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**NUTRIENT UPTAKE AMONG URBAN AND NON-URBAN STREAMS WITHIN THE
PIEDMONT PHYSIOGRAPHIC PROVINCE OF VIRGINIA**

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science
at Virginia Commonwealth University.

by

Joseph T. Famularo
Bachelor of Science
B.S. in Water: Resources, Policy, and Management
Virginia Polytechnic Institute and State University, 2017

Major Professor:
Dr. Paul A. Bukaveckas
Professor, Department of Biology and Center for Environmental Studies

Virginia Commonwealth University
Richmond, Virginia
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Abstract

NUTRIENT UPTAKE AMONG URBAN AND NON-URBAN STREAMS WITHIN THE PIEDMONT PHYSIOGRAPHIC PROVINCE OF VIRGINIA

By: Joseph T. Famularo

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biology at Virginia Commonwealth University.

Virginia Commonwealth University, 2019.

Advisor: Dr. Paul A. Bukaveckas, Professor, VCU Department of Biology and Center for Environmental Studies

Stream ecosystem nitrogen (N) and phosphorous (P) retention is an important ecosystem service, especially in coastal urban centers where the downstream transport of N and P to sensitive estuarine and marine environments has increased. To assess how urbanization impacts stream nutrient uptake, a series of instantaneous (i.e. slug) nutrient additions were conducted in 3 urban and 3 non-urban streams during open and closed canopy conditions. Single additions of N, P, and combined additions of N and P were performed at each site. These data were used to test the hypothesis that high N:P concentrations in urban streams would result in P-limited conditions, and to assess differences in nutrient uptake kinetics (i.e., the relationship between uptake and concentration) between urban and non-urban streams. The results show that there were no consistent differences in N vs. P limitation among urban and non-urban streams suggesting that ambient N:P ratios are not useful predictors of nutrient limitation at the ecosystem scale. Areal uptake rates of N in urban streams were greater than non-urban streams coinciding with elevated N concentrations. Conversely, areal uptake rates of P were similar between urban and non-urban streams because these systems have similar ambient concentrations of P. Urban and non-urban streams demonstrated similar uptake velocity and areal uptake rate responses to increasing nutrient concentrations. However, unique to this study, urban streams had greater uptake velocities at ambient nutrient concentrations. These findings suggest that urban streams could have a greater capacity for nutrient uptake over a broad range of nutrient concentrations, but prior work indicates that this capacity may be constrained by the duration of the nutrient addition. As nutrient additions occur over a longer time period, the relative importance of hyporheic uptake can increase compared to main channel uptake. Yet, hydrologic and geomorphological conditions in urban streams may favor nutrient uptake within the main-channel, which in combination with increased autotrophic biomass may explain their high rates of nutrient uptake and similar uptake velocities when compared to non-urban streams during pulse addition experiments.

Introduction

Human activities throughout the past century have markedly altered nitrogen (N) and phosphorus (P) cycling, with 2 and 5-fold increases in global fluxes, respectively (Falkowski, 2000). These shifts in nutrient availability have changed patterns of nutrient limitation in aquatic systems (Turner et al., 2003; Elser et al., 2009; Glibert et al., 2011), which can alter food web structure and function (Tilman et al., 1982; Stelzer & Lamberti, 2001; Turner et al., 2003; Singer & Battin, 2007; Glibert, 2012). Elevated nutrient fluxes are largely a product of human population growth and landscape alterations to support that growth (Vitousek et al., 1997; Falkowski, 2000). Together, these actions have caused increased N and P export, contributing to the eutrophication of receiving waters (Vitousek et al., 1997; Howarth et al., 2002). As such, this nutrient loading is not spatially uniform, with substantial fluxes originating from anthropogenic land uses, such as urban areas (Puckett, 1995; Carpenter et al., 1998).

Urbanized watersheds contain varying proportions of impervious cover, which prevent water from percolating into soils and thereby generate abnormal quantities of overland flow (Klein, 1979; Feminella & Walsh, 2005; Konrad & Booth, 2005; Walsh et al., 2005). The runoff produced by impervious cover enters urban streams as rapid “pulse” inputs, contributing to the increased fluxes of N and P in these systems (Carpenter et al., 1998; Paul & Meyer, 2001). In addition to elevated nutrient loads, urbanization is known to generate flashier hydrographs, caused by those pulse additions of overland flow, as well as bank incision, reduced habitat quality, and biodiversity loss (Paul & Meyer, 2001; Wang et al., 2001; Roy et al., 2003; Allan, 2004; Meyer et al., 2005; Walsh et al., 2005). These symptoms have been collectively described as the “Urban Stream Syndrome” (Meyer et al., 2005). The consequences of this ecological condition have been shown to manifest when impervious cover constitutes as little as 10% of total watershed area (Paul & Meyer, 2001; Allan, 2004). This is especially problematic, as urban land use has been predicted to triple in area between the

years 2000 and 2030 (Seto et al., 2012), while globally, a majority of humans are now living in urban centers (United Nations, 2016).

Documenting stream functioning among urban and non-urban landscapes will help to further characterize and provide a regional context of the urban stream syndrome, which can benefit efforts to abate urban land use impacts on stream ecosystems (Kaushal & Belt, 2012; Kaushal et al., 2014; Booth et al., 2016). One measure of stream functioning is nutrient spiraling, which is a Lagrangian metric used to describe the simultaneous effects of biotic uptake, abiotic sorption, and the mineralization of nutrients (e.g. N and P) as they move downstream (Webster & Patten, 1979; Newbold et al., 1983). Nutrient spiraling methods allow for the quantification of uptake length (S_w ; m), areal uptake rate (U ; $\mu\text{g m}^{-2} \text{min}^{-1}$), and uptake velocity (V_f ; mm min^{-1}) (Stream Solute Workshop, 1990). Uptake length describes the distance a given nutrient molecule travels in the stream channel until it is removed by biotic or abiotic uptake. Areal uptake rate is used to describe the mass of nutrient retained by a given area of stream benthos in a given period of time. Uptake velocity is the speed at which a nutrient molecule moves towards the benthic environment, which is dependent on both areal uptake rate and nutrient concentrations. Uptake velocity is used to compare the efficiency of nutrient uptake among streams differing in nutrient concentrations (Covino et al., 2010).

Quantifying these processes allows for inter-stream comparisons of biogeochemical functioning, and responses to environmental disturbance at the ecosystem scale (Stream Solute Workshop, 1990; Mulholland et al., 2008; Valett et al., 2008; Von Schiller et al., 2008). Thus, assessments of nutrient spiraling can be used to illustrate the impacts of urbanization on stream ecosystem nutrient uptake (Grimm et al., 2005; Meyer et al., 2005; Mulholland et al., 2008). Prior studies have demonstrated greater uptake rates but reduced uptake velocities in urban streams (Grimm et al., 2005; Meyer et al., 2005; Mulholland et al., 2008) due to elevated nutrient concentrations. However, more recent work has found that forested streams experience rapid reductions of uptake velocity as a function of increasing nutrient concentrations, while urban streams have greater uptake

velocities at higher concentrations (Covino et al., 2012). Prior studies that have observed impaired nutrient uptake functioning in urban streams used constant rate additions of nutrients, which occur over longer time-periods than pulse additions, whereas Covino et al. (2012) found comparable nutrient uptake between urban and non-urban streams when using an instantaneous pulse addition of nutrients. This distinction is important, as hyporheic uptake is more dependent on longer nutrient residence times when compared to main-channel uptake (Weigelhofer et al., 2018). Urbanization can simultaneously reduce the effective size of the hyporheic zone (Bernhardt & Palmer, 2007), and increase autotrophic biomass in the main channel (O'Brien & Wehr, 2010). Thus, urban streams may be more efficient at taking up nutrients during pulse additions due to uptake by enhanced communities of periphyton in the main channel. Yet, there have been no published research efforts in urban streams using pulse additions since Covino et al. (2012), so there is currently insufficient evidence to determine whether urban and non-urban streams differ in their nutrient uptake capacity, specifically during pulse events.

