CrossMark

Quantifying ambient nitrogen uptake and functional relationships of uptake versus concentration in streams: a comparison of stable isotope, pulse, and plateau approaches

Matt T. Trentman · **Walter K. Dodds** · **Jane S. Fencl** ·
Kayla Gerber · **Jay Guarneri** · **Sean M. Hitchman** · **Zach Peterson** ·
Janine Rüegg

Abstract Nutrient releases and spiraling metrics are frequently used to quantify the downstream transport of nutrients and to better understand the effects of anthropogenic inputs to downstream waters. Ambient uptake rates in streams can be measured through stable isotope enrichments, while pulse and plateau additions can estimate such rates via extrapolation and modeling techniques, respectively. Data from these releases can be used to estimate ambient uptake rates from nutrient additions and possibly determine the functional relationships between nutrient concentrations and uptake rates. Here, we compared estimated ambient rates calculated from established pulse and plateau approaches, results obtained from new modeling approaches, and rates at ambient concentrations from stable isotope enrichments. Comparative releases of NH_4Cl and $^{15}NH_4Cl$ were conducted in four experimental reaches across the grassland Kings Creek and urban Campus Creek, KS. Nutrient uptake was predominantly linear with increasing ammonium. Estimated ambient uptake rates varied among sites, release methods, and data analysis approaches.

However, plateau ambient rates from new modeling approaches matched closely with measured ambient rates from isotope enrichments at three sites, suggesting that modeled plateau data may be best for a first look at determining nutrient uptake rates at an individual site. Limitations and benefits of each approach vary; however, baseflow discharge may be a key driver when choosing a method. If possible, multiple methods should be attempted at each location and under each novel set of conditions to determine the best approach prior to designing and implementing a more extensive series of measurements.

Keywords Nutrient spiraling · TASCC · Uptake length · Ammonium uptake

M. T. Trentman (✉) · W. K. Dodds · J. S. Fencl ·
K. Gerber · J. Guarneri · S. M. Hitchman ·
Z. Peterson · J. Rüegg
Division of Biology, Kansas State University, Manhattan,
KS 66506, USA
e-mail: mtrentman@ksu.edu

Introduction

In-stream nutrient uptake and other nutrient cycling processes dictate the downstream movement of nutrients, especially those derived from terrestrial runoff and ground water input (e.g. Peterson et al. 2001; Mulholland et al. 2008). Quantifying nutrient uptake, its functional relationship with nutrient concentrations, and its effect on downstream transport is important for assessing the effects of nutrient enhancement by anthropogenic sources (Bernot and Dodds 2005). Specifically, nitrogen delivery to coastal areas from agriculturally impacted watersheds can cause

eutrophication, indirectly leading to hypoxic "dead zones" that impair water quality and fisheries (Diaz and Rosenburg 2008). Downstream transport of nitrogen may be greater if nutrient uptake is saturated under chronic enhancement (Bernot and Dodds 2005). The extent of saturation determines how river networks respond to nutrient loading (Mulholland et al. 2008). Understanding the functional relationships among nutrient concentrations in streams, uptake by aquatic microorganisms, and downstream transport of nutrients has become an essential part of characterizing biogeochemical processing in streams.

The study of stream nutrient dynamics was advanced by the concept of nutrient spiraling, which is characterized by uptake length (S_w), the cycle length of the average dissolved molecule moving down stream (Newbold et al. 1981). Potentially the most important component of uptake length (and the easiest to measure) is the movement of the reactive portion of nutrients in the water column to the benthic zone, which is influenced by discharge, water velocity, and depth (Davis and Minshall 1999). Initial experiments estimated S_w by elevating stream nutrient concentrations above ambient levels at a steady rate for short-time periods (i.e., plateau addition) because it was straightforward and inexpensive (Webster and Ehrman 1996; Payn et al. 2005). However, this method can overestimate S_w and uptake rates since uptake rates can potentially increase non-linearly with increasing nutrient concentrations (e.g. Hart et al. 1992). Similarly, net uptake (the uptake rate measured at a new plateau) and gross uptake rates are different parameters with different meanings (see below), as verified by stable isotope tracer methods (e.g., ^{15}N, Dodds et al. 2002; Mulholland et al. 2002). Stable isotopes can be used to directly measure ambient uptake length ($S_{w,\ amb}$) and ambient uptake rates without significantly increasing ambient nutrient concentrations, but the equipment needed to analyze the samples is expensive (Payn et al. 2005).

Stream uptake rates vary according to stream nutrient concentrations. Many streams exhibit a linear increase in uptake with equal increases in dissolved nutrients. However, nutrient uptake can become saturated due to limits on nutrient transformation rates (Bernot and Dodds 2005). This relationship can be described with Michaelis–Menten (M–M) uptake kinetics. Knowing the concentration of nutrients necessary to saturate uptake is important for understanding how increasing

anthropogenic inputs might alter the downstream transport of nutrients. Functional relationships between nutrient concentration and uptake also allow for the estimation of ambient uptake rates.

Several investigators have employed a series of steady-state nutrient additions with increasing concentrations (multiple plateau additions) to estimate functional relationships and estimate ambient uptake rates (Dodds et al. 2002; Payn et al. 2005; Earl et al. 2007). However, attaining equilibrium for multiple levels of plateau addition is time consuming; each new plateau takes time to come to equilibrium and only produces one data point for each concentration level, offering modest information on uptake kinetics. Similarly, plateau additions are only practical in small streams with a limited discharge range due to the high injectate volumes necessary to create equilibrium at high discharge (Doyle 2005; Tank et al. 2008).

An instantaneous pulse of highly concentrated solution has been employed with (Dodds et al. 2008) and without stable isotopes (Tank et al. 2008) as an alternative to the plateau addition approach. Covino et al. (2010) refined the pulse method, referred to as the tracer additions for spiraling curve characterization (TASCC) method, taking advantage of the variable concentration of a highly concentrated pulse injectate to estimate uptake kinetics as the injectate moves over a given distance. Samples are taken at the end of the experimental reach as the injectate moves through the reach. In the TASCC method, each data point from the pulse breakthrough curve (BTC) can be used to estimate an S_w and net uptake rates at each concentration given the rate of disappearance of the nutrient compared to a conservative solute. Each data point (i.e. concentration) is then treated as a new (although temporary) plateau parameter, with an S_w calculated for every sample along the curve. Therefore, pulse additions provide an approach to quantify functional relationships across a range of concentrations (Covino et al. 2010). Pulse additions have the extra advantage that they are easier to use in large streams (Tank et al. 2008; Alvarez et al. 2010). But, they may be biased toward conditions in the thalweg (Dodds et al. 2008), particularly points from the leading edge of the peak where the injectate solution may not be fully mixed vertically (in the water column and in the subsurface) and horizontally throughout the reach.

To our knowledge, no one has assessed which of the standard addition methods, plateau or pulse, allows

better estimation of the ambient uptake rate as directly measured by stable isotopes. Some previous studies compared plateau and pulse additions to each other (e.g. Payn et al. 2008; Gooseff et al. 2008; Powers et al. 2009; Alvarez et al. 2010), or plateau additions to stable isotopes (e.g. Dodds et al. 2002; Mulholland et al. 2002; Payn et al. 2005; Earl et al. 2007). Standard addition methods may be incorrect, leading to incorrect nutrient export estimates (Mulholland et al. 2008). Thus, current methods should be refined to properly estimate uptake at ambient nutrient concentrations. Our objective here is to use plateau enrichments of $^{15}NH_4Cl$ as a baseline with which to compare the use of pulse and plateau additions and modeling approaches to estimate ammonium uptake at ambient nutrient concentrations in both urban and prairie streams. Subsequently, we outline updated methods for characterizing functional relationships between net uptake and concentration, which offer more explicit recognition of the processes of net and gross uptake than some previous approaches. We compared these updated methods against our results and previously published work.

