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Nitrogen spiraling in streams: Comparisons between stable isotope tracer and nutrient addition experiments

Abstract—A common method to quantify stream nutrient uptake length is to enrich the stream at an upstream point and monitor the downstream decline in the concentration of the added nutrient. However, increasing the nutrient concentration of the stream alters nutrient uptake, and uptake length quantified using this amendment approach does not necessarily reflect uptake length under ambient conditions. We conducted a series of $^{15}\text{NO}_3^-$ tracer and $^{14}\text{NO}_3^-$ nutrient amendment experiments to (1) assess the overestimation of amendment-derived uptake length in streams spanning a gradient of ambient nitrogen concentration, and (2) evaluate a technique to estimate ambient uptake length using data from multiple amendments conducted at different concentrations. Single amendment-derived uptake lengths were consistently longer than ambient uptake lengths quantified using the stable-isotope tracer. The ratio of single amendment-derived to ambient uptake length ranged more than sixfold across streams but was not related to ambient stream water nitrogen concentration or amendment concentration. Estimated ambient uptake length calculated from the multiple amendment technique was a better predictor of ambient uptake length than single amendment-derived uptake length in three of five experiments. However, with this technique, calculated uptake length was negative in two streams. Our results suggest that nitrogen limitation was weak in these two streams and that the multiple amendment technique may be ineffective in streams where the nutrient of study is not limiting.

The nutrient spiraling concept (Webster and Patten 1979) provides a useful theoretical framework to describe the paired processes of nutrient cycling and advective flow in stream ecosystems. Nutrient uptake length is the average distance traveled by a nutrient in dissolved form before being immobilized through abiotic or biotic processes into particulate form (Newbold et al. 1981) and is the main component of spiraling length in headwater streams.

Uptake length is a function of the longitudinal loss rate of a nutrient and can be quantified by the downstream decline in the concentration of a nutrient tracer (Newbold et al. 1981). The preferred method for quantifying uptake length is to add a tracer in the form of a radioactive or stable isotope of a nutrient. Using an isotopic tracer maintains the extant nutrient concentration of the stream and measured uptake length reflects uptake under background (i.e., ambient) conditions. However, safety and regulatory concerns prohibit the use of radioactive isotopes

in most stream studies, and stable isotope studies are costly and labor intensive (Mulholland et al. 1990, 2002). As an alternative, many investigators have used short-term nutrient additions (amendments) to elevate the nutrient concentration of a stream and monitor the downstream decline of the added nutrient to quantify uptake length (e.g., Munn and Meyer 1990, Davis and Minshall 1999, and many others).

Several studies have documented that uptake length quantified using the nutrient amendment approach consistently overestimates ambient uptake length measured at ambient concentration using isotopic tracers (Mulholland et al. 1990, 2000a, 2002). This discrepancy occurs because nutrient uptake may increase with increasing stream water nutrient concentration, but uptake lengths measured under ambient and nutrient-amended conditions will only be equal if nutrient uptake increases proportionately to the increase in stream water nutrient concentration (Mulholland et al. 2002).

Calculating ambient uptake length from multiple amendments—Uptake length reflects both the downstream flux of nutrients and nutrient removal through uptake, including denitrification, and any forms of autotrophic and heterotrophic immobilization. The Michaelis–Menten (M-M) model, in which uptake increases with increasing nutrient concentration until asymptotically approaching maximum uptake (U_{\max}), describes nutrient uptake dynamics at a broad range of scales, including stream ecosystems (Dodds et al. 2002; Earl et al. 2006). Using this model, Mulholland et al. (2002) demonstrated that the relation between amendment concentration and uptake length is linear and Payn et al. (2005) derived the equation for this relation:

$$S_{w(\text{add})} = \frac{ud(K_m + C_{\text{amb}})^2}{U_{\max}K_m} + C_{\text{add}} \left[\frac{ud(K_m + C_{\text{amb}})}{U_{\max}K_m} \right] \quad (1)$$

In Eq. 1, $S_{w(\text{add})}$ is the uptake length observed in an amendment experiment (S_w' of Mulholland et al. 2002), u is stream water velocity, d is stream depth, C_{amb} is ambient stream water nutrient concentration, C_{add} is the nutrient amendment concentration above ambient, and K_m is the half-saturation constant (the concentration at which uptake is one-half of U_{\max}). On the basis of this relation, Payn et al. (2005) proposed a method whereby the ambient uptake