Nutrient addition experiments are increasingly being conducted as pulse additions, using the Tracer Additions for Spiraling Curve Characterization (TASCC) method (Covino et al., 2010). This method has allowed for the assessment of nutrient limitation at the ecosystem scale based on individual and combined nutrient uptake responses of multiple, potentially co-limiting nutrients (Covino et al., 2010; Piper et al., 2017). Nutrient stoichiometry has been used to indicate the form of nutrient limitation experienced by primary producers (i.e. N vs. P limitation), and is known to affect ecosystem dynamics from the cellular to the whole ecosystem level (Tilman et al., 1982; Glibert, 2012; Bracken et al., 2015; Welti et al., 2017). There is a growing body of evidence that freshwater systems are commonly co-limited by N and P (Francoeur, 2001; Elser et al., 2007; Bracken et al., 2015). Urban streams, however, are known to be chronically loaded with proportionally more N than P (Paul & Meyer, 2001; Glibert, 2012; Kaushal & Belt, 2012). Increasing N:P ratios have been observed in a number of human dominated aquatic systems as a result of increased wastewater N, improved

wastewater P removal, and a ban on P containing detergents (Glibert et al., 2011). Nutrient spiraling studies have historically not considered potential nutrient interactions, such as the co-limitation of nutrient uptake, as the TASCC method has only recently facilitated multi-nutrient, nutrient spiraling studies (Schade et al., 2011; Gibson et al., 2015; Piper et al., 2017; Griffiths & Johnson, 2018; Tromboni et al., 2018). This has limited our understanding of how stoichiometric relationships affect ecosystem scale nutrient uptake and retention in streams. For example, the disproportionate loading of N relative to P in urban streams could increase the prevalence of P limitation of N uptake throughout the urban landscape, but this has not yet been investigated.

To date, no studies have been performed using the TASCC method in urban streams to estimate nutrient uptake metrics derived from the addition of multiple, potentially co-limiting nutrients. This work aims to compare nutrient uptake in urban vs. non-urban streams in order to test two hypotheses. I predict that high ambient N:P ratios in urban streams will result in P-limited conditions. In this scenario, co-additions of N and P will result in higher N uptake rates in comparison to additions of N alone. Second, I predict that urban streams will have greater areal uptake rates of N and P, but lower uptake velocities when compared to non-urban streams.

Materials and Methods

Study Area

Stream nutrient uptake metrics were determined using single and combined pulse additions of N and P in 6 streams within the Piedmont physiographic province of Virginia (Figure 1). Three streams were located in the Richmond metropolitan area (Reedy Creek, Rattlesnake Creek, and Broad Rock Creek), draining catchments comprised of more than 26% impervious cover (Table 1). All urban sites were in residential areas and displayed classic features of the urban stream syndrome, including; elevated N and chloride concentrations, incised banks, channelization, and riparian thinning. Bonbrook Creek, Holiday Creek, and Medely Branch were

selected as reference non-urban sites for this study. Each of these systems occur in nearby state forests and have catchments with more than 74% forested cover (Table 1). Bonbrook Creek, Holiday Creek, and Medely Branch each have fully enclosed forested channels. Reedy Creek occupies a concrete channel with no riparian cover and is therefore fully exposed to sunlight (Figure 1). Broad Rock Creek and Rattlesnake Creek both have riparian buffers of varying density; Rattlesnake Creek has a closed canopy throughout the study reach with the largest riparian buffer among urban sites, while the upper portion of Broad Rock Creek is surrounded by residential land use with relatively fewer trees compared to the lower reach, which is fully enclosed and forested (Figure 1). All sites occur within a 109 km radius of downtown Richmond.

Study reaches were selected to maximize similarities among system sizes, however, identifying 6 reaches with equivalent contributing watershed areas was impractical due to constraints to urban stream access and injection experiment suitability (i.e. piped inputs, accessibility, etc), and limited availability of suitable non-urban sites within Virginia's Piedmont. These constraints resulted in the selection of 3 sets of paired watersheds based on contributing watershed areas between the urban and non-urban sites (Table 1). Among these paired sites, reach lengths were established with the goal of producing similar median transit times (MTT), which describes the time required to achieve $\frac{1}{2}$ of the peak solute concentration (Runkel, 2002). This was done so that nutrient uptake metrics among paired urban and non-urban sites were derived from experiments that exposed the benthos to reactive solutes for similar periods of time.

Nutrient Injection Experiments

The Tracer Additions for Spiraling Curve Characterization (TASCC) method, which uses a pulse addition of solutes, allowed us to conduct 3 experiments (N addition, P addition and N+P addition) at a given site on the same day. Conducting the 3 experiments during the same day was desirable because we aimed to compare uptake metrics between injections to assess nutrient uptake and limitation, and therefore hoped to limit variability among ambient conditions (discharge, temperature, solar radiation, etc.). Experiments were

conducted during baseflow conditions, which was a challenge due to high precipitation during the period of study (August-December 2018; Supplementary Figs. 1 & 2). The TASCC method allows for the quantification of nutrient uptake metrics using a curve integrated approach whereby all samples collected during the nutrient pulse are used to derive a single set of metrics (see Tank et al., 2008; Covino et al., 2010). This approach also allows for the derivation of nutrient metrics from each sample collected during the passage of the nutrient pulse, thereby allowing for an examination of nutrient uptake kinetics (i.e., the relationship between uptake rate and concentration; see below).

Experimental Design

There were 2 sets of experiments conducted between August and December of 2018; 6 during the summer and 6 during the winter, in order to gather data across open and closed canopy conditions. During both seasons, there were 3 injections performed on a single date at each site: 2 individual injections (N, P), followed by a dual-nutrient injection (N+P). Injection experiments always proceeded in the following order: N, P, N&P. This resulted in 36 injection experiments with the potential to generate a suite of 48 spiraling metrics (for N alone, N with P, P alone and P with N) across sites and seasons. The P and N&P injections were initiated only after the stream returned to background conditions, as measured by specific conductivity, which was typically 1 hour after the last sample was taken during the previous injection. Injections were initiated at relatively similar times throughout each day across sites, accounting for changing seasonal light conditions, in an effort to limit inter-site variation in light intensity.

Field Methods

Measurement of Stream Physical Properties

Discharge was calculated using the velocity-area method at the head and base of each reach using a Hach portable velocity meter (FH950) before starting injection experiments. Discharge and average cross-

sectional velocity were used to select a reach length that would generate similar MTT's between paired urban and non-urban sites. These data were then used to estimate the quantity of non-conservative solutes (NCS) and conservative solutes (CS) needed for each slug addition (see below). Wetted width was characterized via 5 equidistant cross sections after the 3 injections had been completed at a given site, in order to limit disturbance of the benthos. Reach length was measured on site using a 100 m measuring tape and checked for consistency using line shapefiles collected at each site with a Trimble Geo 7x GPS. Reach lengths were kept constant between the summer and winter experiments in order to include the inherent differences in seasonal hydrology in the estimation of nutrient uptake metrics across sites.

