and the final volume of injectate (at end of the ^{15}N release) to be able to determine the amount of ^{15}N added during each experiment. **Also, be sure to wear disposable gloves when handling the ^{15}N salt or the ^{15}N injection solution and discard the gloves when finished**. Contamination by ^{15}N is a major concern.

Sampling stations. A total of 6 sampling stations will be chosen along the stream reach downstream from the injection location based on the results of the preliminary nitrate injection experiment. The injection location and 1st station (designated as station 1) are the same as used for the preliminary nitrate injection, assuming there appeared to be good mixing of the injectate at the 1st station (**if not, move station 1 downstream so that good mixing is assured**). The other stations should be located where the background-corrected nitrate:conservative tracer ratio is approximately: 95% (station 2), 85% (station 3), 75% (station 4) , 60% (station 5), and 40-50% (station 6) of that at station 1. Remember, $^{15}NO_3$:conservative tracer ratios are likely to be a good deal lower than those determined for the preliminary nitrate injection. Ideally, we would like to produce a $^{15}NO_3$:conservative tracer ratio at station 6 that is about 20-30% of that at station 1. If the nitrate:conservative tracer ratios from the preliminary injection don't decline to as low as 40-50% within the study reach (and for many streams they probably won't), then adjust the stations accordingly to ensure 6 stations with the last being as far downstream as possible without encountering large (> 25% of discharge) surface water inputs or other problems. It helps to choose sampling locations at points of channel constriction if possible to ensure a well-mixed sample at each station. Once sampling stations are located, take GPS readings for the injection location, station 1, and station 6.

If there is little or no decline with distance in the nitrate:conservative tracer ratio during the preliminary nitrate injection, then sampling stations will have to be located based on other criteria. In such cases, try to establish as long an experimental reach (distance between stations 1 and 6) as feasible. It is best that this reach be long enough to have an average water travel time of at least 1 hour. We need a reach that is long enough to observe at least a 10-20% decline in tracer $^{15}NO_3$ flux to determine at least crude estimates of nitrate uptake length and rates.

For the measurements of ^{15}N-gas, we will add 4 additional sampling stations within the reach: one between the stations 1 and 2 (designated as station 1B), one between stations 2 and 3 (station 2B), one between stations 3 and 4 (station 3B), and one between the stations 4 and 5 (station 4B). This will allow the additional resolution of the longitudinal pattern in ^{15}N-gas needed to estimate denitrification rate. We want to make sure our sampling stations are adequate to characterize the rising limb and the location of the peak in the spatial ^{15}N-gas pattern, thus we have added the additional sampling stations mostly in the upper portions of the study reach. However, for low gradient streams with high concentrations of nitrate and presumably long nitrate uptake lengths, it might be better to add the last extra sampling station between stations 5 and 6 (call it 5B) or downstream of station 6 (call it 6B) rather than between stations 4 and 5.

Streamwater samples. Water samples for a variety of ^{15}N and chemical analyses are collected at each station just prior to, during, and 24, 72 hours, and 1 week after the ^{15}N addition ends as summarized in Table 1. The samples collected prior to the ^{15}N addition (PRE) are to determine background levels. The samples collected 24 hours, 72 hours, and 1 week after the ^{15}N addition ends (POST) are to determine whether there is a rapid regeneration of ^{15}N taken up during the experiment (and in what form). The samples collected during the ^{15}N addition (plateau and diurnal samples) are to determine nitrate uptake length and rates ($^{15}NO_3$ samples), and denitrification rates (^{15}N-gas samples).

During the ^{15}N addition, the sampling schedule is designed to: (1) provide two estimates of nitrate uptake length and rates using the entire longitudinal network of stations and (2) provide an indication of how nitrate uptake length and rates vary over the diurnal cycle using one sampling station. Sampling of the full longitudinal network of stations for all types of samples should occur at about 2 a.m. and again at noon on day 2. The noon sampling is designed to provide a good measure of mid-day nitrate uptake, but it should be completed before termination of the ^{15}N addition. Thus, it is best not to begin this sampling too much before noon and to sample from top to bottom of the reach rapidly (one person could be collecting the solute ^{15}N samples while another person is collecting the ^{15}N-gas samples. The diurnal sampling is conducted every 2 hours at station 4. Station 4 was chosen for the diurnal ^{15}N-solute sampling because $^{15}NO_3$ levels should be substantially reduced at this station relative to station 1 and it is anticipated that the peak in $^{15}N_2$ and $^{15}N_2O$ will occur near this station.

Table 1. Summary of sampling schedule and sample types

Time	Stations	Sample types (reps)
Well sampling:		
Day before	8 streamside wells, 1 rep	$^{15}NO_3$ (1)
Longitudinal sampling of stream:		
Day 1, PRE (prior to 1 pm)	6 stations (10 for ^{15}N-gas)	$^{15}NO_3$ (2), ^{15}N-gas (2), $^{15}NH_4$ (1), $TD^{15}N$ (1)
Day 2, 1st plateau (1 am)	6 stations (10 for ^{15}N-gas)	$^{15}NO_3$ (2), ^{15}N-gas (2)
Day 2, 2nd plateau (noon)	6 stations (10 for ^{15}N-gas & N_2O)	$^{15}NO_3$ (2), ^{15}N-gas (2), N_2O (2)
Day 3, POST 24 h (1 pm)	7 stations (1 up, 6 down) (10 stations for ^{15}N-gas)	$^{15}NO_3$ (2), $^{15}NH_4$ (1), $TD^{15}N$ (1)
Day 5, POST 72 h (1 pm)	2 stations (1 up, station 6)	$^{15}NO_3$ (2), $^{15}NH_4$ (1), $TD^{15}N$ (1)
Day 9, POST 1 wk (1 pm)	2 stations (1 up, station 6)	$^{15}NO_3$ (2), $^{15}NH_4$ (1), $TD^{15}N$ (1)
Diurnal sampling of stream (OPTIONAL):		
Day 1 and 2, every 2 hours (beginning day 1 at 3 pm)	Station 4	$^{15}NO_3$ (1), ^{15}N-gas (2)

The above sampling schedule will then produce the following number of samples of each type per stream (note this doesn't include standards, blanks, etc – see sections 7B and 7C):

$^{15}NO_3$ – 78 samples
$^{15}NH4$ – 17 samples
$TD^{15}N$ – 17 samples
^{15}N-gas – 60 samples

If it is necessary to reduce the sample processing load, the replicate $^{15}NO_3$ samples collected during the longitudinal samplings at all but stations 1 and 5 could be stored (refrigerated or frozen in the case of the $^{15}NO_3$ samples) until it is determined that they are needed. Replicate samples at stations 1 and 5 should be processed regardless because these data are used in the calculation of nitrification rate as well as the other rates.

In summary, each of the above listed sample types consists of the following to be filtered in the field using the geopump:

$^{15}NO_3$: 1 L of filtered water for ^{15}N (for streams with nitrate concentrations > 50 μgN/L smaller sample volumes of 0.5 L or less are enough)
Additional filtered water (separate small bottle) for NO_3 and conservative tracer analysis.
$^{15}NH4$: 4 L of filtered water for ^{15}N (although see text in next subsection)
Additional filtered water (separate small bottle) for NH_4 analysis.
$TD^{15}N$: 1 L of filtered water for ^{15}N (for streams with nitrate concentrations > 100 μgN/L smaller sample volumes of 0.5 L or less are enough).
^{15}N-gas: 13-14 mL of headspace gas injected into Exetainer.
N_2O: 13-14 mL of headspace gas injected into Exetainer.

Note: The additional filtered sample for NO_3, conservative tracer and NH_4 analyses can all be taken out of the same bottle if desired.

The actual volume of filtered sample to be processed for $^{15}NO_3$ and $TD^{15}N$ analysis will depend on the amount of N in the sample and the N pool to be analyzed. These volumes are given in Table 2 in section 7B (processing $^{15}NO_3$ samples) and Table 3 in section 7E (processing $TD^{15}N$ samples) based on the lower and upper N mass requirements for the mass spectrometer analysis.

Collection of stream water samples and filtration in field. It is important to ensure a sufficient number and size of bottles are available for sample collection at the station (cubitainers work well) and to place samples into after filtration. Good "bottle management" is essential here and will help to ensure that the proper samples are collected. For collecting $^{15}NO_3$ samples, a set of 1-gal cubitainers for each station (or smaller if you only need small volumes due to high nitrate concentrations) and one 1-L collection bottle works well. Use the 1-L bottle to carefully collect water from the stream (being careful to avoid stirring up benthic sediments) and pour into cubitainer. Record time (both experimental time – minutes since beginning of ^{15}N injection, and clock time), water temperature, and conservative tracer concentration (conductivity to 0.1 μS/cm

for Cl, ion-specific electrode for Br). It's best to have field data sheets set up beforehand for this. Cubitainers with samples are then returned to a central location for immediate filtration.

Samples are filtered using a large capacity field filtration apparatus (e.g., battery operated Geopump with large filter holder). When filtering samples, always work in order of stations with increasing ^{15}N and make sure to put enough water through the filter and pump at the start of each sample to ensure complete flushing of previous sample. **For the PRE and POST samples (which will have very low ^{15}N levels) use a separate bottle and set of cubitainers to collect in field (clearly marked) at each station to ensure no ^{15}N contamination of these samples**. Make sure the pump and filtration apparatus are well rinsed (with "uncontaminated" water) prior to filtering the PRE and POST samples. It is recommended that you flush about 0.5 L of a high nitrate solution (or 0.1N HNO_3 solution) through the filter holder and geopump tubing and rinse very well afterward to ensure absolutely no ^{15}N contamination of the filtration apparatus. Because we are using very high ^{15}N enrichment levels, contamination will be a big problem if we do not pay close attention to rinsing and using separate collection bottles for the PRE and POST samples. It is best to have a different set of bottles for each of the three streams studied each year. It is probably possible to reuse sampling bottles, cubitainers, and sample holding bottles for streams the following year, but they need to be rinsed very thoroughly with uncontaminated stream water after each use (and preferably acid washed as well).

For $^{15}NO_3$ samples filter into a 1 L bottle (0.5 L is enough for streams with nitrate concentrations > 50 ug/L) and filter an additional sample into a smaller bottle (e.g., 125 or 250 mL) for nitrate and Cl or Br analysis. We need highly accurate conservative tracer concentration measurements with every $^{15}NO_3$ sample collected and it is best to determine these by ion chromatography rather than rely on the less accurate ion-specific electrode or conductivity measurements made in the field when the sample was collected. The "small bottle sample" can also be used for conservative tracer analysis, for NH_4 analyses for those times when $^{15}NH_4$ samples are collected, and for SRP and TSP analysis for one sampling. Processing of the filtered $^{15}NO_3$ samples is described in section 7B. **Note that the first step in sample processing requires the addition of a nitrate spike to the plateau samples (and for some streams with very low nitrate concentrations a smaller spike to PRE and POST samples as well) and this should be done within 1 day of sample collection (see section 7B).**

For the $^{15}NH_4$ samples, larger sample volumes are needed (4 L, or larger if possible in streams with NH_4 concentrations < 2 μgN/L) because ammonium concentrations are likely to be quite low. However, we only will collect 1 replicate sample from each station. In addition to the bottles needed to store the large volume samples, it is best to have one or two additional large bottles to filter samples into and then transfer back to the storage bottles. If possible, it is best to begin the processing of the $^{15}NH_4$ samples in the field as soon as possible after filtration (see section 7C for $^{15}NH_4$ sample processing details). If it is not possible to begin processing the samples within 1 day of collection, they can be frozen until processing can begin. As indicated above, the sample in the small bottle used for chemical analysis of nitrate is also used for ammonium analysis at the times when $^{15}NH_4$ samples are collected.

If it is not possible to process 4L samples for $^{15}NH_4$ analysis (or if NH_4 concentrations are so low that 4L samples are unlikely to contain at least 20 μg NH_4-N), a NH_4 spike of about 20 to 25 μg NH_4-N can be added to 1 L samples (the minimum N needed for mass spectrometer analysis is 20-25 μg N). However, this will reduce the tracer ^{15}N signal that can be observed. If the spike approach is used, then a similar spike must be added to several deionized water

samples of the same volume and these processed for $^{15}NH_4$ along with the samples. A mixing model similar to that for the $^{15}NO_3$ samples will then be used to calculate actual $^{15}NH_4$ values in the stream prior to using these in other calculations (see Appendix A-1, equation A2 for calculations).

For TD^{15}N samples, collect and filter about 1 L of sample (0.5 L should be enough for streams with NO_3 concentrations > 100 μg/L). This is in addition to the $^{15}NO_3$ and $^{15}NH_4$ samples also collected at these times. Also collect a separate small filtered sample (approx. 60 mL) to be used for TDN analysis. Note that we are collecting samples for TD^{15}N only during the PRE and POST sampling to avoid the high $^{15}NO_3$ values. We will actually be determining the ^{15}N level in total dissolved N (TD^{15}N) and getting dissolved organic N (DO^{15}N) using a mixing model and the ^{15}N values of nitrate, ammonium, and total dissolved nitrogen (see section 7E for TD^{15}N sample processing details).

The filtered streamwater samples for the various ^{15}N analyses should be returned to the lab and refrigerated as soon as possible after field collection (preferably within a few hours). The two sets of filtered plateau samples and the set of diurnal samples for $^{15}NO_3$ analyses will need to be **spiked with nitrate** within a day or two of collection (see Section 7B). They are then stable for at least 3 weeks in the refrigerator. If it is not possible to process the $^{15}NO_3$ samples within 3 weeks of collection, they should be frozen until ready to be processed (but add the nitrate spike before freezing the samples requiring the spike). For streams with very low background NO_3 concentrations (< 25 μgN/L) a small NO_3 spike of up to 25 μgN/L should be added to each PRE and POST $^{15}NO_3$ sample in order to have enough N to get a good mass spectrometry measurement (see section 7B). **If the small NO_3 spike is added to PRE and POST $^{15}NO_3$ samples, the same spike must also be added to the TD^{15}N samples**. But only add the small NO_3 spike to the PRE and POST samples if it is absolutely necessary because this will make it more difficult to observe production of tracer DO^{15}N. Processing of the samples for $^{15}NH_4$ analysis must begin within 1 day of collection or frozen (see Section 7C). The samples for TD^{15}N should be oxidized using persulfate within a couple of days of collection or frozen until oxidation can be performed (see Section 7E). The "small bottle" samples for chemical analysis should be frozen if analyses are not going to be run within a week (see next paragraph for analytical methods). **Remember that analysis of nitrate, ammonium, and TDN concentration is required for all samples for which $^{15}NO_3$, $^{15}NH_4$, and TD^{15}N analysis is to be performed, respectively. Analysis of conservative tracer concentration is also required for all $^{15}NO_3$ samples.**

<u>Collection of ^{15}N-gas samples in field</u>. The samples for ^{15}N-gas (^{15}N in N_2 and N_2O) dissolved in water will be collected in duplicate three times: prior to the ^{15}N release (PRE, morning of day 1) and during each of the plateau samplings (1 am and noon of day 2) at 10 stations over the stream reach**. Remember that you are working in an atmosphere of contamination – minute amounts of air entry present a very big problem with N_2 contamination of these samples so it is critical to follow the sampling protocol precisely** (we have conducted several pilot studies and have developed the sampling protocol based on these results). The sampling technique involves a headspace equilibration of stream water with ultra-high purity helium in the field, with precautions designed to avoid air entry.

Sites sampling at high altitudes will follow a slightly modified protocol (details inserted below) to ensure that gas samples retain positive pressure when they are shipped to lower altitude labs.

If there are spare Exetainers at the end of the experiment (Day 3), use 9 of them to prepare blanks in which 14 mL of the He from the tank gas source used for the headspace is injected directly into each Exetainer from the gas delivery syringe. Use the water bath and syringe needle extender as described below so the blanks will include any contamination during handling. Send three blanks to the isotope lab at UC-Davis and six to Hamilton at Michigan State (MSU) for N_2O concentrations and $^{15}N_2$ measurements.

Supply list and approximate costs (* = recommended to be provided by Steve Hamilton; you will subsequently be billed for reimbursement of consumable costs; see step 1 below):

* Labco 12-mL Exetainers, pre-evacuated (Vial Type 3, Order code 839W/GL), 1 per sample (cost $273 for 1000 from Labco: www.labco.uk.co)
* Corning 50-mL polypropylene centrifuge tubes, plug seal, in racks (Corning no. 430290), 1 per sample (cost $238 for 500 from Fisher Scientific)
* Plastic "jammer" to hold Exetainer underwater inside centrifuge tube (actually just a tubing connector)
* Needle extender made with 15 cm of 1/16" O.D./1/32" I.D. Teflon-FEP tubing (Cole-Parmer 6406-60), a B-D 20G 1½ (305176) needle with its point filed off and a B-D 27G ½ (305109) needle, and a Chemfluor Miniature fluid flow fitting (Mini M Luer adaptor, Part no. 06391-90; available from Cole-Parmer as A-06391-90). Note: Sites may want to construct several of these for simultaneous sample processing.
* Becton-Dickinson general use sterile hypodermic needles, regular wall, regular bevel (only a few of each type will be needed): 27G ½ (B-D 305109) (for injection into Exetainers). Also need another similar narrow, stiff needle for transfer of He from the tank source via a septum to the gas delivery syringe. Gauge is not critical but carry some spares in case of clogging.

Monoject 140-mL polypropylene syringes for the 15N-gas samples (the large volume is desirable to maximize sample size). These are available from various vendors; see for example http://sciencekit.com/category/category.asp?c=477002), approximately $160 for a case of 50. Or alternatively, Becton-Dickinson 60-mL disposable syringes (Cole-Parmer cat. no. A-07940-26), two packs of 30 ($37.50 x 2). Note: Use separate sets of syringes for PRE/POST samples and samples collected during the ^{15}N addition (will require a minimum of 20 syringes for the PRE/POST sampling and at least 20 syringes for sampling during the ^{15}N addition, all equipped with the 1-way stopcocks described below).

60-mL or 30-mL disposable syringes (e.g., B-D brand: Cole-Parmer cat. no. A-07940-26). Need just one for the gas delivery syringe (a 30-mL syringe also works well for this purpose) and 20 for the MSU N_2O samples (the larger 140-mL syringes could be used for this purpose but they are more expensive and the 60-mL syringes work just as well; just be sure to note which size you use).

Polycarbonate 4-way male Luer stopcock (Cole-Parmer cat. no. 30600-03), one pack of 10 ($19)(but only need a couple)

Polycarbonate 1-way male Luer stopcock (Fisher cat. no. 30600-01), one pack of 10 ($17.50)(may need a few more than 10 but 4-way stopcock can do the job as well)

1) Pure helium (e.g., 99.995%) in a field-transportable cylinder, with regulator. A single tank of 60 L or more should suffice for all three experiments in a year.

Flexible tubing extension (50-75 cm of 1/8" OD TFE tubing works well) from the tank source to the water bath, equipped at the end with a Swagelock union (1/8" to ¼"), to which is affixed a cutoff 1-cc tuberculin syringe barrel tip (~1/4" dia.) and a plastic 4-way stopcock

Buckets, Ziplok bags or other container to transport and temporarily store syringes in stream water.

Thermometer to measure water temperature.

Procedure:

(1) Preparation of Exetainers: **We strongly recommend that you ask Steve Hamilton to order and prepare your Exetainers**. He will ensure that they are evacuated to <50 mTorr to minimize contamination, and ship a supply of them to each site in time for the experiments. Each one will be labeled with a code and submerged underwater in a larger 50-mL centrifuge tube to reduce gas leakage, and they should remain this way until the final isotopic or GC analysis. Hamilton will monitor the stability of each batch over time. Sites must contact Hamilton and inform him of their anticipated needs as far in advance as possible. You will be billed by Hamilton only to cover the costs of purchasing Exetainers and centrifuge tubes (including MSU's indirect charge of 49%), which in 2003 was $1.12 for each sample. Hamilton can be reached at hamilton@kbs.msu.edu; his lab technician is David Weed (weedd@msu.edu; phone: 269-671-2218). If you plan to prepare the Exetainers yourself, you will need a high-vacuum line or at least a very good rough vacuum pump, a pressure gauge capable of resolving $P < 0.01$ atm, and a manifold; you should also consult Hamilton for advice gleaned from his pilot studies.

(2) Water sample collection (note: for the ^{15}N-gas samples it is best to use 140-mL syringes to provide higher water:headspace ratio and thus higher gas sample sizes, but 60-mL syringes will suffice if the 140-mL syringes cannot be obtained). Slowly draw about 130 mL (55 mL if using 60-mL syringe) of stream water into a 140-mL plastic syringe (with 1-way stopcock valve but without the needle attached) and expel all bubbles by inverting and tapping on the upward-pointing syringe while expelling almost all of the water (expel the final few mLs underwater). Then slowly draw in about 130 mL of stream water again (pull plunger out slowly when drawing in water) and point it upward, tap on the syringe to remove any bubbles that may still in clinging to the syringe plunger, and expel water to achieve the target volume of 120 mL. Close the stopcock.

(3) Keep syringes containing water samples submerged in a container of stream water while other stations are being sampled to avoid air contamination and keep them at stream

temperatures. It is best to avoid long delays before the next step but if they occur record the time and conditions of storage.