Methods

Study areas and site selection

We conducted a series of comparative releases of NH_4Cl in test reaches located within Kings Creek and Campus Creek (Table 1) to assess the merits of the plateau and pulse addition methods against stable isotope plateau enrichments. We use the term "release" as a general term to compare methods, "enrichment" when referring to the stable isotope plateau method, and "addition" when referring to un-labeled plateau and pulse methods. Kings Creek is a low-order stream located on Konza Prairie Biological Station (KPBS) in the Flint Hills ecoregion (Omernik 1987) near Manhattan, Kansas. Kings Creek has been extensively studied in nutrient uptake experiments (e.g., Dodds et al. 2002; O'Brien et al. 2007; O'Brien and Dodds 2008), making it an ideal location to compare methods. The Kings Creek watershed is subject to frequent and severe floods and drought (Dodds et al. 2004), common among low-order streams in the region. Campus Creek is located on the Kansas State University campus. Campus Creek is a highly impacted stream with substantial impermeable cover in the watershed, greenhouse runoff and groundwater influenced by historical animal holding facilities. Experimental additions were conducted in two reaches within the Kings Creek watershed and one reach in Campus Creek. In June 2012, experiments were conducted in Lower Kings and K2 (both within the Kings Creek watershed). In October 2013, experiments were conducted in Campus Creek and again in the Lower Kings site. The study reaches were selected by ease of access, availability of prior data, conservative tracer travel time, and a lack of permanent tributaries, large groundwater input or large pools that would interfere with the addition experiments. More detail on the study reaches can be found in Table 1.

Table 1 Site characteristics for reaches in Kings and Campus Creek

Site	Location (latitude, longitude)	Stream-bed characteristics	Discharge (L s^{-1})	Reach length (m)	Mean wetted width (m)	Mean velocity (m s^{-1})		Ambient (µg [NH$_4$-N] L^{-1})	
						Pulse	Plateau	Pulse	Plateau
Lower Kings 2013	39.105330, 96.603446	Cobble/bedrock	0.689	41	1.30	0.027	0.021	11	10
Campus Creek 2013	39.192239, 96.578779	Cobble/sand/clay, heavy leaf litter	9.01	32	2.60	0.015	0.011	19	189
K2 2012	39.100160, 96.574491	Cobble/bedrock	0.370	33	1.95	0.011	0.018	14	14
Lower Kings 2012	39.105330, 96.603446	Cobble/sand	9.85	60	3.55	0.038	0.032	32	32

Nutrient releases

Pulse and plateau additions and stable isotope enrichments were done over one (Lower Kings 2012 and K2 2012) or 2 days (Lower Kings 2013 and Campus Creek 2013). In 2012, a pulse addition occurred first, followed by the stable isotope plateau, and finally multiple plateau additions. The stable isotope plateau was started after the conservative tracer of the pulse addition was no longer detectable. In 2013, pulse additions were conducted the day prior to the plateau additions and stable isotope enrichments. Addition solutions for both pulses and plateaus were added in the thalweg of the stream in a constricted area above the reach where the turbulence of the water would maximize mixing before the first sampling station. Rocks were moved to improve the constriction at the injectate-release location. Injectate concentrations were based on ambient ammonium concentration ($[NH_4^+{}_{amb}]$) and discharge (Q) within each study reach (Table 1). All water samples were filtered with a Whatman GF/F 0.7 µm filter within 2 h of collection and stored in plastic bottles. An aliquot of each sample was reserved for analyses for bromide ion concentration $[Br^-]$, while the rest was frozen until analysis of $[NH_4^+]$. $^{15}NH_4^+$ samples were treated immediately for concentration by diffusion (see below).

Pulse

A conservative tracer of NaBr was used in each pulse addition to follow the BTC and account for dilution downstream (Covino et al. 2010). The $[Br^-]$ was measured using an ion-selective probe at the downstream end of the stream reach and dictated the timing of sample collection. Water samples were collected across the full range of the $[Br^-]$ BTC (Covino et al. 2012), with more intense sampling occurring when

$[Br^-]$ began to change rapidly (i.e. leading edge through the rapid decline following the peak). Samples were taken periodically after the peak of the BTC, until $[Br^-]$ returned to within 10 % of ambient levels.

Plateau

A single ^{15}N plateau enrichment and multiple NH_4^+ plateau additions were conducted after the pulse addition within each study reach. Each $^{15}NH_4Cl$ injectate was enriched with ^{15}N (>98 mol%), which produced a negligible increase in ambient $[NH_4^+]$ in the streams. Approximate values of 1000–6000 δ ^{15}N (‰) were achieved in these enrichments at the uppermost stations. Following the isotope enrichment, two or three successive unlabeled NH_4Cl plateau additions were completed using increasingly concentrated solutions of NH_4Cl (5.30–21.30 g L^{-1}) to achieve approximately two-times, four-times, and eight-times (four and eight times only in Campus Creek 2013) ambient $[NH_4^+]$ (Table 2). Discharge measurements for experiment calculations were measured during plateau isotope enrichments using the dilution of $[Br^-]$ (Webster and Ehrman 1996). Wetted widths (approximately 10) were measured at equally spaced points along each study reach after solute additions. Widths were averaged for calculations of ammonium uptake for each field method.

Lab analysis

Total $[NH_4^+]$ were determined colorimetrically with the phenol-hypochlorite method (APHA (American Public Health Association), 1995). Isotopic ^{15}N samples were analyzed using a NH_4^+ diffusion technique to concentrate NH_4^+ from the water samples onto glass fiber filters (Holmes et al. 1998) for isotopic analyses by the Stable Isotope Mass Spectrometry Laboratory at Kansas State University. All standards

Table 2 Geometric mean across stations of $[NH_4^+]$ addition and associated S_w for each successive plateau addition

Site	Plateau 1		Plateau 2		Plateau 3	
	Enriched µg N L^{-1}	S_w (m)	Enriched µg N L^{-1}	S_w (m)	Enriched µg N L^{-1}	S_w (m)
Lower Kings 2013	58.5	42.9	115.2	51.0	276.6	84.0
Campus Creek 2013	240.5	39.8	346.0	44.4		
K2 2012	797.8	62.9				
Lower Kings 2012	425.8	277.8	735.9	294.1		

and unknown samples of $[Br^-]$ were measured using an ion-selective probe at room temperature while stirring to account for diffusion and temperature-sensitivity of the probes. A standard curve was established by fitting a 2nd-order polynomial to multiple calibration points in order to convert electrode mV readings to concentrations. Similar $[Br^-]$ were found when comparing a subset of samples analyzed using Ion Chromatography with the ion-selective probe (data not shown).

Data analysis and calculation approaches

Basic equations relating stream physical parameters to S_w, uptake rate (mass per unit area per unit time, U), and uptake velocity (distance per time, V_f) can be found in the Stream Solute Workshop (1990). Here we describe methods for estimating ambient uptake rates for ammonium (U_{amb} though they can apply to any inorganic nutrient), and functional relationships of uptake with nutrient concentrations. The calculations allow cross comparison of uptake rates and spiral lengths across methods.