Determining the Quantity of Added Nutrient

Potassium nitrate (KNO₃) and potassium phosphate (KH₂PO₄) were used as NCS, with sodium chloride (NaCl) serving as the CS. The quantities of NCS were determined with the goal of generating a peak concentration that was double the ambient nutrient concentration across all sites. The mass of CS added to the injectate was selected to maintain a detectable signal throughout the experiment without impacting biological uptake. Because these experiments were slug additions, and not continuous injections, which achieve a plateau concentration, an equation from Kilpatrick & Cobb (1985) was modified to estimate the mass of NCS and CS needed to achieve desired concentration increases (Eq. 1).

$$Eq. 1) S_{inj} = \left(\frac{T \times Q \times S_{amb} \times S_{Inc}}{M_{atomic}} \right) \times M_{molecular}$$

Where S_{inj} is the estimated mass of NCS or CS required to achieve the desired concentration increase, T is the estimated time to peak solute concentration, Q is discharge (L s⁻¹), S_{amb} is ambient solute concentration (µg L⁻¹), S_{Inc} represents the multiple required to achieve the desired concentration increase (i.e., 2x), M_{atomic} is the atomic mass of the chemical species in question (g mol⁻¹), and $M_{molecular}$ is the molecular weight of the NCS

molecule used in the injection (g mol^{-1}). Injections containing only the CS were conducted the day before nutrient experiments at each site, and conductivity data were used from these injections to estimate T for the nutrient additions that occurred the following day. Once equation 1 had been parameterized, quantities of NCS and CS were measured in the field using an Ohaus scale (CS200) and added to a discharge-dependent volume of stream water to form the injectate.

Sampling Design and Chemical Analysis

Immediately before each injection, 4 grab samples were collected to obtain ambient NCS and CS concentrations. These data were used to background correct the solute concentrations of grab samples gathered during each injection experiment. The change in concentration of CS as it moves past the base of a study reach is termed the breakthrough curve (BTC), which was monitored using specific conductivity readings from a YSI Multi Parameter Water Quality Sonde (6600EDS V2) and logged using a HOBO conductivity logger (U24-001). A minimum of 18 grab samples were collected at the base of the study reach throughout the BTC using pre-acid-washed 200 mL high-density polyethylene bottles. The pace of sampling was determined by monitoring specific conductivity readings and varied across sites based on hydrologic conditions. The objective was to collect 8 samples on the rising limb of the BTC, at least 2 samples at the peak, and 8 samples on the falling limb of the BTC. All grab samples were syringe filtered in the field using 25mm Whatman glass microfiber filters (934-AH), stored in 50 mL high-density polyethylene centrifuge tubes, placed on ice for transfer to the lab, and then frozen until analysis.

Equal emphasis was placed on sampling the rising and falling limbs because it has been proposed that the rising limb represents nutrient uptake in the thalweg, whereas the falling limb is indicative of nutrient uptake within transient storage zones (Trentman et al., 2015; Weigelhofer et al., 2018). Thus, nutrient uptake metrics derived from an equal-emphasis sampling approach to the rising and falling limbs should be representative of uptake throughout the system (Trentman et al., 2015). Intervals between samples were shorter during the rising

limb, whereas sampling intervals during the falling limb were longer, because of the differential transport between the thalweg and transient storage zones. Additionally, sampling the very beginning and end of the BTC was avoided due to variability in background grab sample solute concentrations, which tended to produce negative background corrected grab sample concentrations early or late in the BTC. All grab samples were analyzed using a Skalar San++ Automated Wet Chemistry Analyzer, with chloride, nitrate, and orthophosphate being analyzed using automated colorimetry (EPA 352.1, 353.2, 365.1).

Data Analysis

The BTC integrated method compares the ratio of total mass recovery between the NCS and CS relative to added masses of NCS and CS to develop a single integrated uptake metric for each injection. The TASCC method uses ratios of NCS and CS relative to the injectate ratio to derive uptake metrics for each grab sample. Deriving metrics across a gradient of nutrient concentrations provides sufficient data to characterize the relationship between nutrient uptake and concentration and to extrapolate the predicted uptake rate at ambient concentrations. These regressions between nutrient concentrations and uptake metrics are referred to as dynamic spiraling curves, and the uptake metrics derived from individual grab samples are described as dynamic uptake metrics (Covino et al., 2010).

BTC Integrated Approach

To account for variance in the grab sample chloride data, background corrected specific conductivity curves for individual experiments were regressed against chloride grab sample values for the corresponding injection. Outliers were identified and removed if cook's distance was $> \frac{4}{n}$ for a given point. Only those regressions with a significant relationship between chloride and specific conductivity ($p < 0.05$) were retained for analysis. These regressions were subsequently used to produce injection-specific corrected chloride concentration curves. Ambient chloride concentrations were inferred using the y-intercept of these regressions.

All equations used for the BTC integrated approach are contained in Table 2 while terms used in equations are defined in Table 3.

Among those experiments that had significant relationships between chloride and specific conductivity, nutrient uptake was initially assessed by determining the percent of chloride and nutrient that were recovered downstream relative to what was added upstream (Eq. 2). Nutrient uptake metrics, uptake length (S_w ; m), areal uptake rate (U ; $\mu\text{g m}^{-2} \text{min}^{-1}$), and uptake velocity (V_f ; mm min^{-1}) were estimated using methods similar to Ruggiero et al. (2006) and Tank et al. (2008), but adapted for a 1 station pulse addition. Uptake length of the added nutrient ($S_{w-add-int}$) was estimated by calculating the differences in tracer mass recovery between the NCS and CS, in relation to the total mass of added solutes (Eq. 3). From this, $U_{add-int}$ and $V_{f-add-int}$, which represent the areal uptake and uptake velocity of the added nutrients, were calculated using equations 4 & 5, where $NCS_{add-int}$ represents the concentration of added nutrient for which the integrated uptake metric is derived, and is calculated as the geometric mean of integrated observed and conservative NCS concentrations (i.e. $[NCS_{cons}]$; Eq. 6), using equation 7. $[NCS_{cons}]$ is the NCS concentration of a given grab sample if it had behaved conservatively (i.e. no uptake), which is based on observed CS concentrations for the corresponding grab sample. The geometric mean is used rather than the arithmetic mean, because taking the geometric mean of $[NCS_{add-obs}]$ and $[NCS_{cons}]$ provides a more representative estimate of the concentration of added NCS experienced throughout the study reach when compared to grab sample values (Covino et al., 2010).

TASCC Approach

All equations used to estimate uptake metrics with the TASCC method are contained in Table 4. To generate the data for dynamic spiraling curves, the natural log of NCS:CS ratios ($\mu\text{g NCS L}^{-1} [\mu\text{s cm}^{-1}]^{-1}$) for the injectate (i.e. $[NCS_{inj}]$, $[CS_{inj}]$) and each of the background corrected grab samples were regressed against reach length to determine longitudinal uptake rates ($K_{w-add-dyn}$), which were calculated by taking the slope of each regression for a given background corrected grab sample. Uptake length of the added nutrient ($S_{w-add-dyn}$)

was then calculated by taking the negative inverse of $K_{w\text{-add-dyn}}$ (Eq. 8), from which areal uptake of the added nutrient ($U_{\text{add-dyn}}$) was calculated using equation 9. $[NCS_{\text{add-dyn}}]$, calculated using equation 10, is the geometric mean of background corrected nutrient concentrations from grab samples ($[NCS_{\text{add-obs}}]$) and the concentration of the non-conservative solute if it had traveled conservatively (i.e. $[NCS_{\text{cons}}]$; Eq. 6). Uptake velocity of the added nutrient was then calculated using $U_{\text{add-dyn}}$ (Eq. 11). These data were then used to develop dynamic curves of S_w , U , and V_f as a function of total nutrient concentration ($[NCS_{\text{tot-dyn}}]$; Eq. 12).