(4) Headspace helium preparation: When all syringe samples have been collected for a particular sampling period, transport the samples to a central location to add the helium headspace gas (keep syringe samples submerged when transporting). **It is critical to avoid air contamination when preparing and adding the headspace gas.** This step must be done underwater by submersing the sample syringe in a large container such as a cooler (ideally at least 50 x 40 x 25 cm for length x width x depth). Using a second 30- or 60-mL "gas delivery syringe" fitted with the 4-way stopcock, obtain 20.0 mL of He from the source tank as follows:

   a. Rig the flexible tubing extender from the source tank so it stays underwater and attach the gas delivery syringe with 4-way stopcock to the needle.

   b. Slowly and briefly open the He tank valve to flush the tubing and stopcock, and immediately attach the gas delivery syringe/stopcock (be careful not to use too much gas – exit of a few bubbles will indicate ample flushing);

   c. Carefully open the tank valve just enough to push about 20 mL of He into the syringe (beware of plunger expulsion!);

   d. Expel this He through the side port of the stopcock;

   e. Repeat this flushing once more, then collect about 25 mL of He, close the stopcock, and remove the gas delivery syringe/stopcock from the He line;

   f. While holding the syringe tip underwater, expel excess He to obtain 20.0 mL at ambient pressure. Keep this syringe underwater for the next step.

   g. HIGH ALTITUDE SITES (Wyoming, Southwest, Oregon): Add 5 mL to above volumes to obtain a final volume of 25.0 mL He.

(5) Transfer of helium to sample syringe: Submerse the sample syringe and tap out any air bubbles from the stopcock. There should be exactly 120 mL of water sample in the 140-mL syringe (HIGH ALTITUDE: 115 mL). Any bubbles that formed during storage should not be expelled. Connect the sample syringe to the gas delivery syringe via the 4-way stopcock. Transfer 20 mL of helium into the sample syringe (HIGH ALTITUDE: 25 mL), simultaneously pushing on the gas syringe and pulling on the sample syringe plunger to reach 140 mL, and then close the 1-way stopcock and remove the sample syringe. Keep the sample syringe submersed as much as possible to avoid air contamination and maintain at stream temperatures (or a stable, known temperature), although brief transfers through air are OK if stopcocks are shut.

(6) Headspace equilibration: The samples are now **vigorously** shaken for 5 minutes to allow equilibration of the dissolved gases in the sample with the headspace helium. This step need not be done underwater. Avoid increasing the temperature of the sample (e.g., avoid gripping the syringes tightly) and do not open the stopcock (slight pressure differentials from ambient will produce fast gas exchange if it is opened). **Record temperature** of gas-liquid equilibration (this will likely be approximately the storage water temperature if samples have remained submerged most of the time since

collection). Also **record the volumes of sample water and gas** during the equilibration. These normally should be 121 and 19 mL, respectively, because there is some dead space in the stopcocks (HIGH ALTITUDE: should be 116 and 24 mL, respectively).

(7) Injection of headspace gas into Exetainer: Attach the flexible needle extender (without the needle on its end) to the stopcock on the sample syringe. Hold the syringe upright pointing down and flush air out of the extender with a little of the sample water. Attach the 27G ½ needle and push just a few more drops of water through it as well (that narrow needle is subject to clogging so avoid passing much water through it). Open the centrifuge tube containing the submersed Exetainer and jam the Exetainer in place with the "jammer" so it is covered with at least 1-2 mm of water. Place the needle under the water in the outer tube and, with the syringe now pointing upward, expel some of the headspace gas (ca. 4-5 mL) to replace the liquid in the needle extender and to leave ~14 mL of gas in the sample syringe (HIGH ALTITUDE: ~19 mL). While maintaining the syringe vertical and the needle underwater, push the needle through the septum into the Exetainer. Inject the remaining volume of headspace gas into the Exetainer from the syringe (we want to inject slightly more gas than the 12.2-mL volume of the Exetainer to ensure a slight positive pressure in the Exetainer, and we need to know the volume that is injected), but take care to avoid injecting the sample water into the Exetainer. Quickly pull the needle out of the Exetainer septum; you should see some bubbles escape as you do that, indicating positive pressure. Close the Exetainer in its centrifuge tube without any air bubbles; this is most easily accomplished in a bucket of water (not ^{15}N-enriched stream water, however). **Record the gas volume injected** if not equal to ~14 mL (actually in that case about 13 mL will end up in the Exetainer and 1 mL in the stopcock and tubing). **Record the Exetainer code and corresponding sample identity** (you should also label the centrifuge tube on the outside if not already done; use a Sharpie marker and leave room for later relabeling).

(8) Storing Exetainers: The Exetainer samples, each submersed in its individual centrifuge tube, can now be stored at room temperature for a few weeks, although it is best to send them off for analysis of stable isotopes and gas concentrations as soon as possible. Keep the racks upside down if large bubbles form that could contact the septa (or, open and reseal them underwater). The isotope analysis laboratories will determine the ^{15}N:^{14}N ratio as well as the mass of N_2 and N_2O in the Exetainer samples (see section 7D). Hamilton will analyze some samples for dissolved N_2O concentrations (see below).

Collection of N_2O samples. To get an estimate of the rate of N_2O production in each of our streams, we will collect **duplicate** samples at each of our longitudinal series of 10 stations at which we collect ^{15}N-gas samples during the final plateau sampling of the ^{15}N experiment (noon of **day 2**). Note that these samples are in addition to the ^{15}N-gas samples described in the subsection above. Dissolved N_2O will be extracted in the field using static headspace equilibration – use the same sampling procedure and precautions as described above for ^{15}N-gas samples, except adjust the liquid volume from 120 to 40 mL (HIGH ALTITUDE: 36 mL) if you use the 60-mL syringes. Because of the potential of even trace amounts of SF_6 to interfere with N_2O analysis, note whether SF_6 has been used in the stream in the days preceding sampling. Samples will be *immediately* shipped to S. Hamilton's lab at KBS where N_2O will be analyzed

within several days by gas chromatography with a ^{63}Ni electron capture detector. An economical, standard 5-day mail or courier service will suffice if samples are shipped immediately. These data hopefully will allow us to calculate N_2O flux from the stream. Shipping address: Steve Hamilton, Kellogg Biological Station, 3700 E. Gull Lake Drive, Hickory Corners, MI 49060-9516; Tel. 269/671-2354 (administrative office).

Field measurements of conservative tracer concentration. Frequent measurements of the conservative tracer concentration during the ^{15}N addition are needed at the most downstream station (station 6) to calculate the average water velocity through the study reach and to apply a transient storage model for estimation of transient storage zone size and exchange rates. If Cl is used as the conservative tracer, they can be made with a conductivity meter that read to 0.1 μS/cm, whereas if Br is used, they can be made in the field with an ion-specific electrode or on samples returned to the lab using ion chromatography. Conservative tracer concentration measurements are made just prior to the injection, throughout the injection, and during the falling limb of the injection. If using a datalogger, then record at about 2 min intervals. If Br measurements are used, then collect samples or make measurements at about 2-min. intervals until plateau is reached, then one each hour until the injection is terminated, then at about 2-min intervals (lengthening the interval with time) until background conductivity is again reached (also record the precise experimental time with each reading). At station 1 we also need measurements prior to the addition, a few times during plateau, and after the injection when background is reached (if conductivity is measured use the same conductivity meter as used at station 6 unless you have carefully inter-calibrated the meters). Finally, make a reading of the injectate conductivity or Br concentration as follows: fill a 1-L bottle with exactly 1 L of streamwater (background), measure background Br or conductivity, add 0.1 mL of the injection solution and mix thoroughly, then remeasure conductivity or Br and record the increase for this 1:10,000 dilution of the injection solution. This information is used with the injection rate and plateau measurements to calculate discharge rate (Q) as follows:

$$Q = [(I/60)(\Delta C_{1:10,000dil})(10)]/\Delta C_{stream} \qquad (2)$$

where I is the injection rate in mL/min, $\Delta C_{1:10,000dil}$ is the Br or conductivity for a 1:10,000 dilution of the injectate (corrected for background conductivity), and ΔC_{stream} is the Br or conductivity of the stream at plateau (corrected for background conductivity). This bottle should also be saved in case there is a need to determine 15N in the injectate.

The time course measurements of the conservative tracer at station 6 will be used to calculate the **average water velocity** over the N15 experiment reach by dividing the total distance between the injection location and station 6 by the elapsed time until the maximum slope of the rise in conservative tracer concentration at station 6. The maximum slope of the conservative tracer concentration increase is used instead of the half-height (1/2 the concentration at the plateau) because it gives a more accurate measure of surface water velocity in streams with large hyporheic zones (the two methods will give very similar travel times for streams with minimal hyporheic zone size). These measurements also will be used to calculate discharge at station 1 and station 6 to determine groundwater input for the model (and for the calculations of nitrification rate).

The time course conductivity (or Br concentration) data are used with a transient storage model to compute the water exchange rates and size of the transient storage zone. Mulholland will send each site a copy of the Hart et al. model and instructions for running it. The Hart et al. model can be run on a PC and uses the conductivity data to iteratively calculate the exchange rate parameters that best fit it. You will need to create a two-column tab-delimited file ("xxx.prn") with the time since the beginning of the 15N addition (in minutes) in the left column and background-corrected conservative tracer concentration in the right column for use as the input file for the model.

Analysis of conservative tracer, NO_3, NH_4, TDN, SRP, TSP, and DOC concentrations in streamwater samples. From the small bottle samples (see subsection Streamwater sample collection and filtration in field) the following analyses must be run. Conservative tracer (Cl or Br) concentrations are determined by ion chromatography. Nitrate concentrations are determined by ion chromatography or automated Cd reduction/azo dye colorimetry (Standard Methods). Ammonium concentrations should be determined by automated phenate colorimtery (Standard Methods) or by the new fluorometric technique (Holmes, R. M., A. Aminot, R. Kerouel, B. A. Hooker, and B. J. Peterson. 1999. A simple and precise method for measuring ammonium in marine and freshwater ecosystems. Can. J. Fish. Aquat. Sci. 56:1801-1808.) on those samples that are to be analyzed for $^{15}NH_4$. The TDN samples (17 per stream taken at times when TD^{15}N samples are collected) are sent frozen (FedEx one day delivery) to Bill McDowell at UNH where he will analyze them using an automated high temperature combustion technique (Shimadzu CN analyzer). Bill will also report DOC concentrations using the same instrument. Soluble reactive phosphorus (SRP) using the standard molybdate procedure and total soluble phosphorus (TSP) using persulfate digestions followed by SRP analysis should be performed on one set of samples (suggest using the PRE set) in order to compute water TN:TP ratios. The persulfate digestion method should be that of Ameel et al. (Ameel, J. J., R. P. Axler, and C. J. Owen. 1993. Persulfate digestion for determination of total nitrogen and phosphorus in low-nutrient waters. Am. Environ. Lab. 10/93:7-11).

3D. Sampling for ^{15}N in suspended particulate organic matter (SPOM)

Field Equipment:
2-L bottles for standing stock samples (if filtering in lab) or field filtration apparatus
1-L bottles for ^{15}N samples

Laboratory Equipment:
Pre-combusted and tared glass fiber filters (Whatman GFF, 47 mm diameter)
Pre-combusted and tared (only need to be tared if going to include entire filter in sample to send to isotope lab – see Section 7A) glass fiber filters (Whatman GFF, 24 mm diameter)
Filtration set-up for 47 mm filters (for filtering standing stock samples in lab)
Filtration set-up for 24 mm filters (for ^{15}N samples)
Oven for drying material (60 C)
Oven for combusting material (500 C)

Samples of suspended particulate organic matter are collected at the same time the water samples are collected at 24 hours (**day 3**), 72 hours (**day 5**), and 1 week (**day 9**) after the ^{15}N addition ends. Two water samples are required, one for standing stock (i.e., concentration; about 2 L or greater, depending on amount of SPOM in water) and the other for ^{15}N analysis (for the ^{15}N sample it might be best to filter sample in field until it clogs to get enough material for isotope analysis). For standing stock, samples of stream water are collected (carefully so as not to stir up material from the stream bottom) from each station and a known volume is filtered through precombusted and tared glass fiber filters (Whatman GFF, 47 mm diameter), dried (60 C), weighed, combusted (500 C) and reweighed for determination of ash-free dry mass per unit volume. For the POST 24 hour sampling (day 3), samples are collected from the 6 downstream stations and 1 upstream station (2 replicate samples for ^{15}N are collected at the upstream station and station 6, one sample from the other stations). For the POST 72 hour (day 5) and 1 week (day 9) sampling, standing stock and ^{15}N samples are collected only from 1 upstream station and station 6, but the ^{15}N sample is collected in replicate from both stations. Samples for ^{15}N are filtered through precombusted glass fiber filters (Whatman GFF, 24 mm diameter), dried (60 C) and placed in labeled scintillation vial and capped tightly for later processing (see section 7A).

3E. Sampling for ^{15}N and %N in detrital benthic organic matter

Field Equipment:
Cylindrical sampling template (about 30 cm diameter, same as used for standing stock measurements – see section 4D)
1 mm mesh net
Sample bags for leaves and wood (7 each for leaves and wood)
Sample bottles/cups for FBOM (14 or more if >1 type of surface FBOM, 100 mL size is sufficient)

Laboratory Equipment:
Pre-combusted and tared (only need to be tared if going to include entire filter in sample to send to isotope lab – see Section 7A) glass fiber filters (Whatman GFF, 24 mm diameter)
Filtration set-up for 24 mm filters (for FBOM samples only)
Oven for drying material (60 C)

Measurements of ^{15}N and %N (these are both from the mass spectrometry lab) will be made on samples of detrital benthic organic matter (BOM), including leaves, wood, and surface and subsurface FBOM, collected at each of the 6 water sampling stations (within about 5-10 m of the station) on the afternoon of **day 3** after all streamwater samples are collected. Samples are also collected at one station upstream from the ^{15}N addition to correct for background ^{15}N values. The detrital BOM measurements are used with measurements of BOM standing stocks (see section 4D) to compute rates of ^{15}N-nitrate uptake associated with each of these stream compartments. The compartment-specific ^{15}N-nitrate uptake measurements will be used with the measurements of total nitrate uptake determined from the longitudinal decline in streamwater $^{15}NO_3$ to do a mass balance on nitrate uptake. Separate samples for ^{15}N are required for each

type of material treated separately in the standing stock measurements. For example, surface FBOM may be divided into 2 categories if it is thought to be very different in character/biomass in different habitat types (such as detritus-rich fine sediments in pools and algae-rich sandy sediments in areas with greater flow). Note that we are sampling fine sediments that are algae-rich as FBOM rather than as epilithon unless the algae forms a mat over the fine sediments in which case we will sample it as filamentous algae (see section 3G).

For the detrital BOM sampling it is best to begin at the most downstream station (station 6) and work upstream because sampling could result in resuspension and downstream transport of material. Collect leaves and wood by hand from each station (collect and combine material from 3-5 locations near station). Collect surface and subsurface FBOM using the cylindrical template as follows. Place the cylinder as deeply into the sediments as possible and remove all coarse material. Collect surface FBOM by swirling water to fully suspend all surface sediments and collect a sample of the suspension in a bottle. Then remove the cylinder and allow the suspended surface material to be flushed downstream (it may be necessary to use your hand to resuspend material). After all the surface material has been flushed downstream, reset the cylinder into the sediments at the same location, agitate sediments as deeply as possible suspending them thoroughly within the water in the cylinder (in most streams it should be possible to agitate and suspend sediments to a depth of about 20 cm), and collect a sample of the suspension in a bottle. Remember, we are interested in the organic matter, so don't worry if some of the inorganic materials resettle before you can collect the sample of the suspension.

In the laboratory, dry the leaves and wood samples (60 C). After drying, place and seal in a plastic bag for later grinding (see Section 7A). For the FBOM samples, filter a separate portion of each FBOM sample through a pre-combusted glass fiber filter (Whatman GFF, 24 mm diameter) making sure there is a thick sediment "cake" on filter. Process samples in order of increasing ^{15}N level (background first, then most downstream station working upstream) in order to minimize possibility for cross contamination of samples. Also process the FBOM material prior to processing the epilithon since the latter is likely to have higher ^{15}N levels. Finally, use a separate filtration device for the upstream samples to avoid potential ^{15}N contamination of these samples. Dry filters (60 C) and set aside in tightly capped scintillation vials (labeled appropriately). Note that we will be getting ^{15}N values per unit dry mass of material sent for analysis (and %N content of dry mass). Therefore, in order to calculate ^{15}N uptake rates, we need measurements of the standing stock of each organic matter compartment in terms of dry mass per unit area of stream bottom (see section 4D).

Finally, if there is a category of BOM that is thought to be important in N uptake in the stream and it is not adequately sampled by the procedures described above (e.g., large wood), a method should be devised to measure its ^{15}N content on samples collected near as many of the 6 water sampling stations downstream as it can be found and at 1 location upstream from the ^{15}N injection.

3F. Sampling for ^{15}N and %N in epilithon

Field Equipment:
Wire brush for scrapping
Container with wide mouth to collect epilithon slurry

Squeeze bottle filled with stream water to slurry scrapings into container
Sample bottles/cups to hold individual epilithon samples (e.g., 100-mL specimen cup)
Plastic bags or bottles for core samples from fine-grained sediment habitats
Sample template for sampling on bedrock (approx. 2-inch or 4-inch diameter PVC connector with neoprene ring attached to bottom end with silicone sealant)

Laboratory Equipment Needed:
Pre-combusted and tared (only need to be tared if going to include entire filter in sample to send to isotope lab – see Section 7A) glass fiber filters (Whatman GFF, 24 mm diameter)
Filtration set-ups for 24 mm filters
Oven for drying material (60 C)

Measurements of ^{15}N and %N will be made on samples of epilithon collected at each of the 6 water sampling stations (within about 5-10 m of the station) on the afternoon of **day 3** after all streamwater samples are collected. Samples are also collected at one station upstream from the ^{15}N addition to correct for background ^{15}N values. It is best to collect these samples at the same time that detrital BOM samples are collected for ^{15}N (section 3E above). The epilithon ^{15}N and %N measurements are used with measurements of epilithon standing stock (see section 4E) to compute rates of ^{15}N-nitrate uptake associated with epilithon.

One epilithon sample of each habitat category having significantly different type or biomass of epilithon (e.g., bedrock, cobble) should be collected at each sampling station. However, it is possible that a particular habitat type is not present within 5-10 m of some sampling station and therefore epilithon samples from all habitat types may not be collected at each station (although make sure epilithon samples from each habitat type are collected from the upstream station, even if it is necessary to go well upstream of the station to find a suitable area). It is best to collect several subsamples from each habitat type at each station and combine these so as to obtain a composite sample of epilithon material from several locations near each station for ^{15}N measurement (e.g., brush several rocks chosen from different locations near each station and combine, or scrape 3 bedrock locations near each station and combine). Collect the samples from each habitat type in the same way the standing stock samples will be collected (see section 4E), but it is not necessary to measure rock areas sampled since we are only trying to get a representative sample of material for ^{15}N analysis.

In the laboratory (< 5 hours after collection), process samples in order of increasing ^{15}N level (background first, then most downstream station working upstream) in order to minimize possibility for cross contamination of samples. Also, process epilithon samples after the FBOM samples have been processed because epilithon material is likely to be much higher in ^{15}N content than FBOM. Finally, use a separate filtration device for the upstream samples to avoid potential ^{15}N contamination of these samples. Mix the epilithon sample thoroughly and filter a portion through a pre-combusted glass fiber filter (Whatman GFF, 24-mm diameter), dry the material at 60 C, and place into a scintillation vial, label, and cap tightly. Use a large enough subsample of material to get a relatively thick "cake" of material on each filter (but it is not necessary to record volume filtered). Clean the filtration devices thoroughly after completing filtrations of all samples for each stream.

3G. Sampling for ^{15}N and %N in macro-autotrophs

Field Equipment:
Sample bags (e.g., zip-lock plastic bags)

Laboratory Equipment:
Containers for holding material to be dried (e.g., small aluminum pans or trays)
Oven for drying material (60 C)

Measurements of ^{15}N and %N will be made on samples of all important types of macro-autotrophs in the stream (e.g., bryophytes, filamentous algae, macrophytes). These samples are collected at each of the 6 water sampling stations (within about 5-10 m of the station) and one station upstream from the ^{15}N addition on the afternoon of **day 3** after all streamwater samples are collected (best to sample at same time as epilithon ^{15}N sampling). Because we are collecting material about 1 day after termination of the ^{15}N addition, we will largely eliminate the potential for ^{15}N contamination of biomass from inadvertent inclusion of highly enriched stream water clinging to materials. For each type of macro-autotroph, collect and combine material from 3 - 5 locations within 5-10 m of each station. The collected material is returned to the lab and dried (60 C), ground or fragmented, placed in a scintillation vial, labeled and capped tightly, and stored for later processing (see section 7A).

It is important to collect macro-autotrophs for ^{15}N in the same way standing stock will be sampled (see section 4F) so that we can calculate ^{15}N mass balances by simply multiplying the dry mass values per unit area by ^{15}N content for each plant type. In most cases, it is best to collect the entire plant, rather than only new growth. Although this may result in somewhat lower ^{15}N contents for some plant types (by including some senescent or inactive material), it reduces chances that ^{15}N uptake will be underestimated.

3H. Summary of organic matter ^{15}N samples

In summary, the sampling schedule described in sections 3D, 3E, 3F, and 3G above will produce 7 samples for ^{15}N analysis (1 sample of each material type from 1 station upstream and 6 stations downstream of the ^{15}N addition) of the following material types:

Leaves
Wood
FBOM – surface (possibly 2 or more types from different habitat types)
FBOM – subsurface
Epilithon (possibly 2 or more types from different habitat types)
Bryophytes (if present)
Filamentous algae (if present)
Other macrophytes (if present, possibly more than one type)

In addition, there will be 17 SPOM samples for ^{15}N analysis. Finally, each site may want to collect separate samples of key individual species or groups within each of the above categories, but remember to determine standing stock (dry mass) for any type of material that you determine ^{15}N content on separately.