Pulse (extrapolated)

For each pulse addition, an S_w was calculated from the slope of the background-corrected $[NH_4^+]$ from each point in the BTC to the injection solution (after accounting for dilution with conservative tracer, Covino et al. 2010). All S_w's from a single addition were regressed with their equivalent nutrient concentration. The y-intercept of this regression represents $S_{w,\ amb}$, which was used to calculate U_{amb} using standard equations (Stream Solute Workshop 1990; Covino et al. 2010). This method uses extrapolation of measured data to estimate $S_{w,\ amb}$ and will be referred to as the pulse (extrapolated) method below.

Isotope (measured)

Stable isotope and conservative solute additions were used to calculate U_{amb} (Webster et al. 2003). The natural logarithm of the dilution corrected ^{15}N flux rate was calculated at each sampling station along a reach using discharge, $[NH_4^+]$, and the atomic ratio of $^{15}N{:}^{14}N$. The slope of the dilution and ambient corrected ^{15}N flux rate versus distance between sampling locations gives the uptake constant (k,

m^{-1}), which is the negative reciprocal of S_w. This method directly measures $S_{w,\ amb}$ and will be referred to as the isotope (measured) method below.

Pulse and plateau (modeled)

In this paper we adopt a terminology consistent with prior ecosystem and biogeochemical work (e.g. primary production) to distinguish between net uptake and gross uptake, as these two values are expected to vary differently with concentration as we discuss here. We took an alternative modeling approach to calculate gross ambient uptake rate ($U_{gross,\ amb}$) from net uptake (U_{net}) data from both pulse and plateau addition methods. Our approach uses two assumptions, which may seem evident, but are defined here and used because they offer more information that is not clearly described in approaches so far. We took this approach because the extrapolation approach by Covino et al. (2010) to estimate the intercept could be prone to error (which can be calculated by standard regression models) and does not clearly take advantage of logical assumptions based on the relationship between stream nutrient concentrations with U_{net}, U_{gross}, and mineralization (M; Eq. 1; Fig. 1).

$$U_{net} = U_{gross} - M \qquad (1)$$

First, we assume that $U_{gross} = 0$ when $[NH_4^+] = 0$ (actual $[NH_4^+]$, not ambient corrected). This is logical because if there is no nutrient available to take up, then there is no gross uptake. The second assumption is that at ambient NH_4^+ concentration $U_{net} = 0$ (Dodds 1993; Payn et al. 2005; Fig. 1). This assumption holds if concentration does not change over time at a specific location (e.g. if stream concentration before and after a pulse addition are the same) or if ambient concentrations do not vary spatially along a stream (e.g. the longitudinal sampling stations for a plateau addition). In other words, if nutrient concentrations in the water column are constant over space or time, then loss (i.e., biotic uptake and abiotic processes; U_{gross}) and input (primarily mineralization, but also groundwater input and transport from upstream; M) must be equal (Dodds 1993). While it is possible to see diel changes in biotic and/or abiotic portions of U_{gross} due to changes in light and temperature, we assume that these changes are minimal, especially over the time of field additions (a few hours). Given that the assumption of constant ambient nutrient concentration in the absence
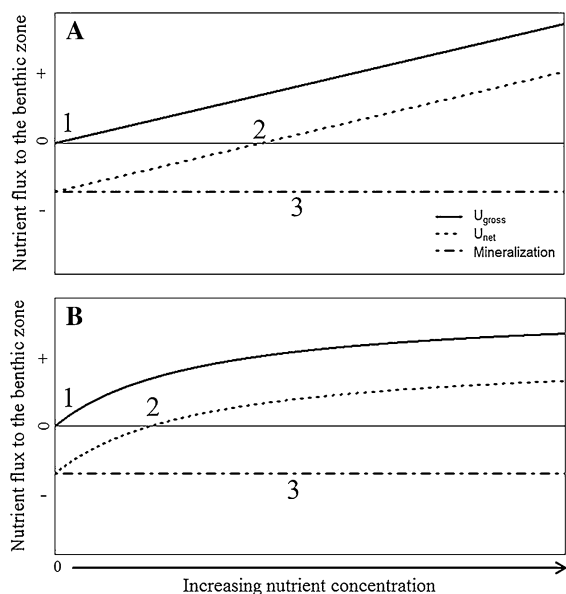
Fig. 1 Conceptual diagram of hypothesized relationships of net uptake (U_{net}), gross uptake (U_{gross}), and mineralization with stream nutrient concentration (not background corrected) for linear (**a**) and Michaelis-Menton (**b**) uptake kinetics. Conceptual points that are used in this paper are labeled with numbers on the figures. (*1*) U_{gross} = zero when stream nutrient concentration = zero. (*2*) U_{gross} and mineralization tend to stabilize at ambient nutrient concentration with equilibrium at the point where U_{gross} = mineralization and U_{net} = zero (Dodds 1993). (*3*) Mineralization does not change with stream nutrient concentration

of perturbation (e.g. a pulse or plateau nutrient addition) is met, $U_{gross,\ amb}$ occurs at the same rate as other constant fluxes at ambient concentration (M_{amb}, Eq. 2, Fig. 1).

$$U_{net,amb} = 0 = U_{gross,amb} - M_{amb} \qquad (2)$$

Thus, in the framework of Fig. 1, pulse or plateau approaches can be viewed as perturbing the nutrient concentration by increasing it, and the direct measurement of decrease in water column nutrient concentrations corrected for dilution leads to estimates of net uptake. This net uptake represents the system dynamics causing it to return to the equilibrium state where net uptake is zero.

For the successive NH_4^+ plateau additions and single pulse addition, each value of S_w represents the uptake length at the new reach-averaged $[NH_4^+]$ (expressed as the geometric mean $[NH_4^+]$ across the range of longitudinal samples, Covino et al. 2010) or from each point of the BTC, respectively. Each S_w can

then be used to calculate a U_{net} at that concentration. From these data we assessed the relationship between concentration and uptake by comparing the best-fit functional relationships between calculated U_{net} and observed $[NH_4^+]$ using both Michaelis–Menten uptake kinetics and a first order response (i.e. linear relationship between concentration and uptake).

Equations 3 and 4 describe these alternative functional relationships of nutrient uptake at various nutrient concentrations (i.e. U_{gross}). M–M uptake is assumed by many to be true when uptake is a function of nutrient concentration based on maximum uptake rates (V_{max}) and the half saturation constant for uptake (K_s).

$$U_{gross} = V_{max} \times \frac{[NH_4^+]}{K_s + [NH_4^+]} \qquad (3)$$

We know from prior data (Dodds et al. 2002) that a linear (first order) relationship between uptake and ambient nutrient concentration is often more relevant than M–M models to observed data (and requires estimating one less functional parameter) such that:

$$U_{gross} = m \times [NH_4^+] + b \qquad (4)$$

where m = the slope and b = the y-intercept. In this case, b = zero to fulfill the assumption that there is no gross uptake when concentrations are equal to zero.