While $[NCS_{\text{add-dyn}}]$ is used to estimate the concentration of added NCS experienced throughout a reach for an individual grab sample, $[NCS_{\text{tot-dyn}}]$ is inclusive of ambient conditions and is used to estimate the total NCS concentration experienced throughout a reach, rather than just the added nutrient concentration, for an individual grab sample. Only those data that met the previous inclusion criteria for the BTC-integrated approach were considered for TASCC, but additionally, only those regressions with a significant relationship between $S_{w\text{-add-dyn}}$ and $NCS_{\text{tot-dyn}}$ were analyzed using the TASCC method. Outliers were identified in these regressions and removed if cook's distance was $> \frac{4}{n}$ for a given point. For those regressions with significant slopes between $S_{w\text{-add-dyn}}$ and $NCS_{\text{tot-dyn}}$, ambient uptake length ($S_{w\text{-amb}}$) was estimated by back extrapolating to the y-intercept. U_{amb} and $V_{f\text{-amb}}$ were calculated using $S_{w\text{-amb}}$ with equations 13 and 14, respectively.

Ambient uptake length (i.e. $S_{w\text{-amb}}$) occurs where $[NCS_{\text{tot-dyn}}] = 0$, which is suggested to be equivalent to what has been previously referred to as the negative ambient NCS concentration (Payn et al., 2005). The negative ambient NCS concentration is used in plateau experiments, because back extrapolating to the y-intercept, which in traditional plateau experiments is the point at which $[NCS_{\text{add}}] = 0$ (i.e. $[NCS_{\text{amb}}]$), had been shown to consistently overestimate uptake length, and underestimate uptake rate when compared to isotopic tracer experiments (Dodds et al., 2002; Mulholland et al., 2002). This observed overestimation of $S_{w\text{-amb}}$ at $[NCS_{\text{add}}] = 0$ occurs because the addition of nutrients to a system affects biological and physical uptake, often times non-linearly (Payn et al., 2005). However, because the plateau derived expressions of net uptake length

and ambient uptake length are equal when $[NCS_{add}] = - [NCS_{amb}]$ (Eq. 15), ambient uptake length should be estimated at the negative ambient concentration, rather than the y-intercept, when using non-isotopic tracers (see Payn et al., 2005).

$$Eq. 15) S_{w-amb} = \frac{Q \times NCS_{amb}}{w \left(\frac{U_{max} \times NCS_{amb}}{C_{half} + NCS_{amb}} \right)} = S_{w-add} = \frac{Q \times NCS_{add}}{w \left(\left(\frac{U_{max}(NCS_{amb} + NCS_{add})}{C_{half} + (NCS_{amb} + NCS_{add})} \right) - \frac{U_{max} \times NCS_{amb}}{C_{half} + NCS_{amb}} \right)}, [NCS_{add}] = - [NCS_{amb}]$$

Because the TASCC method uses $[NCS_{tot-dyn}]$, which combines ambient and added NCS concentrations to estimate whole reach NCS concentrations, rather than the concentration of just the added nutrient, $[NCS_{tot-dyn}] = 0$ is taken to be equivalent to what Payn et al. (2005) referred to as the negative ambient NCS concentration.

Total dynamic uptake velocities and areal uptake rates were estimated if ambient uptake length was greater than 0, and were calculated by combining added and ambient metrics (Eqs. 16 & 17). A Michaelis-Menten (i.e. M-M) model was then used to further assess stream responses to nutrient additions for uptake velocity (Eq. 18). Total uptake velocities ($V_{f-tot-dyn}$) were used for M-M kinetic modelling because they are representative of nutrient uptake efficiency and can be compared across sites due to normalization by discharge, nutrient concentration, and wetted width (Earl et al., 2006; Ensign & Doyle, 2006; J. M. O'Brien et al., 2007). However, models were still applied to $U_{-tot-dyn}$ to compare areal uptake responses. Multiple models can explain areal uptake responses to nutrient additions (Rodríguez-Cardona et al., 2016; Weigelhofer et al., 2018), so M-M (Eq. 19), power (Eq. 20), and linear models (Eq. 21) were applied to each $U_{-tot-dyn}$ curve. M-M or power models were only used in favor of linear models if AIC (i.e. δ AIC) values had a difference of > 5 compared to the linear model and if r^2 values were > 0.1 when compared to the linear model for a specific injection.

Results

Physiochemical and Hydrologic Differences Across Sites and Seasons

Reach lengths were shorter among all non-urban sites relative to urban sites in order to obtain similar median transit times (i.e. MTT), due to higher reach-averaged water velocities in urban streams (Table 5). MTT's were not significantly different between urban and non-urban streams during injections for both the summer and winter (t-test; $p > 0.05$). For urban and non-urban streams, discharge and reach-averaged water velocity were greater and MTT was reduced in the winter when compared to the summer. There was less than a 10% difference between the means of wetted widths for urban and non-urban streams across seasons, and all sites had larger average wetted widths in the winter. Water temperatures were significantly higher in urban streams relative to non-urban streams throughout all experiments (t-test; $p < 0.05$), even though air temperatures were not significantly different between urban and non-urban streams in summer or winter (t-test; $p > 0.05$). Ambient N and Cl concentrations, as well as N:P ratios, were consistently higher in urban streams during the summer and winter compared to non-urban streams (Figure 2). However, ambient P concentrations were greater at two of the non-urban sites (Bonbrook Creek and Medely Branch) when compared to their paired urban sites (Broad Rock and Rattlesnake Creek, respectively). Ambient P was greater at Reedy Creek (urban) when compared to Holiday Branch (non-urban; Figure 2). Ambient N increased by 36% and ambient P decreased by 47%, on average, in urban streams between the summer and winter experiments. Non-urban streams experienced inconsistent shifts in ambient N and P concentrations from the summer to the winter. Changes in ambient Cl concentrations were variable between summer and winter in both urban and non-urban streams.

Nutrient Uptake

BTC Integrated Approach

Well-resolved break-through curves were obtained from all sites (Figure 3). However, in three experiments we were unable to obtain significant relationships between grab sample chloride concentrations

and specific conductivity (the winter N & P co-addition at Bonbrook Creek, summer P addition at Holiday Creek, and summer N & P co-addition at Broad Rock Creek; Figure 4; Supplementary Table 1; $p > 0.05$). Data from these experiments were not used in subsequent analyses. Additionally, summer nutrient uptake was not detected at Holiday Creek during the single addition of N, or for N and P during the co-addition experiment. In the winter, N uptake was not detected during the single N addition at Medely Branch, while N and P uptake were both not detected during the N and P co-addition at Holiday Creek (Figure 5). Thus, nutrient uptake data could not be calculated for 2 nutrients in urban streams and 3 nutrients in non-urban streams due to the lack of a relationship between chloride and specific conductivity, while uptake was not detected for an additional 6 nutrients in non-urban streams (Tables 6 & 7). Of those experiments where nutrient uptake detection was possible, meaning there was a significant relationship between chloride and specific conductivity, nutrient uptake was detected for all injections in urban streams, and for 71% of injections in non-urban streams. Five of the 6 non-urban experiments where uptake was not detected occurred at Holiday Branch.

TASCC Approach

For the experiments where nutrient uptake was detected through BTC integration, there were 17 dynamic spiraling curves where nutrient concentration and dynamic uptake length had a significant relationship (Table 8; $p < 0.05$), which indicates that estimation of ambient uptake length is appropriate. Of these curves, 9 were during urban injections, 6 in the winter, and 3 in the summer. There were 8 non-urban injections that produced significant relationships between nutrient concentration and uptake length; 5 during the summer, and 3 during the winter. Among these regressions, 11 produced positive ambient uptake lengths, and because total nutrient uptake metrics must be used for kinetic modelling, only 11 kinetic curves could be generated.