4. ADDITIONAL FIELD MEASUREMENTS

A number of additional measurements must be made in conjunction with the ^{15}N experiment in each study stream. Information on in-channel, riparian, and watershed characteristics will be collected. Measurements of whole-stream metabolism (including reaeration rate), photosynthetically active radiation (PAR), and standing stocks of detrital benthic organic matter (BOM), epilithon, and macro-autotrophs (e.g., bryophytes, filamentous algae, macrophytes) are also needed. Sediment samples for denitrification assays will be collected and sent to Stuart Findlay. N limitation assays will be performed in the 2- to 3-week period prior to the ^{15}N addition experiment. Finally, microscale anoxic zone surveys can be conducted in each stream if personnel are available. The methods for these additional measurements are given in this section.

4A. In-channel, riparian, and watershed characteristics (contacts: Linda Ashkenas, Sherri Johnson, Stan Gregory).

Rationale

The protocols outlined below are designed to try to determine parameters most likely to explain our results from the $^{15}NO_3$ addition. There are three main classes of potential explanatory variables: (1) within-channel structure and complexity; (2) riparian vegetation type and shade cover; and (3) degree of human influence on (1) and (2). Depending on complexity of channel structure and riparian vegetation, ease of access, and degree of detail, we estimate these protocols will take 2 people 4-8 hours to accomplish for each site. A set of field sheets and data summary spreadsheets is included in the excel file "LINX 2 Data spreadsheets_Inchannel and riparian characteristics.Rev1.xls". A page of notes is also included.

General principles

We will be sampling both channel characteristics and riparian vegetation over the entire experimental reach. Total reach length will be based on the results of the short-term uptake releases done prior to the isotope addition. Some of the measurements will be used as general site descriptors and others will be useful for examining N dynamics and scaling from point measurements to the reach.

The general structure of all the in-channel surveys will be a series of transects, perpendicular to the direction of flow. The transect measures will be combined with point samples as needed for each particular site (see below). Transect locations should coincide with water sample sites and major channel habitat unit breaks. Sampling for riparian vegetation will occur at extensions of a reduced number of transects onto right and left banks. Much of the sampling design is based on protocols for the EPA--EMAP, which were developed for wade-able streams in the Western U.S. See Figure 1 for an overall transect schematic.

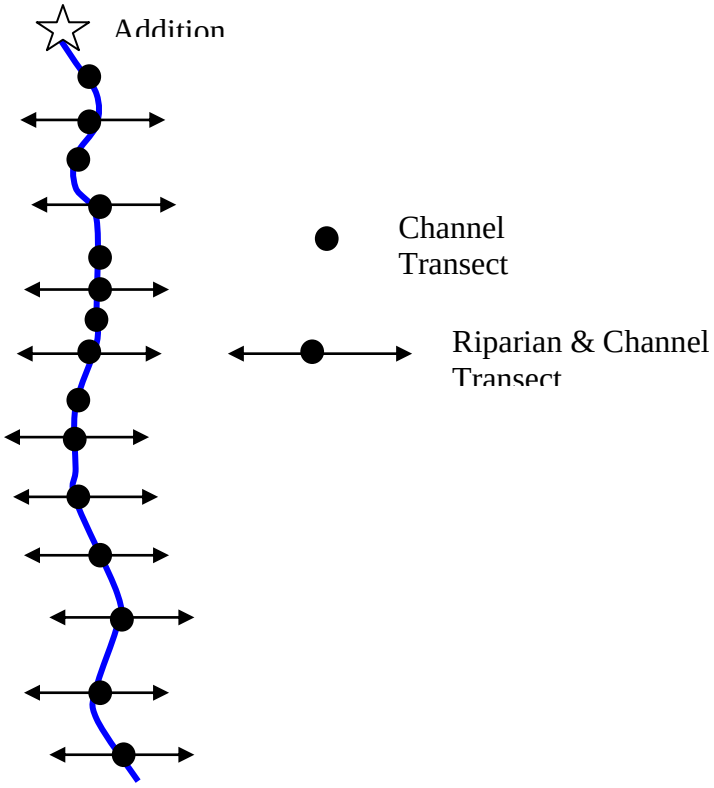


Figure 1. Diagram of possible overall transect layout. Relative position of channel-only vs. riparian and channel transects to be determined on-site.

In-Channel Measurements

Location of site: GPS coordinates taken at the top and bottom of the experimental reach. These locations also correspond to the sites for discharge measurement (see below). Sites with heavy canopy cover or in deep canyons may not be able to obtain accurate GPS readings.

A minimum of fifteen transects should be evenly spaced within the reach at locations representative of general channel conditions (Figure 1). Transect location should coincide with sampling sites and breaks in channel unit type (i.e. transitions from pool to riffle and vice versa). **If you feel your reach needs additional transects, please feel free to add them.**

At each transect, the following will be measured and recorded:
- Distance downstream from ^{15}N addition site (i.e., distance between transects)
- Habitat type (i.e., pool or riffle)
- Wetted channel width
- Width of any emergent bars or islands
- Maximum water depth in the transect
- 10 measurements of depth in each transect. The distance of each depth measurement from left bank (facing upstream) should be noted so cross sectional profiles can be estimated, hydraulic radius and average water depth calculated.

- Diameter of inorganic substrate recorded at each of the 10 water depth points. We will measure diameters with a gravelometer to obtain mean particle size ($30 from USFS, see http://www.stream.fs.fed.us/streamnt/oct97/oct97a5n.html for details on purchase, http://www.stream.fs.fed.us/streamnt/apr96/apr96a1.htm for information on use). These numbers will provide us with 150 measurements of substrate size, from which we can derive D_{80} and D_{50} estimates. (Note that these same points can be used for the bryophyte abundance).
- Densiometer readings (see Appendix for methods and sources) from the center of the channel (facing upstream, downstream, left bank, right bank).
- Line transect for wood (see method below)
- Bank slope and incision. Measure the difference in height between the water level and points in the riparian area, 2.5 m and 5m away from stream edge on each bank (see Figure 2).

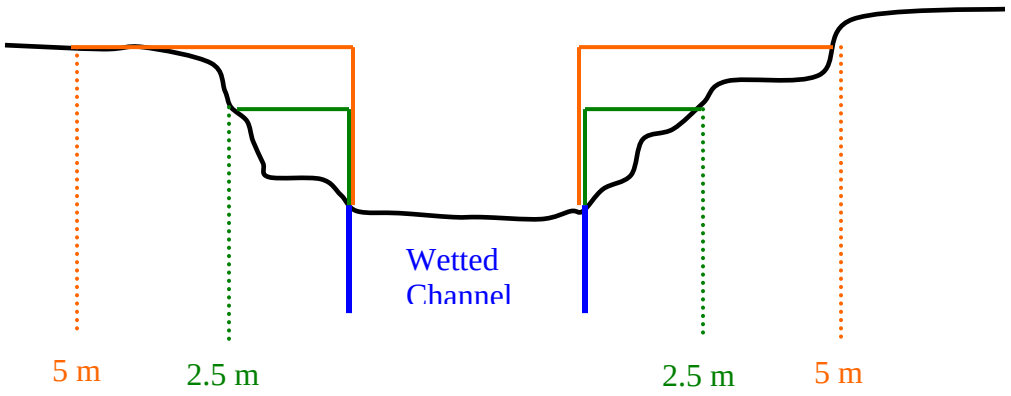


Figure 2. Bank slope measurements.

Between each transect the following should be measured or estimated:
- Beginning and end of major channel unit types (pools/tranquil units, riffle/broken water surface, steps or abrupt changes in elevation) should be noted as a distance downstream from addition site.
- Location of wetted wood accumulations. Measure distance downstream and estimate number of pieces. An accumulation is defined as having 5 or more pieces of large wood (minimum diameter of 5 -10 cm and 1 m length) that touch one another. The definition of "large wood" may vary with site-potential vegetation and stream size.
- Gradient – change in elevation is valuable for both the intensive and extensive reaches. However, this measurement may be time consuming, and perhaps each site should decide whether to measure it. Clinometers give poor measurements in low gradient streams. In such systems, string and line levels or true surveying could be used to note change in elevation between transect.
- Condition of bank, extending 5 m from water edge. The percent cover of each bank between transects in the intensive reach should be categorized as follows: Banks should be classified initially as vegetated (>25% cover) or unvegetated (<25% cover). They should then be categorized as either natural or anthropogenically-modified; within these

categories, the bank may be stable (vegetation covered or non-erosive), unstable (eroding and or bare soil) or hardened (concrete, riprap or bedrock). Total percent cover for a given bank may not exceed 100% (See Figure 3 for a matrix).

Figure 3. Matrix for classification of percent bank cover.

	VEGETATED		UNVEGETATED	
	NATURAL	MODIFED	NATURAL	MODIFIED
STABLE				
UNSTABLE				
HARDENED				

Wood measurements should be made only in the wetted channel (this is the portion most likely to have some type of biofilm actively processing nitrate). We suggest using the string transect method described in Wallace & Benke 1984 (Quantification of wood habitat in subtropical coastal plain streams, *CJFAS* 41:1643-1652) or Swanson et al 1984 (Organic debris in small streams GTR PNW166; see also Van Wagner 1968, *For. Sci.* 14:20-26). These string transects should be conducted at each of the channel measurement transect sites AND through each of the major accumulations, if present. At each transect, caliper measurements of wood diameters are made ONLY for the wood actually in the wetted channel. These measures can then be converted to surface area.

Locations of anthropogenic channel influences between each transect should be noted. These features include factors such as bridge pilings, appliance dams, slumps (number and estimated dimension), buildings, withdrawal pipes, outfall pipes, etc. Discharge shouldl be measured at the upstream and downstream ends of the experimental reach.

Discharge calculations require 10 water depth and velocity measurements; these may be obtained in conjunction with the in-channel transect measurements. Flow meters should be used to determine velocity. The distance of each measurement from the left (looking upstream) wetted edge of the stream should also be recorded. We recommend taking these measures as a backup to the chloride releases, particularly in ungaged streams.

It is also suggested that you obtain the following: (1) A sketch map of each study reach; for each site. Sketch maps can include channel configuration, location of important natural or anthropogenic features (debris dams, irrigation pipes, patches of emergent vegetation, location of nitrogen-fixing plants, sampling sites, etc.). (2) Aerial photos (if they exist). Study sites on federal lands (USFS, BLM) and often on state lands should have aerial photos available for some time within the past 5 years. Agricultural lands are usually photographed annually by county by the USDA/AREC/NRCS to determine which crops were planted (for subsidy payments, etc). Many large municipal areas also have aerial photos available. There are also on-line sites where these can be viewed and/or downloaded (e.g. TerraServer).

Riparian Measurements

A minimum of 10 riparian transects will be needed. Each transect will extend 25 m on each side of wetted channel into potential riparian areas (see Figure 4). This distance is the minimum spatial resolution for Landsat TM imagery, which will probably be used in the landscape analysis.

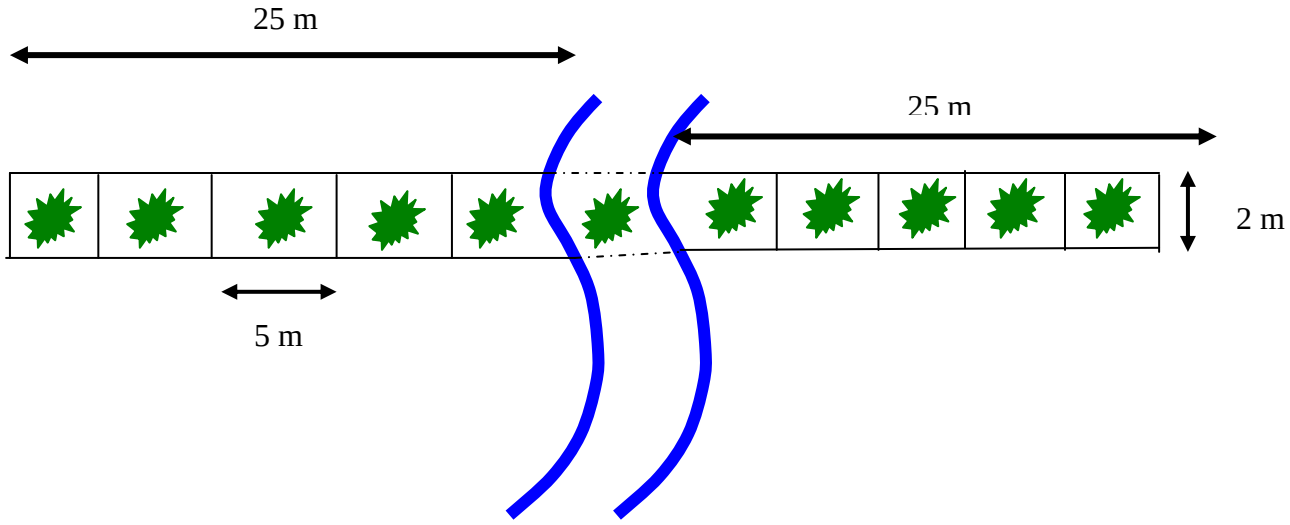


Figure 4. Riparian belt transect detail (not to scale!).

Ground cover – riparian transects for ground cover will be 2 m wide. Percent ground cover will be estimated in each transect for segments 2 m wide and 5 m long (0-5m, 5-10m, etc., into the riparian zone). Possible cover types include bare dirt, concrete, miscellaneous (cows, car bodies, etc.), shrub, grass/forb, nitrogen fixers. The cover types will vary from site to site, but at a minimum should include some estimate of unvegetated (natural vs. concrete), vegetation (shrub, grass/forb), and whether the vegetation is a source of fixed nitrogen (such as clover or alder).

Overstory vegetation and shade - Densiometer measures (see Appendix) will be taken at 4 points (facing upstream, downstream, toward stream, away from stream) within each transect 12.5 m and 25 m from the edge of water on both banks. Type of overstory cover should be noted, such as: conifer, deciduous, buildings, nitrogen fixers. Height of dominant vegetation should be estimated at each transect. If a riparian buffer is present, estimate how far out from the stream it extends, up to a maximum distance of 100 m. This will help quantify buffer widths in human-impacted landscapes.

Timing of Sampling

It will be up to the individual sites to determine when best to take the channel and riparian measurements. Some parameters, such as wetted width, depth and discharge are quite time-sensitive and should be collected as close to the time of the ^{15}N release as possible. However, the transect measurements potentially involve substrate disturbance; sites with fine, easily disturbed sediments may want to take the in-channel measurements 4 or 5 days before or after the ^{15}N addition. More temporally stable parameters such as riparian transects or bank conditions could be sampled earlier or later.

Checklist of Measurements

In-Channel:

15 transects total (minimum). At each transect:
- distance from previous transect
- channel unit type (e.g., pool, riffle)
- wetted channel width
- 10 depths (left to right facing upstream) plus maximum depth
- 10 substrate diameters
- woody debris (string transect method)
- densiometer (from center of channel)
- width of any dry areas within channel (islands, bars, etc.)

Between each transect:
- channel unit lengths (as a function of distance below addition site)
- location of large wood accumulations; if transects do not run through major accumulations, consider doing additional string transects.
- estimates of bank cover and condition

Optional:
- gradient (optional—up to site to decide if needed)
- discharge at 2 locations
- location of anthropogenic channel influences
- sketch map of reach

Riparian Cover & Landuse:

10 transects total. Each transect extends 25 m on BOTH sides of the stream
- estimates of percent ground cover in 2 m x 5 m segments (10 for each transect, 5 on each side)

densiometer at 12.5 and 25 m both sides
type and estimated height of dominant overstory cover
estimate of width of riparian vegetation, if present
estimate of bank slope and incision

Watershed characteristics

An estimate of the catchment area drained by the study stream at the 15N addition point is needed. In addition, estimates of the % of catchment in each of the major land use categories (native vegetation, agricultural, urban/suburban) is needed. More detailed information on the type of land uses is also helpful (e.g., dominant types of native vegetation, type of agriculture, % of urban land that is impervious). Finally, an estimate of the average catchment slope would be valuable if available. The first worksheet in the excel file "LINX 2 Data Spreadsheets_Field 15N addition experiment.xls" is provided for recording the watershed characteristics data.

4B. Whole-stream metabolism and gas exchange rate measurements

Field Equipment:
Dissolved oxygen (DO) sondes (e.g., YSI 6000 series) or Winkler equipment and thermometer
Barometric pressure sensor (or source of information at nearby location)
Propane (or SF6) tank and regulator with flow meter for constant flow addition
Diffuser stone (e.g., Ultra fine bubble diffuser, 12 1/8 x 2 3/8 inches, Cat. No. AS303, cost approx. $150, Aquatic Ecosystems, Inc., 800-422-3939)
Propane sampling equipment (either 60-mL plastic syringes with 3-way stop cock or sampling syringe with needle and freshly evacuated serum vials – 10 or 15 mL size)
Pump and conservative tracer solution (same as used in nitrate injections, see Section 2A)
Conductivity meter for conservative tracer injections (or Br ion electrodes)

Laboratory Equipment:
GC (FID) for measurement of propane or GC(EC) for measurement of SF6

Overview of method. Whole-stream metabolism should be measured during the same dates as the ^{15}N injection (**days 1 and 2**). Metabolism can be measured using the two-station or the one-station method (I recommend the two-station method if you have 2 DO meters). If measurements are to be made using datalogging sondes (e.g., YSI or Hydrolab), record water temperature and dissolved oxygen concentration at 5-min intervals (if using the one-station method, 10- or 15-min intervals are best), beginning the evening before the ^{15}N experiment (about 6 pm on day 0) and continuing until the day after the experiment (about 8 am on day 3). All DO sondes must be calibrated initially and then the calibration should be checked sometime during the measurement period (e.g., afternoon of day 2 while doing salt/propane injection) and again at the end of the measurement period (morning of day 3). A good way to calibrate the DO sondes (or check the calibration) is to let them sit in moist air with the solid cap loosely attached

and partially sitting in the stream water (temperature near that at which the measurements are taken). Make sure to record barometric pressure at the site you are calibrating (you will need this to calibrate the sonde) or checking the calibration.

At the end of the measurement period, download the data immediately so that if there are problems you can re-deploy the sondes within a day or two to ensure a good measurement under conditions similar to those during the ^{15}N experiment. A measure of barometric pressure is needed for each time a measure of DO is recorded during the period. If you do not have a recording barometer that you can place near the stream, you can get these data from a meteorological station nearby but you need to convert values to your site based on the elevation difference between the met. station site and the stream (also note that many regional barometric pressure records are normalized to sea-level and thus the conversion to your stream site is based on the difference between sea-level and the stream elevation). If the DO measurements are to be made manually (using Winkler titrations), take samples at 1-h intervals (30-min intervals would be even better) beginning at midnight prior to the experiment and continuing until midnight on the day the experiment ends (a 48-hour period). Remember to record water temperature and barometric pressure when each DO sample is collected.

The reach over which metabolism is measured will likely be somewhat shorter than that used for the ^{15}N sampling. It would be best to use the upper portion of the ^{15}N reach (beginning at station 1). If using the 2-station method, the downstream station should be chosen to provide a water travel time of about 20-30 minutes between station 1 and this station (for very steep streams, this may need to be shorter). Ideally, the reach should be a length such that about 66 to 75% of the propane added in the gas exchange rate experiments has degassed. For streams like Walker Branch (5-10 L/s, gradient of 0.035 m/m) a reach length of about 100 m works well. If using the one-station method, then choose a station about 100-200 m downstream from station 1.

Discharge, water travel time between stations, and air-water gas exchange rate measurements must also be made in conjunction with the metabolism measurements. The discharge measurements will be made as part of the ^{15}N injection. The water travel time and gas exchange rate measurements are made using a salt/propane injection as described in the next subsection below.

If the one-station method is being used, a measure of average water depth is also needed. Average water depth (D) can be calculated from the discharge (Q), average stream wetted width (W, from measurements made every 1-2 m along the stream reach), and average water velocity (V, calculated from water travel time between stations) as follows:

$$D = Q/(V \times W).$$

The two-station method requires an accurate measure of stream area that can be determined from measurements of wetted width (at 1-2 m intervals along the reach) and reach length.

Air-water gas exchange rates, water travel time, and depth measurements (needed for metabolism calculations). Measurements of air-water gas exchange rates are needed for two reaches: (1) the reach over which the metabolism measurements are being made (station 1 to the downstream dissolved oxygen measurement station or to the only dissolved oxygen measurement station if using the 1-station method), and (2) the entire study reach (station 1 to 6) over which the N_2 and N_2O production rates are being calculated. Short-term (2 – 4 hours) injection of propane (or SF6) and a conservative tracer (Cl or Br) will be used to determine gas exchange rates. There are several alternatives as to when to perform this experiment. If propane

is used, then this experiment should be performed after the conservative tracer from the ^{15}N addition has completely cleared out of the study reach (i.e., values at station 6 are back to background levels). This will likely be too late on day 2 to conduct the experiment so it will probably be necessary to wait until the afternoon of day 3 after water and biomass samples are collected or until the morning of day 4. If SF6 is used, then it could begin at the same time as the ^{15}N addition (1 pm on day 1) using the same injection location and using the conservative tracer in the ^{15}N solution. However, the SF6 injection must be completed within 3-4 hours to allow the reach to be completely flushed of any residual SF6 because SF6 interferes with the N_2O analysis on the 2nd plateau samples. A detailed procedure for the use of SF6 provided by Bob Hall is included as Appendix D.

Propane (or SF6) and a conservative tracer (either NaCl to increase the Cl concentration by about 10-15 mg/L, or NaBr to increase the Br concentration by about 50 ug/L) are injected at the same location as the ^{15}N addition. Measurements of dissolved propane (or SF6) and Cl (either collect samples to analyze for Cl or measure conductivity) or Br (either collect samples to measure by IC, or measure by ion-specific electrode in field) are made at 3 stations: station 1, the lower DO measurement station, and station 6.

The following procedures are for an injection of propane (see Appendix D for procedures for a SF6 injection). The propane injection consists of bubbling in propane (common gas grill tank is fine, but it is best to attach a gas flow regulator to the tank outlet to achieve a constant gas injection rate) through a large gas diffusion stone. The conservative tracer injection involves pumping at a constant rate a concentrated solution of NaCl or NaBr into the stream at the same location as the propane injection. Begin the two injections at the same time.