Table 1. Site characteristics and ambient (S_w) and single amendment-derived ($S_{w(\text{add})}$) uptake lengths. Nitrate–nitrogen (NO_3^- -N), ammonium–nitrogen (NH_4^+ -N), and soluble reactive phosphorus (SRP) are reach-averaged ambient concentrations before releases. Nutrient concentrations $<3 \mu\text{g L}^{-1}$ were below detectable limits (bdl). C_{add} is the NO_3^- -N amendment concentration above ambient and $C_{\text{add}}:C_{\text{amb}}$ is the ratio of C_{add} to ambient stream water NO_3^- -N concentration (C_{amb}). Standard error ($\pm\text{SE}$) of $S_{w(\text{add})}$ at Greenbrier Creek could not be determined.

Stream	Experiment dates (2003)	Reach length (m)	NO_3^- -	NH_4^+ -	SRP	C_{add} ($\mu\text{g L}^{-1}$)	$C_{\text{add}}:C_{\text{amb}}$	$S_{w(\text{add})} \pm\text{SE}$ (m)	$S_w \pm\text{SE}$ (m)	$S_{w(\text{add})}:S_w$
			($\mu\text{g L}^{-1}$)							
Sammy Creek I	29 Apr–02 May	50	5	5	bdl	42	8.5	168 ± 37	128 ± 21	1.31
Sammy Creek II	15–16 Jul	50	3	bdl	bdl	138	57.0	104 ± 8	42 ± 3	2.48
Hugh White Creek	08–11 Oct	80	3	3	bdl	50	15.4	223 ± 7	23 ± 2	9.70
Alta Creek	25–28 Aug	190	182	bdl	7	41	0.2	$1,088 \pm 217$	587 ± 81	1.85
Stoncrop Creek	10–13 Sep	147	298	5	20	68	0.2	$1,047 \pm 158$	632 ± 24	1.66
Greenbrier Creek	14–15 Aug	322	983	bdl	5	101	0.1	4,548	$3,652 \pm 1,037$	1.25

length is estimated using multiple nutrient amendments to characterize the relation between C_{add} and corresponding uptake lengths for a given stream. From this relation, ambient uptake length is estimated by extrapolating to the negative ambient stream water nutrient concentration ($-C_{\text{amb}}$). M-M uptake parameters (i.e., K_m and U_{max}) can also be determined from the relation. In addition, the slope of the relation between $S_{w(\text{add})}$ and C_{add} is indicative of the severity of nutrient limitation, where steeper slopes correspond to less nutrient limitation (Mulholland et al. 2002).

Here we compare ambient and amendment-derived nitrate (NO_3^-) uptake lengths in streams that span a gradient of dissolved inorganic N (DIN) concentration. In each stream, uptake length was quantified under ambient conditions using a stable isotope ($^{15}\text{NO}_3^-$) tracer and during a short-term NO_3^- amendment. Following Mulholland et al. (2002), we use the ratio of amendment-derived uptake length to ambient uptake length ($S_{w(\text{add})}:S_w$) to reflect the degree to which amendment-derived uptake length overestimates ambient uptake length. In addition, we conducted NO_3^- amendment experiments at multiple concentrations to calculate uptake length on the basis of the theoretical method proposed by Payn et al. (2005).

Methods—Study sites: We examined NO_3^- uptake in five first- or second-order headwater streams in the southern Appalachian Mountains of southwestern Virginia and western North Carolina that span a gradient of DIN concentration (Table 1). We selected a single study reach in each stream with length depending on accessibility, stream water travel time, and distance appropriate to quantify uptake length (Table 1).

Solute addition experiments: We conducted a series of short-term (3–5 h) releases in which uptake length was first quantified under ambient conditions using a stable isotope ($^{15}\text{NO}_3^-$) tracer followed by a series of amendments where stream water NO_3^- concentration was successively elevated. Releases were conducted on consecutive days except at Greenbrier Creek, where four releases were conducted over 2 d. Releases were conducted during periods without storms to address similar conditions. Before each release,

background samples were collected from three to seven points distributed longitudinally along the study reach and analyzed for $^{15}\text{NO}_3^-$, NO_3^- , and chloride (Cl^-). A subset of the background samples was analyzed for ambient concentrations of ammonium (NH_4^+) and soluble reactive phosphorus (SRP).