Urban and Non-Urban Stream Nutrient Uptake Metrics

BTC Integrated Approach

Mean areal uptake rate was greater in urban streams relative to non-urban streams for N and N-CO experiments, but neither urban or non-urban streams had consistently greater P areal uptake rates during single or co-additions across seasons (Figure 6). Mean non-urban stream areal uptake rate for N during single and co-additions was $17.85 \mu\text{g m}^{-2} \text{min}^{-1}$ (coefficient of variation (CV) = 176%), and $325.93 \mu\text{g m}^{-2} \text{min}^{-1}$ (CV = 64%) in urban streams (Figure 6). Mean non-urban stream areal uptake rate for P during single and co-additions was $31.62 \mu\text{g m}^{-2} \text{min}^{-1}$ (CV = 115%), and $34.62 \mu\text{g m}^{-2} \text{min}^{-1}$ (CV = 55%) in urban streams (Figure 6). Uptake velocity was significantly higher in urban streams relative to non-urban streams when considering data across seasons (Figure 7 ; Wilcoxon rank sum test; $p < 0.05$), and specifically during the summer (Wilcoxon rank sum test; $p < 0.05$), but not during the winter (Wilcoxon rank sum test; $p > 0.05$). However, both uptake velocity and areal uptake rates for N and P were greater in urban streams 68% of the time among comparable experiments (Tables 6 & 7). The mean of non-urban stream uptake velocity for N during single and co-additions was 0.41 mm min^{-1} (CV = 166%), and 0.52 mm min^{-1} (CV= 60%; Figure 7) in urban streams. Mean uptake velocity for P during single and co-additions in non-urban streams was 0.65 mm min^{-1} (CV = 98%), and 1.15 mm min^{-1} (CV= 42%; Figure 7) in urban streams. The CV for mean uptake velocities and areal uptake rates were consistently higher in non-urban streams when considering summer and winter separately (Figs. 6 & 7). In urban streams, areal uptake rate was greater for N relative to P during 5 of 6 single nutrient additions, and for all co-additions. However, P uptake velocities during single and combined P additions were consistently greater than N uptake velocities. This was not the case in non-urban streams, where greater areal uptake rate tended to correspond with greater uptake velocity. Mean uptake velocity and areal uptake rate increased for all nutrients in urban streams from the summer to the winter. In non-urban streams, all mean uptake velocities and areal uptake rates increased from summer to winter except for P during single P additions, which were greater in the summer.

Uptake velocity in Reedy Creek decreased for N and P during the summer co-addition experiment relative to the additions of N and P individually, but during the winter, N and P co-addition uptake velocities increased relative to single N and P additions, which implies co-limitation of nutrient uptake. Rattlesnake Creek also experienced contrasting seasonal responses to co-additions, but during different seasons, with an increase in uptake velocities for both N and P in response to the co-addition of nutrients during the summer, and a decrease in uptake velocity during the winter co-addition experiment. Uptake velocities increased during the co-addition of N and P in Medely Branch during the winter, while in the summer, N uptake velocity increased, and P uptake velocity decreased during the co-addition of N and P. The uptake velocity of P was enhanced in the presence of N during the winter Broad Rock co-addition, suggesting N limitation of uptake, while N uptake velocity was enhanced by the presence of P during the summer Holiday and Medely Branch co-additions, indicating P limitation. These patterns occur for areal uptake and uptake length as well, because areal uptake rate and uptake velocity are both derived from uptake length.

TASCC Approach

All urban ambient uptake velocities in the summer were negative (i.e. zero ambient uptake; Table 8), but the mean of dynamic uptake velocities of the added nutrient was significantly greater than zero for each of these experiments (t-test; $p < 0.05$). In non-urban streams, 2 out of 3 ambient uptake velocities were positive during the summer (Table 8). Ambient uptake velocities were positive for all urban sites during the winter, and for 3 out of 5 injections that occurred in non-urban sites during the winter (Table 8). Of these 3 experiments, the mean of dynamic uptake velocities for N during the co-addition at Bonbrook Creek was not significantly greater than zero (t-test; $p > 0.05$). There were only 2 comparable ambient uptake metrics across paired sites; winter ambient P uptake velocities and areal uptake rates were greater at both Reedy and Broad Rock Creek when

compared to winter ambient P uptake velocities and uptake rates at Holiday and Bonbrook Creek, respectively (Table 8). The mean of ambient uptake velocities was also greater in urban streams (Table 8).

M-M and linear models produced similar δ AIC and r^2 values when characterizing uptake velocity and areal uptake rate responses across most sites where total dynamic spiraling curves could be generated. M-M models better described responses for P uptake velocities during the co-addition at Broad Rock and for the single additions of P at Holiday Creek in the winter and Medely Branch in the summer (Supplementary Figure 3). Differences between δ AIC and r^2 values for all dynamic areal uptake rate curves were minimal, so linear models were used to describe each injection (Supplementary Figure 4). Total P uptake velocity during the winter single P injection at Holiday Creek produced a linear response to increasing nutrient concentrations, whereas Holiday Creek followed a M-M response (Supplementary Figure 3). Areal uptake rates during these injections had linear responses with similar slopes, although total dynamic areal uptake rates values were greater at Reedy Creek (Supplementary Figure 4). Because only 11 out of a possible 39 experiments could be used to generate total dynamic spiraling curves, added dynamic nutrient uptake metric curves were used in favor of total dynamic uptake curves for comparative analyses.

Co-limitation of nutrient uptake was clear only at Reedy Creek during the winter injection experiments (Figs 8 & 9). Winter P injections at Medely Branch showed that P uptake was enhanced by the presence of N (Figure 8), but co-limitation could not be assessed because more nutrient was recovered than chloride during the single injection of N (Figure 5). At Reedy Creek and Bonbrook Creek during the summer, and Rattlesnake Creek during the winter, single additions of N produced greater uptake metrics when compared to co-additions (Figure 9). Similarly, the summer single P addition at Medely Branch produced greater uptake metrics when compared to the co-addition experiment (Figure 8). Nutrient limitation inferred from areal uptake rates were consistent with those derived from uptake velocities (Supplementary Figs. 5 & 6).

Discussion

Data from summer and winter experiments in both urban and non-urban streams within Virginia's piedmont indicate that there was substantial inter-site and seasonal variability among the form of nutrient limitation. Co-limitation was confirmed only at Reedy Creek during the winter, and no form of nutrient limitation was sustained across seasons (Figs. 8 & 9). Urban and non-urban streams experienced reductions of uptake velocity and increased areal uptake rates for individual nutrients during certain co-additions, relative to single nutrient additions, indicating that nutrient demand had been reduced after single nutrient injections (Figs. 8 & 9). Also, in some cases, nutrient uptake metrics indicated that there was single N or P limitation of added nutrient uptake (Tables 6 & 7). The variability of both urban and non-urban uptake responses to single and co-additions of N and P, irrespective of consistently elevated urban N:P ratios (Figure 2), provides further evidence that N:P ratios are insufficient indicators of the form of nutrient limitation in streams at the ecosystem scale (Gibson & O'Reilly, 2012; Keck & Lepori, 2012; Tromboni et al., 2018).