Background measurements of the conservative tracer (Cl, conductivity, or Br) just prior to the injections are needed at each station to allow calculation of tracer Cl, conductivity increase, or tracer Br by subtracting the background levels from the values measured during the injections. The decline in steady state tracer Cl (or conductivity or Br) between stations is used to correct the downstream propane concentration for dilution due to groundwater input. The average water travel time between stations is computed using the time to reach the mid-point of the maximum slope of the rising limb of the conservative tracer concentration profile between the two stations defining the metabolism reach (if there is a relatively large transient storage zone this approach works better than using the half-height approach). It is a good idea to also collect propane samples (3 per station) prior to the experiment as well, although background propane concentrations are likely negligible.

Propane samples are collected in syringes once steady state is reached (or nearly reached – it is not necessary to wait until complete steady state), being careful not to entrain any air while collecting the samples. Record the water temperature at the time the water samples are collected. Collect 10 samples at each station (more if you feel the variability will be high). The water sample is equilibrated with a headspace gas (air is usually fine) and propane in the headspace gas is measured on a gas chromatograph with a flame ionization detector (GC-FID). It's not necessary to run standards if you know when the propane peak comes off - just record the relative peak areas. Depending on the sample size needed for the GC, for sample collection in the field either: (1) use large syringes (e.g., 60 mL size, collecting 45 mL of streamwater and then adding 15 mL of headspace air – this will provide enough headspace sample to flush and fill a 1-3 mL sample loop on a GC), or (2) use small syringes and inject the sample (about 5-10 mL) into an evacuated vial (vacutainer, about 10 or 15 mL volume) and add headspace air using a

needle afterward (this will provide a headspace sample of 100 – 200 uL that can be easily removed and injected into the GC). In either case, wait to add the headspace air to the syringes or vials until all samples have been collected and you are in a location with no possibility of propane contamination. After adding the headspace to the syringe and closing off the stopcock, agitate gently for about 2 hours at room temperature to equilibrate water with the headspace (or shake vigorously for 5-10 minutes). If you are using the large syringes, it is best to place these on a shaker table for about 2 hours prior to measurement. If you are using the small, evacuated vials, shaking is not necessary since you are injecting water sample into a vacuum and this will promote degassing.

We calculate gas exchange rate in terms of the fractional decline in propane concentration (corrected for dilution due to groundwater input between stations) between two measurement stations (difference between the Ln of the propane concentration at the upstream station minus the Ln of the dilution-corrected propane concentration at the downstream station). The O_2 air-water exchange rate is then calculated as 1.396 times the calculated propane air-water exchange rate**. The N_2 gas exchange rate is calculated as 1.335 times the propane rate and the N_2O gas exchange rate is calculated as 1.308 times the propane rate**. **If SF_6 is used as the tracer gas, then the O_2, N_2, and N_2O conversion factors will be 1.345, 1.285, and 1.258, respectively.** Steve Hamilton can provide details on how these conversion factors were determined. If the one-station method is used to determine metabolism, we need to compute the fractional decline in propane per unit water travel time between the two stations (difference between the Ln of the propane concentration at the upstream station and the Ln of the dilution-corrected propane concentration at the downstream station divided by the water travel time) and convert this rate to an O_2 rate. The water travel time is determined as the elapsed time between the maximum slope of the rise in conservative tracer concentration at the two stations being considered.

In summary, propane samples are collected at 3 stations, and the propane air-water exchange rate is computed for two overlapping reaches: the reach used for the metabolism measurements and the longer reach sampled for ^{15}N measurements. A worksheet for recording data and calculating metabolism using the two station method is given in the excel file "LINX 2 Data Spreadsheets_Field 15N addition experiment.xls" (see section 6). An example from Walker Branch is entered. A separate worksheet for recording data from the propane or SF6 injections to determine gas exchange rates is also provided in the excel file.

4C. PAR measurements

Equipment:
PAR sensor and datalogger (e.g., Li-Cor 1000)

PAR is measured at one representative location either within the stream channel (e.g., on flat rock projecting out of water) or on the stream bank during the ^{15}N addition experiment and metabolism measurements (**days 1 and 2**). Ideally, use a PAR sensor and datalogger (e.g., Li-Cor LI-1000) recording readings every minute or less if possible (or 5-min averages of measurements made every minute or less). Choose a location for monitoring PAR that is representative of the canopy cover (shading) conditions for the stream reach used for the

experiment. Also, record the general weather conditions during the experiment (e.g., clear, partly clear, overcast, etc.).

4D. Detrital benthic organic matter – standing stock measurements

Field Equipment:
Cylindrical sampling template (approx. 30 cm in diameter – e.g., bottomless spaghetti pot)
Sample bags for leaves and wood (approximately 40, 2 per sample)
Sample bottles for FBOM subsamples, approx. 250 mL size (approximately 40, 2 per sample, more if >1 habitat type of surface FBOM)

Laboratory Equipment:
Pre-combusted and tared glass fiber filters (Whatman GFF, 47 mm diameter)
Filtration set-up for 47 mm filters (for FBOM processing)
Oven for drying material (60 C)
Oven for combusting material (500 C)

Measurements of standing stocks of detrital benthic organic matter (BOM) are made on the morning or afternoon of **day 4** (after salt/propane injections are complete). These measurements will be used with the BOM ^{15}N measurements to partition total ^{15}N uptake among the BOM compartments. Because the BOM standing stocks are likely to be substantially different in different types of habitats (channel units), a stream habitat survey must be conducted prior to sampling for standing stocks.

Stream habitat survey. The strategy for determining the standing stock of different organic matter compartments (e.g., CBOM, FBOM, epilithon, etc) in each stream involves first identifying the relative abundance (% of study reach) of the different habitat types (i.e., channel unit types) and then conducting stratified random sampling of these different habitat types for each organic matter compartment. The habitat survey can be conducted as part of the in-channel characteristics measurements described in Section 4A (mapping of channel unit types), preferably before the ^{15}N experiment begins. The number of habitat categories to use in the survey depends on the compartment being measured. Separate habitat categories are needed if the standing stock for a particular compartment varies substantially among them. At a minimum and at the coarsest hierarchical level, two habitat categories (riffle/run and pool) should be used because it is clear that many types of organic matter vary in abundance between them. For some organic matter compartments, these categories should be further divided into sub-categories. For example, the riffle/run category (and perhaps also the pool category) might need to be subdivided by the dominant substrata (e.g., bedrock, cobble/gravel, and fine-grained sediments). If the fine-grained sediments have very different types or biomass of organic matter (e.g., organic rich detritus versus algae-rich sands) this category may need to be subdivided for FBOM measurements (remember that we are sampling algae-rich fine sediments for ^{15}N as part of the FBOM rather than as epilithon – see section 3E and 3F). However, it is best to minimize the number of sub-categories because multiple samples will need to be collected from each sub-

category. We don't need to sub-divide to account for small variations in standing stock between categories, only the large ones.

Detrital BOM sampling. Samples for determination of coarse benthic organic matter (CBOM, > 1 mm, separated into leaves and wood) and fine benthic organic matter (FBOM, < 1 mm, separated into surface and subsurface materials) standing stocks, are collected using a technique similar to that used during LINX I. A stratified random sampling scheme is used based on the results of the habitat survey described above. Multiple samples are randomly collected from each habitat type and means of standing stock values (g/m^2) for each habitat type are weighted by the fractional contribution of that habitat type to total stream area to calculate whole-stream standing stock values. The number of samples per habitat type depends on the relative abundance of that habitat type (e.g., 3 for habitat types that comprise a low % of the reach, 6-8 for habitat types that comprise a high % of the reach). It is suggested that a total of about 20 samples be collected for each organic matter compartment within the study reach.

For each sample, place the metal cylinder (e.g., 30 cm diameter) as deeply into the sediments as possible and remove all coarse material. Collect surface FBOM by swirling water to fully suspend all surface sediments and collect a sample of the suspension in a bottle. Measure the depth of water in the cylinder in order to calculate the total volume of suspended surface FBOM. This volume will be used together with the mass of the filtered subsample to compute the FBOM mass per unit area (area within the cylinder). Next, subsurface FBOM is sampled as follows. Remove the metal cylinder and allow the suspended surface material to be flushed downstream (it may be necessary to use your hand to resuspend material). After all the surface material has been flushed downstream, reset the cylinder into the sediments at the same location, agitate sediments as deeply as possible suspending them thoroughly within the water in the cylinder (in most streams it should be possible to agitate and suspend sediments to a depth of about 20 cm), and collect a sample of the suspension in a bottle. Remember, we are interested in the organic matter, so don't worry if some of the inorganic materials resettle before you can collect the sample of the suspension. Again, measure the depth of water within the cylinder in order to calculate the volume from which the sample was collected. Return samples to the lab for filtration.

In the lab, separate the wood and leaves and dry (60 C). Record dry weight and subsample for ash-free dry mass determination (500 C). For surface and subsurface FBOM samples, shake the sample well and pour a subsample into a graduated cylinder, record the volume, and filter through a pre-combusted and tared glass fiber filter (Whatman GFF, 47 mm diameter). Make sure there is plenty of material on the filter to obtain a good mass value. Dry filters at 60 C, weigh, combust (500 C) and reweigh to determine ash-free dry mass. Calculate surface and subsurface FBOM standing stock for each sample by multiplying the AFDM/volume filtered values by the total volume within the cylinder used in the field and then divide by the area of the cylinder. To compute standing stocks of each BOM compartment for the entire stream, weight the average standing stocks for each habitat type by the relative proportions of each habitat type in the study reach. Calculate all standing stocks **both in terms of AFDM and dry mass per unit area** (the latter is used with the 15N data because the mass spectrometer analysis will give N content as % of dry mass).

For the algae-rich FBOM samples, also filter a known volume of subsample onto a pre-combusted glass fiber filter (Whatman GFF, 47 mm diameter) and process for chlorophyll *a*

measurement as described in Section 4E. Calculate chlorophyll *a* standing stock for this type of sample by multiplying the chlorophyll *a*/volume filtered values by the total volume within the cylinder used in the field and then divide by the area of the cylinder. To compute standing stocks of chlorophyll *a* within the FBOM compartment for the entire stream, multiply the average standing stocks for this compartment by the relative proportions of this habitat type in the study reach.

4E. Epilithon – standing stock and chlorophyll measurements

Field Equipment:
Wire brush for scrapping
Container with wide mouth to collect epilithon slurry
Squeeze bottle filled with stream water to slurry scrapings into container
Paper to trace rock planar areas (or bring rocks back and do planar tracings in lab)
Sample Bottles/cups to hold individual epilithon samples (either a 100-mL specimen cup or larger bottle, depending on volume of slurry - see text below)
Plastic bags or bottles for core samples from fine-grained sediment habitats
Sample template for sampling on bedrock (approx. 2-inch or 4-inch diameter PVC connector with neoprene ring attached to bottom end with silicone sealant)

Laboratory Equipment Needed:
Pre-combusted glass fiber filters (Whatman GFF, 47 mm diameter)
Filtration set-ups for 47 mm filters
Hot ethanol (95%) for extraction of chlorophyll
Spectrophotometer for measurement of chlorophyll
Oven for drying material (60 C)
Oven for combusting material (500 C)

Samples for epilithon standing stock and chlorophyll *a* are collected on the morning or afternoon of **day 4** (depending on whether the salt/propane injection is performed in the morning) using a stratified random sampling design based on the number of habitat types (channel units) having substantially different epilithon biomass or type. Within each habitat type that contains epilithon we need to collect a number of random samples within the study reach to calculate an average epilithon standing stock value for each habitat type. However, the sample collection procedures differ depending on the habitat type. For bedrock habitats, use a small cylinder of known area (e.g., a short piece of 2-inch PVC pipe) with a foam gasket attached to one end as the sampling template. Push this template firmly against bedrock, vigorously scrape material using a wire brush, and suction scraped material into a bottle (using a turkey baster or large plastic syringe with tip cut off). For rocks that can be picked up (e.g., cobble), choose 1-5 rocks (equivalent to a total planar area of about 100-200 cm^2) and brush the rock surface thoroughly with a wire brush to loosen the epilithon and slurry the loosened material into a container. After the epilithon has been removed, measure the approximate planar area of each rock brushed by tracing on a piece of paper (use paper from the same batch that has the same weight per unit area), cutting the tracings and weighing them. If you are brushing more than 1

rock to obtain the desired sample area, combine all rocks collected at a particular sampling location into a single "pooled slurry" sample. For fine-grained sediments the epilithon will be sampled as FBOM as described in the previous section (4D), unless it exits as a mat in which case it will be included as filamentous algae (see section 4F).

In the laboratory (< 6 hours from sample collection in the field) the total volume of each slurry sample is determined and recorded. Then one well-mixed subsample is measured out and filtered through a pre-combusted and tared glass fiber filter (47-mm diameter, Whatman GFF). This filter is then dried at 60 C, weighed to determine dry mass, combusted (500 C) and reweighed to determine ash-free dry mass. To compute standing stocks of epilithon for the entire stream, weight the averages for each habitat type by the relative proportions of each habitat type in the study reach. Calculate all epilithon standing stocks **both in terms of AFDM and dry mass per unit area** (the latter is used with the ^{15}N data because the mass spectrometer analysis will give N content as % of dry mass).

A second well-mixed subsample of the slurry is then measured out and filtered through another pre-combusted, 47-mm diameter GFF filter for chlorophyll a determination (place filter in aluminum foil packet, label, and freeze until analysis).

Chlorophyll *a* measurement. Chlorophyll *a* will be determined by hot ethanol extraction using the method of Sartory and Grobbelaar (Sartory, D. P. and J. E. Grobbelaar. 1984. Extraction of chlorophyll *a* from freshwater phytoplankton for spectrophotometric analysis. Hydrobiologia 114:177-187). This method has the benefit of extraction without grinding and avoiding toxic methanol or acetone exposure. Keep samples in the dark at all times, at least in low light when working on them. If the sample is kept in the dark, only 1.3% of the chlorophyll degrades with the 5 min. hot extraction and 24-hour storage. Use test tubes that have the tube numbers scribed on the side with a diamond pencil (ethanol eventually extracts tube numbers written in sharpie, diamond pencils are about $15 at Carolina Biological Supplies) for filter extraction. Place the filter, scrapped material from known area, or even an entire rock, in a container with a known volume (10 mL ethanol in screw cap tubes works well) of 95% ethanol. Mark the location of the meniscus on the side of the tube with sharpie, and place a marble or loose cap on top of the tube. Heat the tube at 79^{o}C for 5 minutes, then mix and cool for 24 h in the dark (can be at room temperature, and can be sealed once cooled if screw cap tubes are used). After extraction, use additional 95% ethanol to bring up to mark on side of tube if ethanol has evaporated, then mix. Clear sample by centrifugation, filtration, or settling. Analyze sample with spectrophotometric analysis at 665 and 750 nm using a 1 cm spectrophotometer cuvette (method in Standard Methods with different extinction coefficient for ethanol and conversion for chlorophyll per unit area). If adsorption is over 1.5 absorbance units, dilute sample. Add 0.1 mL of 0.1 N HCl for each 10 mL of extractant after the first reading and let sample sit for 90 s to phaeophytinize all chlorophyll before reading. Amount of acid is important, too much causes precipitates that you don't want. Calculations are made as follows:

$$\text{Chlorophyll } a \text{ (mg/m}^2\text{)} = (28.78(665_0\text{-}665_a)*v/(A*l) \quad (3)$$

where 665_0 = absorption at 665 before acid addition with absorption at 750 nm subtracted out, 665a = absorption at 665 nm after acid addition with absorption at 750 nm subtracted out, v =

volume of extractant used (in liters), A = area of benthos sampled in m^2 and l = path length of cell in cm (usually 1 cm).

$$\text{Phaeophytin } (mg/m^2) = 28.78\ [1.72(665_a)-665_0]*v/(A*l) \quad (4)$$

The chlorophyll a data will be used for two purposes: (1) to compute dry mass:chlorophyll a and AFDM:chlorophyll a ratios for those compartments where each of these measurements is made (this will provide a measure of the autotrophic component of epilithon), and (2) to estimate AFDM and dry mass of epilithon in the sand or other habitats where we cannot measure these directly using dry mass:chlorophyll a and AFDM:chlorophyll a ratios.

4F. Macro-autotrophs – standing stock measurements

Field Equipment:
Cylindrical template (or other template for area to be sampled)
Sample bags (e.g., zip-lock plastic bags)

Laboratory Equipment:
Containers for holding material to be dried (e.g., aluminum pans or trays)
Oven for drying material (60 C)
Oven for combusting material (500 C)

We will determine the standing stock of all important macro-autotrophs (bryophytes, filamentous algae, macrophytes) on the morning or afternoon of **day 4**. If the distribution of these components is not too heterogeneous, then the standing stock measurements can be made by collecting all material of each type within a known area (e.g., cylindrical template used for BOM analysis) at about 10-20 random locations in each stream (may need to stratify sampling by habitat type if standing stock of macro-autotroph varies significantly by habitat type – see stream habitat survey in section 4A). The material collected is then dried (60 C), weighed, combusted (500 C) and reweighed to determine dry mass and AFDM per unit stream bottom area for each type of material. If the distribution of a particular macro-autotroph is highly heterogeneous, then a point/transect survey method is preferable for determining standing stock. Set up about 20 lateral transects across the stream along the study reach and determine presence or absence at about 10-20 evenly spaced points across each transect. Then sample all material from about 5-10 locations of 100% coverage and measure the area from which the sample was collected. Process the material for dry mass and AFDM as described above, and calculate standing stock per unit stream bottom by multiplying the standing stock in areas of 100% coverage by the % coverage over the entire stream reach.

4G. Collection of sediment samples for denitrification assays

Sediment samples for denitrification assays will be collected on the morning of **day 4** at the same time samples for BOM standing stocks are collected and sent to Stuart Findlay at IES.

Denitrification is a highly variable process requiring the co-occurrence of at least three factors (available organic carbon, low oxygen and nitrate). Our assay is targeted at the denitrification **potential** to assess the activity of the population capable of carrying out denitrification under "ideal" conditions. This potential measurement is less sensitive to the perturbations inherent in sample collection and shipment. If a sub-set of sites wish to attempt closer to in situ estimates the acetylene block can be applied without sparging, glucose/nitrate addition.

Given the heterogeneous nature of denitrification, the sampling allocation issue is very important. The alternatives are: 1) target "hot spots" under the assumption that a small proportion of the area is contributing the bulk of the DEA activity vs. 2) habitat-weighted sampling where the likely habitats supporting DEA (fine sediments, organic accumulations, etc.) are sampled in proportion to their abundance (c.f. Kemp and Dodds, Ecol. Applications 2002). For sites that get underway even in a preliminary fashion in year 1, I propose a hybrid approach where the sites submit 12 samples per stream type. Four samples are FBOM, 4 are CBOM and 4 are at the investigators discretion (e.g. bryophyte mats, rotting wood etc.). For whatever sample is submitted we'd need reach-scale estimates of habitat abundance to allow comparison.

Collect materials from depositional zones at 12 locations within the study reach of each stream. Samples are collected with a small core (1-2" ID) to a depth of 10 cm (where possible). [Each sample should be 10-50 g Wet wt. Lower end if high in fines and OM, higher if gravelly. Gravel > about 1-2 cm won't fit in the flasks we use, we can change if this substrate type is likely to be a problem. Material with whatever overlying water goes in a 250 mL wide-mouth bottle shipped on ice to IES. [Stuart hopes to have some info on allowable "holding times" by NABS.] At IES we will determine denitrification potential (Ar sparged, glucose and NO3-amended), sediment organic matter and dry mass/volume.

4H. N limitation assays using N diffusing substrata (contact: Jen Tank)

In order to determine whether biofilms at each of our study streams are nutrient limited, we will do N limitation assays using N diffusing substrata. A set of nutrient diffusing substrata will be sent to each site by Jen Tank for deployment about 3 weeks prior to the ^{15}N experiment. The substrata should be placed in riffle/run locations in each stream (to ensure good water flow). Jen will send instructions as to when to pick up the samples and how to send them back to her for processing..

4I. Microscale sediment oxygen surveys (Optional) (contact: Walter Dodds)

To determine the relative distribution of microscale anoxic zones within the sediments, microelectrode surveys can be conducted in each stream using equipment provided by Walter Dodds. These can be conducted either several days before or after the ^{15}N addition experiment, preferably several days before, so the substrata are not disturbed too much. This work is not required for each stream, but is encouraged for as many streams as possible, particularly if there is someone on site who is interested (e.g., a student). Walter Dodds may have a student who could do this if travel costs could be borne by the site.

<u>Materials needed at each site:</u>

Lab tape, anoxic mud, thermometer, voltmeter, acetone (10 mL), macro O_2 meter or Winkler reagents, several scintillation vials, ring stand with cement base (*Note: need to prepare this ahead of time*)

Materials shipped to each site:
Probes, micro-manipulator (2), pico-ammeter, power source, connection wires, metal rods (2), membrane filters, large syringe barrel, reference probe solutions/syringe, reference probe

Preparation

Several weeks in advance of the first set of measurements, need to make a heavy based ring stand. The best way to do this is cut the bottom 1/3 from a 5 gal bucket, tape a 3/8" diameter, 1 m long piece of metal rod so it is standing vertically with the bottom in the bottom of the bucket (alternatively, just take an old ring stand, and set the base in the bucket). Mix enough concrete (quickcrete is available at hardware stores) to fill the cut off bucket, pour it in, and let it set for 2 weeks.

