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Developing a Method for Quantifying Nitrogen Transformation Rates Using In Situ Benthic Chambers Dosed with Isotopically Labeled Nitrate

Chelsea A. Stewardson
Utah State University

Utah State University
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DEVELOPING A METHOD FOR QUANTIFYING NITROGEN TRANSFORMATION

RATES USING *IN SITU* BENTHIC CHAMBERS DOSED WITH

ISOTOPICALLY LABELED NITRATE

by

Chelsea A. Stewardson

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

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Approved:

_____   _____
R. Ryan Dupont, Ph.D.   Bethany T. Neilson, Ph.D.
Major Professor   Committee Member

_____   _____
Joan E. McLean, M.S.   Mark. R. McLellan, Ph.D.
Committee Member   Vice President for Research and
                                                                       Dean of the School of Graduate
                                                                       Studies

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ABSTRACT

Developing a Method for Quantifying Nitrogen Transformation Rates Using *In Situ*

Benthic Chambers Dosed with Isotopically Labeled Nitrate

by

Chelsea A. Stewardson, Master of Science

Utah State University, 2016

Major Professor: R. Ryan Dupont, Ph.D.
Department: Civil and Environmental Engineering

The transport and transformation of nitrogen within streams receiving high nitrate

loads has become increasingly important as restrictions on water quality continue to

tighten due to an increased awareness that pollutant loading impacts the environment at

concentrations lower than previously recognized. Silver Creek in Park City, Utah, is one

of many streams in the state of Utah being evaluated for its ability to process high nitrate

loads coming from a water reclamation facility. Previous modelling techniques at Silver

Creek revealed that the lack of information on site-specific nitrogen transformation rates

left modelers unable to represent the true processes occurring within the system. To

address these concerns, six *in situ* benthic chambers were installed in Silver Creek

downstream of the confluence with the Silver Creek Water Reclamation Facility

discharge. Isotope dilution and pairing techniques using labeled nitrate ($^{15}NO_3^-$) were

used to track the transport and transformation of nitrogen within the chambers. Samples

were collected from the sediment, water, and plant material within the chambers over 21 hours. A suite of laboratory methods was compiled and modified as needed to quantify the mass of labelled nitrogen incorporated as nitrate, ammonium, organic nitrogen, and dissolved nitrogen gas from the collected samples. The rate of denitrification was only able to be determined from one chamber. A complete data set was unable to be obtained from the other chambers due to: 1) leaking chambers, 2) procedural error within the laboratory and 3) error in data reported from samples sent to external laboratories. The rate of assimilation was determined using data from three chambers containing macrophytes. The rate of denitrification and assimilation were 0.032 mg L^{-1} d^{-1} and 0.205 mg kg^{-1} d^{-1}, respectively, and were comparable to those found in literature. Rates of nitrification, anaerobic oxidation of ammonium, and dissimilatory nitrate reduction to ammonium were also considered, but were found to be non-detect. Overall, the study resulted in a proposed methodology for collecting and processing data to determine site-specific nitrogen transformation rates required for improving water quality modeling techniques.

(116 pages)

PUBLIC ABSTRACT

Developing a Method for Quantifying Nitrogen Transformation Rates Using *In Situ*

Benthic Chambers Dosed with Isotopically Labeled Nitrate

Chelsea A. Stewardson

Nitrogen, a nutrient required for biological growth, is a common water quality parameter of concern as too much nitrogen can cause a strain on aquatic environments and even death of plants and animals. One way high levels of nitrogen are introduced into aquatic systems is by anthropogenic sources such as effluent from water reclamation facilities. A method was designed to observe the fate of nitrogen and measure the rates of its transformation into different nitrogen species within a stream receiving the effluent from the Silver Creek Water Reclamation Facility in Park City, Utah.

Sealed chambers were designed and installed along the streambed to create smaller, individual ecosystems across the width of the stream. Water, sediment, and plant samples were taken from the chambers to measure the concentration of nitrate, ammonium, and organic nitrogen within each compartment over time. The rate of production and/or loss of each nitrogen species was then calculated.

This study showed that the installation of chambers within a stream has the potential to produce the data needed to determine the transport and transformation of nitrogen within an aquatic system. Such studies could lead to a better understanding of how to control anthropogenic nitrogen sources and improve water quality in high mountain Utah streams.

ACKNOWLEDGMENTS

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INTRODUCTION

Nitrogen (N) is an essential nutrient required for growth and development within aquatic environments. There are numerous biochemical processes within these environments that transform, mobilize, immobilize, and deposit nitrogen as it moves among water, sediment, biota, and air compartments. Such processes include: nitrogen fixation, assimilation, ammonification, nitrification, denitrification, anaerobic ammonium oxidation (ANAMMOX), and dissimilatory nitrate reduction to ammonium (DNRA). Determining the effects anthropogenic nitrogen loading has on the terrestrial nitrogen cycle continues to be an important area of research (Schlesinger, 2009; Norton and Stark, 2011). The Utah Department of Environmental Quality (DEQ) is currently developing site-specific nitrogen transformation rates in streams affected by the effluent of water reclamation facilities. By determining site-specific rates of transformation, and using river and stream water quality models, such as the EPA's QUAL2K model (United States Environmental Protection Agency, 2013), conclusions can be drawn as to the processes that dominate the fate and transport of nitrogen within a stream.

Silver Creek, a small stream located near Park City, Utah, is one of many streams being modeled and studied by the Utah DEQ, as it receives the effluent from the Silver Creek Water Reclamation Facility (SCWRF). A tributary of the Weber River, Silver Creek begins near Deer Valley Resort in Park City, Utah (elevation 7,200 feet). The creek first meanders through Richardson Flat Tailings from the Ontario Mine (closed 1981) and continues through agriculture and grazing lands where it receives the SCWRF effluent before merging with the Weber River just northeast of Wanship, Utah. It is

currently listed as a Class 3A (cold water fishery) impaired water body for zinc and cadmium (Utah Department of Environmental Quality Division of Water Quality, 2004). The SCWRF is designed to treat 2.0 million gallons per day (MGD) and is currently nearing its treatment capacity. In addition to the expected daily discharge fluctuations from the plant, seasonal fluctuations are also observed during the winter months when tourism in Park City is at its peak. The treatment plant is currently run such that nitrogen entering the system is completely nitrified by the time it is discharged, resulting in very high concentrations of nitrate (4 to 16 mg NO_3^--N/L) and very low concentrations of ammonium (< 0.02 mg NH_4^+-N/L) in the effluent stream (Utah Department of Environmental Quality Division of Water Quality, 2007).

Neilson et al. (2013) previously monitored Silver Creek and modeled the section below the water reclamation facility using the Qual2Kw model (Chapra et al., 2004) to support nutrient criteria development and waste load analyses. They verified that the facility releases nitrogen principally in the form of nitrate, as the facility is able to achieve total nitrification by the end of their treatment process. It was also reported that upstream flow, surface water runoff, and infiltration from groundwater introduce very small amounts of nitrate and ammonium into Silver Creek, supporting the idea that the SCWRF is the main contributor of nitrogen to the system. They also observed large fluctuations in dissolved oxygen downstream of the confluence. Although they were able to explain some of the oxygen sinks by attributing it to plant production during their study of Silver Creek, they contributed the remaining oxygen consumption to sediment oxygen demand, but also stressed the importance of determining the nitrogen

transformation processes being consistently observed within the system in order to properly predict response variable thresholds for some constituents, such as dissolved oxygen and algal growth. Based on their findings, they recommended that site-specific nitrogen transformation rates be measured for future modeling efforts, which in turn would help determine whether important nitrogen transformation mechanisms, if any, were missing from their modelling efforts. This led to the need to develop a method to determine the dominant nitrogen transformation processes and the site-specific rates at which they occur in Silver Creek. To do this, the following two research objectives were developed:

1. Develop a closed system to monitor the possible nitrogen species and transformation processes *in situ* at Silver Creek.

2. Develop a sampling procedure and compile the methodology needed to establish a mass balance and rates of nitrogen transformation.

LITERATURE REVIEW

The Nitrogen Cycle

Traditional components of the nitrogen cycle were considered including assimilation, nitrification, denitrification, and ammonification, as well as nontraditional components including DNRA and ANAMMOX (Figure 1).
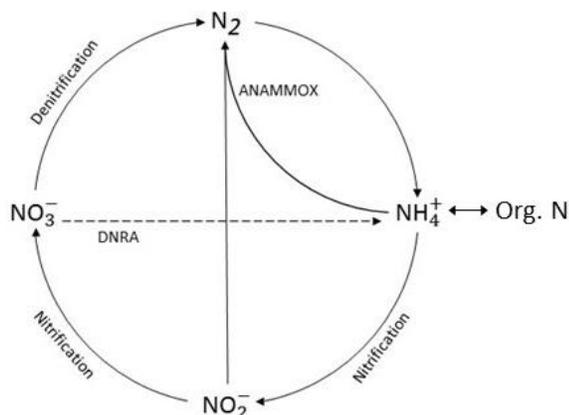
Figure 1. Traditional nitrogen cycle with non-traditional DNRA and ANAMMOX components.

Organic nitrogen was calculated using the nitrogen components shown in Equation 1 (Sawyer et al., 2003):

$$\text{Total Nitrogen} = NO_3^- + NH_4^+ + \text{Organic Nitrogen} \qquad (1)$$

Assimilation

Nitrogen assimilation is the uptake of available nitrate, ammonium, dissolved nitrogen gas, or organic nitrogen by living biomass from the sediment and/or water. This

process is highly dependent on the amount of available carbon within the system. Excess

carbon within a system will cause nitrogen to be utilized until the C:N ratio favors the net

mineralization of nitrogen, otherwise it will lead to a release of organic nitrogen to the

system (Keeney, 1973). Recent studies have shown that the majority of nitrate removed

from stream water is a result of assimilation by stream biomass and not due to direct

denitrification within the water column (Mulholland et al., 2004; Smith et al., 2009;

O'Brien et al., 2012). Smith et al. (2009) found in their study of Sugar Creek, Illinois,

that nitrogen uptake by assimilation occurred at a rapid rate, but acted as a short-term

sink for nitrogen assuming that the return of nitrogen to the system as nitrate occurred

just as rapidly in order to keep nitrate concentrations from decreasing or disappearing

with downstream transport. Because of rapid transformation, these assimilation processes

acted as a temporary sink for nitrogen (Böhlke et al., 2004; Smith et al., 2009) where it

was later remineralized and released back into the water column as nitrate (NO_3^-) and/or

ammonium (NH_4^+) in as little as several hours depending on stream conditions. Lower

temperatures typically result in slower rates of assimilation as decomposition is favored

at higher temperatures (Keeney, 1973).

Nitrification

Autotrophic nitrification by bacteria occurs in two steps: ammonium (NH_4^+) is

oxidized to nitrite (NO_2^-) by bacteria such as *Nitrosomonas*, then this nitrite is oxidized to

nitrate (NO_3^-) by bacteria such as *Nitrobacter* (Chapra, 1997; Sawyer et al., 2003). Nitrite

is usually at very low concentrations, but can accumulate under low temperature or high

pH conditions (Keeney, 1973). It is common to see severe oxygen depletion in

ecosystems where nitrification is dominant because of the high oxygen demand required

to convert ammonium to nitrite and nitrate (Durand et al., 2011), however the reaction

can still proceed down to 0.3 mg/L dissolved oxygen (Keeney, 1973). Conversion of the

ammonium cation to the nitrite or nitrate anion via nitrification increases the mobility of

nitrogen through negatively charged sediments. Once in the form of nitrate, nitrogen is

more likely than ammonium to be assimilated via mass flow by plant material or to be

denitrified within the sediment (Norton and Stark, 2011). This is opposite of nitrogen

within a water column which is more available for uptake as ammonium.

Denitrification

Denitrification is the removal of NO_3^- primarily as N_2. Denitrification occurs

primarily within anoxic sediment layers (where nitrate is the terminal electron acceptor),

and is able to remove NO_3^- from both the water column and NO_3^- resulting from

nitrification in oxic sediment layers (Nielsen, 1992; Durand et al., 2011). The rate of

denitrification is influenced by pH and is much slower in acidic environments. It is also

highly affected by temperature (Keeney, 1973). Many studies have focused on

denitrification within the upper few centimeters of sediment only (Stelzer and Bartsch,

2011) due to the assumption that electron donors and acceptors are more readily available

in this surface sediment than at lower depths within the sediment layer (Lansdown et al.,

2012). Denitrification within the water column only occurs when the dissolved oxygen

concentration goes below approximately 1 mg/L (Durand et al., 2011). However, several

studies using both field and lab techniques have shown that NO_3^- from surface water can

be reduced to N_2 at the water-sediment interface in a process known as benthic

denitrification (Seitzinger, 1988; Nielsen, 1992; Jensen et al., 1994; Cornwell et al., 1999; Herbert, 1999; Kemp and Dodds, 2002; Böhlke et al., 2004). Most researchers have found that the rate of denitrification is independent of nitrate concentration and more dependent on environmental conditions (Keeney, 1973). Denitrification to N_2 is important to quantify because it represents a permanent removal of nitrogen (Böhlke et al., 2004) resulting in a decrease of total available nitrogen from the system (An and Gardner, 2002).

ANAMMOX

Anaerobic oxidation of ammonium (ANAMMOX) is a unique process by which NH_4^+ and NO_2^- are anaerobically transformed to N_2 without the consumption of oxygen. ANAMMOX is important to quantify because it also removes nitrogen permanently from the system. The process of ANAMMOX can be determined by adding enriched nitrate to a system and observing the production of $^{15}NH_4^+$ (An and Gardner, 2002; Hou et al., 2012). Recent studies of the ANAMMOX process within marine and estuarine systems have shown that ANAMMOX has a significant impact on the removal of nitrogen within such environments (Dalsgaard et al., 2003; Trimmer et al., 2003; Trimmer and Nicholls, 2005; Smith et al., 2009). However, little is known about the potential for ANAMMOX within freshwater systems, especially in systems where anoxic water containing ammonium and oxic water containing nitrite are mixed (Smith et al., 2009). A study of the Delaware and Potomac Rivers by Seitzinger (1988) reported that ANAMMOX within the sediment layer contributed to more than 75% of the total nitrogen removed within the system. It is important to determine whether or not this process is present on a stream-by-

stream basis as the accumulation or presence of NO_2^- is vital to making this reaction

occur.

DNRA

Dissimilatory nitrate reduction to ammonium (DNRA) is a process by which NO_3^-

is transformed directly to NH_4^+ (An and Gardner, 2002; Lansdown et al., 2012). DNRA is

important to quantify as this transformation reintroduces nitrogen into the system in a

different, bioavailable form either to be taken up for cell production (Koike and Hattori,

1978; Jørgensen, 1989; Omnes et al., 1996; An and Gardner, 2002) or reoxidized for

energy production through remineralization processes (O'Brien et al., 2012). This

transformation back to ammonium can result in an additional or renewed oxygen demand

as the ammonium is transformed back to nitrate via conventional nitrification. Lansdown

et al. (2012) suggested that DNRA was more prevalent in the upper, gravel sediment

layers of the permeable river the group studied, and decreased with an increase in

sediment depth (more clayey sediment) based on the production of enriched ammonium

from an enriched nitrate spike. Sulfide within a system has been known to inhibit

denitrification, however, it fuels DNRA processes by acting as the electron donor for the

organisms carrying out DNRA (An and Gardner, 2002). Analyzing this nitrogen

transformation pathway is important in assessing which processes could be contributing

to nitrogen transport in streams.

Ammonification

Ammonification is the mineralization of organic nitrogen to NH_4^+ by bacteria.

Lower temperatures usually promote a measureable rate of ammonification (Keeney,

1973). Streams with higher organic matter content usually have a higher rate of biofilm growth, which increases biological activity within the system and can in turn reduce oxygen concentrations (Boulton et al., 1998). Stream contamination due to ammonium release can be qualitatively described by quantifying the rate of bacterial production within the water column (Durand et al., 2011) as heterotrophic bacteria drive the ammonification process. Ammonification is important to quantify when tracking DNRA as the creation of the unlabeled ammonium could dilute any enriched ammonium created from DNRA processes (Dugdale and Wilkerson, 1986). This process also reintroduces nitrogen to the system in a form that is more bioavailable and able to undergo processes such as nitrification, therefore creating the potential to consume oxygen within the system.

Isotope Methods for Observing Nitrogen Transformation

Several different methods and analytical techniques have been used to quantify rates of nitrogen transformation. These include, but are not limited to: mass balance approaches (Knowles, 1982), acetylene inhibition (Sørensen, 1978; Revsbech et al., 1988), use of ion specific electrodes (de Beer and Sweerts, 1989), ^{15}N tracer techniques such as isotope dilution (Nishio et al., 1983; Goeyens et al., 1987; Nielsen, 1992), and measurement of N_2 flux via isotope pairing (Seitzinger et al., 1980; Devol, 1991; Nielsen and Glud, 1996; Van Luijn et al., 1996).

Isotope Pairing

The isotope pairing technique is a sensitive and robust process (Nielsen, 1992; Rysgaard et al., 1993; Risgaard-Petersen et al., 1994; Rysgaard et al., 1994). In this technique, a stable isotope (^{15}N) is added to the water column and the rates of formation of $^{28}N_2$, $^{29}N_2$, and $^{30}N_2$ are measured (Nielsen, 1992; Rysgaard et al., 1993; Nielsen and Glud, 1996; Van Luijn et al., 1996; An and Gardner, 2002; Revsbech et al., 2005). This technique has produced successful results in a variety of marine and freshwater sediments (Nielsen, 1992). Although time consuming and labor intensive, isotope pairing appears to successfully produce rates for ANAMMOX that mass balance or acetylene methods are unable to differentiate. It is important to be mindful of the temperature sensitivity of biological processes and make adjustments when comparing laboratory experiments with field conditions (Van Luijn et al., 1996). Limitations have been found with the isotope pairing method used for laboratory incubated sediment cores including: 1) week-long incubation in the laboratory which can lead to non-realistic *in situ* conditions (Nielsen, 1992; Van Luijn et al., 1996); 2) possible underestimation of ANAMMOX rates (Van Luijn et al., 1996); and 3) risk of introducing atmospheric nitrogen, therefore causing contamination (Van Luijn et al., 1996). Such interferences/limitations are lessened by ensuring uniform mixing between nitrate species in both the overlying water column and upper sediment layer and/or performing experiments *in situ* in the field (Nielsen, 1992; Van Luijn et al., 1996). One main advantage of the isotope pairing technique is its ability to measure denitrification of NO_3^- from both the water column and upper sediment layer (Nielsen, 1992).

Isotope Dilution

Only a few studies have been performed to examine the use of isotope tracers and the isotope dilution method in streams with high NO_3^- loads (Nielsen, 1992; Risgaard-Petersen and Rysgaard, 1995; Böhlke et al., 2004). This technique also requires the addition of a stable isotope (^{15}N) to the water column or sediments within a system, and the rate of $^{15}NO_3^-$ and $^{15}NH_4^+$ production or transformation are measured (Risgaard-Petersen and Rysgaard, 1995). It is important that tracer injections are large enough to create elevated levels of isotopic nitrogen without altering natural transformation rates (Böhlke et al., 2004). It is recommended, and has been shown in multiple experiments, that an isotopic enrichment of approximately 25 to 50 atom% ensures sufficient levels for detection without altering natural processes (Böhlke et al., 2004; Norton and Stark, 2011; O'Brien et al., 2012). As nitrification, denitrification and assimilation occur, the bacteria will preferentially use the lighter ^{14}N (Kendall et al., 2007) and the added ^{15}N will be "diluted out" by ^{14}N species produced during these reactions (Norton and Stark, 2011; Ribot et al., 2012). It has been suggested that if enriched nitrogen is added only to the overlying water column, the incubation time needs to be sufficient to allow diffusion of the isotopic nitrogen into the sediments so as to not underestimate total denitrification rates (Mulholland et al., 2004). Addition of $^{15}NO_3^-$ to a system has been known to stimulate denitrification and DNRA, but mostly favor denitrification over DNRA (Sørensen, 1978; An and Gardner, 2002). In an experiment done by Smith et al. (2009) in which $^{15}NO_3^-$ was used to measure rates within a small creek, they found that this method

was best at determining rates for DNRA and measuring turnover rates within the nitrogen

pool when compared to results obtained using the acetylene method.