The lack of correspondence between N:P ratios and the observed ecosystem responses to single and co-additions of nutrients is likely a function of the complex suite of biotic and abiotic factors that influence nutrient uptake in streams (Newbold et al., 1983; Stream Solute Workshop, 1990). For example, dissolved organic carbon (DOC) availability affects the nutrient uptake capacity of heterotrophic organisms in streams (Bernot & Dodds, 2005; Bernhardt & Likens, 2008; Gibson & O'Reilly, 2012; Rodríguez-Cardona et al., 2016; Stutter et al., 2018), and stream ecosystem responses to nutrient additions can differ depending on variations among the relative abundancies of heterotrophic and autotrophic organisms (Schade et al., 2011), which also vary within and between urban and non-urban streams (Chetelat et al., 1999; O'Brien & Wehr, 2010; Hassett et al., 2018). Additionally, hydro-morphological characteristics, such as streambed composition and the resultant capacity for P adsorption, could contribute to deviations from estimates of nutrient limitation inferred using N:P ratios (see Griffiths & Johnson, 2018). This is likely because N:P ratios and their relation to nutrient limitation were

developed through observations of the biological uptake of nutrients by oceanic phytoplankton (Redfield, 1958). Therefore, it is unsurprising that recent studies conducted in forested systems exhibited similar dynamic responses to single and combined nutrient additions across seasons when compared to expected limitation based on ambient N:P ratios (Griffiths & Johnson, 2018; Tromboni et al., 2018), while results from traditional nutrient diffusing substrate experiments in streams have also been shown to diverge from predictions made using N:P ratios (Capps et al., 2011). Prior studies have identified either N, P, or co-limitation of nutrient uptake across multiple sites (Schade et al., 2011; Gibson et al., 2015; Piper et al., 2017), but were constrained to summer experiments only. Taken together, these outcomes suggest that forested and urban streams are likely to experience varying forms of ecosystem-scale nutrient limitation of nutrient uptake across a broad range of spatiotemporal scales (Tromboni et al., 2018).

Areal uptake rates of N in urban streams were greater than non-urban streams (Figure 6), but this was driven by elevated N concentrations (Figure 2), and is representative of the differences in N availability between systems (Dodds et al., 2002). Conversely, areal uptake rates of P overlapped between these urban and non-urban streams because these systems have similar ambient concentrations of P (Figs. 2 & 6). Uptake velocity increased for most additions across sites during the winter when compared to the summer. This seasonal increase in uptake velocity was likely caused by greater water velocities, which reduces the size of the diffusive boundary layer in the benthos, allowing nutrient uptake to occur more readily (Ensign & Doyle, 2006). P uptake velocities were consistently greater than N uptake velocities during single and co-additions in urban streams, which indicates that these systems preferentially use P across seasons. Contrarily, non-urban streams showed elevated uptake velocities for P during the summer, but uptake velocity was variable across sites and nutrient additions during the winter (Figure 7). As a whole, urban stream uptake responses varied less when compared to non-urban streams, which suggests that elevated N concentrations in urban streams may have a homogenizing effect on nutrient uptake responses.

Results from the BTC-integrated method indicated that uptake velocities were greater in urban streams when compared to non-urban streams. However, BTC-integrated metrics are representative of cumulative uptake throughout a nutrient addition, whereas the TASC dynamic spiraling curves provided a characterization of stream responses to nutrient additions across the range of conditions experienced during the addition. Total areal uptake rates followed linear models across sites, indicating that nutrient uptake did not saturate during injections at both urban and non-urban streams (Supplementary Figure 4). Additions of N that co-occurred with P also produced linear P uptake responses in urban streams, suggesting that P uptake can be sustained irrespective of ambient or added N concentrations (Supplementary Figure 4). This was especially evident at Reedy Creek in the winter, where co-limitation of nutrient uptake occurred regardless of the elevated ambient N concentrations (Figs. 8 & 9). Urban stream total P uptake responses were also relatively similar when compared to the non-urban stream P dynamic areal uptake rates (Supplementary Figure 4), further indicating the potential for urban stream nutrient uptake to remain similar to non-urban streams regardless of persistent nitrate enrichment (Figure 2). Dynamic uptake responses during pulse additions in urban and non-urban streams have been observed to not follow saturation kinetics elsewhere (Covino et al., 2012), while nutrient concentrations have also previously been shown to be poor predictors of nutrient uptake dynamics (Covino et al., 2012; Gibson et al., 2015).

The greater integrated uptake velocities, reduced variability among integrated nutrient uptake metrics, and similar dynamic nutrient spiraling responses to increasing nutrient concentrations in urban streams relative to non-urban streams could represent a functional shift driven by the relative speeds at which typical additions of dissolved nutrients occur within these systems. Functional resilience has been observed in streams with chronically elevated nutrient concentrations (Covino et al., 2012; García et al., 2016), and there is a growing body of evidence demonstrating inter-site variability among how streams respond to nutrient additions that occur over different time scales (i.e. pulse vs. plateau). Weigelhofer et al., 2018 conducted a series of TASC

(i.e. pulse) and plateau nutrient additions in order to assess how oligotrophic, eutrophic, and poly-eutrophic streams respond differently to nutrient additions that occur for variable periods of time. Ambient N and P concentrations were at, or above, average N and P concentrations at the urban sites considered herein (see Weigelhofer et al., 2018; Figure 2), and trophic level was determined using the Trophienidex, which uses diatom communities to infer trophic state (Kelly et al., 2009). Weigelhofer et al., 2018 found evidence of increased uptake responses during 75% of pulse additions relative to plateau additions throughout their study sites. Conversely, in a comparison of pulse and plateau additions of P in streams with phosphorous concentrations lower than our non-urban sites, constant rate additions of nutrients resulted in significantly greater uptake rates (Álvarez et al., 2010). This provides further evidence for a potential shift in how nutrient uptake in streams with chronically elevated nutrient concentrations may respond differently to nutrient additions depending on the timescale through which the nutrient addition is made.

Differential responses to nutrient additions are also a function of biotic community compositions, and the suite of abiotic factors that influence nutrient retention, such as water residence time and adsorption (Weigelhofer et al., 2018). For example, urban streams typically have greater water velocities, reducing water residence times, and often experience riparian thinning, or have open canopies altogether (Walsh et al., 2005; Booth et al., 2016). Yet, urban streams commonly have greater periphyton biomass (O'Brien & Wehr, 2010), and light has been shown to be the dominant factor limiting nutrient uptake for primary producers when comparing streams across an urbanization gradient (Von Schiller et al., 2007). Streams that experience chronic nutrient loading have been observed to have greater uptake rates in the main channel relative to the hyporheic zone (Weigelhofer et al., 2018). Main channel nutrient uptake relies less on nutrient residence time than hyporheic uptake and is therefore likely to be more important in urban streams due to increased water velocities. Nutrient uptake in the main channel is also likely to be more important in urban streams due to enhanced periphyton biomass and the clogging of hyporheic pores through sedimentation, and, or, the concrete

channelization of streambeds. While main channel uptake may be the central pathway for nutrient retention in urban streams, microbial communities, which dominate the hyporheic zone, have demonstrated functional resilience to urban stressors across a gradient of urbanization in streams (Hassett et al., 2018), with greater nutrient areal uptake rates occurring in systems with higher nutrient concentrations without any indication of saturation of nitrate uptake (Niyogi et al., 2004; Ribot et al., 2013). The combined effect of enhanced autotrophic communities and heterotrophic functional resilience to urban disturbances could produce greater, or similar, ambient and dynamic uptake velocities and areal uptake rates during short term pulse events when compared to non-urban streams.

Nutrient uptake was detected less frequently among non-urban streams. The majority of experiments where nutrient uptake was not detected were located at Holiday Creek, but also during an injection at Medely Branch. Chloride recovery during the summer Holiday injections was consistent, but more nutrient was recovered relative to the chloride (Figure 5). There was less than a 4% decrease in background N concentrations between the single N and co-addition experiments, and although there was still no detectable uptake based on the recovery of nutrient relative to chloride, there was a 28% decrease in the quantity of N recovered downstream during the co-addition experiment. The transport of greater quantities of nutrient relative to chloride could indicate that Holiday Creek was experiencing net mineralization along the reach, but that N uptake during the summer co-addition of N and P may have been enhanced after the initial, single addition of P. Given the presence of a deep pool with decomposing woody debris, it is possible that this segment of Holiday Creek was a net source of nutrients during the summer. MTT during the winter additions at Medely Branch was lower than expected and was caused by morphological changes in the study reach. When comparing sampling points along each BTC between the single N, P, and co-addition experiments (Figure 3), the 1st sample was taken later during the single additions relative to the combined addition. This could explain the relatively lower tracer mass recoveries during the single additions, as those missed initial samples accounted for roughly 20% of

the specific conductivity increase. Still, when uptake was measured at Medley Branch, uptake velocity was relatively high. This site had many large deep pools, clear biofilms occurring among those pools, and a silty bottom. Each of these components could have contributed to the relatively high uptake metrics seen here.