For the first release, a solution of K^{15}NO_3 (98 atom %; Cambridge Isotope Laboratories) and NaCl (Cl^- to serve as a conservative tracer) was released at a constant rate designed to increase stream water $\delta^{15}\text{N}-\text{NO}_3^-$ by 500‰. In the two streams with background NO_3^- -N concentrations $<80 \mu\text{g/L}$ (Sammy Creek and Hugh White Creek; Table 1), background and plateau samples were supplemented with a known amount of NO_3^- to ensure a minimum mass ($\geq 80 \mu\text{g}$) of N required to measure $^{15}\text{NO}_3^-$ in a reasonable sample volume (i.e., ≤ 1 liter). Thus, the target $\delta^{15}\text{N}-\text{NO}_3^-$ of the field release was elevated such that the sample $\delta^{15}\text{N}-\text{NO}_3^-$ would reflect an enrichment of 500‰ after supplement addition. Tracer $^{15}\text{NO}_3^-$ additions elevated stream water NO_3^- -N concentrations by less than $0.45 \mu\text{g L}^{-1}$, corresponding to an increase of 11% above ambient at Hugh White Creek, 9% and 3% at Sammy Creek II and I, respectively, and $<0.6\%$ at all other sites.

Three replicate water samples were collected under well-mixed conditions (i.e., tracer plateau as indicated by steady-state conductivity in channel water) at points downstream of the release site corresponding to locations where background samples were collected. Water samples for the analysis of $^{15}\text{NO}_3^-$ were field-filtered (Whatman GF/F, pore size = $0.7 \mu\text{m}$) into clean bottles and refrigerated ($\sim 4^\circ\text{C}$) until analysis within 1 week. An additional set of samples ($n = 3$) for the analysis of NO_3^- and Cl^- was collected, field-filtered (Gelman A/E, pore size = $1.0 \mu\text{m}$) into clean bottles, and stored frozen until analysis within 1 week.

For subsequent amendment releases, a solution of NaNO_3 and NaCl was released into the study reach at a constant rate. Replicate water samples were collected at plateau for the analysis of NO_3^- and Cl^- . Amendment concentrations of NO_3^- -N varied from ~ 40 to $600 \mu\text{g L}^{-1}$ (Table 1).

Discharge (Q) at each transect was determined by dilution gauging using the conservative tracer (Cl^-) and assumed to be constant throughout sampling.

Laboratory methods: Anions (NO_3^- and Cl^-) were analyzed on a Dionex DX500 ion chromatograph. Because of low NO_3^- -N concentrations ($<10 \mu\text{g L}^{-1}$), samples collected at Sammy Creek and Hugh White Creek were analyzed colorimetrically after reduction by Cd (Wood et al. 1967; American Public Health Association [APHA] 1998) on a Technicon autoanalyzer. Samples were analyzed for NH_4^+ using a modified phenol-hypochlorite method (Zhang et al. 1997) and SRP using the molybdate antimony method (Zimmerman and Keefe 1997).

$^{15}\text{NO}_3^-$ was measured by headspace diffusion following Sigman et al. (1997). This method involves removing ammonium by boiling the sample under basic conditions, converting nitrate to ammonia by adding Devarda's alloy, and capturing the ammonia on an acidified glass-fiber filter. Acidified filters with captured ammonia were shipped to the UC Davis Stable Isotope Facility for analysis of ^{15}N on a Europa Integra mass spectrometer (Sercon).

Data analysis: Uptake length was calculated for both NO_3^- amendment ($S_{w(\text{add})}$) and $^{15}\text{NO}_3^-$ tracer (S_w) releases by fitting an exponential decay model (SigmaPlot, SPSS) to background- and dilution-corrected NO_3^- concentration and $^{15}\text{NO}_3^-$ flux (Mulholland et al. 2000b), respectively, versus distance downstream (Newbold et al. 1981). The standard error (SE) of uptake length was determined as the SE of the uptake coefficient of $^{15}\text{NO}_3^-$ (tracer) or NO_3^- concentration (amendment) as described by a modified exponential decay model (Eq. 2), where a is equal to the upstream $^{15}\text{NO}_3^-$ flux (tracer) or dilution-corrected NO_3^- concentration (amendment) at the uppermost transect and x is distance downstream.