Quantifying Nitrogen Species

Colorimetric Methods

The concentration of $NO_3^--N + NO_2^--N$ can be determined using the cadmium

reduction method by which nitrate is reduced to nitrite in the presence of cadmium. The

nitrite then couples with N-(1-naphthyl)-ethylenediamine dihydrochloride to form an azo

dye that is measured colorimetrically at 540 nm (APHA, AWWA, and WEF, 1989;

Sawyer et al., 2003).

The concentration of NH_4^+-N can be determined using a phenate method by which

ammonium first reacts with hypochlorite to form chloramine. The chloramine then reacts

with phenol in the presence of nitroferricyanide to from a blue indophenol dye that is

measured colorimetrically at 660 nm (APHA, AWWA, and WEF, 1989).

Diffusion Procedure

Analysis of isotopically enriched samples from the isotope dilution process

includes quantifying NO_3^- concentrations using colorimetric analysis and quantifying

$^{15}NO_3^-$ by isotope-ratio mass spectrometry (Norton and Stark, 2011). Samples are

processed with a diffusion procedure that transforms enriched nitrogen to ammonium and

captures the concentrated $^{15}NH_4^+$ on acidified filter paper disks prior to analysis on an

isotope-ratio mass spectrometer (Stark and Hart, 1996; Sigman et al., 1997; Mulholland

et al., 2004; Norton and Stark, 2011; Ribot et al., 2012). The diffusion method is usually

chosen because it is less labor-intensive, it eliminates cross-contamination when disposable containers are used, and it can be carried out in a variety of vessels and configurations. However, there are several limitations including variable mass recovery due to contamination from chemicals used within the diffusion procedure, low salinity, and low pH (Stark and Hart, 1996). The sample must also contain at least 20 µg of N for the enriched N mass recovery to not be affected by the dilution from contaminating N sources (Stark and Hart, 1996). Modification of samples to ensure correct chemistry prior to diffusion, such as pH and ionic strength adjustments, reduces these limitations.

Digestion Procedure

     Digestion procedures for the determination of total nitrogen can be used in combination with the diffusion method to determine the amount of enriched total nitrogen. Persulfate digestion methods such as EPA Method 350.1 (United States Environmental Protection Agency, 1993) for water samples and LG602 (United States Environmental Protection Agency, 2004) for sediment samples transform nitrogen species within the sample to nitrate. The liquid extract from the digestion procedure can then be processed using the nitrate diffusion method for the analysis of labeled nitrogen, paying special attention that the sample is adjusted for pH and salinity to ensure sufficient recovery, or using the cadmium coil reduction method for the analysis of the produced nitrate. The resulting masses correspond to the amount of total nitrogen within the sample, and organic nitrogen is determined by difference.

Measuring Isotope Ratios of Dissolved Gasses

Goering and Pamatmat (1971) were one of the first groups to use isotope pairing to quantify denitrification within sediments. Their method has been used and improved upon many times (Nishio et al., 1983; Binnerup et al.,1992; Rysgaard et al., 1993, 1994; Risgaard-Petersen et al., 1994) resulting in the general method used today (Risgaard-Petersen and Rysgaard, 1995). Glass sample vials sealed with aluminum crimp caps and rubber septa are evacuated using a vacuum pump and stored underwater to prevent contamination (Hamilton and Ostrom, 2007). A sample is collected by inserting a double headed needle to pull water into the evacuated vial until completely full and/or the pressure equalized. After sample collection, a He headspace is created within the vial by allowing a steady stream of He to enter the vial as water is slowly extracted from it (Risgaard-Petersen and Rysgaard, 1995; Dalsgaard et al., 2000; Hamilton and Ostrom, 2007). The headspace within the sample is then analyzed on a trace gas isotope ratio mass spectrometer for the ratios of $^{29}N_2$ to $^{28}N_2$ and $^{30}N_2$ to $^{28}N_2$. This method is very robust, however, the biggest limitation of this procedure occurs if care is not taken to prevent contamination from atmospheric nitrogen due to leaks.

Chamber and Reach Scale Methods

Designing chambers for *in situ* stream measurements that do not disrupt the sub-surface and surface flow at the sediment-water interface where denitrification processes commonly occur can be challenging (Grimm and Fisher, 1984; Boulton et al., 1998; Jones and Mulholland, 1999; Mulholland et al., 2004). Chambers can also be difficult to

install in streams with variable, especially course, sediments, fast flows, or large depths. However, installation of chambers allows for parcels of water and sediment to be controlled, and *in situ* measurements to be taken directly from the stream. Chambers allow for small fluxes to be measured over a specific area, therefore requiring minimal disruption of the environment that is usually associated with the removal of sediment cores. Incubation times should be kept short as to limit disruption of the environment as well as prevent excessive buildup of nutrients within the chamber that differ significantly from background. One of the best ways to limit the disturbance of sediments is to place a collar into the sediment in which the chamber is attached during incubation.

Smith et al. (2009) designed a dome-shaped incubation chamber fitted with pore water samplers, water column samplers, and mixing ports to model nitrification rates in Sugar Creek, Illinois and Indiana. Made from 0.6-cm-thick clear acrylic, the hemispheres were installed 5 to 10 cm into the stream sediments. The domed chambers were dosed with enriched nitrogen and samples were taken at different intervals within a 44-hour incubation/sampling period.

O'Brien et al. (2012) designed a two-piece rectangular chamber to measure nitrification rates at Augusta Creek, Michigan. The frames, constructed from aluminum sheeting, were installed 10 cm into the stream sediments. A 0.6 m $\times$ 0.3 m $\times$ 0.1 m (l $\times$ w $\times$ h) acrylic chamber "lid" was attached to each aluminum frame using a foam rubber seal and elastic chording, creating a closed system. Chambers were also fitted with a sampling port and dissolved oxygen sensor. Each chamber was enriched with $^{15}NO_3^-$ to achieve a target enrichment of 50 atom%. On the first day, the chambers were left in place for 22

hours. The chambers were then removed to allow natural flow within the stream. On subsequent sampling days, the chambers were reattached to the frames for 3 hours, and samples were collected at the beginning and end of the sampling period. O'Brien et al. (2012) found that 88% of the NO_3^- uptake they observed was due to assimilation by algal and microbial biomass and 12% from direct denitrification at Augusta Creek, Michigan. Background nitrate concentrations at the Augusta Creek study site were approximately 0.11 mmol/L. The team reported that enrichment of the chambers was most likely too high and was suspected to alter the natural conditions and nitrogen transformation rates of the stream within the study site. Enriched nitrogen was gone at the end of the 24-day study.

Reach scale experiments have also been used to quantify nitrogen rates along sections or entire lengths of streams (Böhlke et al., 2004; Ribot et al., 2012). These experiments can have more unknowns or variations depending on groundwater interactions, surface water interactions, and/or changes in vegetation along the reach being studied compared to *in situ* measurements. These techniques can give an idea as to the overall processes within the stream, but may overlook small-scale, important nitrogen cycling processes occurring within the system (Böhlke et al., 2004). Reach scale studies can provide a good estimation of transport and removal, but may not accurately predict the rates of transformation of nitrogen (Böhlke et al., 2004), especially if those rates vary spatially within the system.

Methods for Monitoring Groundwater Influences

Monitoring the interaction between surface water and groundwater flow within the hyporheic zone is important in determining upwelling or downwelling conditions within *in situ* chambers. Upwelling conditions can supply organisms in the stream with nutrients while downwelling conditions supply dissolved oxygen, nutrients, and organic matter to microbes within the hyporheic zone (Boulton et al., 1998). Downwelling or upwelling conditions can result in the dilution or loss of isotopically enriched nitrogen within the chamber and/or changes in oxygen concentration that can influence nitrification and denitrification within the system. To determine upwelling or downwelling conditions, the nearby vertical hydraulic gradient (VHG) is measured by installing piezometers in several sections of a stream.

In a study by Baxter et al. (2003), minipiezometers were constructed using 5/8 in. PVC pipe with approximately 30 small holes drilled into the lower 15 cm of the pipe and stoppered at the bottom with a rubber cork (Freeze and Cherry, 1979). The piezometers were installed at an equal depth within the sediment to determine the VHG at the chosen depth. The VHG was measured by finding the ratio $\Delta h/\Delta l$, where Δh is the difference in head between the water inside the piezometer and the stream water surface and Δl is the length from the sediment surface to the top of the piezometer holes. In similar methods used by Käser et al. (2009) and Schmadel et al. (2014), Δl is defined as the length from the sediment surface to the middle of the piezometer perforations (Kalbus et al., 2006).

MATERIALS AND METHODS

In order to meet the objectives of this study, it was important that a method be developed in which the relationship between nitrogen and dissolved oxygen could be observed while quantifying the transport and transformation of nitrogen within a closed system. Nitrogen transformation cannot be the sole cause of large fluctuations in dissolved oxygen, but has the potential to contribute to some extent depending on the transformation processes occurring. For example, if DNRA is occurring within a system (transforming nitrate to ammonium) it reintroduces nitrogen in a form that can undergo nitrification, creating a higher oxygen demand than that of the low natural abundance of ammonium within the stream. In order to observe and determine which nitrogen transformation processes occur and how they relate to oxygen demand within a stream, it was decided that a method using isotope pairing and dilution within an *in situ* chamber similar to that used by O'Brien et al. (2012) should be developed (also see generic standard operating procedure for setup and sampling methodology in Appendix A).

Chamber Construction

Benthic chambers were constructed from two parts: an aluminum frame and a clear acrylic top (chamber). Frames were constructed at Mountain View Machine and Welding (Logan, UT) by bending aluminum sheeting to form a 12"x24"x6" box with a 1" lip around the top, with the open seam welded closed. Chambers were constructed from 3/8" acrylic sheeting and measured 12"x24"x6" with a 1" lip around the bottom. A 1.25" (inner diameter) hole was cut in the downstream end (12" side) of the chamber and

fitted with a custom designed watertight compression fitting to hold a Dissolved Oxygen probe (Milwaukee Instruments, Inc., Rocky Mount, NC; Figure 2). An Onset® HOBO® TidbiT Waterproof Temperature Data Logger (Onset® HOBO® Data Loggers, Bourne, MA) was wrapped in aluminum foil and secured with a piece of metal wire to the base of the dissolved oxygen probe to measure water temperature within the chamber. A single 5/8" hole was drilled on the upstream and downstream ends of the chamber and barbed tube fittings attached to create the tubing connection allowing for circulation of the water within the chamber when closed. Rubber foam weather-strip tape was glued to the lip of each chamber, creating a watertight seal between the chamber and frame.

(a)   (b)
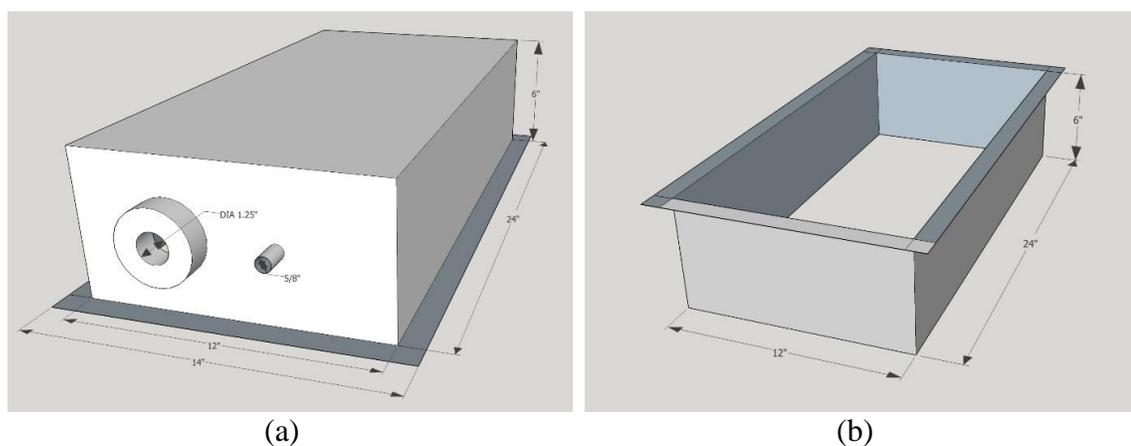
Figure 2. (a) Acrylic chamber with dissolved oxygen probe fitting and tubing ports, (b) Aluminum frame.

Tubing on the downstream end of the chamber was fitted with a plastic 3/8" tee joint containing a rubber stopper through which a 16-gauge side port needle was inserted. A two-way valve with Luer-Lock attachments was secured to the needle, allowing a syringe to be screwed into the other end to inject dosing solutions. This tubing was then

attached to the submersible pump inlet. Tubing on the upstream end of the chamber was fitted with two tee joints: one containing a rubber stopper for dissolved gas sample collection using a double headed needle and one containing tubing that could be opened or closed using a tubing clamp for the collection of water samples. This tubing was then attached to the submersible pump outlet. Magicfly DC brushless submersible pumps (Amazon, Seattle, WA) were run using a 12V gel cell lead acid battery. Batteries powered two pumps each. The completed chamber setup, including frame, chamber, tubing attachments, sampling ports, pump, and battery can be seen in Figure 3.
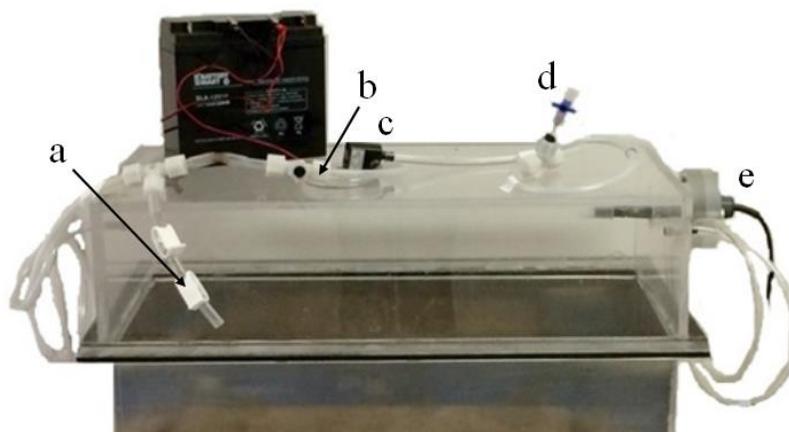
Figure 3. Fully assembled chamber including an acrylic lid with a waterproof seal, aluminum frame, 12 V battery, water sampling port (a), dissolved gas sampling port (b), submersible pump (c), injection port (d), and dissolved oxygen probe fitting (e).

The use of benthic chambers allowed for the nitrogen cycle to be analyzed within two compartments: the aerobic sediment layer and water column (Figure 4). The anaerobic sediment layer occurred at depths deeper than the 5 to 6 inch depth reached by the installed benthic chamber.
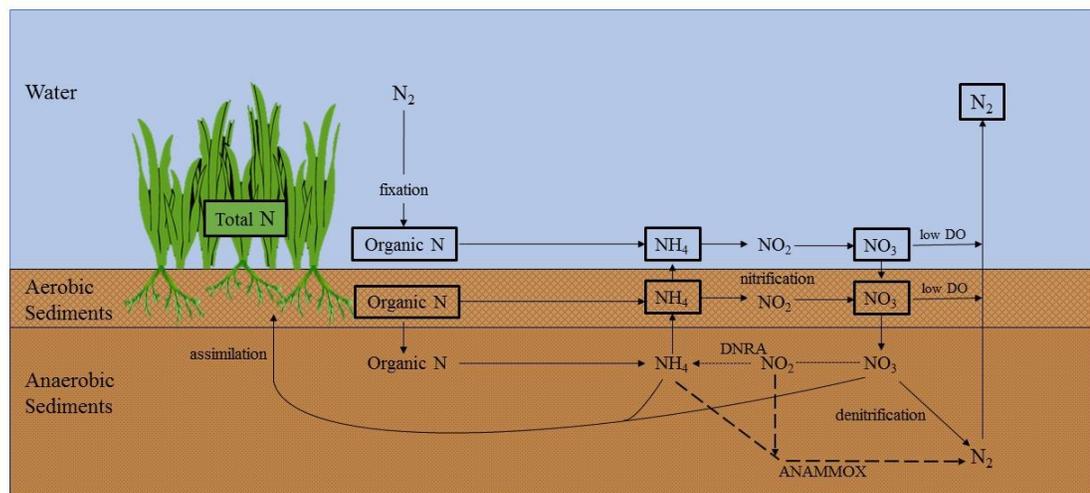
Figure 4. Nitrogen cycle within the anaerobic sediment, aerobic sediment, water, and plant compartments. Water, aerobic sediment, and plant samples were collected from the chambers. The boxed nitrogen species were analyzed for the amount of labeled and unlabeled nitrogen by splitting a single sample based on the mass or volume required by the analytical laboratory method.

Site Installation

Frames and piezometers were installed at least a week before sampling began to ensure they were fully incorporated into the stream bed and were stable. Frames were placed such that flow from the stream did not cross more than one plot, therefore minimizing the chance of contamination between plots. Frames were also placed such that the plots could be defined by plant density (if the site contained visible macrophytic growth), with some plots containing no visible macrophytic material and others containing visible macrophytic material. Piezometers were placed such that groundwater influences could be observed across the entire study site while still allowing adequate room to move the chamber lids around during installation.

Sample Collection

The initial incubation (sampling day) began by collecting a single sediment core using a 3" x 1.25" (length x inner diameter) custom made PVC corer and size 7 ½ rubber stopper. Cores were emptied into Ziploc bags and stored in a cooler on ice for transport back to the laboratory for the analysis of background $^{15}NO_3^-$, NO_3^--N + NO_2^--N, $^{15}NH_4^+$, NH_4^+, Total ^{15}N, and Total N. A small plant sample from each plot (if the site contained visible macrophytic growth) was also collected to determine background nitrogen content in this plant material. Three to four randomly selected blades of plant material were cut near the base within the plot, cut into smaller fragments, and stored in Ziploc bags over ice for transport back to the laboratory for the analysis of Total-N and atom% ^{15}N.

The chambers were then submerged in the stream at the deepest part of the study site, filled with water, and attached to the frames with 10-1" metal spring clamps. Tubing was fully submerged before being attached to the chambers and submersible pumps to try and eliminate air from entering the chambers. Pumps were then connected in pairs to a single 12 V battery, and the tubing, pumps, and batteries carefully arranged on the bank (Figure 5).

Water 5 feet upstream of the chambers was collected at three randomly chosen times during the installation of the chambers for the analysis of background NO_3^- and NH_4^+ by collecting 60 mL volumes using a syringe, filtering through a 0.45 µm filter, and preserving with H_2SO_4 to a pH<2. These samples were also used to observe any variations in nitrogen content over the installation period due to varying discharge from the water reclamation facility.

Figure 5. Installed chambers in the field. Pumps and batteries are arranged on the bank in order to keep the batteries dry and allow easy access to the sampling ports.

Once chambers were fully installed, the temperature and dissolved oxygen within the chambers were recorded. Temperature within the chambers was programed to be recorded by the HOBO® TidbiT every minute. The dissolved oxygen within the chambers was recorded manually by reading the Milwaukee probe every 10 minutes. The dissolved oxygen within the chambers was not allowed to go higher than 10% of the dissolved oxygen outside of the chambers for the entire incubation period. Dissolved oxygen levels within the chambers were decreased by securing black plastic bags around the chambers to reduce sunlight penetration into the chamber and inhibit autotrophic oxygen production until they reached background levels.

Any air trapped within the chambers was recorded by measuring the dimensions of the air bubbles and calculating the volume. The amount of air measured immediately after installation was assumed to be from air that was not properly cleared from the tubing lines before being attached to the chambers.

A water sample for the analysis of background $^{15}NO_3^-$, NO_3^--N + NO_2^--N, $^{15}NH_4^+$, NH_4^+, Total ^{15}N, Total N, and Br$^-$ was collected in a 125 mL Nalgene bottle from each chamber and stored in a cooler on ice. Another water sample was also collected for the analysis of dissolved $^{15}N_2$ using an evacuated 20 mL vial with a grey butyl rubber septa and a double-headed needle. This completed the collection of all background samples.