Several ambient nutrient uptake metrics were less than zero, which indicated that there was no net nutrient uptake occurring at that length of reach under ambient conditions. However, during the addition of nutrients, each of these sites had significant, positive relationships between uptake length and nutrient concentration, and therefore are likely to be utilizing nutrients when experiencing ambient conditions. Negative ambient uptake lengths have been observed when using this method (Gibson et al., 2015; Rodríguez-Cardona et al., 2016), and typically occur as a function of back-extrapolating past observed nutrient concentrations to the y-intercept. This can be associated with large error in ambient uptake estimates (Gibson et al., 2015; Brooks et al., 2017). Furthermore, because a linear model is used to estimate ambient uptake length, which is then used to estimate areal uptake rate and uptake velocity, the cumulative error associated with the multiple measurements of discharge, wetted width, chloride, and nutrient concentrations are not accounted for. A recent Monte Carlo approach has been proposed for addressing this cumulative error and could potentially be used to better constrain estimates of ambient nutrient uptake metrics in streams with elevated ambient concentrations. TASCC has also been known to produce hysteresis, as a function of differential transport during the rising and falling limbs of pulse additions. This has been used to assess uptake in the main channel versus the hyporheic zone (Trentman et al., 2015; Rodríguez-Cardona et al., 2016; Weigelhofer et al., 2018). However, there are currently no generally accepted best practices for TASCC with regards to adjusting for these issues, and yet TASCC is growing in use due to the greater quantity of data points produced per experiment, and because of its capacity to estimate ambient uptake metrics. Experiments across a range of ecosystem types can help to promote a commonly accepted suite of guidelines for using this method. This has occurred with prior nutrient spiraling methods (Stream Solute Workshop, 1990) and should occur when practicable with TASCC.

The urban stream syndrome has increasingly fewer commonalities when compared across biomes (Brown et al., 2009; Booth et al., 2016), although it can be broadly characterized by physical, chemical, and biological degradation (Paul & Meyer, 2001; Walsh et al., 2005). As such, there is a growing paradigm that the effects of urbanization operate along a continuum (Kaushal & Belt, 2012; Kaushal et al., 2014; Booth et al., 2016). This work has provided additional support for a continuum of urban stream impacts, in that while there is ample evidence of urbanization negatively affecting nutrient uptake capacity in streams (Grimm et al., 2005; Meyer et al., 2005; Gibson & Meyer, 2007; Mulholland et al., 2008), these results demonstrate the potential for urban streams to experience resilience in their capacity to maintain uptake efficiency during pulse additions of nutrients and at ambient conditions based on greater ambient uptake metrics, larger integrated nutrient uptake metrics, and observed similarities between nutrient uptake velocities across the dynamic nutrient spiraling curves. However, earlier studies in urban streams that found reductions of nutrient uptake velocity used constant rate additions, which occur over longer timespans relative to pulse-based experiments, and do not reflect how an urban stream would experience typical nutrient additions. Thus, future research should utilize both constant rate and instantaneous pulse additions while considering autotrophic and heterotrophic communities and the relative importance of hyporheic and main channel nutrient uptake as a function of time. This, in turn, could have important implications for how we approach the management of pollutant loads, and the restoration of urban streams.

Tables

Table 1. Watershed contributing areas at sampling points, reach lengths, percent impervious cover, and forested areas, with coordinates for all sites.

Classification	Site	Contributing Area ha	Reach Length m	Impervious %	Forested %	Coordinates DMS
Urban	RS	305	251	26.2	3.7	37° 32' 50" N 77° 32' 40" W
	BR	704	222	43.2	6.2	37° 30' 53" N 77° 28' 30" W
	RD	1010	268	40.9	5.8	37° 29' 14" N 77° 26' 24" W
Non-Urban	MD	295	59	1.6	94.0	37° 09' 06" N 78° 20' 38" W
	BB	622	171	1.6	74.3	37° 31' 54" N 78° 13' 44" W
	HD	940	213	0.6	86.7	37° 25' 24" N 78° 39' 38" W

RS= Rattlesnake Creek, BR= Broad Rock Creek, RD= Reedy Creek, MD= Medely Branch, BB= Bonbrook Creek, HD= Holiday Creek.

Table 2. Equations used to determine breakthrough curve integrated metrics.

Variable	Equation	Number
Tracer Mass Recovery	$T_{mr} = \frac{Q \int_0^t [S_{add-obs}](t) dt}{S_{inj}} \times 100$	2
Integrated Uptake Length	$S_{w-add-int} = \frac{-L}{\ln \left(\frac{Q \int_0^t [NCS_{add-obs}](t) dt}{Q \int_0^t [CS_{add-obs}](t) dt} \right) - \ln \left(\frac{NCS_{inj}}{CS_{inj}} \right)}$	3
Integrated Areal Uptake Rate	$U_{add-int} = \left(\frac{Q \times [NCS_{add-int}]}{S_{w-add-int} \times w} \right) \times 60$	4
Integrated Uptake Velocity	$V_{f-add-int} = \frac{U_{add-int}}{[NCS_{add-int}]}$	5
Conservative Nutrient Concentration	$[NCS_{cons}] = \left(\frac{[NCS_{inj}]}{[CS_{inj}]} \right) \times [CS_{add-obs}]$	6
Integrated Nutrient Concentration	$[NCS_{add-int}] = \sqrt{\frac{Q \int_0^t [NCS_{add-obs}](t) dt}{\int_0^t Q(t) dt} \times \frac{Q \int_0^t [NCS_{cons}](t) dt}{\int_0^t Q(t) dt}}$	7

Table 3. Definitions of terms used in breakthrough curve integrated (BTC_{-int}) and TASCC equations.

Term	Definition	Units
Q	Discharge	L s ⁻¹
w	Reach averaged wetted width	m
S _{-add-obs}	Observed grab sample concentrations for either NCS or CS, used to calculate tracer mass recovery	µg L ⁻¹
S _{-inj}	Mass of either NCS or CS added, used to calculate tracer mass recovery	µg
NCS _{-inj}	Mass of non-conservative solute injected	µg
CS _{-inj}	Mass of conservative solute injected	µg
NCS _{-add-int}	Added non-conservative solute recovered downstream through breakthrough curve integration	µg
CS _{-add-int}	Added conservative solute recovered downstream through breakthrough curve integration	µg
NCS _{-add-obs}	Background corrected concentration of added non-conservative solute for each grab sample	µg L ⁻¹
CS _{-add-obs}	Background corrected concentration of added conservative solute for each grab sample	µg L ⁻¹
NCS _{-tot-obs}	Total (i.e. non-background corrected) non-conservative solute concentrations for each grab sample	µg L ⁻¹
CS _{-tot-obs}	Total (i.e. non-background corrected) conservative solute concentrations for each grab sample	µg L ⁻¹
NCS _{-amb}	Ambient concentrations of non-conservative solutes	µg L ⁻¹
CS _{-amb}	Ambient concentrations of conservative solutes	µg L ⁻¹
NCS _{-con}	Grab sample concentrations of non-conservative solutes if they behaved conservatively (i.e. no uptake)	µg L ⁻¹
NCS _{-add-dyn}	Concentration of added non-conservative solute experienced throughout the stream reach for individual grab samples	µg L ⁻¹
NCS _{-tot-dyn}	Concentration of total (i.e. added + ambient) non-conservative solute experienced throughout the stream reach for individual grab samples	µg L ⁻¹
K _{-w-add-dyn}	Longitudinal uptake rate of the added nutrient for each grab sample	L ⁻¹
U _{-max}	Maximum areal uptake rate used to constrain M-M kinetic model	µg m ⁻² min ⁻¹
K _m	Half saturation constant used to constrain M-M kinetic model	µg L ⁻¹
a	Constant used to constrain power model for areal uptake rate	NA
p	Constant exponent used to constrain power model for areal uptake rate	NA
m	Slope of linear kinetic model for areal uptake rate vs. NCS	mm min ⁻¹
b	Intercept of linear kinetic model for areal uptake rate	µg m ⁻² min ⁻¹

Table 4. Equations used to determine TASCC uptake metrics.