A few days before the measurements, get entire electrode set up put together in lab and make sure it is working. Make sure picoameter is charged (plug in overnight). Make probe tip solution by dissolving 5 Millipore HA filters to 5 mL acetone in a labeled scintillation vial. To put on membrane (only needle probe, **not reference**), dip in acetone, insert tip into "acetone+membrane" filters vial, allow a few seconds to dry (note: a smooth even thin membrane should result from this, redo the last two steps if the membrane was formed unevenly - e.g., drop of membrane fluid solidified at the end of tip). To prepare the reference probe: A) screw off the cap and pull body back, moving down cord to expose inner section (be careful not to touch the probe tip), B) fill inner section of probe using syringe and green fluid by inserting syringe into port (hole on side) and filling to just below port, C) rinse syringe, D) replace outer body and refill with clear KCl solution in port with syringe, fill to just below port, E) replace spring and screw cap and screw on tightly, F) rinse syringe, G) store with tip immersed in water and with port closed while not using, H) when finished, remove solutions and rinse out probe before shipping back.

Connecting probes: 1) plug BNC adapter to power supply, power supply to reference electrode and microlectrode (leads are labeled), 2) connect manipulator to ring stand, then electrode to micromanipulator (use lab tape to connect to plastic rod that is clamped into the micromanipulator, 3) make sure reference sleeve is up, put reference anywhere in water to be measured, make sure that the hole on the probe body is above the water level, 3) put microelectrode tip into solution to be measured (be careful to keep the electronics away from the water), 4) turn on picoammeter and then power supply.

Calibrating probe: 1) place electrode in anoxic portion of sample, allow to come to steady signal, 2) move to oxygenated location, allow to come to equilibrium, record signal and probe number, ambient O_2 as verified by macroelectrode or winkler, and temperature, 3) move to anoxic location, allow to come to steady signal and record signal, 4) do not let probe or reference leave water or polarity will be broken and probe must be re-calibrated, 5) check oxygenated calibration after every profile.

Making the measurements:
1) Determine where the transect will be (will take 5 transects per stream, 10 profiles per transect), it cannot be too windy when measurements are being made because movement of the wires causes erratic signal, 2) place tape across stream, measure width, determine 10 evenly spaced gradient points across width, 3) set up probe on ring stand, 4) calibrate probe, write calibration information on datasheet, 5) place electrode over the site from which O_2 profile will be taken, 6) slowly lower the probe until it is directly above (just touching) the sediment surface. This may be difficult to see; however, it is very important to know when the probe is touching the surface of the sediment. Lower the probe until the sensing part of the probe in the needle is just at the surface (the distance each sensor is from tip of needle is written on the probe barrels (smallest increment on silver wheel= 10 µm), 7) take measurements every 1 mm until 2 cm, consistent anoxic zone, or rock are reached. Make sure the picoammeter has stabilized before taking readings. Be very careful when lowering the electrode because rock or debris can break the tip. Hitting a rock will cause the signal to jump slightly. A large jump in the signal that does not equilibrate means the tip has been broken, 8) take probe from gradient site, and re-measure oxic water, 9) move electrode to next sampling site (if you don't take reference or electrode from surface of water, then recalibration is not necessary unless you get very different signal from what you had previously.

Trouble shooting: Check wire connections, check membrane, check probe tip for breakage, use multi-meter to check power supply and connections, (power source should put in roughly 0.75 volts across probe), try new probe. Sometimes it takes a probe a while to settle down.

5. SUMMARY OF MEASUREMENTS AND SCHEDULING

Date	Measurement (section described)
≥ 1 month prior	Install 8 streamside wells, optional (2B)
3 weeks prior	Deploy nutrient diffusing substrata (4H)
1 to 2 weeks prior	Preliminary nitrate injection (3A) Measurement of in-channel, riparian and watershed characteristics, including habitat survey for stratifying standing stock sampling (4A) Microscale sediment oxygen surveys, optional (4I)
1 day prior	Begin stream dissolved oxygen measurements about 6 pm (4B) Deploy PAR sensors at same time DO measurements are begun (4C) Collect water samples from wells for ^{15}N and nitrate analysis (3B) Channel wetted width measurements (4A)
Day 1	Morning: Collect PRE ^{15}N addition water samples from stream (3C) Begin conservative tracer monitoring at station 6 (3C) 1 pm: Begin ^{15}N addition (3C) 2 pm: Collect first of 2-h diurnal ^{15}N water samples at station 4 (3C)
Day 2	am: Continue 2-h diurnal ^{15}N water samples at station 4 (3C) 2 am & noon: Plateau ^{15}N water samples (3C) 1 pm: End ^{15}N addition (3C) pm: Begin processing $^{15}NH_4$ samples (7C), spike plateau $^{15}NO_3$ samples with nitrate (7B)
Day 3	9 am: End stream dissolved oxygen (4B) and PAR (4C) measurements 1 pm: POST 24-h ^{15}N water and SPOM samples (3C, D) 2 pm: Detrital BOM (3E), epilithon (3F), macro-autotroph (3G) ^{15}N samples pm: Salt/propane injection for gas exchange rate determination (4B) or Day 4
Day 4	am: Salt/propane injection for gas exchange rate determination (4B) am: Collect sediment samples for dentrification assays (4G) pm: BOM (4D), epilithon (4E) and macro-autotroph (4F) standing stocks
Day 5	1 pm: POST 72 h ^{15}N water and SPOM samples (3C, D)
Day 9	1 pm: POST 1 week ^{15}N water and SPOM samples (3C, D)
Day 5 and later	Complete in-channel, riparian and watershed characteristics measurements (4A) Nitrate, ammonium, DON, conservative tracer, SRP, and TSP analyses on samples collected during experiment (3C) Process organic matter samples for ^{15}N (7A) Process $^{15}NO_3$ samples (7B), TD^{15}N samples (7E) Complete processing of $^{15}NH_4$ samples (7C)

OPTIONAL BUT RECOMMENDED:

3 months	Sampling of standing stock and 15N content of major benthic biomass compartments for long-term retention (3E, 3F, 3G, 4D, 4E, 4F)

Ideally, perform the ^{15}N experiments in all 3 streams within a 6-week period each year (same period for experiments in all 3 years). Conduct synoptic water chemistry sampling in large basin during this period as well.

Note that the above table includes a 3-month sampling of ^{15}N content and standing stocks in all major benthic biomass compartments. This sampling is optional but highly recommended. It will provide one measurement of moderately long-term retention of 15N within the study reach. The sampling procedures described for the day 3 and 4 sampling of benthic organic matter ^{15}N content (sections 3E, F, and G) and standing stocks (sections 4D, E, and F) should also be used for the 3-month sampling. The standing stock of $_{15}N$ in each major biomass compartment can be determined from the standing stock values and a relationship between biomass $_{15}N$ content and distance over the study reach as we did in LINX I.

6. DATA MANAGEMENT

A set of spreadsheets for recording the data from the ^{15}N experiment in each stream is included in the excel file "LINX 2 Data Spreadsheets_Field 15N addition experiment_Rev4.xls". Please create one file for each stream with each file consisting of multiple worksheets for recording the primary data and calculating the various N cycling. Name each file as follows: LINX II Data_Rev4_Region_Land use_stream name.xls. Individual worksheets in this file are:

- Notes on spreadsheet use (PLEASE READ THESE)
- Primary data – general
- Primary data – N15 solutes
- Primary data – N15 gases
- Primary data – Conservative tracer
- Primary data – SPOM conc. and N15
- Primary data – N15 in biomass
- Primary data – Biomass standing stock
- Primary data – Well chemistry
- Primary data – Gas exchange rates
- Primary data – Metabolism and calculations
- Metabolism summary
- Calculation of Sw, Vf, and U
- Calculation of biomass specific uptake rate
- Calculation of nitrification rate
- Calculation of denitrification – N2 production (UC-Davis analysis)
- Calculation of denitrification – N2 production (MSU analysis)
- Calculation of denitrification – N2O production
- Calculation of net ammonification and ammonium uptake rates

Please note that the worksheets beginning with "primary data.." are to record the actual measurements made in the field or in the lab as well as to perform the 1st level calculations of 15N fluxes. The worksheets beginning "Calculation of .." are to perform the calculations of the various rates using data that is automatically linked to one or more of the primary data worksheets. Please note that the spreadsheets for recording field and summary data on riparian and channel characteristics are included in a separate excel file ("LINX 2 Data spreadsheets_Inchannel and riparian characteristics_Rev1.xls") as described in section 4A above.

Each worksheet in the "LINX 2 Data Spreadsheets_Field 15N addition experiment_Rev4.xls" file includes a title and a purpose in column A rows 1 and 2, respectively. Notes or explanations are in red font or imbedded as comments in the cell. Data categories or names are in bold font. Data to be entered in the primary data worksheets are listed as "DATA" in the appropriate cells (in some cases an example is listed in parentheses after the word DATA). In some cells the actual data value is given if it is known (i.e., standardized for all streams). The cell entries "#VALUE!" and "DIV/0!" indicate a formula is entered and the calculation will be automatically performed when the cells with "DATA" are completed. In the worksheets for calculations (titled "Calculation of …"), a number of the cells are linked back to cells in the primary data worksheets (as indicated in the cell entry). Explanations how to do the various N cycling rate calculations performed in these worksheets are included in Appendix A.

7. LABORATORY PROCESSING OF ORGANIC MATTER AND STREAMWATER ^{15}N SAMPLES

7A. Preparing dried organic matter samples for ^{15}N analysis

Laboratory Equipment and Materials:
Wiley Mill with 40- or 60-mesh screen
Microbalance accurate to 0.001 mg
Scintillation vials
Encapsulating tins (holder optional)
96 well microtitre plates for storing encapsulated samples
(note: 96-well plates with tins included can be obtained from Perkin Elmer, Organic Division, 800-762-4000, Item number N2411255 for about $75 each, Although much cheaper to buy empty microplates and separate tins)

Organic matter samples for ^{15}N analysis (described in sections 3D, 3E, 3F, and 3G) must be completely dry and in a uniform fine-grained texture. If there is uncertainty about samples being completely dry, then re-dry (60 C). Samples such as leaves, wood, and macro-autotrophs must be ground to a fine powder (e.g. Wiley mill with 40- or 60-mesh screen, but take care not to cross-contaminate samples – do background samples first and work in ascending order of likely ^{15}N content, cleaning mill between samples). A small amount of material is weighed out into a small tin capsule using a balance accurate to 0.01 mg. Because most labs have an upper limit (300 ug N) and a lower limit (about 20-25 ug N) for ^{15}N samples (optimum is 100 ug N), different masses of material must be weighed out for different sample types (different %N content). We have generally used the following:

Leaves (0.5 to 2% N):	6 – 8 mg
Wood (0.3 to 2% N):	10 – 12 mg
FBOM (0.3 to 2% N) :	10 – 12 mg
SPOM (0.5 to 3% N):	5 – 7 mg
Epilithon (0.5 to 3% N):	5 – 7 mg
Bryophytes/filamentous algae (1.5 to 5% N):	3 – 5 mg

Each site should check these (and modify if necessary) using the expected %N of each sample type. It is important that the weights of a particular type of sample (e.g., leaves) be close to each other (within about 1 mg). This gives more consistent $\delta^{15}N$ results. When weighing out material, use great caution to prevent ^{15}N contamination across samples. Work in order of least enriched to most enriched samples (background samples of all types first, then downstream to upstream stations of each type; it is expected that wood and FBOM would be first because they are expected to have the least ^{15}N and bryophytes/algae last because they would likely have the highest ^{15}N values). Clean all surfaces and utensils by wiping with alcohol. It is best to work over an area that is white (a piece of white plastic tape can be placed down on the table) so that you can see if any material is spilled. Place tin capsule on balance and tare. Then place tin capsule on bench and carefully add material using small spatula. Place back on balance (using

forceps) to record added dry mass (or remove and add more material if mass is too low). Remove tin capsule from balance and place on clean white surface to crimp tin down to small packet (fold over top to close, then crimp sides down so that all dimensions are < 2 mm). Check to see that there is no leakage of material by dropping tin packet (from a height of 2 inches) onto white surface. Tin packet is then placed in one well location (use 96-well microtitre plates) and the well location (e.g., A1…A12, B1…B12, etc), sample type, sampling station, and dry mass recorded.

For samples retained on GFF filters (FBOM, SPOM, epilithon), carefully remove only organic material from filter to add to tin. For samples that do not have a thick "cake" on filter (epilithon, SPOM), be as careful as possible to minimize inclusion of filter fibers within the sample (e.g., use a sharp scalpel or small knife for scraping material from filters). If it is not possible to scrape material from filter without inclusion of filter material in the sample, then it may be possible to encapsulate the entire filter, but record only the dry mass of material on the filter (subtract the filter tare mass) as the sample dry mass. The well plates with samples can then be stored for up to several months before shipment to the ^{15}N analytical lab.

The ^{15}N analytical lab will want to have an electronic file of the well location and dry mass of sample in each tin packet sent to them. The background samples of all types should be grouped together first in the well plates (e.g., first 1 or 2 columns of wells - locations A1 through A12 and B1 through B12) and then the ^{15}N-enriched samples (samples downstream from ^{15}N addition) following these (e.g., well locations C1 and higher). This helps to avoid carry-over effects with the mass spectrometer. Generally, the differences in $\delta^{15}N$ will be greater between sample types than between stations below the dripper. The order of ^{15}N enrichment for sample types is likely to be wood < FBOM < leaves < SPOM < bryophytes < epilithon < filamentous algae, so I would suggest ordering samples in this order in the microplates.

7B. Preparing streamwater $^{15}NO_3$ samples for ^{15}N analysis

Laboratory Equipment and Materials:

Nitrate solution for spiking samples with unlabeled nitrate (see text for concentration)
Hot plates with stir bars (preferably 4 or more)
1L or 2L beakers for boiling samples (need beakers about 1.5 to 2 times larger than sample to be boiled down) (preferably 4 or more)
Weigh boats
NaCl ashed (450 - 500 C for 4 hours)
MgO ashed (450 – 500 C for 4 hours)
250 ml HDPE bottles
Filter packets consisting of a Whatman GF/D filter (1.0 cm diameter, pre-combusted to remove any N) filter pressed between two Teflon filters (Millipore, 10.0 μm pore size, white mitex LCWP, 25 mm diameter). Need one filter packet per sample. **Note: Teflon tape (at least 1 inch wide) can be substituted for Teflon filters.**
Devardas alloy – powdered form (do not ash)
Stock NO_3 solution for spiking samples (nitrate conc. dependent on ambient values; may need a second NO_3 spike solution at lower concentration for spiking PRE and POST samples if ambient NO_3 concentrations are very low – e.g., < 25 μgN/L).

Shaker table
Dessicators
2.5 M $KHSO_4$ (Potassium hydrogen sulfate, low N content)
Scintillation vials
Encapsulating tins (see description in 4A above)
96 well microtitre plates for storing encapsulated samples (see description in 4A above)
Well cap strips (for covering filter samples in well plates; sold as 8 well strip cap, polyethylene, Nalge Nunc International, Naperville, IL 60563)

To analyze for ^{15}N in streamwater dissolved nitrate we use an alkaline headspace diffusion procedure after first reducing nitrate to ammonium. However, first we must add an unlabeled nitrate spike to the plateau streamwater $^{15}NO_3$ samples because they will be very highly enriched with ^{15}N (20,000 per mil is the target at the injection site) and most analytical labs do not want to analyze samples above 4,000 per mil or so. All the plateau streamwater $^{15}NO_3$ samples will receive the unlabelled nitrate spike, however no nitrate spike is added to the PRE and POST streamwater $^{15}NO_3$ samples and the streamside well $^{15}NO_3$ samples. The PRE and POST $^{15}NO_3$ samples do not receive the unlabelled nitrate spike in order to make it easier to determine any ^{15}N released after the end of the ^{15}N addition (rapid recycling). **However, if the background nitrate concentration in streamwater or well water is < 25 µg N/L a SMALL nitrate spike will need to be added to PRE and POST $^{15}NO_3$ samples in order to ensure enough N in the 1-L sample to be processed for mass spectrometric analysis (but NOT the larger nitrate spike added to the plateau samples).** If this is the case, add a nitrate spike sufficient to bring the lowest sample to 25 µg N/L. If this small nitrate spike is needed, add the SAME nitrate spike to all PRE and POST samples (and well samples too if needed). Make the nitrate spike solution from the same lot of nitrate as used to make up the higher nitrate spike solution added to the plateau samples (which we'll be analyzing for $\delta^{15}N$) and record the exact amount of nitrate spiked into each sample.

Within a day after the streamwater $^{15}NO_3$ samples are returned to the lab, add to each PLATEAU sample an amount of nitrate that will increase the nitrate concentration in the sample by 5 times the concentration estimated for the stream and on which the amount of ^{15}N added was based (this will result in a 5-fold decline in $\delta^{15}N$ values). In other words, if the ^{15}N added to the injection solution was based on an estimated ambient streamwater NO_3 concentration of 50 µg N/L, then add a 200 µg N spike of nitrate to each 1 L of sample (or 100 µg N spike to each 0.5 L sample, etc). **Be sure that the volume is measured out precisely for each sample to which the nitrate spike is added** (so that we can accurately calculate the increase in nitrate concentration). Don't worry if the measured nitrate concentrations in the stream are not similar to the nitrate levels assumed for the ^{15}N addition, the nitrate spike is based on the estimated nitrate concentrations used to determine the amount of ^{15}N to add to the stream. The easiest way to add the nitrate spike is to make a stock nitrate solution with 1000 times the nitrate concentration to be spiked (e.g., 200 mgN/L if the spike is to be 200 µgN/L) and then add exactly 1 mL to each 1 L sample (or proportionally less to smaller volume samples – see below). In addition, add the same nitrate spike to 5 samples of deionized water (use the same volumes of deionized water as volumes of samples) and process these with the $^{15}NO_3$ samples. These samples will provide a measure of the ^{15}N content of the nitrate used for the spikes (it should be 0 ± 10 per mil) as well as check for 100% diffusion efficiency. It is important to **add the same**

nitrate spike exactly to each sample (use very careful pipeting technique). The samples should be shaken and a small subsample (approx. 20 mL) removed from the deionized water samples and a few of the other samples and stored for nitrate analysis (to determine the precise concentration of the nitrate spike added to each $^{15}NO_3$ sample). The samples are then returned to the refrigerator for storage until ready for processing for ^{15}N (samples can be stored in the refrigerator for up to about 1 month after the nitrate spike has been added). If a smaller level nitrate spike was added to the PRE and POST samples because of low ambient NO_3 concentrations, add the same nitrate spike to 3 samples of deionized water (same volume as samples) and analyze these for NO_3 concentration and $\delta^{15}N$ as described above (because we need to know the exact amount of N added and the $\delta^{15}N$ of the added N).

If the target stream enrichment is considerably less than 20,000 per mil, the nitrate spike can be adjusted downward accordingly if desired. However, because the sample volumes to process (given in table 2) are calculated based on a nitrate spike of 200 μgN/L, if a smaller spike is used then the sample volumes to process must be increased proportionately.

We will also send to each site several vials of a secondary $^{15}NO_3$ standard (prepared by Lee Cooper and sent to each site). These standards will be enriched in ^{15}N and will serve as a check on diffusion efficiency as well as the accuracy of the mass spectrometer analysis (we will call these check standards). There will be instructions sent with the vials of standard as to how to dilute these and what volume to process after dilution. After dilution, the standards should be processed along with and in the same way as the samples.

The volume of sample to process for $^{15}NO_3$ depends on the concentration of nitrate in the sample. We want to have a final mass of 50 to 200 μgN in approximately 100 mL of sample (to ensure that we are well within the 20 to 300 μgN limits, the optimum amount is 100 μgN). If the nitrate concentration in stream water is < 100 μgN/L (which will be < 500 μgN/L after adding the nitrate spike), then concentrate an appropriate sample volume by boiling it down to about 100-125 mL (to ensure that we have 50 to 200 μgN in the concentrated sample). If the streamwater nitrate concentration is > 100 μgN/L, then there is no reason to concentrate the sample (because after the nitrate spike 100 mL will contain at least 50 μgN). However, to ensure that all NH_4 is removed from the sample prior to further sampling, deionized water (N free) should be added to bring the volume to about 200 mL and then a short boil-down is performed to reduce the sample volume to 100 to 125 mL. Below is a table of sample volumes to process (boil down) depending on the streamwater nitrate concentration prior to adding the nitrate spike. Because the PRE and POST stream samples and the well samples for $^{15}NO_3$ in groundwater do not receive the nitrate spike (or a small NO_3 spike if ambient concentrations are very low), the volumes to process for these samples are greater than for the plateau streamwater samples.

Table 2. Sample volumes to process for $^{15}NO_3$.

NO3-N conc in streamwater *prior to* spike (μgN/L)	Sample volume to process (mL)
Streamwater plateau $^{15}NO_3$ samples:	
< 40 (i.e., < 200 after spike)	1000
40 to 100 (i.e., 200 to 500 after spike)	400
100 to 400 (i.e., 500 to 2000 after spike)	100 *
400 to 1000 (i.e., 2000 to 5000 after spike)	40 *

> 1000 (i.e., > 5000 after spike)	20 *

Well $^{15}NO_3$ samples and PRE and POST streamwater $^{15}NO_3$ samples:

< 50 to 200	1000
200 to 500	400
500 to 2000	100 *
> 2000	40 *

* The boil-down step is not necessary for concentrating these samples. However, to ensure that all NH_4 is driven off before further processing, deionized water (N free) should be added to bring the volume to about 150 mL and then the sample reduced to 100 to 125 mL using a short boil-down.