Chambers were each dosed with 30 mL of a 3.77 g/L K$^{15}NO_3^-$ and 139.4 g/L Br$^-$ enrichment/tracer solution at the start of the incubation period. The purpose of the bromide tracer was to quantify any losses from the chamber. A 125 mL water and a 20 mL dissolved gas sample were collected from each chamber after dosing (Time 0.25 hours), after allowing time for the pump to circulate two chamber volumes, therefore, fully mixing the chamber water. One additional round of 20 mL dissolved gas and 125 mL water samples was collected to measure the rate of denitrification at 4.3 hours (time randomly chosen) into the incubation to get an intermediate data point.

The water level within the installed piezometers and stream depth outside of the piezometers was measured using a lockable measuring tape and Kolor Kut® Water Finding Paste or a well sounder. The exposed and total length of the piezometer was also measured to determine the installed depth within the sediment.

Overnight, the dissolved oxygen within the chambers was not controlled to within the 10% of background limit. Observations were recorded until about an hour before sundown, 2 hours after sundown, and just before sunup before returning to the 10 minute observation schedule about an hour after sunup.

Chambers were incubated for 21 hours. After the 21-hour incubation period, final air bubble measurements were recorded as well as 125 mL water and 20 mL dissolved gas samples taken from each chamber, the chambers removed, and final sediment and macrophyte samples were taken from each plot. The frames and piezometers were left in place and plots were left exposed to natural stream conditions.

Chambers were re-installed three more times after the initial incubation as described above to quantify the rate of transformation from any remaining incorporated label. Each day consisted of dosing each chamber with 30 mL of a 139.4 g/L Br^- tracer solution at the start of the incubation period followed by collection of initial (Time 0.25 hours) water and/or dissolved gas samples 15 minutes after dosing. Chambers were left in place for 3 hours before taking final water and/or dissolved gas samples after which the chambers were removed and final sediment and macrophyte samples collected. Piezometer measurements were recorded each day in the same manner as Day 0.

Site Removal

At the end of the study, macrophytic density was determined in each plot (if plant samples were collected) by harvesting the entire plot, and drying and weighing the harvested plant material at the UWRL. Frames and piezometers were removed from the stream bed and all equipment was removed from the study site.

Sample Analysis

Unlabeled Nitrogen Analysis

Ten mL of water were pulled from each 125 mL Nalgene bottle to determine the

Br$^-$ concentration using an Orion Model 94-35 Halide electrode (Orion Research

Incorporated Laboratory Products Group, Boston, MA; Appendix A). Fifteen mL of

water were pulled from each 125 mL Nalgene bottle for the analysis of unlabeled nitrate

+ nitrite and ammonium using an AQ2 Discrete Analyzer (Seal Analytical, Inc., Mequon,

WI) and EPA Methods 114-A Revision 7 and 103-A Revision 7, respectively. An

additional 10 mL of water were pulled from each 125 mL Nalgene bottle, digested using

EPA Method 350.1 (Appendix A), and analyzed on the AQ2 Discrete Analyzer with EPA

Method 114-A Revision 7 to determine the concentration of unlabeled total nitrogen.

Sediment cores were mixed in their Ziploc bags to create a homogenized sample.

Five grams of the homogenized core were added to a 60 mL glass sample vial and run

through a KCl extraction (University of Colorado Boulder Aridlands Ecology Lab

Protocol, 2009; Appendix A) and the extract was measured on the AQ2 Discrete

Analyzer for unlabeled nitrate + nitrite and ammonium using EPA Methods 114-A

Revision 7 and 103-A Revision 7, respectively. An additional 0.2 to 0.4 g of sediment

were added to a digestion vial and digested using EPA Method LG602 (Appendix A) and

the digested solution analyzed on the AQ2 Discrete Analyzer using Method 114-A

Revision 7 to determine the concentration of unlabeled total nitrogen. Water content of

the sediment was determined by drying approximately 10 g of wet sediment and

recording the wet and dry weights of the sample.

Labeled Nitrogen Analysis

Water samples for the analysis of $^{15}NO_3^-$ and $^{15}NH_4^+$ were processed using a modified diffusion procedure (Appendix A) described in Stark and Hart (1996). The diffusion procedure was performed using 45 to 60 mL of water from each 125 mL Nalgene bottle, 15 to 30 mL for the nitrate diffusion and 30 mL for the ammonium diffusion. Diffusion volume was chosen such that the diffused sample contained approximately 90 µg of nitrogen. Five to 6 grams of potassium chloride were added to each diffused water sample to increase the ionic strength of the sample to about 2.5 M. Magnesium oxide (MgO) was also added to the jars to raise the pH above 9 ($NH_{3(g)}$ pKa = 9.3). Acid traps (acidified filter paper disks enclosed in Teflon tape, Appendix A) were added to the ammonium diffusions to capture the ammonia gas and the jars sealed. Nitrate diffusions were put in a 60 °C oven for 2 hours in place of leaving the jars open to the atmosphere for 2 days to volatilize all of the ammonia from the sample. Devarda's Alloy was then added to the nitrate diffusions to convert the remaining nitrate to ammonia and an acid trap added to capture the converted ammonia gas. Diffusion jars were stored inverted for 7 days to make detection of leaks easy to observe. Water samples were also analyzed for labeled total nitrogen by pulling 30 mL of water from each 125 mL Nalgene bottle and digesting the samples according to EPA Method 350.1. The final extract was diffused using the Stark and Hart method (1996) with the modification as described above. Processed samples were sent to the Utah State University Stable Isotope Laboratory for analysis.

Two 20 g aliquots of the homogenized sediment core were each added to a 60 mL glass sample vial, one for the analysis of $^{15}NO_3^-$ and one for the analysis of $^{15}NH_4^+$. These homogenized samples were run through a KCl extraction procedure (University of Colorado Boulder Aridlands Ecology Lab Protocol, 2009) and the extract used in the modified Stark and Hart method (1996). Total labeled nitrogen was determined by digesting 0.2 to 0.4 g of homogenized sediment using EPA Method LG602 and the extract diffused using the modified Stark and Hart method (1996). Processed samples were sent to the Utah State University Stable Isotope Laboratory for analysis.

Standards (laboratory control samples) for diffused water and sediment samples were prepared each sampling day and once more at the end of sampling, for a total of five replicates, to quantify and track the amount of nitrogen contamination introduced to the field samples via the chemicals added during the procedure (Appendix A). One hundred milliliters of standard solution with a target mass of 50 µg N at 5 atom% (Stark & Hart, 1996) were prepared at the start of the experiment. Non-diffused standard samples were used to determine the true mass and atom% of the standard solution and were not introduced to any of the diffusion chemicals, resulting in no contamination. Aliquots of the initial solution were then run through the same diffusion procedures as samples collected in the field. The average mass of contamination due to the addition of KCl, MgO, and Devarda's Alloy was calculated and the corrected atom% of the samples were determined using the method described in Stark and Hart (1996).

Standard samples had to meet three criteria for the procedure to be considered valid: 1) The difference between the average measured mass of the standard sample and

the mass of contamination due to the procedure must be greater than 20 µg, as anything lower is below the instrument calibration; 2) the corrected mass must be above 50% recovery of the non-diffused sample (or the starting sample mass); and 3) the corrected atom% must be above 75% recovery of the non-diffused sample (or the starting atom%).

The mass of contamination determined from the diffusion standards was applied to the field data and the reported masses and atom% values adjusted for the procedural contamination. Next, the atom% was further adjusted for losses within the individual chambers based on the bromide data (Equation 2).

Water samples collected in 20 mL vials for the analysis of dissolved $^{15}N_2$ had a headspace of 35 mm created in each vial using the setup described in Dalsgaard et al. (2000) by allowing pure helium to flow freely into the sealed sample while pulling out approximately 11 mL of sample water using an 18 G needle attached to a Luer-Lock 35 mL syringe (Figure 6).

Figure 6. Setup for creating the helium headspace in vials used to measure $^{15}N_2$. Helium is constantly bled into the vial through the tubing on the left while water is pulled from the vial using the syringe on the right. Helium is also allowed to flow out of the tubing inserted in the beaker of water so that the vial does not become pressurized during creation of the helium headspace.

Samples were sent to the University of California, Davis Stable Isotope Facility for

analysis using a Sercon Trace Gas Isotope-Ratio Mass Spectrometer.

Plant samples were freeze dried for at least 2 days and then ground into a fine

powder (Appendix A). A mass of 5 to 20 mg of the crushed sample were added to an 8x5

mm tin pressed capsule (EA Consumables, Pennsauken, NJ), the weight of the sample

was recorded, and the samples were then sent to the Utah State University Stable Isotope

Laboratory for analysis of total nitrogen and percent ^{15}N enrichment.

A summary of the field samples collected, methodology used, and nitrogen

species determined within each sample is summarized in Table 1.

Table 1. Summary of the types of field samples collected, collection vessels used, analytical methods used, and nitrogen species determined from each sample.

Sample Type	Collection Vessel	Laboratory Methods	Nitrogen Species Analyzed
Water	125 mL Nalgene bottle	EPA Method 114-A Revision 7	NO_3
		EPA Method 103-A Revision 7	NH_4
		EPA Method 350.1, EPA Method 114-A Revision 7	TN
		Diffusion Procedure (Stark and Hart, 1996)	$^{15}NO_3$ or $^{15}NH_4$
		EPA Method 350.1, Diffusion Procedure	^{15}TN
	20 mL evacuated vial with septa	Headspace created (Dalsgaard et al., 2000)	$^{29}N_2$ and $^{30}N_2$
Sediment	custom made PVC corer	KCl extraction, EPA Method 114-A Revision 7	NO_3
		KCl extraction, EPA Method 103-A Revision 7	NH_4
		EPA Method LG602, EPA Method 114-A Revision 7	TN
		Diffusion Procedure (Stark and Hart, 1996)	$^{15}NO_3$ or $^{15}NH_4$
		EPA Method LG602, Diffusion Procedure	^{15}TN
Plant	Ziploc bag	Freeze dried, ground	^{15}TN and TN

Correcting For Losses

All atom% data was corrected for flow in or out of the chambers by applying the

percent loss within each chamber based on bromide data and assuming that the lost

volume was replaced by water outside of the chamber whose composition was measured

by the initial background sample (Time 0 hours). The mass balance equation for this

correction is shown in Equation 2

$$A_{measured} = A_{actual}(\%_{remained}) + A_{background}(\%_{loss}) \qquad (2)$$

where $A_{measured}$ is the atom% measured by the instrument and reported, A_{actual} is the

atom% that would be in the chamber if no leaks had occurred, $A_{background}$ is the atom%

measured in the background sample (Time 0 hours), $\%_{remained}$ is the percent of the

bromide tracer that was measured and therefore remained in the chamber over the

incubation time, and $\%_{loss}$ is the percent of the bromide tracer that was lost due to leaks

from the chamber. The actual atom% (A_{actual}) within each chamber was calculated and

used to complete statistical analyses, mass balances, and rate estimations. This equation

assumes the concentration of labeled nitrogen in the stream water, or background, is

equal to that found in the pore water, which is expected for natural systems. In the case of

correcting for ^{14}N, this equation would need to be modified so that the background

concentration corresponds to the concentration in the stream water, if the chamber is

leaking, or in the pore water, if groundwater influences are causing the loss of bromide.

Mass Balance and Rates

A mass balance was performed on each chamber individually by calculating and

summing the mass of ^{15}N (mg) in nitrate, ammonium, organic nitrogen, and/or dissolved

nitrogen gas within the sediment, water, and plant compartments. The actual mass of

$^{15}NO_3^-$-N added to the chambers was determined by calculating the change in NO_3^--N

between Time 0 hours and Time 0.25 hours within each chamber as measured by the

AQ2. The percent mass recovery within each chamber was then calculated by dividing the summed mass of ^{15}N within each chamber by the addition measured by the AQ2.

Rates of nitrification, denitrification, ANAMMOX, DNRA, and assimilation (if macrophytic material was present) were calculated for each chamber individually using the methods and equations outlined in Appendix B. The average of the three macrophytic chambers and the three non-macrophytic chambers were then calculated and used as the overall rate of nitrogen transformation based on plant density. Rates of denitrification based on the labeled nitrate data were compared to the rate of production of nitrogen gas data to test the assumption should be comparable as nitrogen gas production should arise from nitrate reduction.

Statistical Analysis

A statistical analysis of each nitrogen species between plots (after correcting for losses) was performed to determine if the change in concentration as a function of sampling time was statistically significant for each sampling day. A statistical analysis was also done within each individual chamber between sampling times to determine if the change in concentration was statistically significant. Non-macrophytic and macrophytic plots (if plant samples were collected) were also compared at the same sampling time (after correcting for losses) to determine statistical significance between plot type. Statistical analyses were also performed on the calculated rates using data corrected for losses to determine statistical differences within individual plots, between plots, and based on plot density if plant material was collected.

RESULTS

The method described above (and outlined in Appendix A) was applied at Silver

Creek in Park City, Utah on four sampling days over a 16 day period. Data from diffused

samples showed that the label added during the initial 21-hour incubation period washed

away with the removal of the chambers at the end of incubation. Therefore, data collected

on sampling Days 2, 9, and 16 were at background concentrations and are not reported

below. Instead, focus was kept on the transport and transformation of the label over the

initial 21-hour incubation period when the chambers remained in place in the stream.

Site-Specific Application

The sampling site (elevation 6,500 feet) for this study was located approximately

0.4 miles (2,000 feet) downstream from the SCWRF discharge point and approximately

300 feet downstream from a USGS stream gauge station (USGS 10129900 Silver Creek

near Silver Creek Junction, UT; Figure 7). The stream measured 6 to 7 feet across at the

sampling site and flows recorded by the USGS station range from 0.7 cfs to 70 cfs

(median daily over 13 years of 3.4 cfs; USGS, 2015). Cores taken at the study site

revealed that the upper 10 inches of sediment are comprised of gravel and sand and the

lower 2 inches of sandy clay. However, the thickness of the sandy clay layer increases

along the edges of the streambed.

Samples collected during preliminary visits to the sampling site in fall and winter

of 2014 showed that nitrate concentrations ranged from 3.0 to 9.5 mg $NO_3^--N + NO_2^--N/L$.

The nitrate concentration in the water column was highly variable, and changed within

the hour as the effluent from SCWRF varied throughout the day. Very low concentrations of NH_4^+-N (typically below a method detection limit of 0.02 mg/L) were found both upstream and downstream of the SCRWF confluence. Recorded dissolved oxygen levels during the preliminary visits ranged from 10 mg/L to <1 mg/L. It was also observed that dissolved oxygen levels increased as temperature increased, opposite of a typical response in which gas saturation increases with a decrease in temperature.
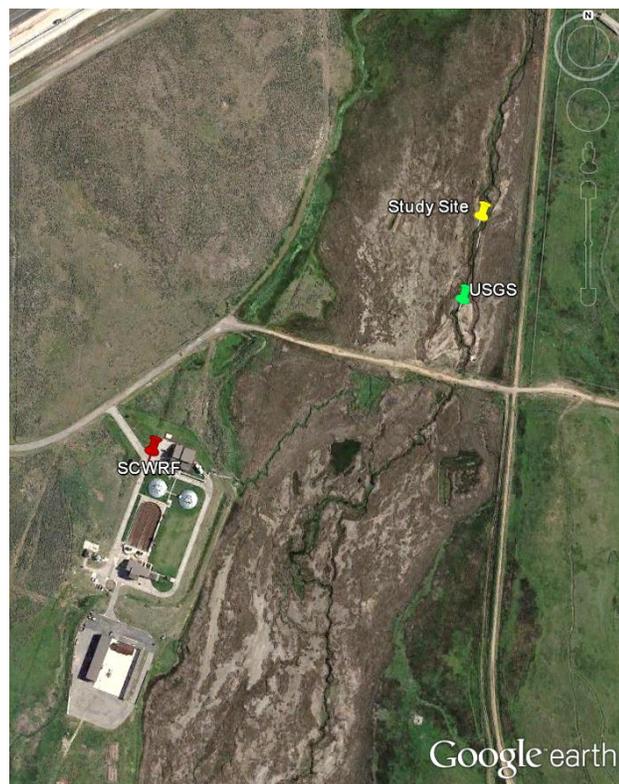
Figure 7. The location of the Silver Creek Water Reclamation Facility, USGS data collection station, and chamber installation. The study site is located approximately 0.4 miles downstream of the discharge point and approximately 100 yards downstream of the USGS station.

Frames and piezometers were installed on June 2, 2015, a week before sampling began. Frames were placed diagonally across the reach of the creek so that three of the six frames contained macrophytes (M) and the other three did not (NM). This minimized flow from one plot to another plot, therefore minimizing the chance of contamination between plots. Piezometers were also installed to monitor influences from groundwater across the reach in which the chambers were deployed (Figure 8), being careful to ensure they would not interfere with chamber installation.
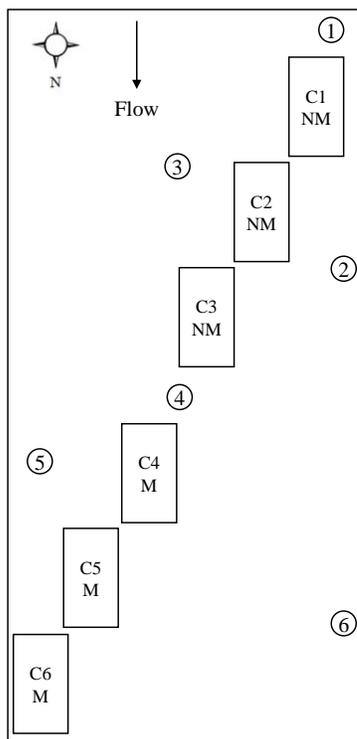
Figure 8. Chamber frame (rectangles) and piezometer (circles) layout at the Silver Creek field site. Chambers were numbered one through six, upstream to downstream. Macrophytic chamber plots are marked with an 'M' and non-macrophytic plots with an 'NM'.

Sampling was conducted as described above, with the initial incubation (Day 0) occurring on June 9 and 10, 2015. The nitrogen label was added in the form of nitrate to

the water within the chamber to achieve a target of 16.63 mg ^{15}N at 10 atom%. Chambers were reinstalled on June 12, 19 and 26, 2015 (Days 2, 9 and 16). Gas samples were not collected on Days 9 or 16, as previous sampling events during the fall and winter of 2014 showed that denitrification measured by ^{15}N incorporation into nitrogen gas only was measureable on Day 0.

USGS and AQ2 Stream Data

Stream discharge, temperature, and dissolved oxygen data were recorded by the USGS gauging station upstream of the sampling site every 15 minutes (Figure 9). A large rain event occurred about 2 days prior to sampling on June 9, 2015, causing a large peak in the hydrograph. Following the large pulse, flows returned to approximately 5 cfs and slowly decreased throughout the rest of the month. The smaller, daily oscillations were due to the variable discharge from SCWRF.

Temperature and dissolved oxygen within the stream peaked and dipped simultaneously, unlike the typical increase in saturation with decreasing temperatures observed in a non-impacted stream. Stream temperature peaked near solar noon and decreased overnight as air temperature and solar radiation influences decreased. Dissolved oxygen levels fluctuated between 5 and 10 mg/L, but did not show signs of supersaturated or anoxic conditions.