$$\text{NO}_3^- = ae^{\left(\frac{x}{S_w}\right)} \quad (2)$$

Linear regression (performed on SigmaStat, SPSS) was used to characterize relations between $S_{w(\text{add})}$ and NO_3^- -N amendment concentration (C_{add}) and to calculate ambient uptake length on the basis of these relations. M-M parameters (K_m and U_{max}) were estimated from these regressions. The slopes of the relations between $S_{w(\text{add})}$ and NO_3^- -N amendment concentration were compared among sites by analysis of covariance using the PROC REG procedure in SAS. Linear regression was used to compare $S_{w(\text{add})}:S_w$ to C_{add} , C_{amb} , and $C_{\text{add}}:C_{\text{amb}}$. Significance level (α) was set to 0.05 for all statistical tests except when Bonferroni correction was needed.

Results—Stream characteristics: The study streams varied in discharge from 2 to 31 L s^{-1} at the time of the releases and mean concentrations of NH_4^+ -N were consistently low ($\leq 5 \mu\text{g L}^{-1}$), while ambient NO_3^- -N (C_{amb}) varied from 3 to 982 $\mu\text{g L}^{-1}$ (Table 1). The concentration of SRP was $\leq 7 \mu\text{g L}^{-1}$, with the exception of Stonecrop Creek (20 $\mu\text{g L}^{-1}$).

Comparison of tracer and amendment studies: The lowest amendment concentration (C_{add}) of NO_3^- -N among streams varied from 41 to 138 $\mu\text{g L}^{-1}$, representing

a threefold change in experimental amendment. However, the ratio of amendment to ambient stream water NO_3^- -N concentration ($C_{\text{add}}:C_{\text{amb}}$) ranged 570-fold and was highest in the low-N streams (Table 1). Because C_{add} was generally similar across streams, $C_{\text{add}}:C_{\text{amb}}$ fluctuated dramatically depending on C_{amb} . Both C_{add} (138 $\mu\text{g L}^{-1}$) and $C_{\text{add}}:C_{\text{amb}}$ (57.0) were particularly high during a single amendment release conducted at Sammy Creek II. In contrast, C_{add} in the streams with relatively higher background concentrations (Alta Creek, Stonecrop Creek, and Greenbrier Creek) translated into exceptionally low $C_{\text{add}}:C_{\text{amb}}$ ratios (Table 1).

Amendment-derived uptake lengths ($S_{w(\text{add})}$) determined from the lowest amendment at each site varied from 104 to 4,548 m and were consistently longer than ambient uptake lengths (S_w) (23 to 3,652 m) (Table 1). The ratio of $S_{w(\text{add})}:S_w$ varied from 1.25 to 9.70 and was markedly higher at Hugh White Creek ($S_{w(\text{add})}:S_w = 9.70$) relative to all other streams (Table 1). Despite a substantial range in the disparity between $S_{w(\text{add})}$ and S_w , there were no clear trends relating the discrepancies to either C_{amb} , C_{add} , or $C_{\text{add}}:C_{\text{amb}}$ ($r^2 \leq 0.14$, $p \geq 0.46$, $n = 6$). Hugh White Creek was an outlier but regressions among these variables were still not significant when Hugh White Creek was omitted from the analyses ($r^2 \leq 0.68$, $p \geq 0.09$, $n = 5$).

Multiple amendments: At least two releases at different amendment concentrations were conducted in each of the study streams except Sammy Creek II. C_{add} among all releases varied from 41 to 636 $\mu\text{g L}^{-1}$ NO_3^- -N and $C_{\text{add}}:C_{\text{amb}}$ varied from 0.1 to 194.4 (Table 2). All measured uptake lengths were statistically significant except the release corresponding to the final and highest amendment ($C_{\text{add}} = 394 \mu\text{g L}^{-1}$ NO_3^- -N; $C_{\text{add}}:C_{\text{amb}} = 2.2$) at Alta Creek ($p = 0.15$); thus results from this release were not included in the analyses.

$S_{w(\text{add})}$ increased consistently with increasing NO_3^- amendment concentration at all sites (Fig. 1). In streams where more than two amended releases were conducted, a linear model described most of the variability ($r^2 \geq 0.94$).