We used the Solver function in Excel 2007 ® to fit modeled U_{net} (Eq. 5) to observed U_{net} from pulse and plateau addition experiments by varying either V_{max} and K_s from Eq. (3) or m from Eq. (4) to minimize the sum square of error between observed and modeled values, with the constraint that $U_{net} = 0$ at ambient concentration. We accounted for M within the stream by subtracting $U_{gross,\ amb}$ from U_{gross} (Eq. 5), assuming that M is constant across all nutrient concentrations (see Eq. 2; Fig. 1).

$$U_{net} = U_{gross} - U_{gross,amb} \qquad (5)$$

Modeled ambient uptake rates (i.e., M–M or linear; 8 preferred models of initial 16) were used to compare extrapolated and measured ambient uptake rates after determining the correct functional relationship based on visual inspection of the plots (see results). The resulting modeled $U_{gross,\ amb}$ was used to compare with the $U_{gross,\ amb}$ from the isotope (measured) and pulse (extrapolated) methods. All sites except K2 2012 have results from both M–M and linear models for pulse and plateau addition experiments (M–M modeling for the

plateau at K2 2012 was not done because there was only one data point; though we could have applied Eq. 7 [see below], we elected not to do so for this comparison). Results from this method will be referred to as plateau (modeled) and pulse (modeled) below.

Equations 3 and 4 can also be used to calculate $U_{gross, amb}$ when only one field measurement of U_{net}, ambient ammonium concentration ($[NH_4^+{}_{amb}]$), and ammonium at measured concentration ($[NH_4^+{}_{meas}]$) are known. This can be done by assuming a 2-point linear relationship between uptake and concentration (U_{net}, $[NH_4^+{}_{meas}]$ and 0,0) and that the slope of the linear equations for U_{net} and U_{gross} are equal. By solving for m in Eq. 4 for both U_{net} and U_{gross} and setting them equal to each other (Eq. 6), it is possible to solve for $U_{gross,}$ at $[NH_4^+]_{amb}$ (Eq. 7).

$$\frac{U_{gross}}{[NH_4^+]} = m = \frac{U_{net,meas}}{[NH_4^+]_{meas} - [NH_4^+]_{amb}} \quad (6)$$

$$U_{gross,amb} = \frac{U_{net,meas}}{[NH_4^+]_{meas} - [NH_4^+]_{amb}} \times [NH_4^+]_{amb} \quad (7)$$

The single plateau extrapolation method could also be useful in calculating ambient uptake from previously published single plateau methods, which can be compared to $U_{gross, amb}$ rates from stable isotopes as we do in the discussion.

Results

NH_4^+ pulse

Overall, uptake lengths were longer at K2 2012 and Lower Kings 2012 and shorter at Lower Kings 2013 and Campus Creek 2013 (Fig. 2). When additions were separated into rising or falling limb of the BTC, the regression slopes (S_w vs $[NH_4^+]$) were different in sign (i.e. positive or negative) for the different limbs in 3 of the 4 pulses. Campus Creek was the only reach to have positive slopes for both the rising and falling limb (i.e. increasing uptake with increasing dissolved nutrient concentration; Fig. 2).

Ambient S_w was estimated by regressing the calculated S_w vs. observed $[NH_4^+]$ across the BTC for each pulse addition. The highest S_w was measured at Lower Kings 2012 and lowest at Campus Creek 2013 (Fig. 3). Similar rankings were found when the rising and falling

limbs were analyzed separately. Error (95 % CI, for the calculated intercept) for extrapolated $S_{w, amb}$ was relatively low for this method; however, higher and lower values were found when considering error from the rising and falling limbs individually (Figs. 2, 3).

^{15}N plateau

Ambient $[NH_4^+]$ ranged from 10–32 µg N L^{-1} within Kings Creek reaches and was 189 µg N L^{-1} in Campus Creek 2013 (Table 1). Isotope (measured) $S_{w\ amb}$ ranged from 21.9 to 26.6 m in Kings Creek reaches and was 74 m in Campus Creek (Fig. 3). Campus Creek had the highest $[NH_4^+{}_{amb}]$ and $S_{w, amb}$ of all sites. Errors (based on the 95 % confidence interval of the slope of flux versus distance) from $S_{w, amb}$ calculations were greatest at Lower Kings 2013 and lowest at Lower Kings 2012 (Fig. 3). The average percentage of error from K2 2012, Lower Kings 2012, and Campus Creek 2013 was 43 %. The error for Lower Kings 2013 was nearly double the estimated $S_{w, amb}$ (Fig. 3).

NH_4^+ Plateaus

In Kings Creek, the successive plateaus elevated the in-stream concentration by ~6–57 times $[NH_4^+{}_{amb}]$ in the combined Kings Creek reaches, and ~1.3–2 times $[NH_4^+{}_{amb}]$ in Campus Creek 2013 (Table 2). Calculated S_w values were highly variable across sites, as would be expected with the large range of steady state plateau $[NH_4^+]$ achieved across all additions. Campus Creek, which exhibited the lowest increase in $[NH_4^+]$ relative to ambient, had the smallest S_w relative to the other sites. The longest S_w occurred during the Lower Kings 2012 addition (Table 2). Net uptake rates were calculated for each estimated S_w and used to model $U_{gross, amb}$.

Ambient uptake rates

Gross ambient uptake rates were derived from multiple release methods and calculation approaches. The isotope (measured) method was the only release that provided a direct measurement of an ambient gross uptake rate. For the successive plateau additions we used the modeling approach outlined in this paper to calculate gross ambient uptake rates. For the pulse experiment, we used both extrapolation and modeling approaches to estimate ambient uptake rates. For