(a)

(b)
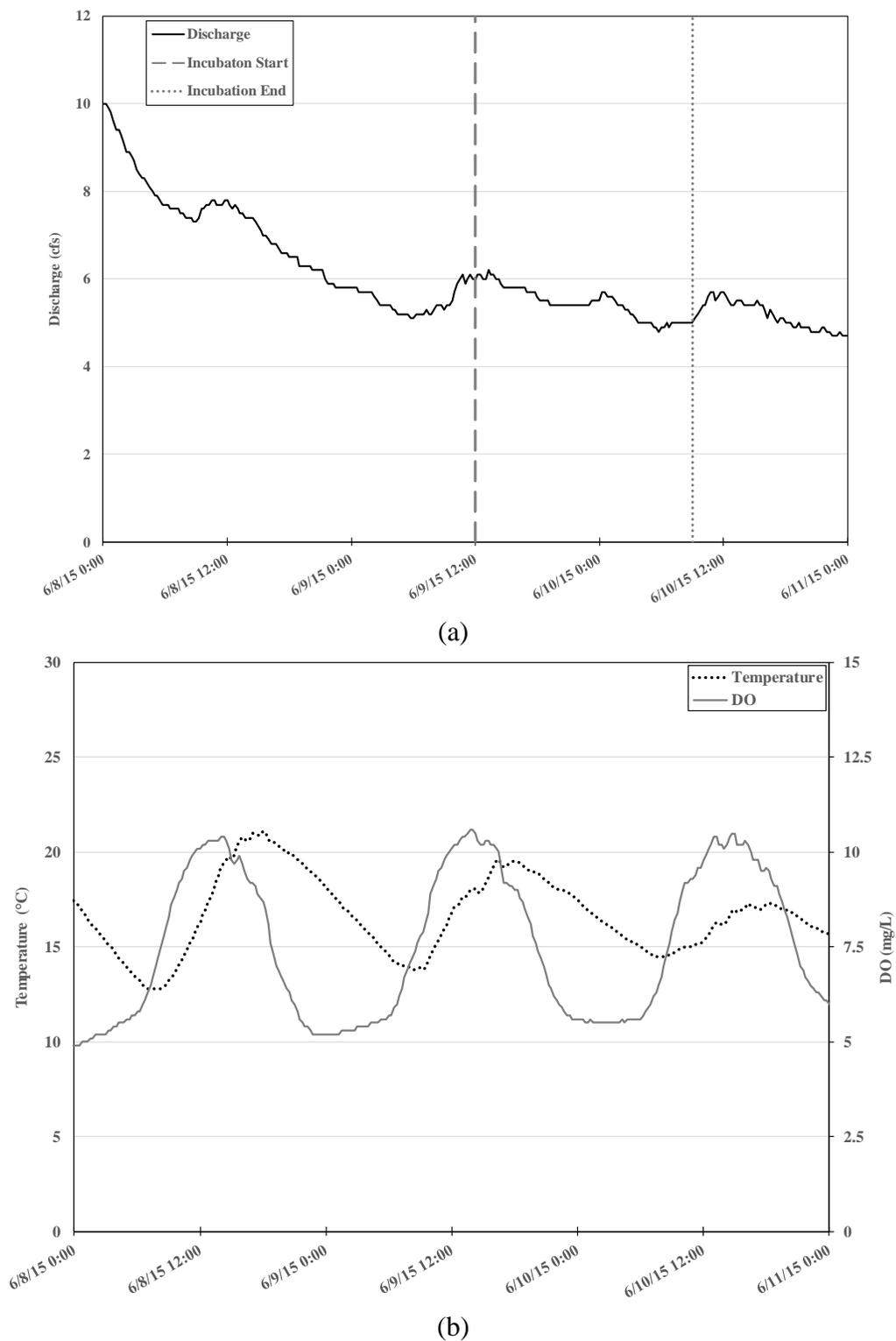
Figure 9. (a) USGS discharge data and (b) USGS temperature and dissolved oxygen data for the initial 21-hour incubation. Sampling started at 6/9/15 12:00 and ended at 6/10/15 9:00.

The amount of $NO_3^--N + NO_2^--N$ and NH_4^+-N from water samples collected upstream of the study site during chamber installation provided an idea of the starting conditions within the chambers. $NO_3^--N + NO_2^--N$ concentration was 3.65 ± 0.18 mg N/L. Ammonium levels were below the method detection limit of 0.02 mg/L.

Bromide Tracer and Piezometer Measurements

Bromide concentration measurements were used to quantify the percentage of water loss, corresponding to the percentage of label loss, from within the chamber and exchanged with water from the hyporheic zone (Figure 10).
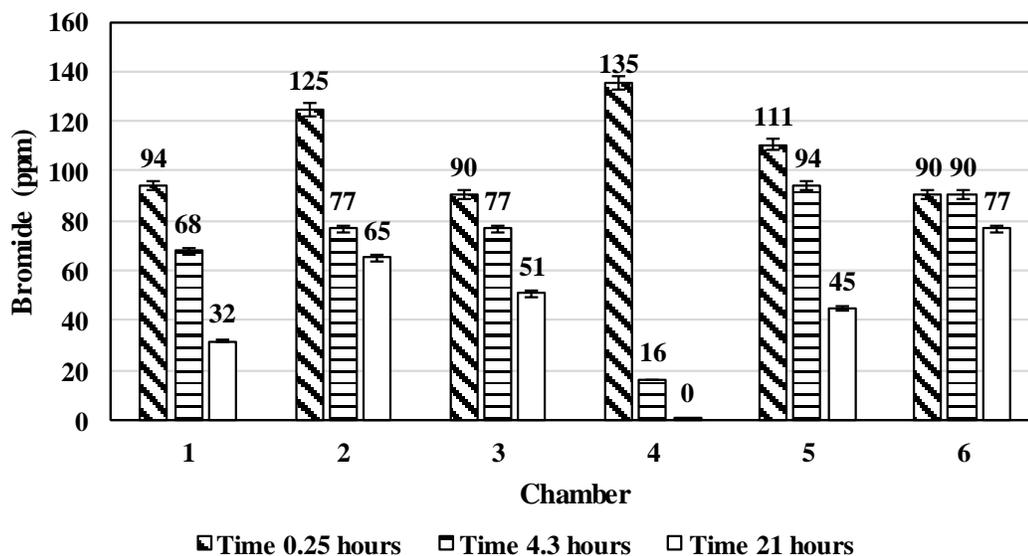
Figure 10. Bromide data during the 21-hour incubation. Error bars represent the 2% error as reported by the manufacturer (Orion Model 94-35 Halide electrode; Orion Research Incorporated Laboratory Products Group, Boston, MA). Chambers 1-3 were non-macrophytic and Chambers 4-6 contained macrophytes. The target dosing concentration was 100 ppm.

The target bromide dose was 100 mg/L. The 0.25-hour sample showed that complete

mixing within the chambers was achieved as the average concentration after dosing of all

six chambers was 108 mg/L $\pm$ 19 mg/L. Bromide concentrations were highly variable

between macrophytic (Chambers 4-6) and non-macrophytic (Chambers 1-3) plots and

within single chambers. Piezometer measurements indicated that mixing between

chamber water and hyporheic flow was mostly due to downwelling conditions (Table 2).

The combined data illustrate that the frames did not serve their purpose of restricting flow

into and out of the chamber as expected due to the streambed substrate consisting mainly

of gravel over the frame depth (Table 2).

Table 2. Bromide and piezometer results for the initial 21-hour incubation. A $\Delta h/\Delta l$
value of zero would indicate no influence from groundwater interactions.

Day	Chamber	% Br loss	Piezometer #	$\Delta h/\Delta l$	flow direction
	1	66	1	0.56	downwelling
	2	48	2	-0.19	upwelling
0	3	44	3	0.49	downwelling
	4	100	4	0.21	downwelling
	5	60	5	0.37	downwelling
	6	15	6	0.01	downwelling

The tubing connected to Chamber 4 had to be disconnected about an hour after

dosing due to free-floating macrophytic material being sucked up into the submersible

pump, contributing significantly to loss in bromide and nitrogen label within the

chamber. Although the bromide and piezometer measurements signify a loss in water

from the chambers, the volume of water within the chambers did not significantly change

over the 21-hour incubation period (Table 3).

Table 3. Ratio of the volume of air bubbles to water volume within the chambers during the initial 21-hour incubation period. All of the air was introduced from the chamber tubing during setup, as the tubing was not fully cleared of air prior to attaching the lines to the chambers and starting the submersible pumps. No additional air was produced within the chambers over the 21-hour incubation.

Air/Water Volume (%)

Chamber 1	Chamber 2	Chamber 3	Chamber 4	Chamber 5	Chamber 6
0.5	1.2	0.7	0.9	1.4	1.6

Note: Air and water volume did not change over 21 hour incubation

The volume percent exchanged over the incubation time was calculated based on changes in bromide concentration within the chambers and used as a correction factor for the production of dissolved nitrogen gas and isotopic nitrate and ammonium calculations.

Dissolved Oxygen and Temperature

Dissolved oxygen was monitored inside the chambers using Milwaukee Dissolved Oxygen Meters and outside of the chambers using an RDO Optical Dissolved Oxygen Sensor and Orion Star Meter (Thermo Scientific, MA). As indicated earlier, dissolved oxygen within the chambers was kept within 10% of the stream concentration by covering the chambers with black plastic bags to lower dissolved oxygen concentration and removing them when concentrations returned to background (Figure 11).

The probes used during the 21-hour incubation had been equipped with new batteries, but were reporting high values despite numerous attempts to recalibrate and fix the probes in the field before chamber installation. However, the observation was made that the change in dissolved oxygen reported by the Milwaukee Dissolved Oxygen Meters was comparable to that reported by the RDO Optical Dissolved Oxygen Sensor.
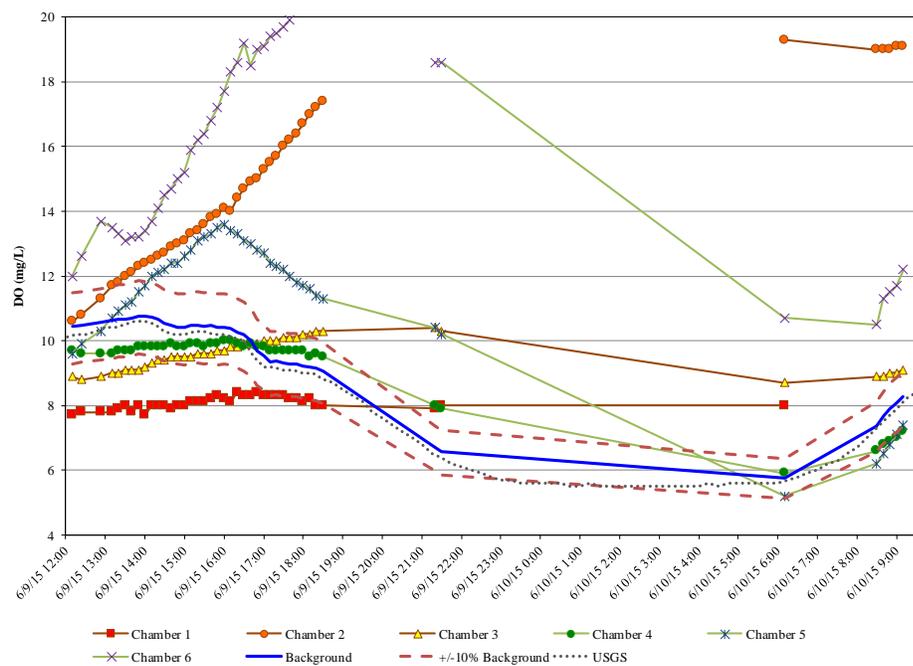
(a)
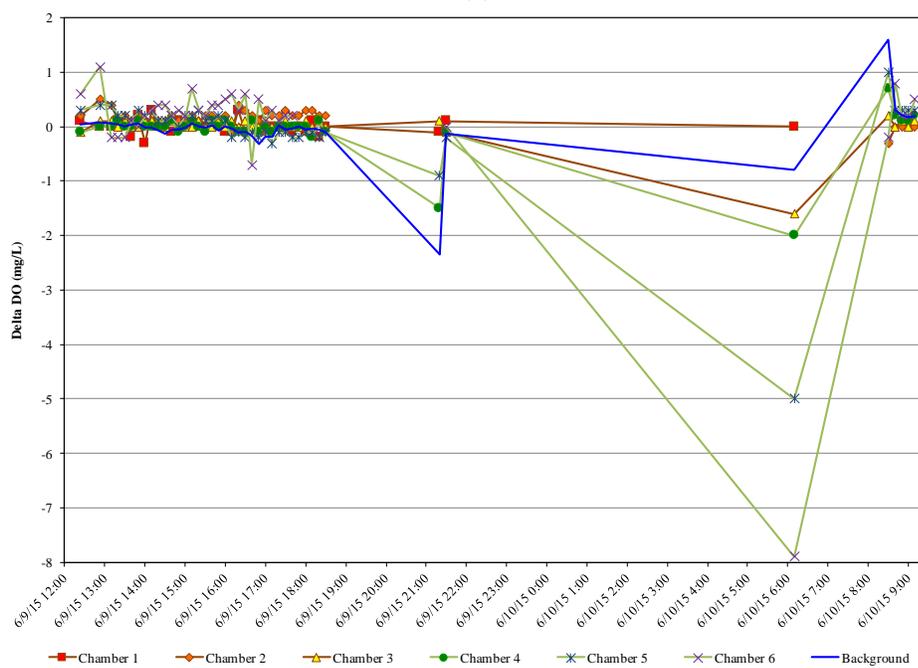
(b)

Figure 11. (a) Measured dissolved oxygen over the initial 21-hour incubation within the six chambers and for the background outside of the chambers and (b) the change in the measured dissolved oxygen between each chamber and the background. Background dissolved oxygen was measured outside of the chambers using an RDO Optical Dissolved Oxygen Sensor and Orion Star Meter.

The decision was made to do one final calibration attempt on the Milwaukee Dissolved Oxygen Meters and then install the chambers regardless of the high reported values. The dissolved oxygen was recorded every 10 minutes within the chambers and upstream (Figure 11a) and the change in dissolved oxygen calculated every 10 minutes (Figure 11b). If the change in dissolved oxygen was significant (+ 0.5 mg/L), a black bag was installed on the chamber to lower the dissolved oxygen production rate. The change in dissolved oxygen shows chambers fluctuating around the measured background dissolved oxygen concentration during the day with Chamber 6 having the highest fluctuations. Negative changes represent a decrease in dissolved oxygen and were only observed overnight when the chambers were not controlled.

The temperature within each chamber was recorded with a HOBO® TidbiT Temperature Logger to see if covering the chambers with black bags significantly affected the temperature within the chamber. Stream temperature outside of the chambers was recorded by the USGS station upstream of the sampling site and verified by monitoring temperature every 10 minutes using the RDO Optical Dissolved Oxygen Sensor and Orion Star Meter at the sampling site (Figure 12).

Dissolved oxygen concentration within the chambers increased and decreased simultaneously with temperature. The probe installed in Chamber 1 was most difficult to recalibrate within the field and therefore gave the most unstable readings. The monitor attached to the probe installed in Chamber 2 was accidentally knocked into the stream causing the monitor to power off around 7 hours into the incubation.
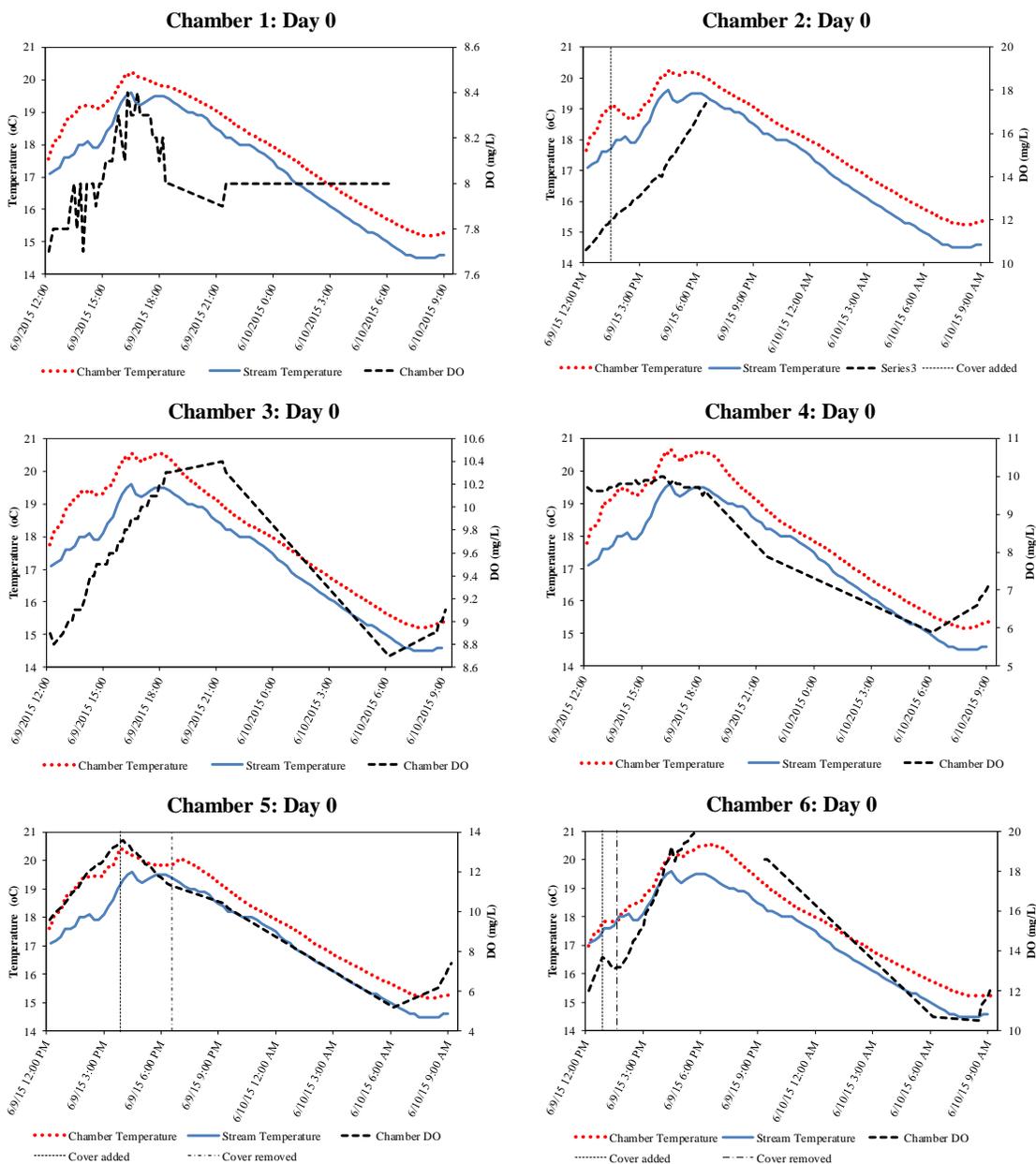
Figure 12. Stream temperature and chamber dissolved oxygen concentration and temperature over the initial 21-hour incubation to assess the relationship between temperature and dissolved oxygen when coving the chambers with black plastic bags. Chamber 2's probe was knocked into the stream 7 hours into incubation, causing the screen to power off. Probes in Chamber 2 and 6 reported higher than expected values of dissolved oxygen due to calibration problems, but were monitored to be within 10% of background by using the difference in dissolved oxygen every 10 minutes as described above.

Chambers 2 and 6 were second most difficult to recalibrate in the field and reported higher values than actually observed within the stream. The monitor for Chamber 6 was able to read a value up to 20 mg/L, but anything higher caused an error message to be shown. Although the monitor reported concentration values upwards of 20 mg/L due to problems with calibration, the actual and expected dissolved oxygen concentration within the chamber was much lower, as anything above 12 mg/L would start to exceed super-saturated conditions. The reading on the monitor, although inaccurate, was still recorded so that the change in concentration could be used to control the dissolved oxygen within the chamber to be within 10% of the background stream concentration. The monitor was able to report values again once the dissolved oxygen dropped to 18 mg/L. Although the data are skewed because of the high readings from the probes, the data were still plotted to show the increase and decrease in dissolved oxygen in relation to the increase and decrease in temperature, especially if the chamber was covered with a black plastic bag.

The addition of the black plastic bags for lowering dissolved oxygen within Chambers 5 and 6 did not increase temperature within the chamber as was expected, rather it decreased or slowed temperature increases within the chamber. Overall, chamber temperatures were warmer than stream temperatures. The acrylic chambers caused a greenhouse-like effect which allowed solar radiation to raise the temperature within the chamber but did not allow excess energy to leave at the same rate. The addition of the black bag completely stopped solar radiation, allowing for a net loss of energy from the chamber, therefore lowering temperature and dissolved oxygen.

Overall Nitrogen Concentration Data

Water and sediment samples collected from the chambers were analyzed on the

AQ2 Discrete Analyzer for the amount of unlabeled Total N, NO_3^--N and NH_4^+-N, with

Organic N being calculated by difference (Table 4).