Calculated uptake lengths were similar to ambient uptake lengths in two of the five experiments. The calculated uptake lengths at Sammy Creek I (111 m) and Stonecrop Creek (605 m) were within the range of S_w (\pm SE) measured at those sites, 128 (± 21) and 632 (± 24) m, respectively (Table 2). The calculated uptake length at Hugh White Creek (139 m) was more than sixfold higher than S_w (23 ± 2 m). Calculated uptake lengths were negative at both Alta Creek and Greenbrier Creek ($-1,342$ and $-12,601$ m, respectively) and thus substantially different from ambient uptake lengths at both sites (Table 2). The calculated uptake length at Alta Creek is based on a two-point regression (Fig. 1); however, including the nonsignificant $S_{w(\text{add})}$ at the highest amendment concentration further decreased the calculated uptake length to $-4,249$ m.

M-M parameters (K_m and U_{max}) varied across streams but both parameters were negative at Alta Creek and Greenbrier Creek. When Alta Creek and Greenbrier Creek

Table 2. Uptake lengths calculated from multiple amendments and Michaelis–Menten parameters (K_m and U_{max}). The range of amendment concentration for each stream is presented as the amendment concentration above ambient (C_{add}) and the ratio of C_{add} to the ambient stream water NO_3^- -N concentration ($C_{add} : C_{amb}$). Uptake lengths were estimated by regressing $S_{w(add)}$ measured from multiple amendments in a given stream to the NO_3^- -N amendment concentration (C_{add}) and extrapolating to the negative ambient concentration. Ambient uptake lengths (S_w) are presented for comparison. Only one amended release was conducted at Sammy Creek II; thus uptake length and Michaelis–Menten parameters could not be determined by this method.

Stream	Amendments		Range of $S_{w(add)}$ (m)	Calculated S_w (m)	$S_w \pm SE$ (m)	K_m ($\mu\text{g L}^{-1}$)	U_{max} ($\mu\text{g N m}^{-2} \text{min}^{-1}$)
	C_{add}	$C_{add} : C_{amb}$					
Sammy Creek I	42–80	8.5–16.1	168–214	111*	128 ± 21	92	129
Sammy Creek II	138	57.0	104		42 ± 3		
Hugh White Creek	50–636	15.4–194.4	223–1,134	139	23 ± 2	90	47
Alta Creek	41–394	0.2–2.2	1,088–8,467	–1,342*	587 ± 81	–123	–11
Stoncrop Creek	68–555	0.2–1.9	1,047–1,719	605	632 ± 24	447	75
Greenbrier Creek	101–588	0.1–0.6	4,548–11,973	–12,601	3,652 ± 1,037	–810	–11

* S_w based on a two-point regression.

were excluded, K_m varied from 90 to 447 $\mu\text{g L}^{-1}$ and U_{max} varied from 47 to 129 $\mu\text{g N m}^{-2} \text{min}^{-1}$ across streams.

The slopes of the relation between $S_{w(add)}$ and C_{add} were significantly greater at Alta Creek and Greenbrier Creek than slopes at Stoncrop Creek and Hugh White Creek ($p \leq 0.01$, $\alpha = 0.02$ after Bonferroni correction). Because of the limited number of data points, experiments at Sammy Creek were not included in the statistical comparison, but the slope at Sammy Creek I was similar to those at Stoncrop Creek and Hugh White Creek.

Discussion—Comparison of tracer and amendment studies: $S_{w(add)}$ overestimated S_w in all streams, consistent with patterns reported by Mulholland et al. (2002). These results suggest that the increase in NO_3^- uptake associated with amendments did not increase proportionately to the increase in NO_3^- concentration even in the low-N streams where N would most likely be limiting.

Despite a relatively large range of $S_{w(add)} : S_w$ across streams, the range primarily reflected the high $S_{w(add)} : S_w$ at Hugh White Creek and there was little variability among the other streams. The ratio of $S_{w(add)} : S_w$ was not statistically related to either stream water N concentration (C_{amb}) or the magnitude of amendment as either C_{add} or $C_{add} : C_{amb}$. These results are contrary to those reported by Mulholland et al. (2002) in which $S_{w(add)} : S_w$ of ammonium was significantly related to the ammonium concentration increase (i.e., C_{add}). The absence of a similar relation in this study likely reflects the limited variability of $S_{w(add)} : S_w$ across streams because of enrichment effects in low-N versus high-N streams. The range of $C_{add} : C_{amb}$ in this study is larger than that reported by Mulholland et al. (2002), reflecting the substantially higher ambient N concentration in several N-rich streams relative to those in the study by Mulholland et al. (2002). Slopes of the relation between $S_{w(add)}$ and C_{add} were highest in the two high-N streams (Alta Creek and Greenbrier Creek) and we would have expected to see the largest overestimation of S_w in these streams because of comparatively longer uptake lengths per unit increase in C_{add} . However, C_{add} was low relative to the ambient concentration, resulting in relatively small increases in $S_{w(add)}$. In contrast, similar C_{add}