Fig. 2 Uptake length (S_w) across various ammonium concentrations from four pulse additions. Rising and falling limbs are separated for comparison. **a** Campus Creek 2013, **b** Lower Kings 2013, **c** K2 2012, **d** Lower Kings 2012
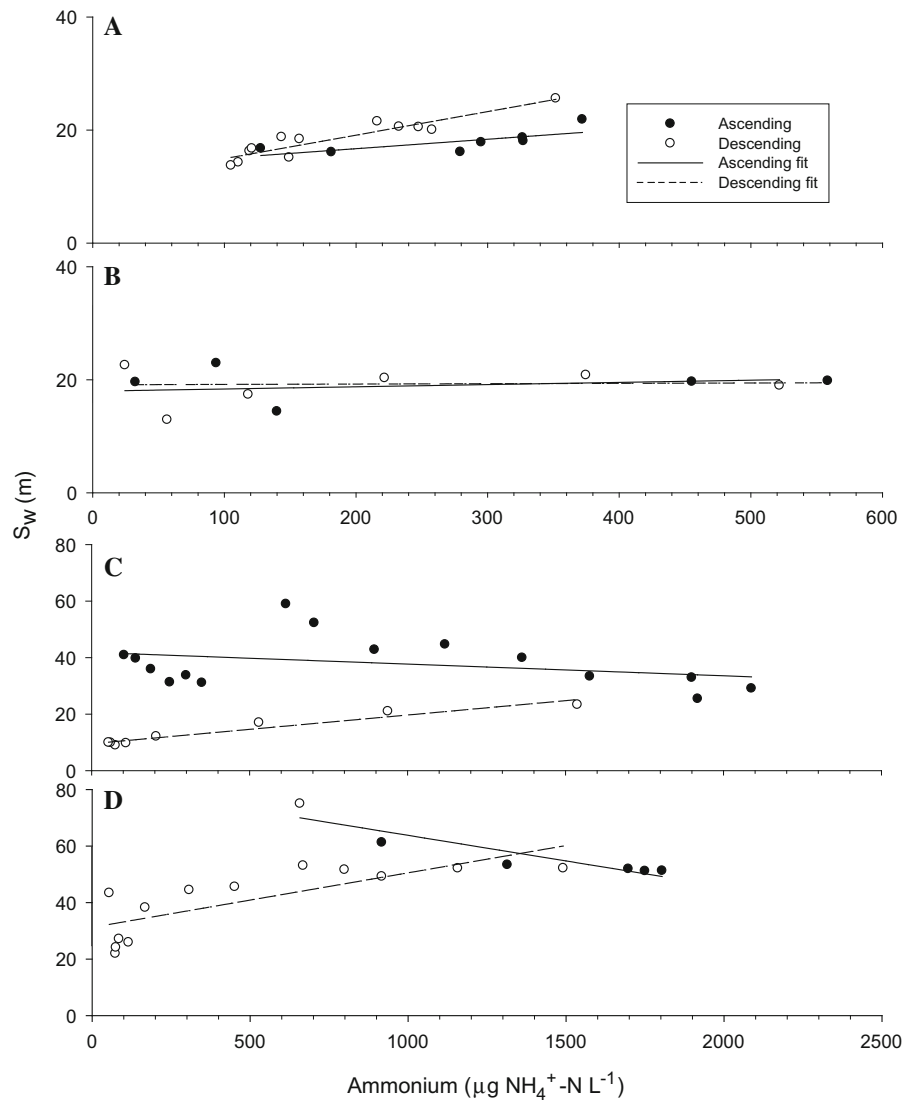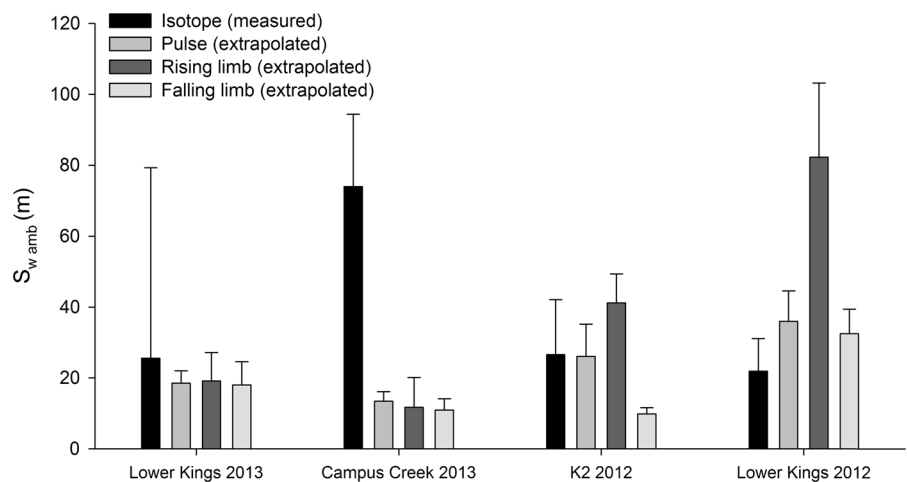

Fig. 3 Estimated ambient uptake length ($S_{w, amb}$) and 95 % CI across sites and method for analyses that used the extrapolation approach (isotope and pulse). Estimates of $S_{w, amb}$ were calculated for the rising and falling limb of the pulse separately for comparison

modeled parameters and rates, results from both M–M and linear models are reported for comparison.

Gross ambient uptake rates varied across sites, experimental methods, and analysis methods. However, only Campus Creek 2013 had a calculated value that was more than one order of magnitude from other values of the same site (Table 4). Isotope (measured) $U_{gross, amb}$ values ranged between 8.77 and 310.08 µg N m^{-2} min^{-1}, and were greatest at Lower Kings 2012 (Table 4). Eight of the 12 modeled and extrapolated rates were more than double or less than half of their respective isotope (measured) rate. In some instances, modeled and extrapolated rates were more similar across methods than to the isotope (measured) rate at an individual site. For example, modeled pulse and plateau rates in Lower Kings 2012 differed by only 2 µg m^{-2} min^{-1}, but both were ~120 µg m^{-2} min^{-1} away from the stable isotope rate (Table 4). Overall, no single field or analysis method was consistently better or worse at predicting rates measured from the stable isotope rate across all sites. However, each site usually had one or two methods that either matched the isotope (measured) or had extrapolation or modeled rates that were similar.

Functional relationships

The M–M model resulted in very high values for K_s and V_{max} at most sites, indicating the relationships were essentially linear within the modeled concentration range (Fig. 4b; Table 3). Campus Creek was the only site that exhibited M–M saturation, and it was only evident with the pulse (modeled) method (Fig. 4a). We still solved for M–M parameters with these high values even though they result in a functional relationship that was essentially indistinguishable from a linear model given the variance in the measured input model values. Given the inflated parameters for V_{max} with M–M models, both models often created similar, if not identical, gross ambient uptake rates (Table 3). This was especially evident when comparing M–M and linear models for the plateau experiments.

Discussion

Error estimates

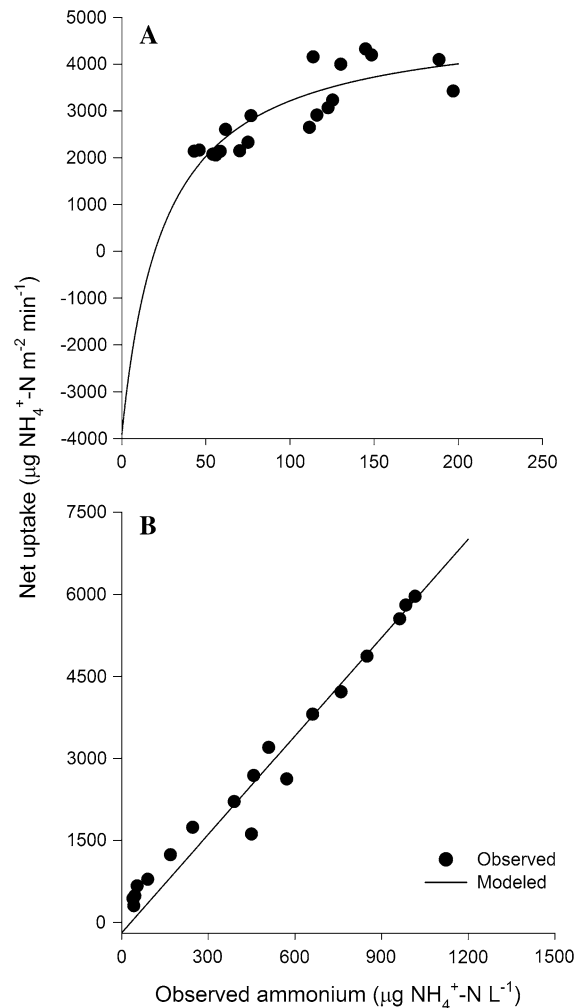Error estimates derived from the 95 % CI can be used to describe the precision of gross ambient uptake