Table 4. Nitrogen data from within the chambers during the 21-hour incubation. These values were corrected based on the loss of bromide from within each chamber.

| Chamber | Time (hours) | Water | | | Sediment |
		TN (mg/L)	NO_3-N + NO_2-N (mg/L)	Org N (mg/L)	NO_3-N + NO_2-N (mg/kg)
1	0	6.45	3.48	2.97	1.44
	0.25	7.39	3.98	3.42	
	4.3	7.16	4.21	2.95	
	21	5.89	2.63	3.26	0.76
2	0	6.30	3.83	2.47	1.59
	0.25	7.19	4.11	3.08	
	4.3	6.46	4.30	2.16	
	21	6.33	3.54	2.79	0.79
3	0	6.57	3.73	2.84	1.04
	0.25	7.03	3.89	3.14	
	4.3	6.59	3.86	2.73	
	21	5.13	3.38	1.75	BD
4	0	6.21	3.70	2.51	1.25
	0.25	6.84	3.95	2.89	
	4.3	9.83	2.48	7.35	
	21	-	-	-	-
5	0	6.27	3.68	2.59	0.50
	0.25	6.42	4.09	2.33	
	4.3	5.90	3.19	2.71	
	21	1.84	0.16	1.68	BD
6	0	6.60	3.72	2.87	0.20
	0.25	6.72	4.12	2.60	
	4.3	6.22	3.90	2.31	
	21	5.72	3.22	2.50	0.08

BD = Below detect values

Total nitrogen for the sediment samples were not recoverable as the chemistry of the sample after digestion interfered with the AQ2 Discrete Analyzer method. Efforts were made to find another method for quantifying total nitrogen within the sediments, but no other analyses were done as the holding time was no longer comparable to the other samples and the amount of $NO_3^--N + NO_2^--N$ in the sediments was insignificant compared to the water samples. Ammonium for both the water and sediment samples were below method detection (0.02 mg N/L).

A detectible increase can be seen between the background nitrate concentration at Time 0 hours and after the nitrate label is added at Time 0.25 hours for the water data. This concentration then decreases as the nitrate is distributed and/or transformed, with the exception of Chamber 4. Chamber 4 samples collected at the Time 0 and Time 0.25-hour marks are comparable to the results measured within the other five chambers. However, the Time 4.3-hour sample does not, due to the 88% water loss from within the chamber. Correcting the data for such a large loss of volume resulted in values that were not comparable to the other five chambers at the same sampling time.

Labeled Nitrogen Data

Water and Sediment Samples

The average mass of contamination due to the addition of KCl, MgO, and Devarda's Alloy was calculated based on the results of the five replicated diffusion standards (Table 5).

Table 5. Standards prepared for the diffusion procedure.

	WATER STANDARDS				
	Average mass (µg N)	**Average atom%**	**Average mass from contamination (µg)**	**Corrected mass (µg)**	**Corrected atom%**
Non-diffused nitrate	39.1	5.35	0		
Diffused nitrate	47.9	4.09	13.2	34.7	5.12
Digested nitrate	19.2	3.86	16.7	2.5	6.90
Non-diffused ammonium	38.8	5.20	0		
Diffused ammonium	43.0	4.85	3.0	40.0	5.17

	SEDIMENT STANDARDS				
	Average mass (µg N)	**Average atom%**	**Average mass from contamination (µg)**	**Corrected mass (µg)**	**Corrected atom%**
Non-diffused nitrate	39.1	5.35	0		
Diffused nitrate	45.1	4.12	12.9	32.3	5.19
Digested nitrate	66.3	3.02	34.2	32.1	4.40
Non-diffused ammonium	38.8	5.20	0		
Diffused ammonium	42.1	4.86	2.9	39.1	5.17

The water standard sample that was digested and then diffused (listed as 'Digested nitrate' above) resulted in 19.2 µg of N being recovered, but 16.7 µg due to contamination, resulting in a corrected mass of 2.5 µg, well below the 20 µg minimum. The corrected atom% was estimated to be 6.90%, higher than the true atom% within the standard solution of 5.35%. Therefore, all field water samples run through a digestion and diffusion procedure were considered invalid, resulting in the loss of labeled Total N data within the water column. All sediment standards were considered valid.

After correcting the water and sediment data based on the standards results and using Equation 2, the total mass of ^{15}N within each chamber was calculated (Table 6). The mass of ^{15}N was highest in the water samples due to the contribution from the added labeled nitrate. Contributions from ammonium within the water column were very small

and almost negligible compared to nitrate. Very little label (at or below the method

detection limit of 0.02 mg/L) diffused into the sediment.

Table 6. Labelled nitrate and ammonium data for the water and plant samples collected over the initial 21-hour incubation. These data were corrected based on the loss of bromide from within each chamber.

Chamber	Time (hours)	Water Samples				Sediment Samples			
		Adjusted atom% ^{15}N-NO$_3$	Total mass (mg^{15}NO$_3$-N)	Adjusted atom% ^{15}N-NH$_4$	Total mass (mg^{15}NH$_4$-N)	Adjusted atom% ^{15}N-NO$_3$	Total mass (mg^{15}NO$_3$-N)	Adjusted atom% ^{15}N-NH$_4$	Total mass (mg^{15}NH$_4$-N)
1	0	0.411	0.4	0.633	2.8E-02	0.478	2.6E-01	0.673	1.1E-01
	0.25	7.77	8.6	0.973	1.4E-02				
	4.3	6.79	9.4	0.909	8.4E-03				
	21	9.55	9.5	0.477	2.1E-02	1.20	2.4E-01	0.007	9.4E-04
2	0	0.466	0.1	0.431	1.0E-01	0.470	4.4E-01	0.618	1.2E-01
	0.25	LS	LS	0.552	1.3E-02				
	4.3	LS	LS	2.98	8.0E-03				
	21	11.1	12.3	0.515	1.8E-02	IM	IM	0.320	3.9E-02
3	0	0.415	0.2	0.681	2.9E-02	IM	IM	0.619	8.4E-02
	0.25	7.70	8.3	0.562	1.7E-02				
	4.3	LS	LS	0.387	2.3E-02				
	21	LS	LS	0.860	4.1E-02	IM	IM	0.270	7.3E-02
4	0	0.386	0.4	0.747	7.6E-03	0.821	1.3E-01	0.719	6.1E-02
	0.25	LS	LS	0.920	7.3E-03				
	4.3	IM	6.8	IM	IM				
	21	LS	LS	163 *	11.9 *	IM	IM	IM	IM
5	0	0.379	0.4	0.890	4.8E-03	IM	IM	0.455	1.9E-01
	0.25	LS	LS	1.77	7.3E-03				
	4.3	10.4	11.7	0.524	2.5E-02				
	21	LS	LS	IM	IM	IM	IM	0.302	2.0E-01
6	0	0.388	0.5	1.18	1.6E-02	7.96 *	13.3 *	0.418	2.0E-01
	0.25	LS	LS	0.456	2.0E-02				
	4.3	LS	LS	0.458	1.6E-02				
	21	26.4 *	2.2	0.316	9.9E-03	IM	IM	0.408	2.2E-01

* Higher than expected values due to accumulation of error during correction

LS = lost due to leaks during the diffusion procedure

IM = insuffiecient mass (< 20 µg)

As discussed earlier, standards for the digestion and diffusion of water samples

for determining Total N showed that the process did not work and was considered

invalid. This made determining the mass of Organic N by difference impossible.

However, the measured atom% before correcting for procedural contamination (3.86 %)

accounted for 72% of the atom% within the sample. Therefore, it was decided to

calculate the relative percent difference (RPD) at each collection time between the

reported atom% values from the digested and diffused water field samples and reported

atom% values from the diffused nitrate water field samples, and see if there was a

significant difference in the amount of ^{15}N (Table 7).

Table 7. Comparison of the reported atom% in TN and NO_3^- from the field water samples over the initial 21-hour incubation and their relative percent difference (RPD). These data were corrected based on the loss of bromide from within each chamber.

Chamber	Time (hours)	NO_3 atom%	TN atom%	RPD
1	0	0.40	0.44	9%
	0.25	6.41	6.12	5%
	4.3	4.30	4.24	1%
	21	2.87	2.57	11%
2	0	0.41	0.44	7%
	0.25	LS	7.45	
	4.3	LS	5.42	
	21	4.98	4.04	21%
3	0	0.40	0.40	1%
	0.25	6.32	6.21	2%
	4.3	LS	5.25	
	21	LS	4.02	
4	0	0.38	0.40	5%
	0.25	LS	7.83	
	4.3	1.71	1.59	7%
	21	LS	0.47	
5	0	0.38	0.42	11%
	0.25	LS	6.99	
	4.3	7.38	6.46	13%
	21	LS	2.99	
6	0	0.38	0.38	1%
	0.25	LS	5.54	
	4.3	LS	5.90	
	21	5.87	5.26	11%

LS = lost due to leaks during the diffusion procedure

The measured atom% from the Total N samples collected after dosing (Time 4.3

and 21 hours) were all lower than the atom% measured in nitrate, and therefore it was

concluded that none of the ^{15}N label was incorporated as organic nitrogen.

Dissolved Nitrogen Gas Results

The measured ratios of $^{29}N_2/^{28}N_2$ and $^{30}N_2/^{28}N_2$ were adjusted for losses within the individual chambers by assuming that the water lost from the chamber (determined from the bromide data) was replaced with background (stream) water (Table 8).

Table 8. Adjusted isotope ratios for samples collected during the initial 21-hour incubation. These data were corrected based on the loss of bromide from within each chamber.

Chamber	Time (hrs)	Raw Isotope Ratios		Raw at% $^{15}N-N_2$	Raw umoles/vial N_2	Vol. Lost %	Adjusted Isotope Ratios	
		29/28	30/28				29/28	30/28
1	0	7.03E-03	6.80E-04	0.366	21.5	0	7.03E-03	6.80E-04
	0.25	8.11E-03	8.26E-04	0.421	34.1	0	8.11E-03	8.26E-04
	4.3	8.61E-03	6.32E-04	0.447	25.8	28	9.22E-03	6.32E-04
	21	8.65E-03	6.12E-04	0.449	15.6	66	1.18E-02	6.12E-04
2	0	7.03E-03	7.34E-04	0.366	32.1	0	7.03E-03	7.34E-04
	0.25	8.15E-03	8.43E-04	0.424	34.9	0	8.15E-03	8.43E-04
	4.3	8.75E-03	6.53E-04	0.454	24.0	39	9.84E-03	6.53E-04
	21	1.05E-02	6.85E-04	0.545	14.2	48	1.37E-02	6.85E-04
3	0	7.03E-03	7.71E-04	0.366	38.4	0	7.03E-03	7.71E-04
	0.25	8.39E-03	8.68E-04	0.436	40.7	0	8.39E-03	8.68E-04
	4.3	9.02E-03	6.66E-04	0.468	22.2	15	9.38E-03	6.66E-04
	21	1.04E-02	6.38E-04	0.540	15.6	44	1.31E-02	6.38E-04
4	0	7.04E-03	7.64E-04	0.366	25.9	0	7.04E-03	7.64E-04
	0.25	8.34E-03	8.73E-04	0.434	36.6	0	8.34E-03	8.73E-04
	4.3	7.42E-03	6.05E-04	0.385	28.0	88	1.02E-02	6.05E-04
	21	7.05E-03	5.73E-04	0.366	25.4	100	2.30E-02	5.73E-04
5	0	7.04E-03	7.48E-04	0.366	19.1	0	7.04E-03	7.48E-04
	0.25	9.20E-03	6.72E-04	0.477	26.0	0	9.20E-03	6.72E-04
	4.3	9.35E-03	7.23E-04	0.485	21.6	15	9.76E-03	7.23E-04
	21	1.04E-02	6.96E-04	0.540	18.9	60	1.54E-02	6.96E-04
6	0	7.04E-03	7.49E-04	0.366	20.3	0	7.04E-03	7.49E-04
	0.25	9.88E-03	6.95E-04	0.512	19.2	0	9.88E-03	6.95E-04
	4.3	7.38E-03	6.33E-04	0.383	19.3	0	7.38E-03	6.33E-04
	21	8.75E-03	6.63E-04	0.454	25.7	15	9.05E-03	6.63E-04

However, the reported mass of N_2 per vial was higher than expected from N_2 saturation calculations and inconsistent within and between chambers. A calculation using a saturation value of 20 mg N_2/L (saturation at the stream temperature during the 21-hour incubation period) and 0.83 atm at the study site showed that at saturation the

concentration in the vial would measure 5.4 µmol/vial. The reported masses were

replaced with the saturation mass of 5.4 µmol/vial and the adjusted measured ratios were

then used with the saturation mass to calculate the molecular fraction of each N_2 species,

the production of $^{28}N_2$, $^{29}N_2$, and $^{30}N_2$, and finally the total mass of ^{15}N within each

chamber (Table 9).

Table 9. Dissolved nitrogen gas total mass for the initial 21-hour incubation samples after correction for calculated N_2 saturation at field conditions.

Chamber	Time (hrs)	total mass mg ^{15}N
1	0	2.0
	0.25	2.4
	4.3	2.5
	21	3.1
2	0	2.1
	0.25	2.4
	4.3	2.7
	21	3.6
3	0	2.1
	0.25	2.4
	4.3	2.6
	21	3.4
4	0	2.1
	0.25	2.4
	4.3	2.8
	21	5.8
5	0	2.1
	0.25	2.5
	4.3	2.7
	21	4.0
6	0	2.1
	0.25	2.7
	4.3	2.1
	21	2.5

Production increased over the 21-hour period except in Chamber 6 which varied over time. Statistical analysis showed that the measured amount of ^{15}N at Time 0 hours and Time 21 hours were significant for all chambers. Atom percent values for all dissolved gas samples collected on sampling Day 2 were at background levels (~0.366%), as expected.

Plant Samples

The mass of total nitrogen and atom% within the harvested plant material was measured by isotope ratio mass spectrometry (Table 10).

Table 10. Plant ^{15}N data over the initial 21-hour incubation. The atom% data were corrected based on the loss of bromide from within each chamber.

Chamber	Time (hours)	Harvested Weight dry wt (g)	Adjusted atom% ^{15}N	Total Mass mg ^{15}N
4	0	7.6	0.369	1.2
	21		19.2 *	9.7 *
5	0	25.9	0.369	0.9
	21		0.586	2.8
6	0	38.7	0.368	3.2
	21		0.439	5.7

* Samples had higher than expected results

Plant material within Chambers 5 and 6 incorporated 13 to 15% of the total added label. Using an average of 14%, the amount of labeled nitrogen within Chamber 4 would drop from 9.7 mg ^{15}N to a more realistic value of 3.5 mg ^{15}N.

Mass Balance

A mass balance was performed only for Chamber 1 as it was the only chamber with a complete data set from the diffused water samples. The total mass of labeled nitrogen added to the chamber was calculated based on AQ2 data to be 13.96 mg instead of the targeted 16.63 mg. Percent recoveries were based on this actual amount added. The background concentration was then subtracted from the measured values to obtain the net mass of ^{15}N (Table 11).

Table 11. Mass balance for Chamber 1 during the initial 21-hour incubation period. The amount of labeled nitrogen in the dissolved gas phase is based off of the calculation in which saturation conditions were assumed. Chamber 1 was a non-macrophytic chamber so there was no assimilation into macrophytic material. All values were corrected for losses based on bromide data.

Time (hours)	water		sediment		gas	Total	%	average
	mg ^{15}NO$_3$-N	mg ^{15}NH$_4$-N	mg ^{15}NO$_3$-N	mg ^{15}NH$_4$-N	mg ^{15}N$_2$-N	mg ^{15}N	recovery	% recovery
0	0.0	0.0	0.0	0.0	0.0	0.0		
0.25	8.2	LB			0.3	8.5	61%	
4.3	9.0	LB			0.5	9.5	68%	67%
21	9.1	LB	LB	LB	1.1	10.2	73%	

LB = value lower than background

Less than 66% percent of the added labeled nitrate remained in the water column. Less than 8% of the added label was denitrified (converted to ^{15}N$_2$-N) over time. Variability within the chamber over time can be seen in the percent recovery which has a range of 61 to 73%. The overall average recovery over the 21-hour incubation was 67%.

The amount of label added was also tracked by looking at the measured atom% values (Table 12).

Table 12. Tracking atom% within Chamber 1.

Time (hours)	Water	Gas	Total	% recovery	average % recovery
0	0.411	0.366	0.78		
0.25	7.77	0.421	8.19	82%	85%
4.3	6.79	0.478	7.27	73%	
21	9.55	0.611	10.2	102%	

The dosing solution was made such that a target enrichment of 10% would be added to each chamber. Again, the variability within the chamber due to assumptions and measurement uncertainty can be seen in the percent recovery which ranges from 73 to 102%. The overall atom% recovery over the 21-hour incubation was 85%.

The final distribution of labeled nitrogen within Chamber 1 was calculated to easily observe the overall transport of label within the 21-hour incubation period (Figure 13).
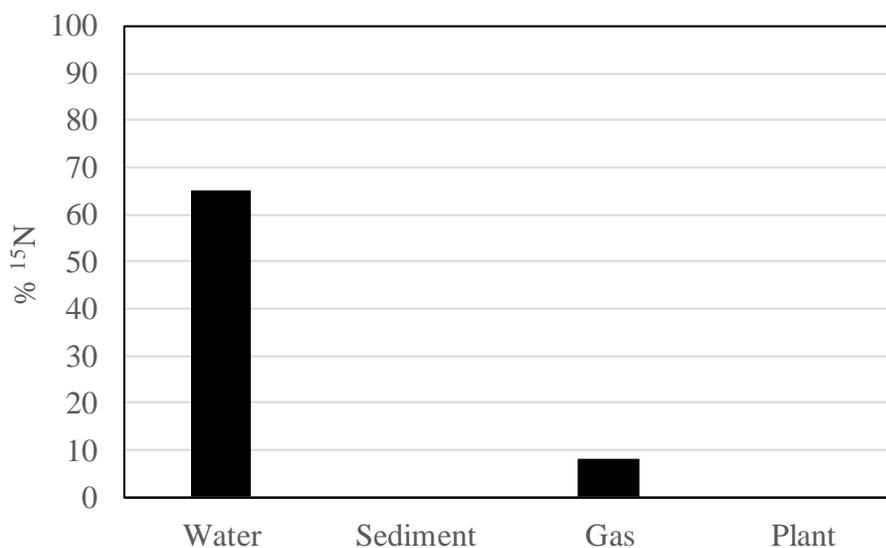
Figure 13. Distribution of labeled nitrogen within Chamber 1 after the initial 21-hour incubation period. Chamber 1 was a non-macrophytic chamber.

Most of the label stayed in the water column. Less than 8% was denitrified and transformed to nitrogen gas. None of the label was incorporated into the sediments and there was no macrophytic material in Chamber 1 for the label to be assimilated.