The samples should be processed as "sampling units" in case there is some systematic variation in recovery efficiency, etc. A "sampling unit" would in consist of all the samples from a stream (PRE, POST, plateau and diurnal samples) plus the spiked deionized water samples, check standards and the Devarda's alloy blanks (see Figure 5 and text below). The idea is to be able to do any corrections that might be needed based on the standards, blanks, etc. for a stream sampling unit. **However, it is critical to use separate glassware for all PRE, POST, deionized water spike, and Devarda's alloy blank samples versus the samples collected during the ^{15}N addition (plateau and diurnal) and the check standards to avoid contamination problems.** Acid wash and thoroughly rinse glassware between samples. It's best to have about 4 or 5 hot plates and boil-down beakers so that 4 – 5 samples can be boiled down simultaneously. Steps for sample processing (boiling to concentrate samples, reduction of nitrate to ammonium, sorption of ammonium onto acidified filters, encapsulating filters) are as follows:

1. Measure out the sample volume to be boiled down and add to a clean, acid-washed beaker or flask.
2. Add about 5g of ashed NaCl (for ionic balance, want final concentration after boil down to be about 50 g/L) and about 3 g ashed MgO (to raise pH) to sample.
3. Add stir bar and place on hot plate with stirring capability. Heat until volume is reduced to roughly 100 ml (doesn't have to be exact, 75-125 mLs should be okay). Aim for a simmering boil, but if just below this then that is fine as well. Stir during the boil. It takes about 3-4 hours of gentle boiling to reduce 1 L to about 100 mL.
4. After cooling somewhat, pour the boiled down sample into an acid-washed 250 mL HDPE bottle (suggest rigid rectangular bottles). These can be stored in refrigerator until you have an entire series (stream) ready for next step (reduction of nitrate to NH_3 and diffusion of NH_3 into headspace).
5. Add about 0.5 g of ashed MgO and then 0.5 g of Devardas alloy to boiled-down sample in 250 mL bottle.
6. Immediately after adding Devardas alloy, place filter pack (see subsection on steps for constructing filter packs below) carefully on surface of water, place piece of parafilm over top, and cap very tightly.
7. Place bottles in oven at 60^{o}C for 48 hours (be careful not to pack too closely in a tray since the bottles will swell slightly).

8. Remove from oven and place bottles on shaker and shake gently for 7 days at room temperature.
9. Open bottles and remove filter pack. Blot water droplets from filter pack and place in labeled scintillation vial and into dessicator. Also place an open vial of 2.5 M $KHSO_4$ (to absorb any ammonium in air) in dessicator.
10. Let filters dry in dessicator for 4 days or so. Remove and cap the scintilation vials containing filter packs very tightly (use parafilm if necessary) and store until ready to encapsulate in tins.
11. Encapsulating filters: Remove filter pack from scintillation vial on clean surface (use alcohol to clean). Using cleaned forceps, open filter pack and remove small glass fiber filter. Place filter in tin capsule and fold opening of tin down once and compress. Crimp sides of tin to form small packet (all dimensions < 2 mm). Place tin packet into a well in the well tray recording the well location and sample ID (station, time). Place well cap strip over wells as soon as possible to minimize any further exposure of encapsulated filter to air. It is recommended that you use a separate well plate for the samples that will be only minimally enriched if at all (PRE, POST, and deionized water samples) and the enriched samples (collected during the ^{15}N addition).
12. Tell the isotope lab to enter a dry mass value of 1 mg for all filter samples and to report a %N value for each sample. This will allow calculation of N recovery during processing of each sample. Poor N recovery may be a reason to eliminate data points.

Reference: Sigman, D. M., M. A. Altabet, R. Michener, D. C. McCorkle, B. Fry, and R. M. Holmes. 1997. Natural abundance-level measurement of nitrogen isotopic composition of oceanic nitrate: an adaptation of the ammonia diffusion method. Marine Chemistry 57:227-242. (Note that the methods outlined for the LINX II project here are based on the Sigman et al. paper, but are not exactly the same. In particular, the LINX II protocol does not go through extra incubation steps to reduce blank effects caused by breakdown of DON. Instead, DON blank effects are minimized by running diffusions at a lower incubation temperature than is used in the Sigman et al. paper.)

Calculation of N recovery:

Calculation of total N recovered in the ^{15}N analysis (calculated from the %N reported by the mass spectrometry lab and the mass values given them for the samples) for the spike samples and the secondary standards allows you to check that your % recovery of N mass is near 100%. At the small diffusion volumes associated with the $^{15}NO_3^-$ method, 100% recovery of N from the sample to the acidified filter is typical if your shaker table is working properly. If you get less than 90% recovery of your standards, this run should be redone (save the portion of the sample not processed by refrigeration or freezing in case you need to rerun some samples). Note that % recovery is a useful check for spike samples and standards, but should be viewed with caution when evaluating the recovery of real samples. When N concentrations are low, cumulative error from concentration measurements and mass determinations during isotope analysis make the percent recovery value only an approximate figure.

Devarda's alloy blanks:

All Devarda's alloy contains some N. The amount of N and the $\delta^{15}N$ in each lot # of Devarda's must be quantified to account for dilution of the ^{15}N signal. This quantification needs to be done only once per season if the same bottle of Devarda's is used throughout. To determine the blank, run a size series of nitrate standards (i.e. 25 µg N, 50 µg N, 100 µg N, 150 µg N, 200 µg N) all made up in 100mL of deionized water through the sample diffusion process along with the other $^{15}NO_3$ samples. The easiest way to do this is to make up a nitrate solution with 25 µg N per mL (25 mg N/L) and add the appropriate number of mL's to 100 mL of deionized water to get this series (it doesn't matter if the final volumes of this standard series are slightly different from one another). The amount of Devarda's added to each of these standards should be the same as used for the samples. With this size series you can then determine the mass and del value of the Devarda's blank, as well as the del value of the nitrate standard (if you use the same nitrate source as used in the nitrate spike, then this will provide another measure of the del value of the nitrate spike). Use the following calculations (M=mass, Del=$\delta^{15}N$):

1) Plot $M_{standard}$ (x-axis) versus M_{mix} (y-axis), and fit a linear relationship. The equation of the line is then $M_{mix} = (slope*M_{standard})+M_{blank}$. In other words, the y-intercept is the mass of your Devarda's blank.
2) Plot $1/M_{mix}$ (x-axis) versus Del_{Mix} (y-axis) and fit a linear relationship. Here the y-intercept is the $\delta^{15}N$ value of your standard ($Del_{standard}$), and the slope = $M_{blank}(Del_{blank}-Del_{standard})$. Rearranging, slope = $M_{blank}(Del_{blank}-Del_{standard})$ gives you $Del_{blank} = Del_{standard} + (slope/M_{blank})$. Note that value that you plug in here for M_{blank} comes from #1 above.

To correct your $^{15}NO_3^-$ sample values for the Devarda's alloy blank, use the following equation:

$$(Del_{mix}* M_{mix}) = (Del_{sample}* M_{sample}) + (Del_{blank}* M_{blank}) \quad (1)$$

or, rearranging gives you:

$$Del_{sample} = [(Del_{mix}* M_{mix}) - (Del_{blank}* M_{blank})] / (M_{mix}- M_{blank}) \quad (2)$$

where:

Del_{mix} = del value from mass spec (represents sample + Devarda's dels)
M_{mix} = N mass from mass spec analysis (represents the sample + Devarda's N mass)
Del_{sample} = value to solve for
M_{sample} = (total N mass – Devarda's N mass)
Del_{blank} = value from Blank diffusion (del value of Devarda's)
M_{blank} = value from Blank diffusion (N mass of Devarda's)

<u>Steps for construction of filter packets</u>:

1. Ash GF/D filters.
2. Spread out aluminum foil over a layer of paper towels and clean by rubbing down with alcohol. Clean forceps with alcohol.
3. Place teflon filter down and GF/D filter centered on top. Pipet 25 µL of 2.5 M KHSO4 onto GF/D filter (it will be completely absorbed by the filter).
4. Place second teflon filter centered on top of GF/D filter.
5. Seal teflon filters holding acidified GF/D filter by rolling the open end of a scintillation vial around the outside portion of the teflon filter, pressing and twisting to seal edges. To ensure that the filter pack remains stuck together, you can also create another ring around the filter with a smaller diameter vial. You should notice a thinning of the teflon filters around the edge where it is sealed. Hold the filter pack up to the light to verify this. **If you press too hard the membrane will tear, but if**

you press too lightly the membranes will not be truly stuck together and may come apart during the diffusion. You will need to practice this. Press really hard and break through a test filter pack so that you know how much is too much. Check your filter-pack-making capabilities by making a batch of dummy filter packs and shaking them with NaCl, MgO, and Devarda's for a week. See if any fall apart. Repeat this until you are confident of your abilities. Remember, a broken filter pack is a lost sample value!

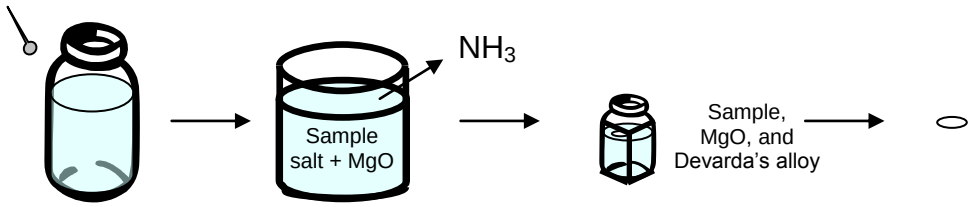
6. Place filter packet into a scintillation vial and cap very tightly. It is best to make up filter packets within a few days of use. If necessary, filter packets can be stored for several weeks, but it's critical that they be tightly capped to prevent any exposure to air.

LINX II Streamwater $^{15}NO_3$ Analysis– standards, blanks, and normal runs

Del and N Determination of Devarda's alloy blanks
(Frequency: once per season)

- 100mL standard
- 5g ashed NaCl
- 3g ashed MgO
- 0.5g Devarda's alloy
- filter packet

Del Determination of Samples (and standards and spikes)
(Frequency: once per run)



STEP 1: Spike sample (Plateau and diurnal samples only)

STEP 2: Concentrate sample and remove NH_4^+ (boil and raises pH)

STEP 3: Convert NO_3 to NH_4 and diffuse onto acidified filter (heat for 2 d and shake for 1 week)

STEP 4: Analyze filter for $\delta^{15}N$ and N mass

So, each run should look like this (after steps 1 – 3):

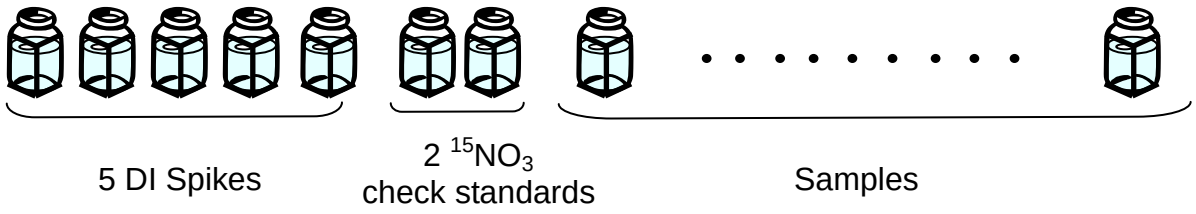


Figure 5. Summary of sample preparation and analysis for $^{15}NO_3$ (thanks to Suzanne Thomas)

7C. Preparing streamwater $^{15}NH_4$ samples for ^{15}N analysis

Laboratory Equipment and Materials:

NaCl ashed
MgO ashed
Standard NH4 solution (concentration similar to expected stream concentrations, volume similar to stream sample volumes)
Filter packets (pre-combusted and acidified GF/D filter between two teflon filters – see section 7B above for construction)
Shaker table with heater (MBL can construct for about $2300, contact: Suzanne Thomas, email: sthomas@mbl.edu, components for construction listed in Appendix C)
Scintillation vials
Encapsulating tins (holder optional)
96 well microtitre plates for storing encapsulated samples (see description in section 7A above)

To analyze for ^{15}N in streamwater ammonium we use a similar type of alkaline headspace diffusion procedure as used for the dissolved nitrate samples described above, except that we do not need to add DeVarda's alloy to the ammonium samples (see Holmes et al. 1998). The biggest problem we will face with the ammonium ^{15}N samples is getting enough N sorbed onto the filter we will have analyzed. We were able to get by with a 4-L sample in LINX and we will attempt to get by with 4 L samples in LINX II as well. However, because we will be using contract labs whose detection levels might not be as low as MBL it would be a good idea to use somewhat larger volumes if possible (6-8 L) for those streams with NH_4 concentrations < 2 μgN/L. If using larger volumes of sample is not practical (e.g., limitations on the shaker table), then it is possible to spike each sample with a known amount of NH_4 (e.g., 25 μg N into a 1 L sample) to bring the N value up above the minimum mass needed for mass spectrometer analysis (20-25 μg of N). If samples are spiked, then it is necessary to make up spike standards (e.g., same spike added to 1 L of deionized water; make up three of these spiked distilled water samples) and process these for ^{15}N in an identical way as the samples in order to determine the ^{15}N value (i.e., the del value) of the spike additions. The sample ^{15}N value is then computed using a mixing model in the same way as for the spiked $^{15}NO_3$ (see equation A2 in Appendix A-1). However, because the NH_4 concentrations are low and the ^{15}N content is likely to be at most only slightly enriched in most of our samples, this spiking procedure will reduce the accuracy of the calculation of tracer $^{15}NH_4$ in the stream and should be done only as a last resort.

The $^{15}NH_4$ samples to be processed should be in large plastic bottles/containers with a good tight screw cap that seals the bottle. The steps to follow are listed below:

1. Add 50 g of NaCl per liter of sample (200 g of NaCl for a 4-L sample).
2. Add 3 g of MgO per liter of sample (12 g MgO for a 4-L sample) and then immediately add a filter pack to float on the surface and cap tightly (would be a good idea to use parafilm under the cap to help seal it).
3. Incubate the samples for two weeks at 40 C on a shaker (or for at least 3 weeks at room temperature).

4. After the incubation remove the filter pack and follow steps #9, 10, 11 and 12 in section 7B above for drying and encapsulating the GF/D filters for ^{15}N analysis. For the samples collected during the ^{15}N addition, rinse the filter pack in deionized water immediately after removing to dilute the ^{15}N-laden water drops adhering to it.

Reference: Holmes, R. M., J. W. McClelland, D. M. Sigman, B. Fry, and B. J. Peterson. 1998. Measuring 15N-NH4 in marine, estuarine and fresh waters: an adaption of the ammonium diffusion method for samples with low ammonium concentrations. Marine Chemistry 60:235-243.

If possible, it is best to begin the processing of these samples in the field soon after samples are collected and filtered (steps 1 and 2 above). However, if this is impractical, processing should begin in the lab at least by day 2.

Determination of ^{15}N fractionation factor:

We need to determine a ^{15}N fractionation factor by including several NH_4 standards (see next subsection below) with each set of samples. These standards are run to correct for ^{15}N fractionation due to incomplete recovery of N (this is an important issue with the $^{15}NH_4$ samples because we will be dealing with low ^{15}N enrichments in these samples). The amount of N in the standards should be enough to recover 70 to 150 µg on each filter. It is not crucial to mimic the concentrations of the samples, since fractionation is independent of concentration. However, the volume of the standards and samples should be identical. Fractionation is volume dependent (see Holmes et al. 1998). The fractionation factor for each run should be determined from the standards and used to correct the $^{15}NH_4$ values of the samples in that run.

NH_4 fractionation standards:

One set of standards for the fractionation determination (natural abundance, not enriched) should be diffused with each set of samples. These standards allow correction for fractionation as well as correction for incomplete recovery of N mass. Fractionation is almost certain for sample volumes > 200mL. Your sample values will be preferentially lighter (more ^{14}N relative to ^{15}N) due to this fractionation and you will need to subtract the fractionation factor from the value you get from the mass spec (positive minus negative value equals even more positive value). The average fractionation factor from the standards will be used to correct the sample del values for each run. If the samples are moderately enriched in ^{15}N, this may not change the del value much, but for most of our samples the ^{15}N enrichment is likely to be low and this correction is critical.

Make up the fractionation standards using a NH_4 salt (e.g., NH_4Cl, $(NH_4^+)_2SO_4$, etc.) to achieve a final concentration similar to that you expect for the stream water samples (e.g., this will likely be within the range 2-10 µgN/L for most streams). The volume of the standards should be the same volume as the samples (e.g., 4 L if you are processing 4 L stream samples). Three to four fractionation standards are processed along with each set of the stream samples (see Figure 6 below) and the ^{15}N values of these standards are referred to as $Del_{standards}$.
Remember to set aside a small subsample of each fractionation standard for NH_4 analysis.

To determine the true del value of the fractionation standards (Del_{true}), run about 5 diffusions of the standard NH_4 (same lot of NH_4 salt) made up at a much higher concentration in

100 mL of dionized/deionized water (see top part of Figure 6) and calculate the mean value. Aim for a concentration of 1,400 µgN/L (so you will have about 140 µg of N in 100 ml for diffusion). With this quantity of N, you can be absolutely sure that any blank effects have been eliminated and there will be no fractionation.

The fractionation factor is then calculated as:

$$\text{Fractionation factor} = \text{Del}_{\text{standards}} - \text{Del}_{\text{true}}. \quad (3)$$

The recovery factor is calculated as:

$$\text{Recovery Factor} = \text{N mass standards/N mass true}, \quad (4)$$

where N mass standards is the calculated mass of N for the fractionation standards (% N × sample mass provided mass spec lab) and N mass true is the calculated mass of N for the Del_{true} samples.

To correct the stream $^{15}NH_4^+$ sample values ($\text{Del}_{\text{observed}}$) for fractionation, the following equation is used:

$$\text{Del}_{\text{sample}} = \text{Del}_{\text{observed}} - \text{fractionation factor}, \quad (5)$$

where the fractionation factor is from equation 3 above. The $\text{Del}_{\text{sample}}$ values will be higher (more positive) than the $\text{Del}_{\text{observed}}$ values because the fractionation factors will be negative (e.g., the fractionation factor for 2 L samples should be approximately –8 per mil).

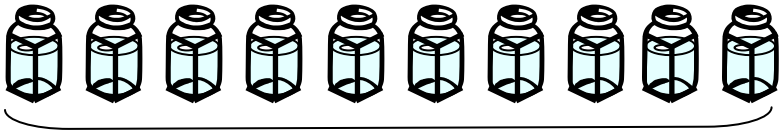
The recovery factor is used to correct the sample N mass values to determine the true N mass of each sample for use in the calculations of tracer $^{15}NH_4$ flux as follows:

$$\text{N mass true sample} = \text{N mass observed sample/Recovery Factor} \quad (6)$$

where N mass observed sample is the mass of N calculated for each sample (% N × sample mass provided mass spec lab). We will only use the sample N mass values calculated using equation 6 (and converted to a NH_4-N concentration by dividing by the sample volume), however, if we do not have good measurements of NH_4 concentration from our wet chemistry analysis. In most cases we will use the NH_4 concentrations determined from the wet chemistry analysis to compute tracer ^{15}N flux from the $\text{Del}_{\text{sample}}$ values.

LINX II Streamwater $^{15}NH_4$ Analysis– standards, blanks, and normal

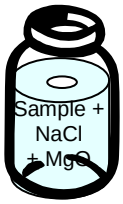
Del Determination of Standard
(Frequency: once per season)



1,400µgN/L

- 100mL standard
- 5g ashed NaCl
- 3g ashed MgO
- filter packet

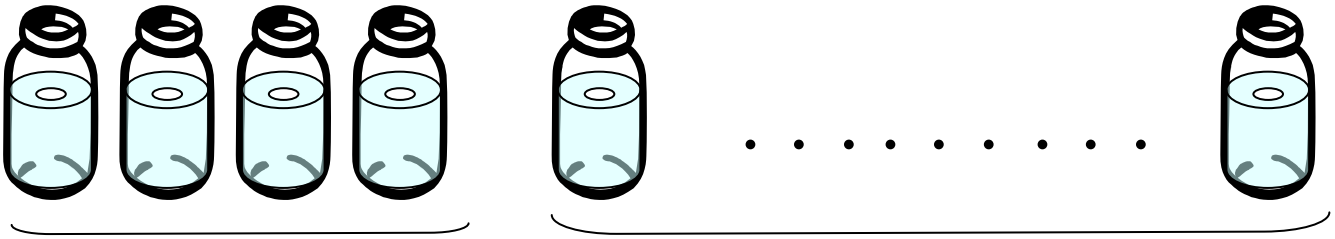
Del Determination of Samples
(Frequency: once per run)



STEP 1:
Diffuse NH_4 onto acidified filter
(shake for 2 weeks)

STEP 2:
Analyze filter for $\delta^{15}N$ and N mass

So, each run should look like this):



4 $^{15}NH_4$ standards
to calculate the fractionation factor

Samples

Figure 6. Summary of sample preparation and analysis for $^{15}NH_4$ (thanks to Suzanne Thomas)

7D. Preparing N-gas samples for ^{15}N analysis

The Exetainer samples of headspace gas (from He-equilibrations done in the field) are ready to send to the mass spectrometer analytical lab for ^{15}N analysis once the field collection, headspace equilibration, and transfer to Exetainers is complete (see subsection on Collection of ^{15}N-gas samples in section 3C). Samples should be sent to the mass spectrometer lab as soon after collection as possible, and **coordination with the lab to ensure timely analysis of the Exetainer samples is critical**. Exetainer samples should be stored and shipped under water to minimize the possibility of air contamination. . Any samples with more than 1 cm of water inside the Exetainer should be considered leaky and discarded.