Fig. 4 Example modeled and observed net uptake relative to observed in-stream ammonium for the Campus Creek 2013 (**a**) and Lower Kings 2012 (**b**) pulse additions. Data from Campus Creek 2013 suggests M–M saturation with higher ammonium concentration, while Lower Kings 2012 data suggests a linear relationship between net uptake and observed ammonium with no apparent saturation

extrapolations from stable isotope and pulse additions. It is important to keep in mind the distinction between precision and accuracy in our current work. Even though the isotope method was less precise than others (i.e. larger CI for the intercept), we view it as more accurate as it is the only method that directly measures uptake at ambient nutrient concentrations, and we compare against the ^{15}N tracer rate for this reason. Similarly, the 95 % CI derived from the isotope (measured) method describes real variance of ambient uptake across the measured reach, while the 95 % CI

Table 3 Modeled gross ambient uptake rates for pulse and plateau additions with model parameters from both M–M and linear models. N gives the number of samples used to model the parameters, $U_{gross, amb}$, V_{max} and M are in µg m^{-2} min^{-1} and K_s is in µg L^{-1}

| Site | Pulse | | | | | | Plateau | | | | | |
| | M–M | | | | Linear | | M–M | | | | Linear | |
	N	$U_{gross, amb}$	K_s	V_{max}	$U_{gross, amb}$	M	N	$U_{gross, amb}$	K_s	V_{max}	$U_{gross, amb}$	M
Lower Kings 2013	11	60.0	708.9	4316	48.4	4.8	3	7.8	1,031,133	861,523	7.8	0.8
Campus Creek 2013	19	3908.2	25	8904	623.0	31.1	2	145.3	928,537	10,010,582	145.3	10.8
K2 2012	20	87.64	171,333	1,007,898	87.15	5.8	1	NA	NA	NA	5.8	0.2
Lower Kings 2012	19	117.6	171,333	1,007,898	190.4	6.0	2	30.8	1,031,133	861,523	19.6	0.5

from the pulse data only describes the variance of samples taken at a single point over time (i.e. a relatively constant integration of uptake from upstream flow paths). The extrapolations from pulse methods may be precise, but we do not know if the assumptions associated with extrapolations lead to accurate estimates. For stable isotope calculations, error from the slope of the isotopic flux rate versus distance was high, indicating low precision. At Lower Kings 2013, the error was larger than the estimated value, rendering that measurement impractical. At the other sites, error was ~25–50 % of the calculated $S_{w, amb}$. High error suggests low confidence for ^{15}N measurements of $S_{w, amb}$; however, we maintain that values for $S_{w, amb}$ estimated from the other methods that fall outside the 95 % CI for the isotope method could be considered inaccurate.

For pulse additions, the 95 % CI of the y-intercept from the S_w versus added NH_4^+ regression provides the error associated with the estimated $S_{w, amb}$. Error from experimental pulse additions was much lower than that of the $S_{w, amb}$ measured from isotopic enrichments. However, 95 % CI overlapped between $S_{w, amb}$ from isotopic and pulse additions at 3 of the 4 sites (Fig. 3). The fourth site (Campus Creek 2013) had a drastically higher $S_{w, amb}$ (a difference of 61 m, the length of the experimental reach).

Estimated ambient uptake rates

Comparisons of methods across all of our study reaches suggested that no one method was consistently better than others at estimating $U_{gross, amb}$. The best method is likely site dependent and no method is best suited for all locations. However, at almost every site, multiple methods converged on a narrow range of possible $U_{gross, ambs}$. In comparisons of rates across extrapolation and modeled methods, and sites, the plateau (modeled) and measured isotopic $U_{gross, ambs}$ had the smallest difference of any inter-method comparison. At 3 of the 4 sites (Lower Kings 2013, Campus Creek 2013, and K2 2012), the modeled plateau $U_{gross, ambs}$ were within ±35 µg m^{-2} min^{-1} of the isotopic $U_{gross, ambs}$. The pulse (extrapolated) method was accurate within ±10 µg m^{-2} min^{-1} at 2 of the 4 sites (Lower Kings 2013 and K2 2012); however, the difference in direction of rising and falling limb for S_w vs [NH_4^+] relationships at these and other sites make these results subject to scrutiny (see

Table 4 Estimated gross ambient uptake rates ($U_{gross,\ amb}$, µg m^{-2} min^{-1}) by site across multiple methods

| Site	Isotope (measured)	Pulse (extrapolated)	Pulse (modeled)	Plateau (modeled)
Lower Kings 2013	8.8	17.3	48.4	7.8
Campus Creek 2013	112.1	294.0	3908.2	145.3
K2 2012	16.9	17.2	87.2	5.8
Lower Kings 2012	310.1	188.5	190.4	19.6

discussion point below). While not consistently accurate, using plateau (modeled) approaches outlined in this paper may be best for a first look at understanding $U_{gross,\ amb}$ at an individual site. Below in the pros and cons section, we consider the merits of the various methods in more detail.

We expanded our tests of plateau modeling approaches by applying them to data from Dodds et al. (2002). Dodds and colleagues had data from 10 sites, spanning Puerto Rico to Alaska, for single plateau additions and the concentration at which those occurred, including gross ambient ammonium uptake measured using $^{15}NH_4^+$ at ambient concentrations. We applied our model (Eq. 6) to those data and found that it provided a statistically significant relationship between predicted ambient uptake rates from a single plateau and stable isotope enrichment across all sites (adjusted $r^2 = 0.49$, isotope (measured) ambient uptake = 0.552* plateau (modeled) ambient uptake +0.466; Fig. 5). The modeled ambient uptake was

about 50 % higher than the measured ambient uptake rate. Thus, single plateau additions can be corrected for ambient uptake rates, but direct measurement is preferable. This approach may be useful, particularly in assessing prior measurements of uptake rates done with a single plateau addition as is commonly reported in the literature.

The number of pulse (modeled) data points ranged from 11–20 while the plateau (modeled) included 2–3 data points (Table 3). In this case, plateau (modeled) rates better predicted isotope (measured) $U_{gross,\ amb}$ than either pulse (modeled) or pulse (extrapolated). This relationship may exist because plateau data points are more accurately representative of uptake at any given concentration compared to pulse additions because they take into account more complete mixing of the release nutrient and conservative solute into hyporheic zones (as opposed to the hysteresis observed with many pulse additions). The observation of accurate results from plateau additions supports the

Fig. 5 Modeled gross ambient uptake as a predictor of measured gross ambient uptake using data analyzed by Eq. (6) from Dodds et al. (2002). Regression equation significant (p < 0.001), slope = 0.552 which is not significantly different from 1 (p > 0.05)
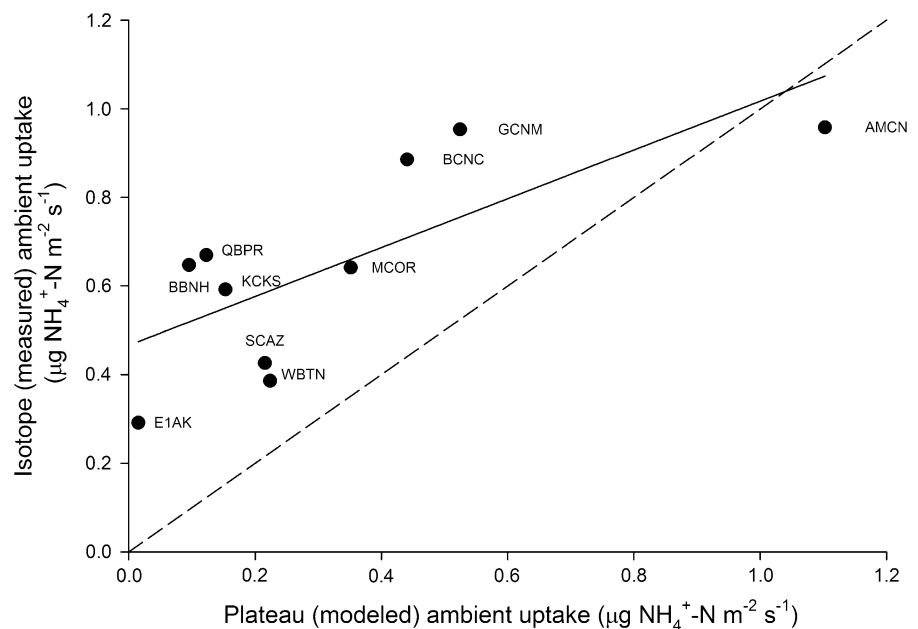
idea that a few relevant data points with complete mixing may be more valuable than the large number of data points potentially less representative of the whole stream generated from pulse approaches when attempting to correctly determine $U_{gross,\ amb}$.