Rates

Gross nitrification and denitrification rates were first calculated using the two-point method shown in Equations 3 and 4 (Table 13; Appendix B).

$$\text{Gross Nitrification Rate } \left[\frac{mg}{L-d}\right] = \left(\frac{P_0 - P_t}{t}\right)\left(\frac{\log\frac{P_0}{P_t}}{\log\frac{I_0}{I_t}}\right) \tag{3}$$

$$\text{Gross Denitrification Rate } \left[\frac{mg}{L-d}\right] = \left(\frac{P_0 - P_t}{t}\right)\left(\frac{\log\frac{P_0}{P_t}}{\log\frac{I_0}{I_t}}\right) - \left(\frac{P_t - P_0}{t}\right) \tag{4}$$

Table 13. Two-point gross nitrification and denitrification rates for Chamber 1

Gross Nitrification		Gross Denitrification	
Timespan (hours)	*rate (mg L^{-1} d^{-1})*	*Timespan (hours)*	*rate (mg L^{-1} d^{-1})*
0 - 4.3	0.093	0 - 4.3	0.087
0 - 21	-0.017	0 - 21	-0.018

Due to the logarithm used in Equation 3 to calculate gross nitrification, a positive rate is representative of the loss of ^{15}N from the water column over time and a negative rate representative of the production of ^{15}N within the water column over time. If the result behaved as expected with P_0 being greater than P_t and I_0 being greater than I_t, Equation 3 would result in a positive value for the rate of gross nitrification, as seen at Time 4.3 hours (Table 13). Equation 4, which is composed of Equation 3 and one additional term

(the change in nitrate concentration over time), would also follow this trend despite the additional term being subtracted. If the system behaved as expected, the equation would contain the positive value from Equation 3 followed by the subtraction of a negative value, resulting in a larger positive value for the rate of gross denitrification. This makes sense as a positive rate would indicate the loss of nitrate from the water column as it is denitrified to N_2. However, because the atom percent at Time 21 hours is greater than at Time 0.25 hours, the Time 21-hour denitrification rate resulted in a negative value instead of a positive one. Therefore, it was decided to estimate the rate of production of N_2 (denitrification) assuming a zero-order reaction rate and plotting the ^{15}N as N_2 concentration from the estimated saturation concentration values within Chamber 1 over time (Figure 14).
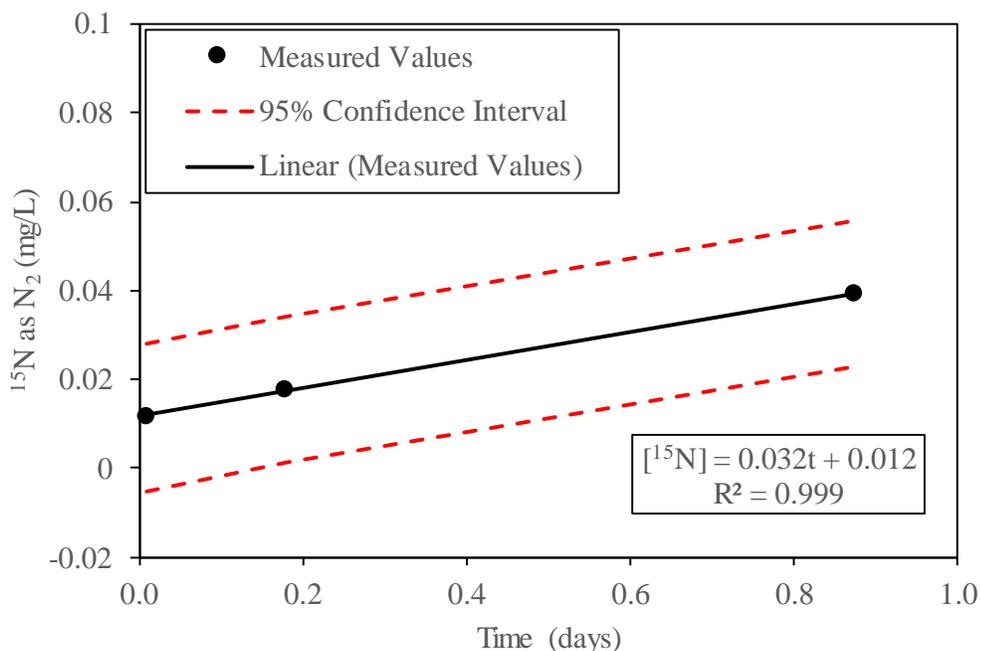
Figure 14. Zero-order rate of N_2 production estimated assuming a saturation concentration and the reported dissolved gas ratios from Chamber 1.

The rate of denitrification within Chamber 1 using both the two-point method and the zero-order graphical method were compared to values from similar chamber experiments reported in the literature as indicated in Table 14. The first-order rate (calculated by converting the zero-order rate using the graphical method above) of denitrification from Chamber 1 based on loss of $NO_3^--N + NO_2^--N$ from the water column as measured in the non-labeled data also compared to values used in previous modeling techniques (Table 14).

Table 14. A comparison of literature values which reported the possible ranges for the rate of denitrification found using various modeling or chamber techniques. See Appendix C for calculations[1, 2].

Denitrification Rate Comparisons

Min. Rate	Max. Rate	^{15}N based	units	Reference	Technique
0.002	1	no	day^{-1}	Bowie et al., 1985	Model
0.05	2	no	day^{-1}	Neilson et al., 2013	Model
0.3	0.41	no	day^{-1}	Silver Creek Non-Labeled Data	Chamber
1.8*		yes	day^{-1}	Silver Creek Chamber 1[1]	Chamber
0.032*		yes	mg L^{-1} d^{-1}	Silver Creek Chamber 1	Chamber
0.012	0.084	yes	mg L^{-1} d^{-1}	O'Brien et al., 2012[2]	Chamber
0.28*		yes	mg L^{-1} d^{-1}	Smith et al., 2009[2]	Chamber

* = only one rate provided or able to be calculated

[1]zero-order rate converted to a first-order rate by dividing by the concentration at each sampling time and taking the average

[2]Rate reported in μmol m^{-2} h^{-1} and converted using the reported area and volume of the chamber

The rate of denitrification was comparable to the rate found by O'Brien et al. (2012) using similar chamber studies. The rate of denitrification at Silver Creek was 10 times smaller than the rate found by Smith et al. (2009) during their study using labeled

nitrate. The non-labeled rate of denitrification and the minimum rate from labeled data within Chamber 1 at Silver Creek was within the possible ranges reported by Bowie et al. (1985) and Neilson et al. (2013), but the maximum rate based on labeled data from Chamber 1 exceeded these ranges.

The zero-order rate of assimilation within the three macrophytic chambers was 0.205 mg kg^{-1} d^{-1}, but was determined from only two observations (Time 0 and 21 hours) as plant samples could only be collected while the chambers were removed.

Rates for ANAMMOX, DNRA, and ammonification were not significant or detectible, as none of the diffused samples were able to detect the production of $^{15}NH_4^+$, a key component required for the verification of all three processes.

DISCUSSION

Discharge measured by the USGS station upstream of the study site at Silver Creek detected a large rain event about 2 days before sampling started. The initial 21-hour sampling occurred after the pulse measured from the rain event passed through the system. Discharge during the initial incubation was approximately 6 cfs, comparable to the discharge recorded before the rain event.

One way to assess impacted streams is to observe how dissolved oxygen concentration changes with temperature. Instantaneous standards are used to assess minimum dissolved oxygen requirements, requiring observations to be made over hourly timescales. It is expected that dissolved oxygen concentrations would be greatest when temperature is lowest as gasses are more soluble and the rate of oxygen consumption by microbes is lower at lower temperatures. Daily dissolved oxygen observations at Silver Creek do not reflect that of a non-impacted stream, as dissolved oxygen increases as stream temperature increases. This pattern is reflective of oxygen generation and consumption by aquatic plants due to photosynthesis and respiration as light and temperature increase and decrease. It appears that the dissolved oxygen in Silver Creek is driven by aquatic plant activity and/or sediment oxygen demand rather than microbial metabolism of carbon and transformation of nitrogen.

The combined bromide tracer and piezometer data showed that the installed frames did not restrict groundwater influences. Significant loss of the added $^{15}NO_3^-$ in the chambers over the initial 21-hour incubation period was indicated by the loss of bromide from the chambers over time. The loss of this label led to a decrease in measureable

transformation of ^{15}N into other nitrogen species and transport to other compartments over the incubation time. The loss was so great in some chambers, such as Chamber 4, that the confidence in the measured concentrations of enriched nitrogen species within the chamber significantly decreased with each correction calculation. O'Brien et al. (2012) used bromide as a tracer for their experiments, but did not comment on the results of bromide recovery and how it related to the added label. Smith et al. (2009) also used bromide and reported a loss of 0.6% h^{-1} to 0.8% h^{-1} (<20% over 21 hours) during their 22-hour incubation experiments due to flux of bromide-free water into the chambers and/or loss by diffusion into the sediment; much smaller than the losses at Silver Creek. All of the data collected from field samples was corrected based on the percent loss of bromide. The assumption that the water lost was exchanged or replaced with background stream water may or may not have been a good assumption. Natural abundance (background concentration) of ^{15}N is known to be 0.366 atom%, which made it easy to calculate the actual atom% within the chambers if there had been no leaks. However, this assumption does not hold when adjusting the unlabeled data, as the source of the exchanged water was not precisely known and the nitrogen concentration could have been highly variable between surface and ground waters. It is recommended that future experiments only be conducted if bromide losses are consistently controlled to be <30%.

Minimizing groundwater influences may be very difficult in streams similar to Silver Creek due to the substrate being mostly composed of sandy gravel. Sidewalls taller than the 6" used in this study that reach the deeper, more clayey, fine-grained sediments may lessen this problem, but may also be significantly harder to install as sidewall depth

increases. However, it is vital that groundwater influences be minimized (e.g. loss of bromide <50%) so that any data collected using the chamber method can be reported with confidence. Preliminary data collection during which piezometers are installed to map the groundwater interactions across the site may aid in the decision of where to install the frames prior to sampling by choosing locations in which groundwater interactions result in the smallest differential pressure head ($\Delta h/\Delta l$). It is critical that losses or exchanges from the chambers be minimized before conducting any experiments with the labeled nitrogen to increase confidence in the collected data.

Conditions within the chambers were kept as close to stream conditions outside the chambers as possible to try to replicate natural conditions so as to not affect transformation rates. Dissolved oxygen within the chambers was controlled such that it did not vary by more than 10% from the stream concentration outside of the chambers, as recommended by O'Brien et al. (2012). Chamber dissolved oxygen concentrations that began to exceed 10% of background were lowered by completely coving the chamber with a black plastic bag. Temperature inside the chambers was also recorded so that any changes within the chambers due to the installation of the black plastic bags could be observed, as there was concern that using black bags would lead to an increase in temperature. Dissolved oxygen concentrations did not respond as quickly as anticipated to the addition of the black plastic bags and took approximately 30 to 45 minutes to reach background concentrations once they were covered. Results could not be compared with literature as O'Brien et al. (2012) did not report the results of controlling dissolved oxygen concentration. Temperatures inside the chambers were higher than stream

temperature over the initial 21-hour incubation by 0-1.5 °C. The use of acrylic chamber lids caused a greenhouse-like effect, allowing solar radiation to add energy to the chamber in the form of heat while preventing excess heat from leaving the chamber at a comparable rate. When the black plastic bag was used to cover the chamber, it blocked all solar radiation from getting to the chamber, resulting in the net loss of heat from the chamber and a decrease in dissolved oxygen. The effect the acrylic chamber lids have on the temperature within the chambers is critical and needs to be controlled or closely monitored during incubation periods. Increases in temperature increase the rate at which chemical reactions occur (Keeney, 1973), including not only the rate at which nitrogen is transformed but also the rate at which dissolved oxygen is consumed and exchanged.

Nitrogen concentration data that were measured on the AQ2 included both the labeled and unlabeled nitrogen within the chambers. Sediment samples subjected to the digestion procedure and tested for nitrate resulted in poor quality control on the AQ2. Bench tests were performed using scaled up quantities of AQ2 reagents and sample to test the color changing reagent and to determine if the sample pH was within the specified limits for the method. The sample pH was in the correct range, however, no significant change in color with increasing concentration was observed. It was hypothesized that the chemistry of the sample due to unreacted persulfate was interfering with the AQ2 reagents and preventing a reliable reading. It is recommended that sediment samples for the analysis of total nitrogen be analyzed through another method, such as a combustion method.

Standards for the diffusion of the labeled nitrogen samples showed that the digested and diffused water samples had a less than acceptable mass recovery ($< 50\%$), but an average recovery (65-75%) of the nitrogen label (atom%). Digested and diffused water samples were corrected for the poor mass recovery by using the mass as measured from digested water samples run on the AQ2, and from the atom% reported from the diffusion procedure. This ensured a more accurate estimate of the mass of nitrogen, both labeled and unlabeled, within the sample. The diffusion standards also showed that the labeled nitrogen recovery (atom%) in the digested and diffused sediment samples was below an acceptable level ($< 75\%$). A bench scale experiment in which the digestion and diffusion method was performed and the pH measured between every step showed that the pH of the sample did not get above 9 with the addition of the magnesium oxide. Therefore, the pH was not high enough to convert the nitrogen that was transformed to nitrate during the digestion procedure into ammonia during the diffusion procedure (NH_3^+ pKa = 9.3). Future digestion and diffusion samples should have the pH adjusted accordingly prior to the diffusion procedure. However, because the amount of total nitrogen within the sediments was much lower than the water or plant samples, it still allowed for the overall mass balance on labeled nitrogen to be completed with acceptable accuracy (>80% recovery).

Ten diffusion samples for labeled nitrate were lost due to leaks ('LS' labeled samples). Diffusion jars were stored upside down such that the liquid slurry lined the lid of the jars and any leaks due to the buildup of gasses within the jars could be easily observed. The leaks occurred because the lids on the diffusion jars were not tight enough.

Water leaking out of the jars was observed approximately 2 days into the diffusion process. Extra care was taken on subsequent sampling days to ensure the lids on the diffusion jars were tightly placed, and no other leaks were observed over the remainder of the experiment.

Holding times for samples being analyzed for labeled and unlabeled nitrate, ammonia, and total nitrogen was set at 48 hours to ensure consistency between analytical sampling methods. Extra care was taken to ensure that all analytical laboratory processes were started within 48 hours of sample collection. However, because samples were collected between 4 to 21 hours apart, there was concern about the holding time being within the same order of magnitude as the time between samples. To address this concern for future experiments, the methods (Appendix C) were revised such that filtering of the samples occurred in the field before transport back to the laboratory to prevent continued reactions within the collected samples.

Dissolved gas data showed that denitrification was occurring as the atom%, delta $^{15}N_2$ versus air, and $^{29}N_2/^{28}N_2$ and $^{30}N_2/^{28}N_2$ ratios of the samples increased between Time 0 hours (before dosing) and Time 0.25 hours (after dosing) and continued to increase over time. However, upon further inspection, the reported mass of nitrogen per sample vial seemed to fluctuate and be inconsistent between similar times and between similar chambers. It was estimated that a fully saturated sample vial could contain between 5 and 7 µmol/vial based on atmospheric conditions and water temperature. However, reported values from UC, Davis ranged from 14 to 41 µmol/vial. Efforts were made to understand how this value was measured and/or calculated based on instrument set up and example

calibration data provided by the lab at UC, Davis, but no conclusions were reached. Calculations were done using the reported mass of N per vial and production determined by subtracting off the Time 0 hour, or background, measurement. This method provided estimates of nitrogen mass as N_2 within the expected ranges despite the inconsistency in the data. Hamilton and Ostrom (2007) discuss the challenges associated with making sure contamination during dissolved N_2 collection is minimized. Preparation of vials in nitrogen free environments and attention to storage time is vital to keeping contamination low. They provide detailed methods and recommendations as to the types of equipment that should be used and ways to detect and correct for small amounts (<2 µmol/vial) of contamination. Some of those methods were used for this study such as evacuating the vials just before sampling and storing the evacuated vials in water during transport to and from the field site. Other techniques that were not used for this study but were studied by Hamilton and Ostrom (2007), such as submerging the vials during evacuation and creation of the helium headspace could be added to the procedure used in this study to further decrease risk of contamination.

Constructing a mass balance on the chambers based on plant density was impossible because of the number of lost samples and the inconsistency in the dissolved nitrogen gas data. Individual chamber mass balances were impossible to construct for all but Chamber 1 because of the amount of lost samples in the diffusion procedure. However, even with the complete set of data for the label in the water and sediment compartments, the dissolved gas data for Chamber 1 needed to be adjusted using the calculated saturation value and bromide data in order to complete the data set resulting in

these values becoming estimates rather than measured values. The percent recovery for each sampling time varied from 61-73%, with an average recovery over the entire 21-hour incubation of 67%.

Reasons for the large variation within plots were investigated by looking at the impact due to dosing volume, chamber volume, sampling volume and chamber tubing volume. A deviation in dosing volume of $\pm$ 1 mL would result in a chamber concentration of $\pm$ 0.13 mg/L, or 23% of the target concentration. A 35 mL syringe with 1 mL increments was used to dose the labeled nitrate solution into the chamber. Although the same syringe was used to dose all chambers and the gradations were clearly visible and marked, a difference of 1 mL could be enough to create large variations in labeled nitrate concentrations among the chambers. Chamber volume was also assessed by calculating the change in total volume due to 1" of the frame being above the stream bed. An inch of exposed frame adds 4.7 L to the total chamber volume, or 17% of the expected/assumed 28.3 L used in the above calculations. Sampling from the chambers during the 21-hour incubation resulted in the removal of 0.58 L, or 2% of the total volume. Finally, the change due to the length of tubing, correlating to the volume of water within the tubing, was assessed by calculating the average volume within the tubing to be 0.2 L. A volume of 0.2 L accounts for only 0.7% of the assumed total volume of 28.3 L, making a variability in tubing volume the least likely source of variation.

The rate of nitrification in Chamber 1 was compared to the rate of denitrification based on the assumption that the consumption of ^{15}N, the source of nitrogen gas, should be proportional to the rate of production of ^{15}N$_2$-N. The two-point method using

Equations 5 and 15 in Appendix B generates an estimate for both gross nitrification and denitrification, making the two rates directly comparable. The calculated rate did show a loss of nitrate from the water column corresponding to a similar positive rate of N_2 production driven by denitrification over 4.3 hours of the incubation, but resulted in unexpected negative values over the entire 21-hour incubation. A zero-order graphical method was used in an attempt to provide a better estimate than the two-point method for the loss of nitrate due to denitrification from the water column using dissolved gas data instead of nitrate concentrations within the water column. However, the dissolved gas data had to be estimated by assuming saturation conditions within the chambers, as the reported values were inconsistent within individual chambers as well as between similar plots. Despite using the calculated saturation concentration, there was a higher level of confidence with the rates estimated using the zero-order graphical method than the rates estimated using the two-point analytical method. The biggest concern with the two-point method is it does not account for the loss of nitrate from the water column due to assimilation into plant material and, therefore, may overestimate the rate of direct denitrification in chambers with significant assimilation rates. The zero-order graphical method can more accurately estimate the rate of direct denitrification by looking at the production of N_2 over the incubation time. Another concern with the two-point method is the equation for the rate of denitrification takes into account the atom% within the water column within the rate of gross nitrification, but does not include it in the second half of the equation in which the change in concentration over time is subtracted from the rate of gross nitrification.

The rate of denitrification calculated using the non-labeled data from Chamber 1 was comparable to the possible range of rates presented by Bowie et al. (1985) and Neilson et al. (2013). The minimum rate of denitrification based on the labeled data from Chamber 1 was also within the ranges reported by Bowie et al. (1985) and Neilson et al. (2013), but the maximum rate exceeded this range. The Chamber 1 rate based on the labeled data was also comparable to rates found in similar chamber studies done by O'Brien et al. (2012), but was 10 times smaller than the rate reported by Smith et al. (2009), making it difficult to conclude whether or not the rates found from Chamber 1 at Silver Creek are representative of the processes occurring specifically at this site. Having a rate from all six chambers would allow the variation within the system to be observed and help make conclusions as to the expected range of denitrification rates within Silver Creek.

CONCLUSION

The overall objective of this project was to develop a method to quantify site-specific rates of nitrogen transformation in order to improve modelling techniques. Chambers were designed so that isotope dilution and isotope pairing methods could be used to track the transport and transformation of nitrogen through the system. Sampling procedures were evaluated and modified as needed to quantify the amount of nitrate, ammonium, total nitrogen, and dissolved nitrogen gas within the water, plant, and sediment compartments. A collection of laboratory methods was established and modifications made in order to process the collected field samples. A list of equations to quantify the rates of transformation based on the collected field data was also compiled. Despite best efforts, a complete data set was not obtained at Silver Creek, preventing rates of transformation to be calculated. Even with all of the missing data, this study provides insights into the challenges that have to be overcome in order to successfully use *in situ* chambers to quantify site-specific nitrogen transformation rates which include but are not limited to:

1. Constructing frames for the chambers that keep fluxes into and/or out of the chamber to a minimum (<25%). The addition of labeled nitrogen to the chambers should not be carried out until such leaks and exchanges are controlled so that data collected from the chambers can be reported with confidence.

2. Performing an initial survey of the study site to study groundwater influences. Piezometers should be installed in multiple sections of the stream to determine areas in which groundwater influences are the most minimal. Doing so will also

aid in minimizing the amount of water exchanged or lost from within the chambers prior to sampling.

3. Practicing laboratory procedures for processing the field data in advance. Sediment and water should be collected from the site and used to determine any shortcomings due to chemical interferences or required modifications to the methodology.

4. Monitoring chamber dissolved oxygen concentration and temperature closely to keep rates comparable to natural rates of transformation. Increases in temperature can lead to an increase in rates. The goal is to keep conditions with the chambers as close to natural conditions as possible. Therefore, it is important the dissolved oxygen concentrations within the chambers be closely monitored to be within 10% of background, as the production of dissolved oxygen can also be dependent on temperature.

5. Filtering samples in the field to prevent further reactions. Filtering samples in the field will also help increase the confidence in the measured data as the time between samples (4-21 hours) is on the same order of magnitude as the holding time for samples (48 hours).