concentrations resulted in substantial increases in N availability relative to ambient in the lower-N streams. This substantial increase in N availability relative to ambient translated into large increases in $S_{w(add)}$. These results suggest that the extent to which nutrient amendment experiments overestimate ambient uptake length is a complex function of both ambient and amendment nutrient concentrations in relation to whole-stream nutrient demand.

S_w calculated from multiple amendments: Calculated ambient uptake lengths were better predictors of S_w than $S_{w(add)}$ in three of the five streams and closely reflected ambient uptake length in two of those streams. Calculated uptake lengths at Sammy Creek I and Stoncrop Creek underestimated S_w by only 13% and 4%, respectively. These results are consistent with Payn et al. (2005), who reported that, compared with $S_{w(add)}$, the multiple amendment technique yielded a better estimate of ambient ammonium uptake for streams in Kansas and Tennessee, and a better estimate of nitrate uptake in a North Carolina stream. Although the calculated uptake length was more than sixfold higher than S_w at Hugh White Creek, the calculated uptake length was still a better predictor of S_w than $S_{w(add)}$.

While the multiple amendment approach proved successful in some streams, the nonsensical results (i.e., negative calculated uptake lengths) at both Alta Creek and Greenbrier Creek suggest that this technique is not appropriate in all cases. The underlying principle of the multiple amendment technique is that the relation between nutrient uptake and concentration follows M-M kinetics (Mulholland et al. 2002; Payn et al. 2005). Although $S_{w(add)}$ increased linearly with increasing amendment concentration, as predicted given M-M kinetics, the negative K_m and U_{max} values suggest that uptake in these streams did not follow M-M kinetics. The slope of the relation between $S_{w(add)}$ and C_{add} was greater at both Alta Creek and Greenbrier Creek relative to all other streams, suggesting weak N limitation (Mulholland et al. 2002) or, possibly, N saturation (Earl et al. 2006). Uptake may deviate from the asymptotic M-M relation when the nutrient of study is not limiting (Dugdale et al. 1981), thus negating the mathe-