The difference in rising and falling limb in many of our S_w versus $[NH_4^+]$ pulse (extrapolated) additions can be visualized by the potential hysteresis effect (Fig. 2, Panels B–D); this effect was used by Thomas et al. (2003) and noted by Covino et al. (2010) and is likely a function of the leading edge of the pulse moving primarily through the thalweg, while the trailing edge has time to come into contact with side channels and hyporheic habitats (contacting a larger area of stream channel per unit distance). One possible explanation for the dramatic hysteresis found in our data may be caused by the exceptionally low discharge in 2 of the experimental reaches of this study (0.4 and 0.6 L s^{-1}). These discharges are much lower than those from similar comparisons of nutrient uptake methods (Covino et al. 2010; Alvarez et al. 2010). With lower discharge (e.g., <1 L s^{-1}) it is possible that even with a well-mixed injection zone at the top of a reach, the rising limb of the pulse may become 'unmixed' as injectate in the thalweg moves faster than water closer to the bottom and sides of the stream that are slowed down by friction. Sampling unmixed areas can lead to potential errors. Three of our four pulse (extrapolated) calculations, including the two reaches with discharge $< 1\ Ls^{-1}$, were subject to abnormally high $NH_4^+:Br^-$ ratios (i.e. higher than any ratio throughout the BTC in the most extreme cases) in the initial samples of the rising limb where ratios would be expected to be lowest (data not shown). These high ratios led to higher S_w's at lower $[NH_4^+]$ and to negative slopes for the rising limb (Fig. 2). This issue is avoided during plateau additions where samples are collected only after the stream is fully mixed and at a new steady state.

Estimated functional relationships

We found that uptake rates were often not well described by the saturation exhibited in Michaelis–Menten equations. While these equations are derived by first principles for enzyme kinetics, they do not necessarily apply to mixed assemblages of microbes in the natural environment. Some species may be adapted to take advantage of high nutrient pulses (Suttle et al.

1991) while others compete well at low concentrations. Alternatively, abiotic adsorption will continue to increase as concentrations increase. In the case of ammonium, nitrification can use ammonium as an energy source, and half saturation constants for nitrifiers can be high (Martens-Habbena et al. 2009). Finally, ammonia could be volatilized from streams at higher concentrations, with rates increasing as concentration increases at higher pH values. Rates of ammonia volatilization from streams are not well characterized.

Campus Creek 2013 was the only site that exhibited M–M saturation, which was only evident with pulse (modeled) data. The observation of saturation in this case reinforces the potential usefulness of the pulse field approach when examining functional relationships in streams. The successive plateau method at this site was not successful in indicating uptake saturation, likely because only two points were available for modeling functional relationships. Furthermore, the highest plateau U_{net} was lower than the point where saturation of U_{net} began to be indicated by the pulse M–M relationship. If functional relationships are the main interest of a study, a pulse addition with a high injectate concentration can be used to make it more likely that uptake rates will approach saturation. This would not be feasible using successive plateaus or isotope tracer methods unless very high release concentrations are used.

Pros and cons of methods assessed

We are not able to suggest a particular addition approach (pulse or plateau) or data analyses (extrapolation or modeling) method that is best for estimating gross ambient uptake rates across all sites. However, discharge is likely to be a key driver when deciding which method to use for a particular site. Multiple plateaus might give more reliable results at low discharges, while both plateau and pulse methods could be useful in reaches with a moderate discharge. Reaches with high discharge will be restricted to pulse methods due to the logistical limitations of plateau methods. Measuring ambient rates with isotopes can be achieved across most discharge gradients in plateau or pulse forms, while more research using isotopic pulse additions across discharge gradients (possibly compared to isotopic plateau enrichments) is warranted.

We assess the pros and cons for each field method based off our field experiences and the results of this and

Table 5 Pros and cons for each uptake method. See text for details

Method	Pro	Con
Isotope (Plateau)	Provides a direct measure of gross ambient uptake	High cost of isotope enriched nutrients and analysis
	Can be used with high ambient nutrient concentrations as uptake may not be evident with nutrient additions	Long processing time for samples (1–2 weeks)
	Reach better defined (i.e. can use conservative tracer to find poor mixing)	Sample processing must begin immediately after collection (i.e. samples cannot be frozen or preserved)
	Can be conducted by one person	Requires extra field equipment and larger sample volumes compared to regular plateau or pulse releases
		Unsuitable for large systems and/or high discharges due to logistical issues and large volumes of injectate. The high cost of stable isotopes will limit maximum injectate volume
Plateau	Reach better defined (i.e. can use conservative tracer to find poor mixing)	Method with longest amount of time in field if conducting multiple plateaus
	Can be conducted by one person	Unsuitable for large systems and/or high discharges due to logistical issues and large volumes of injectate
	Modeled ambient uptake rates can be done with few data points	Unsuitable for long reaches (too long to reach equilibrium)
	Modeled rates from plateau were most accurate of the methods assessed in this paper	Conditions could change during release
		Few nutrient concentrations represented
TASCC	Multiple concentrations represented	Thalweg might have heavy influence on rising limb dynamics, particularly at the top of release, and transient storage can influence later samples, potentially leading to hysteresis
	Suitable for higher discharge sites	Complicated sampling regime, can require several people
	Modeling and extrapolation methods can be used on the same data	Very high nutrient concentrations may not be relevant
	Better characterization of functional relationships than plateau method	Might be dependent on range of NH_4^+ concentrations achieved in BTC
		Could be inaccurate at very low discharge

previous studies (Table 5), which can be used to choose a method based on the question of interest and logistical constraints. Briefly, plateau methods can determine uptake in a more closely defined reach of stream than a pulse method, while pulse methods may take less time (but create a greater sample burden and require more people) than plateau. The recently introduced pulse methods have definite advantages and seem to be increasing in popularity. In general, the preferred method for determining uptake rates depends upon the individual site and research questions. If functional relationships between uptake rates and nutrient concentrations are needed, a single pulse measurement might be suggested. If accurate measurements of U_{amb} are the goal, a combination of isotope enrichments and multiple plateau additions could be used.

It is important to understand the limitations and benefits to each approach before choosing one for a particular application. If possible, multiple methods should be attempted at each location and across seasons or other times when in-stream conditions might change in order to determine the best method prior to designing and implementing a more extensive series of measurements.