Recommendations for Future Work

This study of Silver Creek did not result in a complete data set, however, it does not mean that the techniques and methods described above are not useful for determining

site-specific nitrogen transformation rates. Loss of data in this study was mainly due to:
1) loss of water samples due to human error during the diffusion procedure, 2) loss of
sediment total nitrogen data due to method selection (although sediment concentrations
were typically below detect and ended up not being a significant loss), and 3) incomplete
data analysis of dissolved nitrogen gas samples due to laboratory analytical errors.

Fortunately, all of these shortcomings can be corrected to ensure better results for
future studies. Practice runs for each of the analytical laboratory procedures to ensure
proper set up and technique will help reduce the potential for losing samples, such as the
water diffused samples. It will also help to determine if a new method should be used, as
in the case with the sediment total nitrogen data. Combustion of the sediment samples for
the analysis of total nitrogen would allow for the samples to be analyzed without having
to adjust or worry about chemical interferences between the sample and the AQ2 Discrete
Analyzer. Sediment and water samples were analyzed for total mass of nitrogen by both
the Stable Isotope Laboratory at Utah State University from the diffusion procedure, and
at the Utah Water Research Laboratory using the AQ2 methods. Doing so helped ensure
that any loss in data from one facility would, hopefully, be accounted for by the other.
This was not done, however, for the dissolved gas data. Collecting replicates in the field,
one to be sent to an outside laboratory and one to be analyzed internally, would have
helped with validating the mass of nitrogen within the test vials as reported by the
University of California Davis Stable Isotope Facility.

Another option would be to collect replicates from each chamber while sampling.
However, not only would this increase cost, analytical time, and space requirements, but

would require more technicians and assistance within the laboratory in order to transport and process additional field samples. The sampling procedures listed in Appendix C have been revised to include and account for the updated procedural approaches and recommendations based on the results of this study.

This method for quantifying nitrogen transformation rates, when done correctly, is able to provide a great deal of information on the nitrogen transformation processes occurring within a system. The methods chosen have proven to be robust and provide accurate data over several different experiments and environmental conditions by others. However, this method does require high attention to detail, extensive knowledge and practice of the various methods, and is very time consuming. Even so, the power of producing site-specific transformation rates to be used in models is invaluable when it comes to better understanding ways in which stream quality can continue to improve.

ENGINEERING SIGNIFICANCE

The data collected in this experiment were used to determine assimilation, nitrification, denitrification, ammonification, ANAMMOX, and DNRA transformation rates within the water column, sediments, and macrophytes within Silver Creek. Identifying significant nitrogen transformation processes, quantifying the rate of nitrogen transformation, and understanding nutrient distribution within streams affected by high nitrogen loading from wastewater treatment plants allows for better understanding of the impacts such operations can have on the natural environment, leading to improvements in stream modeling and plant design and/or operations. Site-specific rate constants could be used in models such as Qual2K (Chapra et al., 2004; Neilson et al., 2013) to understand nitrogen transformation along a particular reach of receiving streams. Such models could be used to assess impacts due to sediment type, temperature, storm events, biomass density, and many other predicted environmental changes.

The Utah Department of Environmental Quality is researching nitrogen transformation rates in other streams throughout the state that also serve as the receiving body for wastewater discharges. The methods and results at Silver Creek, modified as suggested, could be implemented at other sites across Utah. These data could then be used to assess operations, methods, and processes at other water reclamation facilities, or for the development of additional nutrient removal requirements at these facilities to improve overall stream water quality.

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APPENDICES

Appendix A

Standard Operating Procedures

Chamber and Piezometer Installation and Sample Collection SOP
Water, Sediment, and Plant Samples

1 week before sampling

Materials:
- Aluminum frames
- Piezometers with stoppers
- Piezometer installer including collar, inner rod, collar cap, and screwdriver or rebar for inner rod opening
- Metal mallet
- Rubber mallet
- Some 2' x 4' or 4' x 4' boards
- Tape measure

Procedure:
1. Layout the frames such that the streamflow does not flow from one chamber into another.
2. Install frames by pushing the frames into the sediment, stopping when the lip of the frame is about 0.5" from the stream bed, or enough to fit a clamp between the lip and the sediment without too much digging. Do not be forceful.
   a. If macrophytes are present, be careful to move them out of the way, keeping them as intact as possible within the plot created by the frame.
   b. Use the boards and a rubber mallet to help drive frames in if needed. Lay one board across the frame and put another board perpendicularly on top (making a T) in order to hammer above the water.
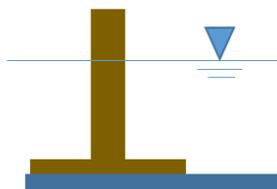
(lay the horizontal board across the strongest points and place the vertical board where the red x is)
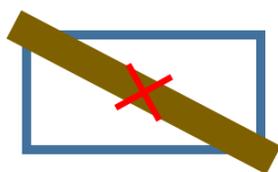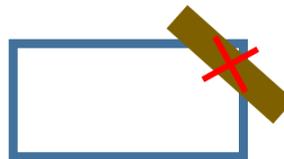
This is ok   This is ok   This is NOT ok

3. Record the total length of the piezometers and the distance to the middle of the mesh/holes.
4. Install piezometers by hammering the inner rod into the sediment to the preferred installation depth. Place the collar over the inner rod and hammer into sediment until the top of the collar is almost flush with the top of the inner rod. Place the collar cap on top of the collar and continue hammering the collar until the top of the collar is below the hole in the top of the inner rod. Slowly remove the inner rod by placing the screwdriver or rebar into the hole and twisting and pulling until the rod releases. Insert the piezo, and slowly remove the outer collar by lifting and twisting while holding the piezometer in place until sediment begins to fill in around the base.
   a. Make sure piezometers are installed such that they will not interfere with the installation of the chamber lids (give yourself room to move those around as needed in the stream as filling them in one location may not always be possible).
   b. Measure the length of the piezo that is above the sediment and calculate the installation depth, making sure it is deeper than the depth of the frames.

Day 0 Sampling

Materials:
- Chambers with calibrated DO probes and programmed temperature sensors
- Chamber tubing: 2 lines per chamber
- Pumps
- Batteries
- Clamps
- 125 mL acid rinsed Nalgene bottles
- Evacuated vials stored in DI water
- Double headed needles
- Dosing syringe (35 mL)
- Collection syringe (60 mL)
- 0.45 µm filters
- Plastic Ziploc bags
- Sediment corers and stoppers
- Metal meter stick
- Tape measure
- Kolor Kut Water Finding Paste or well sounder
- Black trash bags

Procedure:
1. Take background sediment and plant samples.
   a. Use the sediment corer and metal meter stick to collect a full core of sediment. Empty core into a Ziploc bag and store in cooler over ice.
   b. Collect 3-4 blades of plant material by cutting the plant at the sediment surface. Store in Ziploc bag in cooler over ice.
2. Submerge chambers by inverting them and filling them completely with water. There cannot be any air bubbles. Turn right side up and carefully move onto frame making sure to keep all sediments and plant material clear of the rubber seal. The DO probe goes on the downstream end. Secure in place with clamps.
   a. Collect background water samples from the stream upstream of the sampling site to measure background water nitrogen concentrations during chamber installation by collecting 60 mL of water and filtering through a 0.45 µm filter into a Nalgene bottle. Three to four samples will be enough.
3. Attach the tubing to the chamber.
   a. Submerge tubing underwater to get rid of as much air as possible. Have one person hold the battery above the chamber but out of the water, a second person hook the pump to the battery making sure the pump stays submerged, and a third person hook the dosing tubing to the inlet side of the pump and the sampling tubing to the outlet side of the pump. Allow the tubing to completely fill with water before attaching the dosing tubing to the downstream end of the chamber and the sampling tubing to the upstream end of the chamber.
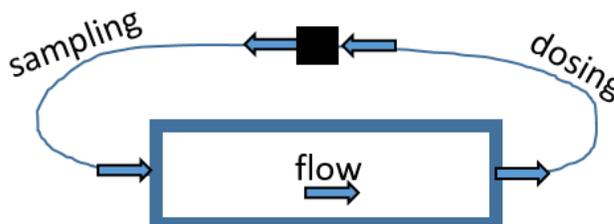
4. Double check that everything is set up correctly.
   a. Flow is going the correct way through the chambers.
   b. DO probes are working.
   c. Sampling lines are closed and there are no obvious major leaks.
5. Take a background water and dissolved nitrogen sample.
   a. Use and syringe and 0.45 µm filter to fill a 125 mL Nalgene bottle completely with water from the sampling tubing. Store over ice.
   b. Insert the double headed needle into the sampling tubing port, making sure water is dripping from the needle before inserting the other end into the evacuated vial. Fill the evacuated vial completely. Remove the vial, then remove the needle from the tubing line. Store over ice.
6. Dose chambers with ^{15}N and Br dosing solution (volume and concentration based on desired dosing conditions).

7. Wait 15 minutes for complete mixing and then take another water and dissolved gas sample from the chambers.
8. Record the DO every 10 minutes, or as often as deemed necessary. Keep the DO in the chamber within 10% of the DO outside the chambers.
   a. Place black garbage bags over chambers to lower DO.
9. Record piezometer information.
   a. Installation depth (measure depth above sediment and subtract from total length)
   b. Water surface depth (from sediment surface to water surface)
   c. Depth of water inside of the piezometer
10. At the end of the incubation (20-24 hours), take a final water and dissolved gas sample from the chambers.
11. Remove chambers carefully so as to limit the disturbance of plant or sediment material.
12. Take a final plant and sediment sample.
13. Carefully clean up the site, leaving the frames in place but taking everything else with you from the site

Additional Sampling Days

Additional sampling days may not be necessary if all of the ^{15}N label washes away at the end of the first day. If additional days are needed, the procedure is similar to that of Day 0 with the only differences being: 1) no pre-dosing samples are collected, 2) no ^{15}N label is added (only Br) and, 3) incubation time is around 3 hours. See the example sample collection chart below (Chambers 4, 5 and 6 have macrophytes while Chambers 1, 2 and 3 do not).

Day	Chamber	Pre-Dosing				Incubation Start		Intermediate		Incubation End			
		Macrophytes	Sediment	Water	Gas	Water	Gas	Water	Gas	Water	Gas	Sediment	Macrophytes
0	1		1	1	1	1	1	2	2	1	1	1	
	2		1	1	1	1	1	2	2	1	1	1	
	3		1	1	1	1	1	2	2	1	1	1	
	4	1	1	1	1	1	1	2	2	1	1	1	1
	5	1	1	1	1	1	1	2	2	1	1	1	1
	6	1	1	1	1	1	1	2	2	1	1	1	1
2	1					1	1			1	1	1	
	2					1	1			1	1	1	
	3					1	1			1	1	1	
	4					1	1			1	1	1	1
	5					1	1			1	1	1	1
	6					1	1			1	1	1	1

Bromide Measurements
Water Samples

Materials:
- Bromide Ion Selective Electrode (Orion Model 94-35 Halide electrode)
- Double Junction Reference Electrode (Cat. No. 900200)
- Inner chamber filling solution (Cat. No. 900002)
- Outer chamber filling solution (Cat. No. 900003)
- Ionic strength adjuster (ISA)
- 0.1 M bromide standard solution
- 0.1, 1, and 10 mL pipette
- 3 – 100 mL volumetric flasks
- Snap caps

Refer to the probe manuals for proper technique on how to use the probes, maintenance, and trouble shooting

Setting up the probe:
1. Refer to the probe manual on how to fill the double junction reference electrode chambers with the correct solutions.
2. Make sure both probes are correctly connected to a meter and the meter set to read the output in mV.

Calibrating the Br Probe:
1. Fill each of the volumetric flasks with ~50 mL of DI water. Make three standards by adding 0.1 mL of the 0.1 M bromide standard to the first flask, 1 mL to the second, and 10 mL to the third. Swirl to mix and then bring to volume.
2. Measure each of the three standards by combining 2 mL of ISA for every 100 mL of standard solution. Make sure both probes are in the solution and the solution is continuously stirred until the reading is stable. Record the output in mV.
3. Calculate the corresponding bromide concentration for each using stoichiometric relationships. Plot concentration (x-axis) vs mV output (y-axis) and fit a linear regression to the data. The slope should be -54 to -60 mV. If it is not, check the setup of the probes, and/or remake the standard solutions and measure again.

Procedure:
1. Add 2 mL of ISA for every 100 mL of sample into a snap cap and gently mix.
2. Insert both probes into sample and stir until reading is stables
3. Record the output in mV.
4. Using the linear equation determined during calibration, solve for the bromide concentration.

KCL Extraction
Sediment Samples

Materials:
- 1 L volumetric flask
- 60 mL glass sample vial with flat bottom and lid
- 2 M KCl (149.1 g/L)
- Shaker table
- Aluminum weigh boats

Removal of excess liquid:
1. Allow the sediment core to settle then decant any excess liquid from the top of the sample being careful not to remove any fines from the sediments.

Soil Moisture Content:
1. Record the weight of an aluminum boat.
2. Tare the boat then add 10 g $\pm$ 0.5 g (record weight) of saturated sediment sample.
3. Place the sample into a drying oven (60-105°C) until mass does not change.
4. Weigh (and record) the dried boat and sediment sample on the same scale.
5. Subtract the weight of the dry boat/sediment sample from the wet boat/sediment sample to determine the amount of water in the sediment.

Extraction Procedure:
1. Weigh out a 10 g $\pm$ 0.5 g (record weight) saturated sediment sample in a 60 mL glass sample vial with flat bottom.
2. Add 40 mL of 2 M KCl to sample vial and cap.
3. Arrange samples in shaker table so that the length of the vial is parallel to the direction of movement of the shaker table. Shake for 1 hour at low speed.
4. Remove sample from shaker table and allow to settle at least 1 hour by placing upright on lab bench.
5. Prepare samples for AQ2:
   a. Pull 15 mL of liquid extract from sample vial using a pipette. Filter sample through a 45µm syringe filter into a 16 mL plastic sample vial and cap.
   OR
6. Prepare samples for diffusion:
   a. Pull liquid extract from sample vial[1] using a pipette and put into a pint sized jar.

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[1] Volume of extract is based on the mass of nitrogen determined during AQ2 analysis and the mass required for the diffusion procedure (see diffusion procedure for mass requirements).

Modified ^{15}N Diffusion Procedure for $^{15}NO_3^-$ and $^{15}NH_4^+$ Water and Sediment Samples

Acid Traps

Materials:
- Half-inch wide Teflon pipe thread tape
- Whatman #1 filter paper
- Buchner funnel
- Hole punch
- 11-mm diam. glass culture tube
- Forceps
- 2.5 M KHSO$_4$ (11 g K$_2$SO$_4$, 35 mL DDW, *then* 3.5 mL H$_2$SO$_4$ – bring to 100 mL)

Procedure:
5. Leach the Whatman #1 filter paper using a Buchner funnel and aliquots of DDW.
6. Dry in a 60°C oven loosely wrapped in aluminum foil.
7. Use the hole punch to create 7-mm diameter disks from the dried filter paper.
8. Cut a piece of Teflon tape at least 6 cm in length.
9. Place two filter paper disks onto one half of the Teflon tape, about 3 mm apart.
10. Add 5 µL of 2.5 M KHSO$_4$ to each disk.
11. Fold the trap in half, covering both filter paper disks and press into place.
12. Press the mouth of the 11-mm diameter glass culture tube around each disk to make a visible circular seal.

Water and Digested Water Samples
NH_4^+ diffusion
1. Add 30 mL of sample[2] into an acid-washed canning jar.
2. Add a 5 g scoop of KCl and mix.
3. Add a 0.2 g scoop of MgO and mix.
4. Add an acid trap.
5. VERY TIGHTLY seal the jar, swirl, and invert.
   a. If the lid pops when tightening, use a different ring.
6. Diffuse for 7 days – swirl the contents of the jar at least daily to remix any condensate.

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[2] Dr. Stark's lab can analyze traps with 20-120 µg of N. If the mass will be outside of this range, dilute the sample so that the total mass will be within this range but keep the total diffusion volume at 30 mL.

7. At the end of 7 days, remove the trap, rinse with DDW, blot dry, and place in 24-well plate (record the well ID and #), and dry in a desiccator containing concentrated sulfuric acid for at least 1 day with the well tray lid off.
8. Once dry, pull apart the trap with a pair of forceps and, using the ends of a bent paper clip, place both disks into a tin capsule. Discard the paper clip.
9. Crimp the tin capsule closed using a pair of forceps, being careful not to rip the capsule (if a tear occurs, wrap the torn capsule in another tin capsule).
10. Place the capsule into the 96-well plate, and record the well ID and #.

NO_3^- diffusion
1. Add 30 mL of sample[2] into an acid-washed canning jar.
2. Add a 5 g scoop of KCl and mix.
3. Add a 0.2 g scoop of MgO and mix.
4. Mark the liquid level on the jar.
5. Leave the jar in a 60°C oven for 3 hours (DO NOT LET THE SAMPLES GO DRY).
6. Remove from oven and immediately bring back to volume with DDW, add an acid trap, another 0.2 g scoop of MgO, and 0.4 g scoop of Devarda's Alloy.
7. VERY TIGHTLY seal the jar, swirl, and invert.
   a. If the lid pops when tightening, use a different ring.
8. Diffuse for 7 days – swirl the contents of the jar at least daily to remix any condensate.
9. At the end of 7 days, remove the trap, rinse with DDW, blot dry, and place in 24-well plate (record the well ID and #), and dry in a desiccator containing concentrated sulfuric acid for at least 1 day with the well tray lid off.
10. Once dry, pull apart the trap with a pair of forceps and, using the ends of a bent paper clip, place both disks into a tin capsule. Discard the paper clip.
11. Crimp the tin capsule closed using a pair of forceps, being careful not to rip the capsule (if a tear occurs, wrap the torn capsule in another tin capsule).
12. Place the capsule into the 96-well plate, and record the well ID and #.

Sediment Samples
NH_4^+ diffusion
1. Pipette 30 mL of the sediment sample extract into an acid-washed canning jar.
2. Add a 0.2 g scoop of MgO and mix.
3. Add an acid trap.
4. VERY TIGHTLY seal the jar, swirl, and invert.
   a. If the lid pops when tightening, use a different ring.
5. Diffuse for 7 days – swirl the contents of the jar at least daily to remix any condensate.
6. At the end of 7 days, remove the trap, rinse with DDW, blot dry, and place in 24-well plate (record the well ID and #), and dry in a desiccator containing concentrated sulfuric acid for at least 1 day with the well tray lid off.

7. Once dry, pull apart the trap with a pair of forceps and, using the ends of a bent paper clip, place both disks into a tin capsule. Discard the paper clip.
8. Crimp the tin capsule closed using a pair of forceps, being careful not to rip the capsule (if a tear occurs, wrap the torn capsule in another tin capsule).
9. Place the capsule into the 96-well plate, and record the well ID and #.

NO_3^- diffusion

1. Add 30 mL of the sediment sample extract into an acid-washed canning jar.
2. Add a 0.2 g scoop of MgO and mix.
3. Mark the liquid level on the jar.
4. Leave the jar in a 60°C oven for 2-3 hours, or until about half of the liquid is gone (DO NOT LET THE SAMPLES GO DRY).
5. Remove from oven and immediately bring back to volume with DDW, add an acid trap, another 0.2 g scoop of MgO, and 0.4 g scoop of Devarda's Alloy.
6. VERY TIGHTLY seal the jar, swirl, and invert.
   a. If the lid pops when tightening, use a different ring.
7. Diffuse for 7 days – swirl the contents of the jar at least daily to remix any condensate.
8. At the end of 7 days, remove the trap, rinse with DDW, blot dry, and place in 24-well plate (record the well ID and #), and dry in a desiccator containing concentrated sulfuric acid for at least 1 day with the well tray lid off.
9. Once dry, pull apart the trap with a pair of forceps and, using the ends of a bent paper clip, place both disks into a tin capsule. Discard the paper clip.
10. Crimp the tin capsule closed using a pair of forceps, being careful not to rip the capsule (if a tear occurs, wrap the torn capsule in another tin capsule).
11. Place the capsule into the 96-well plate, and record the well ID and #.