As of 2004, we will analyze one of each pair of duplicate ^{15}N-gas samples at separate labs: the UC-Davis stable isotope lab and the Michigan State University (MSU) stable isotope lab. One of the duplicate samples will be sent to UC-Davis (http://stableisotopefacility.ucdavis.edu; contact is David Harris, dharris@blue.ucdavis.edu) for $^{15}N_2$ and $^{15}N_2O$ analysis (they are the only lab we know of now that can analyze for $^{15}N_2O$). The UC-Davis lab can measure both $^{15}N_2$ and $^{15}N_2O$ in the same sample, but often we do not have enough ^{15}N enrichment in the N_2 samples to use that measurement. Briefly, the entire content of the sample vial (12 mL) is flushed through a cryo trap to freeze out the N_2O. During the flushing a small sampling loop (15 µL) removes a portion for N_2 analysis that is sent directly to the MS via a molecular sieve GC column while the N_2O is held frozen. After the N_2 measurement, the N_2O is released from the cryotrap, focused in a second cryotrap, then released to the MS via a second GC column. The other duplicate sample will be sent to Michigan State (Nathaniel and Peggy Ostrom's lab, contact is Steve Hamilton) to be run for $^{15}N_2$ using a manual method that provides greater accuracy and precision. The manual measurement at MSU, which is preferable for $^{15}N_2$, is accomplished by connecting the Exetainer directly via a syringe needle to a low-deadspace evacuated line that is equipped with a sample loop on a switching valve. Costs for these analyses in 2004 were $9.50 (Davis) and $25 (MSU) per sample.

7E. Preparing streamwater TD^{15}N samples for ^{15}N analysis (from which DO^{15}N is calculated)

Laboratory Equipment and Materials:
Potassium persulfate (low N reagent)
Boric acid (low N)
Sodium hydroxide (low N)

We will determine tracer ^{15}N flux as DON (DO^{15}N) as the difference between the tracer ^{15}N flux in total dissolved N (TDN) and the tracer ^{15}N fluxes in NH_4 plus NO_3. The ^{15}N level and N concentrations in the NH_4 and NO_3 fractions are determined as described above. The ^{15}N level of TDN (TD^{15}N) is determined using a persulfate oxidation of the filtered sample, then processing the oxidized sample for ^{15}N in the same manner as for the $^{15}NO_3$ samples (see Section 7B above). However, remember that no nitrate spike is added to the TD^{15}N samples unless there is a need to add the small nitrate spike to all PRE and POST samples**. If the small nitrate**

spike (e.g., 25 µg N/L) is needed for the PRE and POST $^{15}NO_3$ samples, then the same nitrate spike should be added to the TD^{15}N samples as well.

We than calculate the tracer DO^{15}N flux is:

Tracer DO^{15}N flux = tracer TD^{15}N flux – tracer $^{15}NO_3$ flux – tracer $^{15}NH_4$ flux.

The persulfate oxidation of TDN follows the method of Valderrama 1981 (Valderrama, J. C. 1981. The simultaneous analysis of total nitrogen and phosphorus in natural waters. Mar. Chem. 10:109-122). Because we are not adding a nitrate spike, it will be necessary to oxidize somewhat larger volumes than for the 15NO3 samples collected during the ^{15}N addition (see table 3 below for sample volumes needed). The oxidized sample should be split into two portions, a small subsample (10 mL) for analysis of nitrate (to calculate TDN concentration) and the remainder to process for ^{15}N in the same way that $^{15}NO_3$ samples are processed (section 7B). The table below gives the sample volumes to process based on the nitrate concentrations in each and assuming that DON is about 100 µgN/L in all samples. Again, we are shooting for 50 to 200 µgN in the final 100 mL sample. If you know that the DON concentration is well above 100 µgN/L, then you will want to use slightly lower volumes than recommended below.

Table 3. Sample volumes to process for TD^{15}N analysis.

NO_3-N conc in streamwater (µgN/L)	Sample volume to process (mL)
< 50	1000
50 to 400	400
400 to 1500	100 *
> 1500	40 *

* The boil-down step is not necessary to concentrate these sample. However, to ensure that all NH_4 is driven off before further processing, deionized water (N free) should be added to bring the volume to about 150 mL and then the sample reduced to 100 to 125 mL using a short boil-down.

The specific procedure for persulfate oxidation is as follows. Dissolve 45 g potassium persulfate and 27 g boric acid into 315 ml 1 M sodium hydroxide. After dissolution bring up solution to total volume of 900ml with DI water. Oxidizing reagent should be made fresh daily. There are a lot of batches of potassium persulfate out there that are horribly contaminated with N. Test yours first before running samples. If anyone has a particularly good (i.e. clean) manufacturer and lot # they can share it. We have had good luck with JT Baker, 3239-01, Lot #J03777. Potassium persulfate can be recrystallized (will provide method on request) to reduce the blank but its probably easier to find a good batch and stick with it (do not heat the persulfate above 100° C or it will loose its oxidizing power. The ratio of the persulfate/boric acid/NaOH oxidizing reagent to add to the samples is 4 mL of oxidizing reagent to 30 mL of sample. For small volume samples do the oxidation in acid washed screw cap test tubes with teflon lined caps. For larger volume samples use screw cap culture bottles whose caps have a teflon (or other inert) liner. Autoclave for at least 1 hour at 121°C and 15 lb/in^2. Run some standards of urea,

nicotinic acid, nitrophenol, or other organic N compound the first couple times to convince yourself the reaction is working fine.

As stated above, the oxidized sample is then processed for ^{15}N in the same manner as the $^{15}NO_3$ samples (boil down, then addition of Devarda's, salt, MgO, and filter pack, heat at 60^{o}C for 48 hours, then on shaker for 1 week, remove and process filter with sorbed ^{15}N).

7F. Analytical Labs for ^{15}N samples

There are several analytical labs capable for analyzing ^{15}N samples (should also request ^{13}C values for all organic matter samples if possible, although make sure labs optimize for ^{15}N). It is recommended that you use the same lab for analysis of samples from all of your experiments. See Section 7D above regarding ^{15}N gas samples. The University of California at Davis can analyze various kinds of samples (website: http://stableisotopefacility.ucdavis.edu; contact: David Harris, email: dharris@ucdavis.edu). In 2004, they charged $9.50 per sample for $^{15}N_2$ and $^{15}N_2O$ (gas samples), $5.00 for highly enriched filter samples, $6.50 for the filter samples at or near natural abundance, $7.00 for highly enriched organic matter samples, and $8.00 for organic matter samples at or near natural abundance.

The isotope lab at Kansas State (website: www.ksu.edu/simsl) can also analyze the solid samples (filters and organic matter). Although the Kansas State lab can analyze $^{15}N_2$ in the ^{15}N-gas samples (but not $^{15}N_2O$), the accuracy and precision is higher at the MSU lab for these samples so we will use the UC-Davis lab and MSU lab for the duplicate ^{15}N-gas samples as described in section 7D.

For solid samples remember to send the isotope lab a list of the dry mass values for each of your samples (by well position in the microtitre plates). Also, remember to tell the lab to use a dry mass of 1 mg for all the filter samples (and to report the %N values) so that we can calculate N recovery using the %N values. For the ^{15}N gas samples, both the Davis and MSU labs need to know the sample size (as indicated by the syringe size). Standards are important to verify the accuracy and precision of the measurements, and ideally they would span the range of N mass and $\delta^{15}N$ found in the samples. This is difficult although not impossible for the gas samples.

APPENDIX A

CALCULATION OF UPTAKE LENGTH AND RATES

Excel worksheets that will perform the calculations described below are included in the excel file "LINX 2 Data Spreadsheets_Field 15N addition experiment_REV 2.xls" and are titled "Calculation of ….". Some of the preliminary calculation steps (e.g., calculation of tracer ^{15}N flues) are performed in the worksheets for recording the primary data ("Primary data ….").

A-1. Nitrate Uptake Length

We will use two methods for calculating NO_3 uptake length using the ^{15}N data: (1) using the water ^{15}N-NO_3 mass flux data (determined from $\delta^{15}N$-NO_3 data, NO_3 concentrations, and flow), and (2) using benthic biomass $\delta^{15}N$ data (epilithon, macro-autotrophs) corrected for downstream dilution. Each of these methods involves the same calculation: a **regression of the natural log of the ^{15}N value (corrected for background, and corrected for dilution if $\delta^{15}N$ data are used) against distance below the ^{15}N dripper (in meters).** The slope of this regression is the distance-normalized NO_3 uptake rate and the inverse of the slope is the NO_3 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 benthic biomass approach should only use compartments that become highly labeled during the experiment and that are stationary (e.g., epilithon, bryophytes, filamentous algae).

Calculation of uptake length using streamwater tracer ^{15}N mass flux data**.** The uptake length of nitrate is calculated from the decline in tracer ^{15}N mass flux over the stream reach. **However, because the measured values of ^{15}N content ($\delta^{15}N$, in units of per mil) are $^{15}N/^{14}N$ ratios we must first convert the $\delta^{15}N$ values to $^{15}N/^{15}N+^{14}N$ ratios (mole fraction of ^{15}N) because our measurements of nitrate include both ^{15}N and ^{14}N.** We did not need to do this conversion in the first LINX project because the $\delta^{15}N$ values were relatively low and thus our measurements of nitrate or ammonium included very little ^{15}N. In LINX II, however, we will be dealing with relatively high $\delta^{15}N$ values (up to 20,000 per mil) and the amount of ^{15}N in the samples will be non trivial (e.g., at enrichment of 20,000 per mil ^{15}N will make up about 7.7% of the nitrate).

The equation to convert $\delta^{15}N$ values to $\frac{^{15}N}{^{15}N+^{14}N}$ is:

$$\frac{^{15}\mathbf{N}}{^{15}\mathbf{N}+^{14}\mathbf{N}} = \frac{\left(\frac{\boldsymbol{\delta}^{15}\mathbf{N}}{1000}+1\right)*0.0036765}{1+\left(\left(\frac{\boldsymbol{\delta}^{15}\mathbf{N}}{1000}+1\right)*0.0036765\right)} \quad . \qquad \text{(A1)}$$

From here on out we will call $\frac{^{15}N}{^{15}N+^{14}N}$ the mole fraction of ^{15}N (MF).

After converting nitrate $\delta^{15}N$ values to MF values using equation A1, the first correction that must be made is for the Devarda's blank ^{15}N. This will result in a relatively small (but not trivial) change in the MF of highly ^{15}N-enriched samples collected during the tracer plateau as well as a small change in the MF of the PRE and POST samples. This is because the mass of N we are inadvertently adding with the Devarda's alloy is small relative to the mass of N in the samples, although the $\delta^{15}N$ value of the Devarda's alloy will be very much lower than the $\delta^{15}N$ values of plateau samples. Nonetheless, for completeness, we will make this correction for all samples. The MF values for Devarda's blank corrected samples (MF_{DCi} are calculated as follows:

$$MF_{DCi} = \frac{(((V_i * [NO_3\text{-}N_i]) + M_{ND}) * MF_{mi}) - (M_{ND} * MF_D)}{V_i * [NO_3\text{-}N_i]} \qquad (A2)$$

where V_i is the original volume of stream water sample at station i boiled down to 100 mL, $[NO_3\text{-}N_i]$ is the measured stream water nitrate N concentration at station i (in μgN/L), M_{ND} is the mass of N in the Devarda's alloy added to each sample (this is determined from the regression described in section 7B in the subsection on Devarda's alloy blanks), MF_{mi} is the measured MF values for station i determined from the $\delta^{15}N$ values using equation A1 above, and MF_D is the calculated MF of N in the Devarda's alloy (determined as described in section 7B in subsection on Devarda's alloy blanks except that we also convert to an MF value).

Next we must correct for the nitrate spike added to all samples collected during the ^{15}N addition (plateau samples and diurnal samples only). As described in section 7B, the nitrate spike was added to increase the sample nitrate N concentration and thereby lower the $\delta^{15}N$ values (into a range the lab would analyze). The following equation is used to calculate MF values representative of the stream water (i.e., nitrate spike corrected) from the MF values initially calculated from the measured $\delta^{15}N$ values using equation A1 and then corrected for the Devarda's alloy blank:

$$MF_i = \frac{[NO_3\text{-}N_i + NO_3\text{-}N_{sp}](MF_{DCi}) - [NO_3\text{-}N_{sp}](MF_{sp})}{[NO_3\text{-}N_i]} \qquad (A3)$$

where $[NO_3\text{-}N_i]$ is the measured nitrate N concentration at station i (in μgN/L), $[NO_3\text{-}N_{sp}]$ is the nitrate N concentration increase from the nitrate spike (in μgN/L, should be the same for all stations), MF_{DCi} is the MF value at station i calculated from the measured $\delta^{15}N$ values at station i using equation A1 and corrected for the Devarda's blank using equation A2, MF_{sp} is the MF value of the nitrate spike calculated from the measured $\delta^{15}N$ value of nitrate in the spike ($\delta^{15}N$ values of samples of nitrate spiked into deionized water), and MF_i is the true MF value of nitrate at station i. A similar calculation is performed for the PRE and POST samples if a small NO_3 spike was added using the appropriate values for $[NO_3\text{-}N_{sp}]$ and MF_{sp} for the low NO_3 spikes.

The tracer ^{15}N mass flux at each station i (tracer $^{15}N_{flux\ i}$, units of μg/s) is then computed by subtracting the background ^{15}N mass flux from the total ^{15}N mass flux using the appropriate MF values, nitrate concentrations, and discharges according to the following equation:

$$\text{Tracer } ^{15}N_{flux\ i} = (MF_i * [NO3\text{-}N_i] * Q_i) - (MF_b * [NO3\text{-}N_i] * Q_i) \qquad (A4)$$

where MF_b is the background MF value determined as the average Devarda's blank and spike corrected (if necessary) MF value for the PRE samples. Please note that in this calculation we use the nitrate concentrations determined during the ^{15}N addition in both the first and second terms of the right side of equation A4 (i.e., the [NO3-N_i] values are the same in the first and second terms). We use the nitrate concentration during the experiment rather than nitrate concentration for the PRE samples to calculate the background ^{15}N mass flux during the experiment because there may be diurnal variations in nitrate concentration and we want to account for these. Stream discharge at each station (Q_i) is determined from the increase in streamwater Cl concentration during the ^{15}N injection as follows:

$$Q_i = (Cl_I)/\Delta Cl_i \qquad (A5)$$

where Cl_i is the Cl injection rate (mg/s) determined as the product of Cl concentration in the injection solution and the injection rate, and ΔCl_i is the increase in Cl concentration at each station i during the injection determined as the measured Cl concentration of samples collected during the ^{15}N injection minus the measured Cl concentration just prior to the ^{15}N injection.

Uptake length is then calculated as the inverse of the slope of the regression of $\ln(^{15}N_{fluxbci})$ versus distance downstream from the ^{15}N addition point. For sampling periods during which samples are collected at all stations (longitudinal plateau samples), we will use data from all stations in the regression to compute uptake length. We will likely use the calculation of uptake length for the 2nd plateau sampling for most comparisons among streams. For the diurnal samples collected only from station 4, we will calculate uptake length for each hour using the station 1 values determined from the 1st plateau sampling (we are assuming values are constant over the diurnal period) and the individual samples collected every two hours at station 4 (essentially a two-point regression).

Calculation of uptake length using biomass ^{15}N values. As an alternative to calculating uptake length using the streamwater fluxes of tracer ^{15}N-NO_3, we can use the biomass tracer ^{15}N standing stocks determined for samples collected just after the experiment ends. Uptake lengths calculated in this way should only use biomass compartments that are relatively stationary and that have relatively high tracer ^{15}N values. Calculation of NO_3 uptake length using the organism/biomass ^{15}N values at each station involves first the calculation of tracer ^{15}N biomass standing stock at each station (these values are calculated in the "Primary data – N15 in biomass" worksheet using the background-corrected MF values, %N values, and dry mass standing stocks for a particular biomass compartment) and then correcting for dilution by multiplying by the ratio of flow at that station to flow at the uppermost stations below the ^{15}N addition point. Then, uptake length is calculated as the inverse of the slope of the regression of ln(dilution-corrected tracer ^{15}N biomass standing stock at station i) vs. distance downstream from the dripper. If highly labeled and stationary biomass compartments are used, this approach should provide a good measure of uptake length. This approach, however, provides an indirect measure of uptake length whereas quantifying the decline in tracer $^{15}NO_3$ flux is a direct measure of uptake length.

A-2. Nitrate Uptake Rates

We can compute two types of NO_3 uptake rates from the ^{15}N 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., μgN m^{-2} s^{-1}).

Whole-stream NO_3 uptake rate. The whole-stream uptake rate is computed from the NO_3 uptake length (S_W), streamwater NO_3 flux (F, computed as the product of stream discharge in L/s and stream NO_3 concentration in μgN/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):

$$\mathbf{NO_3\ \ uptake\ rate\ (\mu gN\ m^{-2}\ s^{-1}) = \frac{F}{S_w * w}} \qquad \text{(A6)}$$

This is the total NO_3 uptake rate (rate of removal of NO_3 from stream water) by all stream compartments (usually designated as U).

Biomass compartment-specific NO_3 uptake rate. To compute compartment-specific NO_3 uptake rates we first compute the tracer ^{15}N standing stock in biomass at each station i using the biomass standing stocks (average stream dry mass standing stock), the %N in biomass, and the MF values determined for biomass samples at the upstream station (MF_b, background value) and the downstream stations (MF_i):

$$\text{Tracer } {}^{15}N_{\text{biomass i}} = \text{Biomass standing stock} \times (\%N/100) \times (MF_i - MF_b) \qquad \text{(A7)}$$

These calculations are performed in the "Primary data – N15 in biomass" worksheet.

The calculations for the biomass compartment-specific nitrate uptake rates are then performed in excel worksheet "Calc of biomass-spec upt rate". Uptake rates of NO_3 by individual stream biomass compartments are computed using the tracer $^{15}N_{\text{biomass i}}$ values, the steady state (at plateau during ^{15}N addition) tracer ^{15}N-nitrate flux in streamwater ($^{15}N_{\text{flux i}}$, computed using equation A4 above), and the total nitrate-N flux in streamwater at each station i ($N_{\text{flux i}}$, computed as the nitrate concentration × discharge at the plateau samplings at each station) as follows:

$$\text{Biomass } NO_3 \text{ uptake rate i} = \frac{Tracer\,{}^{15}N_{biomassi}}{({}^{15}N_{fluxi} / N_{fluxi})} \qquad \text{(A8)}$$

The NO_3 uptake rates at each station i are then averaged over all of the stream stations to get an average stream NO_3 uptake rate for that particular biomass compartment. These values are uptake rates per day because the ^{15}N addition experiment was 1 day in length.

Equation A8 might slightly underestimate NO_3 uptake rate by each compartment because we are assuming that there has been no recycling of ^{15}N (release back to the water) between day 1 (beginning of experiment) and day 3 when we sample biomass. Although we expect that there is likely some release of ^{15}N taken up in the 24 hours between the end of the ^{15}N addition (1 pm on day 2) and the biomass sampling (day 3 pm), this is probably relatively small and thus the underestimate should likewise be relatively small. A much larger source of uncertainty will be the estimates of biomass standing stock for each compartment.

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 (using equation A6). The summed compartment-specific uptake rates probably will be lower than the whole-stream rate from the uptake lengths because: (1) there may be compartments involved in uptake that were not sampled, and (2) underestimation of the true compartment-specific uptake rates because of the assumption of no ^{15}N recycling between days 1 and 3. Nonetheless, the biomass-specific calculations give an indication of relative importance of different biomass compartments in assimilatory NO_3 uptake. Finally, it should be emphasized that we are calculating only NO_3 uptake rate rates, both on a whole-stream basis and for each compartment. If there is appreciable uptake of NH_4 (and perhaps of DON), which is likely, then the total N uptake rate will be greater than the uptake rate of NO_3 alone.

A-3. Nitrification Rate

Total nitrification rate (sum of direct nitrification of ammonium in water and indirect nitrification due to coupled mineralization and nitrification) will be determined using a nitrate mass balance approach for the study reach. This approach uses the measurements of stream discharge and nitrate concentration in stream water (station 1) and groundwater to determine the stream and groundwater inputs of nitrate to the reach ($N_{in\ stream}$ and $N_{in\ gw}$, respectively). If there are not good measurements of groundwater nitrate concentration, then the average streamwater nitrate concentration can be used instead to estimate groundwater nitrate inputs, although this will likely result in an overestimation of nitrification rate. Stream nitrate losses include downstream nitrate losses ($N_{out\ stream}$), determined from stream discharge and nitrate concentration at the bottom of the reach (station 6), and the total nitrate uptake rate flux (Upt). The total nitrate uptake flux (Upt, in units of ugN/s) is calculated from the nitrate uptake length (Sw), the average of nitrate fluxes in water (F_{ave}, which is the average of F_{up} and F_{dn}), and the reach length between the stations (L) as follows:

$$\mathbf{Upt} = \mathbf{F_{ave}} \times \left(\frac{1}{\mathbf{Sw}} \right) \times \mathbf{L} \qquad \text{(A9).}$$

Finally, the nitrification rate flux (Nit) is then calculated from the nitrate mass balance for the reach as follows:

$$N_{in\ stream} + N_{in\ gw} + Nit = N_{out\ stream} + Upt \qquad \text{(A10)}$$

$$Nit = (N_{out\ stream} + Upt) - (N_{in\ stream} + N_{in\ gw}) \quad \text{(A11)}.$$

Comparison of the nitrification N flux (Nit, calculated using equation A11) with the total uptake flux of nitrate (U, calculated using equation A9) is an indication of the importance of nitrification to the nitrate budget.