References

Alvarez M, Proia L, Ruggiero A, Sabater F, Butturini A (2010) A comparison between pulse and constant rate additions as methods for the estimation of nutrient uptake efficiency in-streams. J Hydrol 388:273–279

APHA (American Public Health Association) (1995) Standard methods for examination of water and wastewater, 19th edn. American Public Health Association, Washington, DC

Bernot MJ, Dodds WK (2005) Nitrogen retention, removal, and saturation in lotic ecosystems. Ecosystems 8:442–453

Covino TP, McGlynn BM, McNamara RA (2010) Tracer additions for spiraling curve characterization (TASCC): quantifying stream uptake kinetics from ambient to saturation. Limnol Oceanogr Meth 8:484–498

Covino T, McGlynn B, McNamara RA (2012) Land use/land cover and scale influences on in-stream nitrogen uptake kinetics. J Geophys Res 117:G02006

Davis JC, Minshall GW (1999) Nitrogen and phosphorus uptake in two Idaho (USA) headwater wilderness streams. Oecologia 119:247–255

Diaz RJ, Rosenburg R (2008) Spreading dead zones and consequences for marine ecosystems. Science 321:926–929

Dodds WK (1993) What controls levels of dissolved phosphate and ammonium in surface waters? Aquat Sci 55:132–142

Dodds WK, Lopez AJ, Bowden WB, Gregory S, Grimm NB, Hamilton SK, Hershey AE, Marti E, McDowell WH, Meyer JL, Morrall D, Mulholland PJ, Peterson BJ, Tank JL, Valetti HM, Webster JR, Wollheim W (2002) N uptake as a function of concentration in streams. J N Am Benthol Soc 21:206–220

Dodds WK, Gido KB, Whiles MR, Fritz KM, Matthews WJ (2004) Life on the edge: ecology of Great Plains prairie streams. BioSci 54:205–216

Dodds WK, Beaulieu J, Eichmiller J, Fischer J, Franssen N, Gudder D, Makinster A, McCarthy M, Murdock J, O'Brien J (2008) Nitrogen cycling and metabolism in the thalweg of a prairie river. J Geophys Res 113:G04029

Doyle M (2005) Incorporating hydrologic variability into nutrient spiraling. J Geophys Res 110:G1

Earl SR, Valett HM, Webster JR (2007) Nitrogen spiraling in streams: comparisons between stable isotope tracer and nutrient addition experiments. Limnol Oceanogr 52:1718–1723

Gooseff M, Payn R, Zarnetske J, Bowden W, McNamara J, Bradford J (2008) Comparison of in-channel mobile-immobile zone exchange during instantaneous and constant rate stream tracer additions: implications for design and interpretation of non-conservative tracer experiments. J Hydrol 357:112–124

Hart B, Freeman P, McKelvie I (1992) Whole-stream phosphorus release studies: variation in uptake length with initial phosphorus concentration. Hydrobiologia 235:573–584

Holmes RM, McClelland JW, Sigman DM, Fry B, Peterson BJ (1998) Measuring ^{15}N- NH_4^+ in marine, estuarine and freshwater: an adaptation of the ammonium diffusion method for samples with low ammonium concentrations. Mar Chem 60:235–243

Martens-Habbena WP, Berube M, Urakawa H, de la Torre JR, Stahl DA (2009) Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria. Nature 461:976–979

Mulholland PJ, Tank JL, Webster JR, Bowden WB, Dodds WK, Gregory SV, Grimm NB, Hamilton SK, Johnson SL, Marti E, McDowell WH, Merriami JL, Meyer JL, Peterson BJ, Valett HM, Wollheim WM (2002) Can uptake length in streams be determined by nutrient addition experiments? Results from an interbiome comparison study. J N Am Benthol Soc 21:544–560

Mulholland PJ, Helton AM, Poole GC, Hall RO, Hamilton SK, Peterson BJ, Tank JL, Ashkenas LR, Cooper LW, Dahm CN, Dodds WK, Findlay SEG, Gregory SV, Grimm NB, Johnson SL, McDowell WH, Meyer JL, Valett HM, Webster JR, Arango CP, Beaulieu JJ, Bernot MJ, Burgin AJ, Crenshaw CL, Johnson LT, Niederlehner BR, O'Brien JM, Potter JD, Sheibley RW, Sobota DJ, Thomas SM (2008) Stream denitrification across biomes and its response to anthropogenic nitrate loading. Nature 452:202–205

Newbold JD, Elwood JW, O'Neill RV, Vanwinkle W (1981) Measuring nutrient spiralling in streams. Can J Fish Aquat Sci 38:860–863

O'Brien JM, Dodds WK, Wilson KC, Murdock JM, Eichmiller J (2007) The saturation of N cycling in Central Plains streams: 15 N experiments across a broad gradient of nitrate concentrations. Biogeochemistry 84:31–49

O'Brien JM, Dodds WK (2008) Ammonium uptake and mineralization in prairie streams: chamber incubation and short-term nutrient addition experiments. Freshw Biol 53:102–112

Omernik JM (1987) Map supplement: ecoregions of the conterminous United States. Ann Assoc Am Geogr 77:118–125

Payn RA, Webster JR, Mulholland PJ, Valett HM, Dodds WK (2005) Estimation of stream nutrient uptake from nutrient addition experiments. Limnol Oceanogr Methods 3:174–182

Payn RA, Gooseff M, Benson F, Cirpka O, Zarnetske J, Bowden WB, McNamara J, Bradford J (2008) Comparison of instantaneous and constant-rate stream tracer experiments through non-parametric analysis of residence time distributions. Water Resour Res 44:W06404

Peterson BJ, Wollheim WF, Mulholland PJ, Webster JR, Meyer JL, Tank JL, Martí E, Bowden WB, Valett HM, Hershey AE, McDowell WH, Dodds WK, Hamilton SK, Gregory S, Morrall DD (2001) Control of nitrogen export from watersheds by headwater streams. Science 292:86–90

Powers SM, Stanley EH, Lottig NR (2009) Quantifying phosphorus uptake using pulse and steady-state approaches in streams. Limnol Oceanogr Methods 7:498–508

Stream Solute Workshop (1990) Concepts and methods for assessing solute dynamics in stream ecosystems. J N Am Benthol Soc 9:95–119

Suttle CA, Cochlan WP, Stockner JG (1991) Size-dependent ammonium and phosphate uptake, and N: P supply ratios in an oligotrophic lake. Can J Fish Aquat Sci 48:1226–1234

Tank JL, Rosi-Marshall EJ, Baker MA, Hall RO Jr (2008) Are rivers just big streams? A pulse method to quantify nitrogen demand in a large river. Ecology 89:2935–2945

Thomas SA, Maurice VH, Webster JR, Mulholland PJ (2003) A regression approach to estimating reactive solute uptake in advective and transient storage zones of stream ecosystems. Adv Water Resour 26:965–976

Webster JR, Ehrman TP (1996) Solute dynamics. In: Hauer FR, Lamberti GA (eds) Methods in stream ecology. Academic Press, New York, pp 145–160

Webster JR, Mulholland PJ, Tank JL, Valett HM, Dodds WK, Peterson BJ, Bowden WB, Dahm CN, Findlay S, Gregory SV, Grimm NB, Hamilton SK, Johnson SL, Marti E, McDowell WH, Meyer JL, Morrall DD, Thomas SA, Wollheim WM (2003) Factors affecting ammonium uptake in streams—an inter-biome perspective. Freshw Biol 48:1329–1352