Digested Sediment Samples
NH_4^+ diffusion

1. Pipette 30 mL of the sediment sample extract into an acid-washed canning jar.
2. Add a 5 g scoop of KCl and mix.
3. Add a 0.2 g scoop of MgO and mix.
4. Add an acid trap.
5. VERY TIGHTLY seal the jar, swirl, and invert.
   a. If the lid pops when tightening, use a different ring.
6. Diffuse for 7 days – swirl the contents of the jar at least daily to remix any condensate.
7. At the end of 7 days, remove the trap, rinse with DDW, place in 24-well plate (record the well ID and #), and dry in a desiccator containing concentrated sulfuric acid for 1 day.
8. Once dry, pull apart the trap with a pair of forceps and, using the ends of a bent paper clip, place both disks into a tin capsule. Discard the paper clip.

9. Crimp the tin capsule closed using a pair of forceps, being careful not to rip the capsule (if a tear occurs, wrap the torn capsule in another tin capsule).
10. Place the capsule into the 96-well plate, and record the well ID and #.

NO_3^- diffusion

1. Add 30 mL of the sediment sample extract into an acid-washed canning jar.
2. Add a 5 g scoop of KCl and mix.
3. Add a 0.2 g scoop of MgO and mix.
4. Mark the liquid level on the jar.
5. Leave the jar in a 60°C oven for 2-3 hours, or until about half of the liquid is gone (DO NOT LET THE SAMPLES GO DRY).
6. Remove from oven and immediately bring back to volume with DDW, add an acid trap, another 0.2 g scoop of MgO, and 0.4 g scoop of Devarda's Alloy.
7. VERY TIGHTLY seal the jar, swirl, and invert.
   a. If the lid pops when tightening, use a different ring.
8. Diffuse for 7 days – swirl the contents of the jar at least daily to remix any condensate.
9. At the end of 7 days, remove the trap, rinse with DDW, place in 24-well plate (record the well ID and #), and dry in a desiccator containing concentrated sulfuric acid for 1 day.
10. Once dry, pull apart the trap with a pair of forceps and, using the ends of a bent paper clip, place both disks into a tin capsule. Discard the paper clip.
11. Crimp the tin capsule closed using a pair of forceps, being careful not to rip the capsule (if a tear occurs, wrap the torn capsule in another tin capsule).
12. Place the capsule into the 96-well plate, and record the well ID and #.

Standards
Make Standard Solutions:
10 g/L NH_4^+-N (5 atom%) standard solution

1. Add about 50 mL of DI water to a 100 mL volumetric flask.
2. Add 0.191 g $^{15}NH_4Cl$ and 3.63 g NH_4Cl to flask and mix.
3. Bring to volume with DI water.
4. Store at 4°C.

10 g/L NO_3^--N (5 atom%) standard solution

1. Add about 50 mL of DI water to a 100 mL volumetric flask.
2. Add 0.361 g $^{15}KNO_3$ and 6.86 g KNO_3 to flask and mix.
3. Bring to volume with DI water.
4. Store at 4°C.

Ammonium Standard Samples:
Prepare non-diffused and diffused standards at the same time, alternating between when dosing the 5 uL of standard solution to reduce pipette error
NH_4^+ *non-diffused standard*
1. Pipette 5 uL of $KHSO_4$ onto each disk.
2. Pipette 5 uL the $^{15}NH_4^+$ standard solution onto ONE of the disks.
3. Seal the trap and place in in a 24-well plate and into the H_2SO_4 desiccator to dry (record well ID and #) for 1 day.
4. Once dry, put both disks into a tin capsule, crimp capsule, and place in the 96-well plate (record the well ID and #).

NH_4^+ *diffused standard*
1. Prepare a normal acid trap.
2. Add either 30 mL 2 M KCl or 30 mL DDW to jar.
3. Add 5 g scoop to any DDW diffusion jars and mix.
4. Pipette 5 uL the $^{15}NH_4^+$ standard solution into the sample jar.
5. Add 0.2 g MgO and acid trap and mix.
6. VERY TIGHTLY seal the jar, swirl, and invert.
    a. If the lid pops when tightening, use a different ring.
7. Diffuse for 7 days, dry for 1, and put into tin capsule just like samples.

Nitrate Standard Samples:
Prepare non-diffused and diffused standards at the same time, alternating between when dosing the 5 uL of standard solution to reduce pipette error
NO_3^- *non-diffused standard*
1. DO NOT put $KHSO_4$ onto acid trap disks.
2. Pipette 5 uL the $^{15}NO_3^-$ standard solution onto ONE of the disks.
3. Seal the trap and place in in a 24-well plate and into the H_2SO_4 desiccator to dry (record well ID and #) for 1 day.
4. Once dry, put both disks into a tin capsule, crimp capsule, and place in the 96-well plate (record the well ID and #).

NO_3^- *diffused standard*
1. Add either 30 mL 2 M KCl or 30 mL DDW to jar.
2. Add 5 g scoop to any DDW diffusion jars and mix.
3. Add 0.2 g MgO and mix.
4. Mark the volume on the jar.
5. Leave the jar in a 60°C oven for 2-3 hours, or until about half of the liquid is gone (DO NOT LET THE SAMPLES GO DRY).
6. Prepare a normal acid trap.
7. Remove from oven and immediately bring back to volume with DDW, add an acid trap, another 0.2 g MgO, and 0.4 g Devarda's Alloy.
8. Pipette 5 uL of the $^{15}NO_3^-$ standard solution into the jar.
9. VERY TIGHTLY seal the jar, swirl, and invert.
    a. If the lid pops when tightening, use a different ring.
10. Diffuse for 7 days, dry for 1, and put into tin capsule just like samples.

Digestion Procedure for TN
Sediment Samples
Reference: EPA Method LG602

Materials:
- 50 mL Pyrex/Kimax digestion vials with Teflon lined lid
- $K_2S_2O_8$
- 3.75 M NaOH (149.988 g/L)

Procedure:
1. Add 0.2-0.4 g of uniform wet sample to a 50 mL digestion vial and record the sediment weight.
2. Add 40 mL DI water to digestion vial, 1.0 g of potassium persulfate ($K_2S_2O_8$), and 1 mL of 3.75 M NaOH.
3. Also prepare all QA/QC standards and blanks by adding 40 mL of standard or blank to a 50 mL digestion vial, 1.0 g of potassium persulfate ($K_2S_2O_8$), and 1 mL of 3.75 M NaOH.
4. Cap samples and autoclave for 45 minutes at 121°C and 15 psi. DO NOT LEAVE SAMPLES IN THE AUTOCLAVE OVERNIGHT.
5. Bring the samples to room temperature.
6. Prepare samples for AQ2:
   a. Pull 15 mL of liquid extract from sample vial using a pipette. Filter sample through a 45µm syringe filter into a 16 mL plastic sample vial and cap.
   OR
7. Prepare for diffusion:
   a. Pull liquid extract from sample vial[3] using a pipette and put into a pint sized jar.

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[3] Volume of extract is based on the mass of nitrogen determined during AQ2 analysis and the mass required for the diffusion procedure (see diffusion procedure for mass requirements).

Digestion Procedure for TN
Water Samples
Reference: EPA 350.1 (Nitrogen)

Materials:
- Volumetric flask (volume = total reagent volume required)
- 250 ml beaker (or appropriate size)
- 100 ml graduated cylinder
- Stir bar
- Magnetic hot plate
- 16 mL and 50 mL Pyrex/Kimax digestion vials with Teflon lined lid
- 1 M NaOH (40.0 g/L)

Digestion Reagent Preparation:

For **100 ml**:
- 6.25 g $K_2S_2O_8$ (re-crystallized)
- 3 g $B(OH)_3$
- 35 ml 1 M NaOH

For **200 ml**:
- 12.5 g $K_2S_2O_8$ (re-crystallized)
- 6 g $B(OH)_3$
- 70 ml 1 M NaOH

For **250 ml**:
- 15.625 g $K_2S_2O_8$ (re-crystallized)
- 7.5 g B(OH)3
- 87.5 ml 1 M NaOH

Using clean spatulas, weigh the reagents in separate weighing dishes. Add the NaOH to a 250 mL glass beaker with a magnetic stir bar. Place beaker on hotplate then add potassium persulfate and Boric acid while stirring. The mixture needs to be heated mildly until dissolved to form a clear, colorless solution. Set the temperature to 80°C and the rpm to 350. Cover the beaker with parafilm. A few mL of DDW can be added to aid this process. After dissolution is complete, add the solution to a volumetric flask and, after cooling, bring to volume with DDW and mix well.

Sample digestion Procedure:
1. Prepare samples for AQ2:
   a. Place 10 ml of sample into the autoclavable vials. Add 2 ml of digestion reagent to each vial, seal tight, and shake for a few seconds.
   OR
2. Prepare samples for diffusion:

    a.   Place 30 ml of sample[4] into the autoclavable vials. Add 6 ml of digestion reagent to each vial, seal tight, and shake for a few seconds

3. Autoclave for 90 minutes at 100^0C (Fluid ~94°C). DO NOT LEAVE SAMPLES IN THE AUTOCLAVE OVERNIGHT. Let cool before analyzing on AQ2.

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[4] Volume of extract is based on the mass of nitrogen determined during AQ2 analysis and the mass required for the diffusion procedure (see diffusion procedure for mass requirements).

Plant Samples

Materials:
- Aluminum weigh boats
- Aluminum foil
- 8x5mm tin capsules
- Tweezers

Preparing Plant Samples:
1. Tear the plant clippings into small pieces.
2. Place pieces into an aluminum tin boat and cover in tin foil.
3. Poke 3-4 small holes in the tin foil to allow water to escape.
4. Store in the freezer overnight (longer is ok).

Freeze Dryer Setup:
1. Make sure the vacuum pump is properly attached to the freeze dryer and all ports are closed.
2. Turn refrigeration switch to "Manual Vacuum ON" (up).
3. Wait for collector temperature light to turn green.
4. Turn on vacuum switch (up).
5. Wait for vacuum light to turn green (or gets to the lowest, stable level possible).
6. Add a sample to a vacuum jar, attach the jar to a single port, and slowly open the port.
7. Wait for vacuum light to turn green (or become stable again) before adding additional samples.

Processing Samples:
1. Place a single sample into a glass freeze dryer tube. Attach lid and small glass tube arm/attachment.
2. Place the sample on one of the freeze dryer ports, making sure the sample is upright within the freeze dryer tube.
3. Slowly open the valve, allowing the tube to be vacuum sealed.
4. Wait for the lights on the freeze dryer to turn green again before adding additional samples.
5. Freeze dry for 24-48 hours, or until grass samples can easily crumble.
6. Remove samples from freeze dryer by turning off the freeze dryer and removing samples from glass freeze dryer tubes.
7. Crush samples in their individual weigh boats.
8. Take samples to Dr. Martin's lab where the microbalance is kept.
9. Weigh and record the mass of the aluminum tin capsule.
10. Fill the capsule with the crushed material from one plant sample.
11. Weigh and record the mass of the tin capsule and plant material.
12. Tightly close the capsule, being careful not to lose any plant material.

Appendix B

Rate Equations Using Isotope Data

Nitrification Rates

Results for the analysis of the sediment and water diffusion samples include total mass of nitrogen (μg N) and atom% enrichment of ^{15}N. The gross rate of nitrification within the water column and sediments is determined by measuring the rate at which $^{14}NH_4^+$ is nitrified to $^{14}NO_3^-$, therefore "diluting" the added $^{15}NO_3^-$ (or $\delta^{15}NO_3^-$), using Equation 5:

$$\text{Gross Nitrification Rate } \left[\frac{mg}{L-d}\right] = \left(\frac{P_0 - P_t}{t}\right)\left(\frac{\log\frac{P_0}{P_t}}{\log\frac{I_0}{I_t}}\right) \tag{5}$$

where P_0 = initial $[NO_3^-]$, P_t = $[NO_3^-]$ at the end of the incubation period, I_0 = initial excess atom percent enrichment of ^{15}N (measured minus background, which is assumed to be 0.366 %), I_t = excess atom percent enrichment of ^{15}N at the end of the incubation period, and t = incubation time between the initial dosing and final samples (Norton and Stark, 2011).

Total N_2 Production

Results from the analysis of the nitrogen gas samples include: molecular mass ratios for $^{29}N_2/^{28}N_2$ (^{29}R) and $^{30}N_2/^{28}N_2$ (^{30}R), atom% enrichment of $^{15}N_2$, and total gas-phase concentration (C_{gas}) in μmol N_2/vial. Production of nitrogen gas is determined using Equations 6 through 12 (University of California Davis Stable Isotope Facility, 2015):

1) Determination of molecular fractions

$$^{29}N_2 = {}^{29}R / (1 + {}^{29}R + {}^{30}R) \tag{6}$$

$$^{30}N_2 = {}^{30}R / (1 + {}^{29}R + {}^{30}R) \tag{7}$$

$$^{28}N_2 = 1 - (^{29}N_2 + {}^{30}N_2) \tag{8}$$

2) Specific gas phase concentration

$$^{**}C_{gas}\frac{\mu mol}{vial}=\text{molecular fraction} \times C_{gas} \tag{9}$$

where ** is 28, 29, or 30 depending on molecular fraction being used.

3) Specific aqueous phase concentration

$$^{**}C_{aq}\frac{\mu mol}{vial}= {}^{**}C_{gas} \times K_H \times \frac{V_{aq}}{V_{gas}} \tag{10}$$

where K_H is the unitless Henry's constant of 1.492×10^{-2}, V_{aq} is the aqueous

volume in the vial (mL), and V_{gas} is the gaseous volume in the vial (mL).

4) Specific total vial concentration is:

$$^{**}C_{Total}\frac{\mu mol}{vial} = {}^{**}C_{aq} + {}^{**}C_{gas} \tag{11}$$

5) Production is determined as:

$$^{**}P\frac{mg}{L-d} = {}^{**}C_{Total} \times \frac{1}{V_{aq}} \times MW_N \times \frac{1}{t} \tag{12}$$

where MW_N is the molecular weight of the nitrogen species of interest and t

is the incubation time between initial dosing and final sampling.

Denitrification and ANAMMOX Rates

Denitrification rates of $^{15}NO_3^-$ (D_{15}) and $^{14}NO_3^-$ (D_{14}) to N_2 are described in

Equations 13 and 14, respectively (Nielsen, 1992; Nielsen and Glud, 1996; Dalsgaard et

al., 2000; Risgaard-Petersen et al., 2003).

$$D_{15} = {}^{29}P + 2{}^{30}P \tag{13}$$

$$D_{14} = \frac{^{29}P}{2^{30}P} \times D_{15} \tag{14}$$

where ^{29}P is the production of $^{29}N_2$ in $\mu mol\ m^{-2}\ d^{-1}$ and ^{30}P is the production of $^{30}N_2$ in $\mu mol\ m^{-2}\ d^{-1}$.

Gross denitrification can be calculated by measuring the disappearance of $^{15}NO_3^-$ from the nitrate pool (Norton and Stark, 2011) using Equation 15.

$$\text{Gross Denitrification Rate } \left[\frac{mg}{L-d}\right] = \left(\frac{P_0\text{-}P_t}{t}\right)\left(\frac{\log\frac{P_0}{P_t}}{\log\frac{I_0}{I_t}}\right) - \left(\frac{P_t-P_0}{t}\right) \tag{15}$$

Conversely, the production of $^{28}N_2$, $^{29}N_2$, and $^{30}N_2$ from denitrification of random isotope pairing (D_{28}, D_{29}, and D_{30}, respectively) can be calculated using the equations used by Thamdrup and Dalsgaard (2002) as a function of the fraction of ^{15}N in NO_3^- (F_N) in water. This is assuming a single $^{14}NO_3^-$ or $^{15}NO_3^-$ combines with a single $^{14}NH_4^+$ during ANAMMOX (making only the production of $^{28}N_2$ and $^{29}N_2$ possible) therefore allowing $D_{30} = P_{30}$ (Equations 16 through 19).

$$D_{28} = D_{total}(1\text{-}F_N) \tag{16}$$

$$D_{29} = D_{total} \times 2(1\text{-}F_N) \times F_N \tag{17}$$

$$D_{30} = D_{total} \times (F_N)^2 = P_{30} \tag{18}$$

$$D_{total} = D_{28} + D_{29} + D_{30} \tag{19}$$

Solving Equation 18 for D_{total} as a function of P_{30} (Equation 20) and substituting into Equations 16 and 17 results in denitrification equations as a function of N_2 production and fraction of ^{15}N in NO_3^- (Equations 21 and 22, respectively).

$$D_{30}=P_{30} \tag{20}$$

$$D_{29}=\frac{P_{30}\times2(1\text{-}F_N)}{F_N} \tag{21}$$

$$D_{28}=\frac{P_{30}}{(F_N)^2}(1\text{-}F_N)^2 \tag{22}$$

Production of N_2 from ANAMMOX can be determined using the equations used

by Thamdrup and Dalsgaard (2002) (Equations 23 through 25) and Equation 21 above

(again, assuming a single $^{14}NO_3^-$ or $^{15}NO_3^-$ combine with a single $^{14}NH_4^+$ during

ANAMMOX making only the production of $^{28}N_2$ and $^{29}N_2$ possible).

$$A_{28} = A_{total}(1-F_N) \tag{23}$$

$$A_{29} = A_{total} \times F_N \tag{24}$$

$$A_{total} = A_{28} + A_{29} \tag{25}$$

Because nitrogen gas is produced by both ANAMMOX and denitrification, Equation 23

can be rewritten as

$$A_{29} = P_{29} - D_{29} \tag{26}$$

and Equation 21 substituted in to give an equation for the production of $^{29}N_2$ from

ANAMMOX as a function of total production and the fraction of ^{15}N in NO_3^- (Equation

27).

$$A_{29} = P_{29} - \frac{P_{30} \times 2(1-F_N)}{F_N} \tag{27}$$

An equation for A_{28} as a function of total production and the fraction of ^{15}N in NO_3^- can

be found by substituting Equation 25 into Equation 24, solving for A_{28} and substituting

Equation 27 in for A_{29} (Equation 28).

$$A_{28} = \frac{P_{29}(1-F_N)}{F_N} - \frac{P_{30} \times 2(1-F_N)^2}{(F_N)^2} \tag{28}$$

DNRA Rates

The assumption is made that within the same sediment layer, the rate of DNRA is equal to the rate of denitrification, therefore allowing total DNRA to be calculated as (Equation 29) (Risgaard-Petersen et al., 2003).

$$DNRA_{total} = P_{^{15}NH_4} \times \frac{D_{14}}{D_{15}} \tag{29}$$

where $P_{^{15}NH_4}$ is the production of labeled ammonium and D_{15} and D_{14} and the denitrification rates of $^{15}NO_3^-$ and $^{14}NO_3^-$ to N_2, respectively.

Assimilation Rates

The rate of assimilation (R_{Assim}) by the macrophytes can be calculated using Equation 30

$$R_{Assim} = F_{^{15}NO_3} - D_{Total} - A_{total} - DNRA_{total} \tag{30}$$

where $F_{^{15}NO_3}$ is the total removal flux of $^{15}NO_3^-$ over the incubation time.

Appendix C

Converting Reported Rates

The rate of denitrification at Silver Creek was determined by plotting the ^{15}N-N$_2$ data from Chamber 1 as a zero-order rate, resulting in a rate of 0.32 mg ^{15}N L^{-1} d^{-1}. This rate was converted to a first order rate for easy comparison with the rates reported by Neilson et al. (2013) and Bowie et al. (1985) by diving the zero-order rate by the concentration at each of the three sampling times and taking the average (Table C-1).

Table C-1. Converting the zero-order rate of denitrification from Chamber 1 into a first-order rate.

Time (hours)	Concentration or C (mg/L)	Rate (d^{-1}) (=0.32/C)	Average rate (d^{-1})
0.25	0.011	2.8	
4.3	0.018	1.8	1.8
21	0.039	0.8	

The rate of denitrification reported by O'Brien et al. (2012) and Smith et al. (2009) was 12 to 83 µmol N m^{-2} h^{-1} and 171 µmol N m^{-2} h^{-1}, respectively. These rates were converted to zero order rates using the following equation:

$$\frac{\mu mol}{m^2 h} \times \frac{\text{Chamber area } (m^2)}{\text{Chamber volume (L)}} \times \frac{24h}{d} \times \frac{14\mu g}{\mu mol} \times \frac{mg}{1000\mu g}$$

where the chamber area and volume used by O'Brien et al. (2012) was 0.045 m^2 and 15 L, respectively, and the chamber area and volume used by Smith et al. (2009) was 0.28 m^2 and 59 L, respectively.