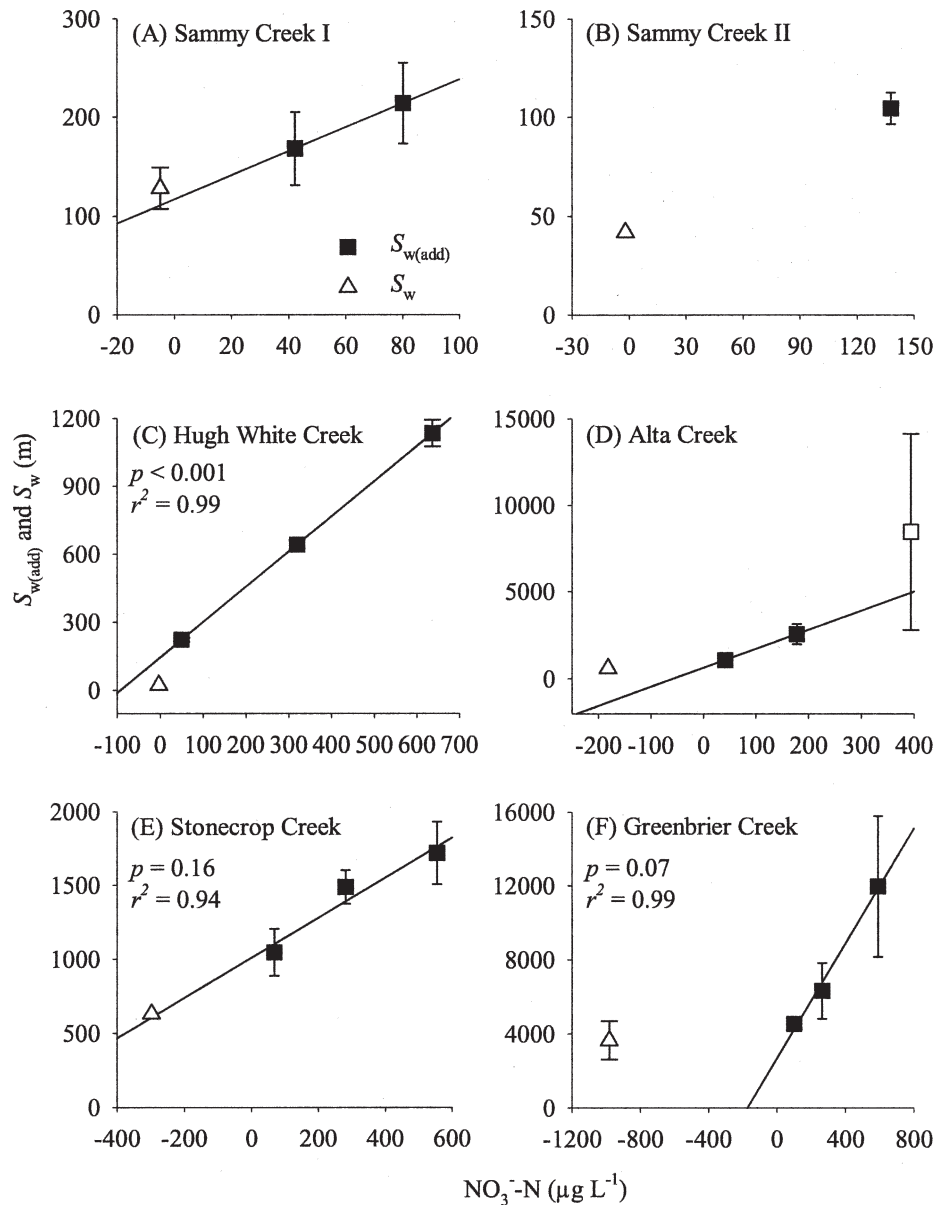


Fig. 1. Calculations of ambient uptake lengths from multiple amendments at Sammy Creek I (panel A), Sammy Creek II (panel B), Hugh White Creek (panel C), Alta Creek (panel D), Stonecrop Creek (panel E), and Greenbrier Creek (panel F). Closed squares are $S_{w(\text{add})}$ (\pm SE) from multiple amendment experiments plotted at the corresponding $\text{NO}_3^- \text{-N}$ amendment concentration (C_{add}). Open triangles are $^{15}\text{NO}_3^-$ tracer-derived ambient uptake lengths (S_w) plotted at the negative ambient stream water $\text{NO}_3^- \text{-N}$ concentration. Standard error of the first amended release at Greenbrier Creek (panel F) could not be determined. The nonsignificant $S_{w(\text{add})}$ at Alta Creek (open square) (panel D) was not included in the analysis. Linear regression results (p , r^2) are presented for those streams in which more than two releases were included in the analysis. These calculations were not possible at Sammy Creek II (panel B), where only one amended release was conducted.

mathematical framework behind the multiple amendment technique. Further, increased nutrient concentration associated with amendments should result in only slight changes in uptake in those streams where that nutrient is weakly or nonlimiting, potentially resulting in poor estimates of uptake. Greater error associated with quantifying uptake in N-rich streams was evident at both Alta Creek and

Greenbrier Creek. Slight changes in uptake with increasing nutrient concentration coupled to greater error around estimates of uptake length in weakly limited or saturated streams could lead to inaccuracy using the multiple amendment technique.

The empirical examples cited by Payn et al. (2005) included only low-N streams and, although the technique

was effective, $S_{w(\text{add})}:S_w$ was relatively low in all cases. They suggested that the multiple amendment technique might be more useful in streams with higher ambient nutrient concentrations. However, in this study, $S_{w(\text{add})}:S_w$ was not directly related to C_{amb} and, thus, the overestimation of S_w by amendment experiments would not necessarily be greater in high-N streams unless exceptionally high amendment concentrations are used. The multiple amendment technique can be a viable option to estimate uptake lengths under nutrient-limited conditions, but our results suggest that it will not work in some cases, perhaps because nutrient saturation or weak nutrient limitation results in a poor fit to the kinetic model.

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