A-4. Denitrification Rates (N_2 and N_2O production rates)

Denitrification rates (production of N_2 and N_2O considered separately) are estimated from the production of tracer $^{15}N_2$ and $^{15}N_2O$ within the study reach. The tracer $^{15}N_2$ and $^{15}N_2O$ fluxes are computed from the $\delta^{15}N$ values and the N_2 and N_2O mass values reported by the mass spectrometry lab in the primary data worksheet ("Primary data_N15 gases"). In order to compute tracer $^{15}N_2$ and $^{15}N_2O$ fluxes, we must first convert the $\delta^{15}N$ values of N_2 and N_2O to mole fractions of ^{15}N (MF) using equation A1 as we did for the $^{15}NO_3$ data. In addition, the headspace mass values for N_2 and N_2O reported by the mass spectrometry lab are not the total mass of N_2 and N_2O in the original water sample because there is not a complete degassing of N_2 and N_2O into the headspace (and the N_2 concentrations may also be high due to air contamination). For N_2, we calculate Bunsen coefficients at the temperature and pressure at which the headspace equilibration was performed and then determine the concentration of N_2 in the original stream water sample from the measured headspace concentrations. We then calculate the expected concentration if there was no air contamination (assuming our stream samples should be at equilibrium N_2 levels w.r.t. the atmosphere), and then use the expected and measured N_2 concentrations to correct the $\delta^{15}N$ values for the air contamination (using a mixing model and assuming that the air contamination has a $\delta^{15}N$ value of 0). It is these air-contamination corrected $\delta^{15}N$ values that we use to compute the MF values for N_2. The tracer $^{15}N_2$ fluxes at each station i are then computed using equation A4, except that we use the expected streamwater concentration of N_2 (that calculated assuming no air contamination) instead of the nitrate concentration.

For the N_2O calculations, we compute the N_2O concentration in the original streamwater sample from the headspace N_2O mass values reported by the mass spectrometry lab and the Henry's Law coefficient for N_2O. You may wish to use the headspace N_2O values from Steve Hamilton's GC analysis rather than those reported for the mass spectrometry lab. This gas partitioning correction will result in a substantial increase in the N_2O concentration relative to the reported values in the headspace gas (because N_2O is a moderately soluble gas). We then use equation A4 to compute the tracer $^{15}N_2O$ fluxes at each station i using the background-corrected MF values, the N_2O concentrations in the original water samples, and the stream discharges.

We now use the longitudinal distribution of tracer $^{15}N_2$ and $^{15}N_2O$ fluxes to determine the N_2 and N_2O production rates within the stream. The excel worksheets for these calculations are "Calculation of Denitrif_N_2" and Calculation of Denitrif_N_2O". Because we will have simultaneous production of $^{15}N_2$ and $^{15}N_2O$ and loss of these via exchange with the atmosphere, we should observe a humped shaped pattern of $^{15}N_2$ and $^{15}N_2O$ flux with distance within our study reach. This situation is similar to that for $^{15}NO_3$ produced by nitrification in the LINX I study, except that the products are now $^{15}N_2$ and $^{15}N_2O$. Thus, we will use a similar two-box

model as used in LINX (see Figure 1 in Mulholland et al. 2000 paper – Ecological Monographs 70:471-493). Figure A1 at the end of this subsection illustrates the model modified for use here.

We will solve for the of $^{15}N_2$ and $^{15}N_2O$ production rates by fitting the following relationship to the longitudinal pattern in tracer $^{15}N_2$ and $^{15}N_2O$ flux (A, in units of ug ^{15}N/s for $^{15}N_2$ and ng ^{15}N/s for $^{15}N_2O$, respectively) with distance x (in units of m) downstream from the ^{15}N addition point:

$$\mathbf{A} = \left(\frac{\mathbf{k_{den}} \times \mathbf{N}_0}{\mathbf{k}_2 - \mathbf{k}_1} \right) \times \left(\mathbf{e}^{-\mathbf{k}1\mathbf{x}} - \mathbf{e}^{-\mathbf{k}2\mathbf{x}} \right) \qquad \text{(A12)}$$

where k_{den} is the denitrification rate (production rate of either of N_2 or N_2O); N_0 is the flux ^{15}N-nitrate calculated at the point of the injection [e.g., calculated from the intercept of the ln (^{15}N-nitrate flux) vs distance regression]; k_1 is the measured rate of decline in streamwater ^{15}N-nitrate flux with distance due to all processes (i.e., assimilatory uptake, k_U, and denitrification; in units of m^{-1}); and k_2 is the N_2 or N_2O gas exchange rate per unit distance (units of m^{-1}). Values of k_2 are determined from the propane or SF6 injection experiment described in Section 4B above. The tracer $^{15}N_2$ and $^{15}N_2O$ flux values at each station i are computed as described in the first paragraph of this section.

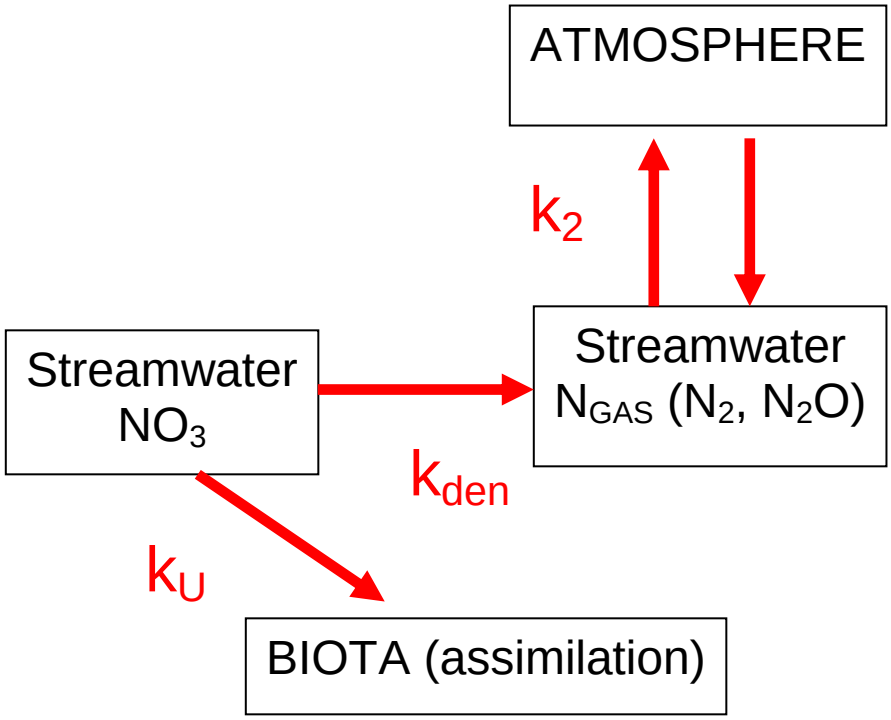
We then use a least squares fitting procedure in Excel (optimization tool "Solver") to determine the values of k_{den} from fitting the model to the longitudinal data on ^{15}N-N_2 or ^{15}N-N_2O flux (A). The steps in the application of "Solver" are listed in the excel spreadsheet "Calculation of Denitrification_N2" or "Calculation of Denitrification_N2O" and are as follows:

1. Set up an area where all model parameters are stored (e.g., rows 21-26, columns A-D) and enter the knowns and an initial estimate for the unknowns (k_{den}).
2. Set up a column for distance and a column that includes the solution of the equation (e.g., for plateau 1 columns K and L rows 21 and on). A column can also be set up for the equation with no ammonium uptake (e.g., column M rows 21 on). Extend this data table to include distances corresponding to the entire reach for which there are $^{15}N_2$ or $^{15}N_2O$ data (probably corresponding to station 6)
3. Set up an area that includes the observed (e.g., cells C33 to C42) and predicted data (e.g., cells D33 to D42). The predicted data needs to refer to the cell calculation in step 2 that corresponds with the observed distance. Calculate the error squared of observed vs distance (e.g., cells E33 to E42).
4. Sum the errors squared (e.g., cell E44). The SSE may need to be multiplied by 1000 or so in order to get enough digits (too many decimal places messes up Solver).
5. Go to the excel tool "Solver".
6. Enter the target cell as the cell with the SSE (e.g., either E44 or E45). Choose to minimize this value.
7. Pick the cells that you want to solve for and enter in the blank space under "By changing cells" (e.g., $C25).
8. Set up the parameter constraints by clicking on Add next to the "subject to the constraints" window (e.g., C25 >= 0, C25<k1 value).

9. Solve by clicking on "solve" button. This should provide an optimum solution for k_{den}.

The calculated value of k_{den} (units of m^{-1}) can then be compared to the measured value of k_1 (inverse of nitrate uptake length) to determine the relative importance of dentrification (either as N_2 or as N_2O production) as a sink for stream nitrate.

Figure A1: Model used in denitrification calculations.



Tracer ^{15}N over distance, x:

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

$d^{15}N_{GAS}/dx = k_{den}{}^{15}NO_3 - k_2{}^{15}N_{GAS}$

Steady state solutions:

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

$^{15}N_{GAS} = [k_{den}(^{15}NO_3)_0/(k_2-k_{den}-k_U)] * [e^{-(kden+kU)x} - e^{-k2x}]$

where:

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

k_{den} is the denitrification rate (N_2 or N_2O production rate

k_2 is the air-water exchange rate of N_2 and N_2O

k_U+k_{den} is the measured rate of $^{15}NO_3$ decline with distance ($1/S_W$)

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

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

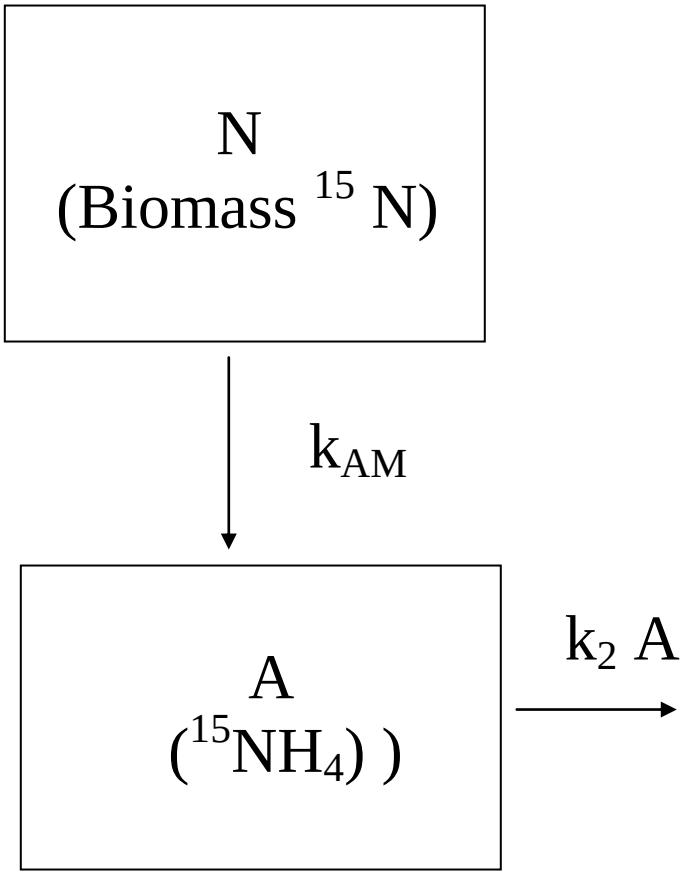
A-5. Net Ammonification and Ammonium Uptake Rates

Net ammonification rates (net ammonium production from mineralization) and ammonium uptake rates are estimated from the production of ^{15}N-ammonium within the study reach as measured at the POST 24-hour sampling. The ammonification rates are net rates because we will not be able to account for the rapid ammonification/nitrification that may occur within interstitial water (this will be accounted for in the indirect nitrification calculations using the POST 24-hour ^{15}N-nitrate data. Because we will have simultaneous production of ^{15}N-ammonium via ammonification and uptake of ^{15}N-ammonium via assimilatory uptake and nitrification, we should observe a humped shaped pattern of ^{15}N-ammonium flux with distance within our study reach. This situation is analogous to the pattern produced by nitrification during the ^{15}N-ammonium additions in the LINX experiments. Thus, we will use the same two-box model we used in LINX (see Figure 1 in Mulholland et al. 2000 paper – Ecological Monographs 70:471-493). The modified two-box model for this application is given in Figure A2. We will solve for both the net ammonification rate and the ammonium uptake rate by fitting the following relationship to the longitudinal pattern in tracer ^{15}N-ammonium flux (A, in units of ug ^{15}N/s) with distance x (in units of m) downstream from the ^{15}N addition point (x=0):

$$\mathbf{A} = \left(\frac{\mathbf{k_{AM}} \times \mathbf{N_0}}{\mathbf{k_2} - \mathbf{k_1}} \right) \times \left(\mathbf{e}^{-\mathbf{k1x}} - \mathbf{e}^{-\mathbf{k2x}} \right) \qquad \text{(A13)}$$

where k_{AM} is the net ammonification rate (units of s^{-1}); N_0 is the total ^{15}N in biomass calculated at the point of the injection (in units of ug^{15}N/m and calculated from the intercept of the Ln (^{15}N-biomass) vs distance regression); k_1 is the measured rate of decline in total ^{15}N in biomass with distance (in units of m^{-1} and calculated from the slope of the Ln (^{15}N-biomass) vs distance regression); and k_2 is the total ^{15}N-ammonium uptake rate per unit distance (in units of m^{-1}). We then use a least squares fitting procedure in Excel (optimization tool "Solver") to determine the values of k_{AM} and k_2 from the longitudinal data on ^{15}N-ammonium flux (A). Note that in this application of "Solver" in step 7 (choice of cells to solve for) you select both the cells for k_{AM} and k_2 so that both are solved simultaneously.

Figure A2. Model used in net ammonification calculations



Model equations:

$dN/dx = k_1 N$

$dA/dx = k_{AM} - k_2 A$

Steady state solutions:

$N = N_0 e^{-k1x}$

$A = (k_{AM} N_0 / k_2 - k_1) ((e^{-k1x} - e^{-k2x})$

where :

N_0 is biomass ^{15}N at x=0

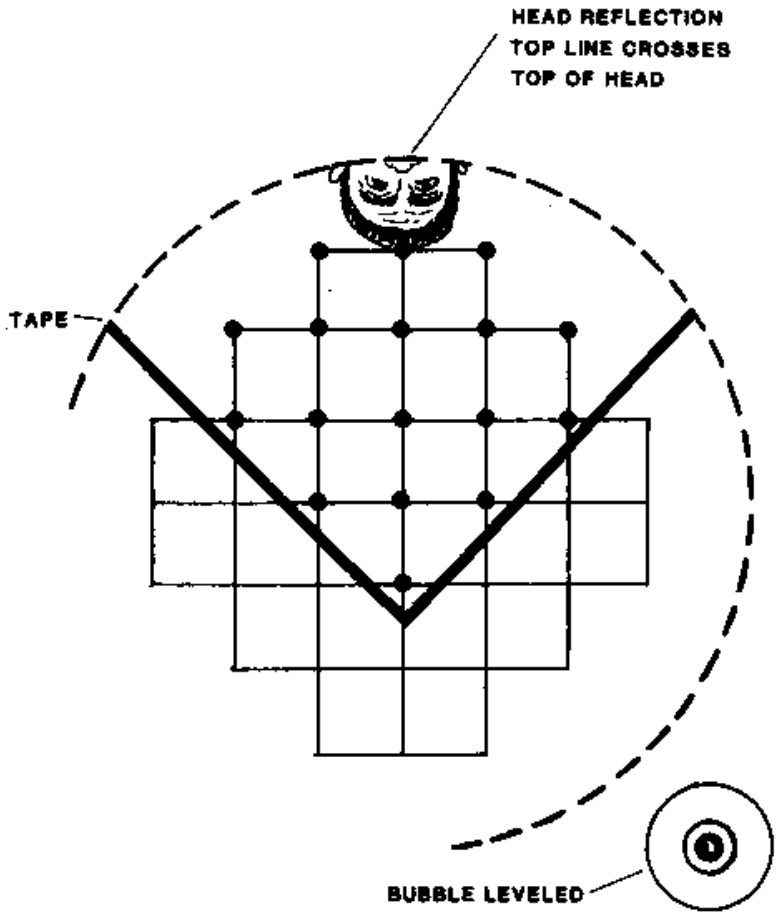
k_{AM} is net ammonification rate

k_1 is rate of biomass ^{15}N decline w/ distance

k_2 is total ammonium uptake rate

APPENDIX B.

Canopy Cover (Densiometer) methods



-The concave spherical densiometer with placement of head reflection, bubble level, tape, and 17 points of observation.

We will be using concave densiometers to measure vegetative canopy cover over the stream and within the riparian transects. Concave spherical densiometers are available from Ben Meadows Company and Forestry Suppliers, among others. The respective web pages are:

http://www.benmeadows.com/store/product.asp?dept_id=1276&pf_id=7789&cat_prefix=2WB

http://www.forestry-suppliers.com/product_pages/View_Catalog_Page.asp?ID=1397 (model C)

Vegetative cover over the stream or in the riparian vegetation transects will be quantified using a Concave Spherical Densiometer, model B (Lemmon, 1957). The densiometer must be taped to limit the number of square grid intersections to 17 (see above). While doing the measurement, it is important that the densiometer be level, using the built in bubble level.

To take a canopy cover density measurement, the observer looks down on the densiometer held just above waist level, concentrating on the 17 points of intersection. If the reflection of a tree or high branch or leaf overlies any of the intersection points, that particular intersection is counted as having cover. The measure to be recorded is the count (from 0 to 17) of all the intersections that have vegetation covering them. At each point, densiometer measures are taken separately in four directions (facing upstream, facing downstream, facing right bank and facing left bank).

APPENDIC C

Shaker/Heater for ammonium 15N sample diffusions (from MBL)

SHAKER OVEN COMPONENTS

Supplier: McMaster-Carr

Gear motor 59825K53 $258.67
- 130v
- 1.8 amp
- ¼ hp
- torque = 75 in lb
- R.P.M. = 125
- Ratio = 20 : 1

Speed control 7793K51 $178.25

Stainless cart 2544T2 $205.00
- *check on the size of cart relative to incubator size

Supplier: Grainger

Heater (1550w 120v)	4E268	$48.00
Blower motor	4C441A	$32.74
Thermostat (60º - 250º F)	3HL19	$111.03
Housing	3HL21	$23.03

**Dimensions of the wood cabinent at Ecosystems : 3 ft wide x 4 ft high x 2 ft deep

Contact at MBL (machinist who has built these and can answer questions on construction):
Rick Langill. (rlangill@mbl.edu, phone: 508-548-3705 ext 7237 or 7776).

Photo of MBL Shaker/heater:

APPENDIX D

USE OF SF_6 AS GAS EXCAHNGE RATE TRACER IN STREAMS
Bob Hall

Materials:

5-10lb tank of SF_6. Price on this varies over short time intervals. It is expensive ($200-$500) but a 10lb tank has lasted me about 5 years (ca.30 reaeration estimates)

SF6 regulator

Tubing

Flowmeter with needle valve. Cole-Parmer variable area flow meter. Tube is N082-03 with glass float. Female threads to which I screw in NPT threaded barbed fittings for tubes. Needle valve to precisely control flow. I don't think it is possible to regulate flow without a needle valve. Cole Parmer will give you the calibration curve for air for this flowmeter, but this won't work with heavy SF6--you will need to calibrate it yourself. My calibration won't work for most users because it is for high elevation.

Airstone. I use a Aquatic Ecosystems Sweetwater AS3 diffuser, which has almost no back pressure. Any fine bubble diffuser will work.

60 ml syringes with 2-way valve

Wheaton 10 ml serum vials (Fisher cat # 06-406D) with butyl stoppers (Supelco 27232) with aluminum crimps (Supelco 27200). Evacuate these yourself.

GC with Electron capture detector set up as follows. Poropak Q column, 6 feet, 80-100 mesh. Detector temp, 320 °C. Oven temp 40 °C. High-purity N2 carrier gas at 39 ml/min

Methods:

In the field bubble SF_6 through the airstone into a stream at a spot good for mixing. I use about 100 ml/min for a 50-200 L/s stream. In a big stream (say 1000 L/s) I will increase to 200-300 ml/min. Also in big, shallow stream mixing may be a problem. This is plenty of gas. At the same time add chloride or bromide tracer to 1-20 mg Cl /L or 50µg Br/L. After equilibrium is reached downstream collect 8-20 gas samples along the study reach. Suck up 45 ml of water into a 60-ml plastic syringe equipped with a 2-way valve ensuring that there a few gas bubbles in the syringe. Later (and far away from the stream), suck in 15 ml of air, shake for 10 min, and then inject all of the air into the evacuated serum vials. Collect water samples for salt tracer and analyze on an ion chromatograph. Gas samples are good to store at this point for at least 2 months. If mixing is a problem or if reaeration is going to be very low, then use the leftover 45 ml of water in the syringes for the salt analysis, by filtering the water into a clean plastic bottle.

In the lab, inject 0.1 ml into the GC This is usually enough for a huge peak. Retention time is about 2 min. I don't use standards since absolute concentration is not necessary to calculate reaeration. In no way is this analysis method optimized for anything. I imitated the approach given in Cole and Caraco (1998), and even had to dumb down the GC to avoid pinning the detector. Anyone who knows anything about a GC could optimize this for much lower concentrations of SF_6, so my method represents almost no trial and error or optimization; it

worked on the first try with the settings above. ECD's are super sensitive to SF_6 so fiddling with the GC is probably not necessary given the large amount of SF_6 in the samples

Reaeration coefficient of SF_6 is calculated based on Wanninkohf et al. (1990), which is analogous to propane. Given K_{SF6} it is possible to calculate the K for any other gas based on the ratio of their Schmidt numbers. I estimate the reaeration rates for O2 as 1.4 times higher than SF_6 at the same temperature.

Cole, J. J. and N. F. Caraco. 1998. Atmospheric exchange of carbon dioxide in a low-wind oligotrophic lake. Limnology and Oceanography 43:647-656.

Wanninkhof, R., P. J. Mulholland, and J. W. Elwood. 1990. Gas exchange rates for a first-order stream determined with deliberate and natural tracers. Water Resources Research 26:1621-1630.

NOTE: IF YOU HAVE ANY QUESTIONS CONCERNING THESE PROTOCOLS, PLEASE CONTACT PAT MULHOLLAND (mulhollandpj@ornl.gov, 865-574-7304).