Variable	Equation	Number
Conservative Nutrient Concentration	$[NCS_{cons}] = \left(\frac{[NCS_{inj}]}{[CS_{inj}]} \right) \times [CS_{add-obs}]$	6
Dynamic Added Uptake Length	$S_{w-add-dyn} = \frac{-1}{K_{w-add-dyn}} = \frac{-L}{\ln \left(\frac{[NCS_{tot-obs}] - [NCS_{amb}]}{[CS_{tot-obs}] - [CS_{amb}]} \right) - \ln \left(\frac{[NCS_{inj}]}{[CS_{inj}]} \right)}$	8
Dynamic Added Areal Uptake Rate	$U_{add-dyn} = \left(\frac{Q \times [NCS_{add-dyn}]}{S_{w-add-dyn} \times w} \right) \times 60$	9
Dynamic Added Nutrient Concentrations	$[NCS_{add-dyn}] = \sqrt{[NCS_{add-obs}] \times [NCS_{cons}]}$	10
Dynamic Added Uptake Velocity	$V_{f-add-dyn} = \frac{U_{add-dyn}}{[NCS_{add-dyn}]}$	11
Dynamic Total Nutrient Concentration	$[NCS_{tot-dyn}] = \sqrt{[NCS_{tot-obs}] \times ([NCS_{cons}] + [NCS_{amb}])}$	12
Ambient Areal Uptake Rate	$U_{amb} = \left(\frac{Q \times [NCS_{amb}]}{S_{w-amb} \times w} \right) \times 60$	13
Ambient Areal Uptake Velocity	$V_{f-amb} = \frac{U_{amb}}{[NCS_{amb}]}$	14
Dynamic Total Uptake Velocity	$V_{f-tot-dyn} = V_{f-add-dyn} + V_{f-amb}$	16
Dynamic Total Areal Uptake Rate	$U_{-tot-dyn} = U_{-add-dyn} + U_{-amb}$	17
Uptake Velocity M-M Model	$V_{f-tot-dyn} = \frac{U_{max}}{K_m + NCS_{tot-dyn}}$	18
Areal Uptake Rate M-M Model	$U_{-tot-dyn} = \frac{U_{max} \times NCS_{tot-dyn}}{K_m + NCS_{tot-dyn}}$	19
Power Model	$U_{-tot-dyn} = aNCS_{-tot-dyn}^P$	20
Linear Model	$U_{-tot-dyn} = m \times NCS_{tot-dyn} + b$	21

Table 5. Median transit time (MTT), discharge (Q), mean water velocity (V), mean wetted width (W), mean water temperature (WT), and mean air temperature (AT) during experiments.

Class	Site	Date	MTT MM:SS	Q L s ⁻¹	V m s ⁻¹	W m	WT C°	AT C°
Urban	RD	27-Aug	23:50	10	0.19	3.23	28.0	32.6
		10-Jan	13:02	31	0.34	5.37	5.9	1.9
	BR	30-Aug	48:34	10	0.08	3.17	25.7	32.7
		7-Dec	29:31	32	0.13	3.65	7.3	6.5
	RS	4-Oct	33:16	12	0.13	3.20	21.9	30.4
		21-Nov	21:28	26	0.19	3.92	10.3	10.0
Non-Urban	HD	3-Oct	20:45	77	0.17	4.16	20.2	29.7
		11-Jan	14:53	125	0.24	4.39	3.1	2.7
	BB	6-Sep	51:19	10	0.06	4.23	24.2	33.0
		8-Dec	23:57	28	0.12	4.91	4.0	3.3
	MD	7-Oct	31:55	2	0.03	1.17	21.7	29.5
		6-Dec	7:40	8	0.13	2.26	4.2	3.7

Table 6. Results from summer injection experiments showing Uptake lengths ($S_{w\text{-add-int}}$), areal uptake rates ($U_{\text{add-int}}$), and uptake velocities ($V_{f\text{-add-int}}$), calculated using the breakthrough curve integrated approach. N.D. indicates that uptake was not detected, while CL represents that there was not a significant relationship between chloride and specific conductivity.

Site	Class	Injection	$S_{w\text{-add-int}}$ m	$U_{\text{add-int}}$ $\mu\text{g m}^{-2} \text{min}^{-1}$	$V_{f\text{-add-int}}$ mm min^{-1}
Reedy Creek	Urban	N	428	422	0.43
		N-Co	534	381	0.35
		P	216	67	0.86
		P-Co	298	43	0.62
Broad Rock Creek	Urban	N	714	132	0.27
		N-Co	CL	CL	CL
		P	518	8	0.37
		P-Co	CL	CL	CL
Rattlesnake Creek	Urban	N	437	261	0.53
		N-Co	373	254	0.62
		P	286	12	0.81
		P-Co	211	14	1.09
Holiday Creek	Non-Urban	N	N.D.	N.D.	N.D.
		N-Co	N.D.	N.D.	N.D.
		P	CL	CL	CL
		P-Co	N.D.	N.D.	N.D.
Bonbrook Creek	Non-Urban	N	513	22	0.28
		N-Co	5037	2	0.03
		P	319	34	0.45
		P-Co	706	10	0.20
Medely Branch	Non-Urban	N	981	3	0.12
		N-Co	688	4	0.17
		P	67	65	1.77
		P-Co	292	11	0.40

Table 7. Results from winter injection experiments showing Uptake lengths ($S_{w-add-int}$), areal uptake rates ($U_{add-int}$), and uptake velocities ($V_{f-add-int}$), calculated using the breakthrough curve integrated approach. N.D. indicates that uptake was not detected, while CL represents that there was not a significant relationship between chloride and specific conductivity.

Site	Class	Injection	$S_{w-add-int}$ m	$U_{add-int}$ $\mu\text{g m}^{-2} \text{min}^{-1}$	$V_{f-add-int}$ mm min^{-1}
Reedy Creek	Urban	N	25998	2	0.01
		N-Co	503	269	0.70
		P	294	25	1.19
		P-Co	270	29	1.30
Broad Rock Creek	Urban	N	469	707	1.12
		N-Co	786	504	0.67
		P	313	56	1.69
		P-Co	250	50	2.11
Rattlesnake Creek	Urban	N	794	539	0.50
		N-Co	3538	100	0.11
		P	291	42	1.35
		P-Co	302	33	1.30
Holiday Creek	Non-Urban	N	1712	20	0.99
		N-Co	N.D.	N.D.	N.D.
		P	1659	26	1.03
		P-Co	N.D.	N.D.	N.D.
Bonbrook Creek	Non-Urban	N	508	40	0.68
		N-Co	CL	CL	CL
		P	1062	13	0.32
		P-Co	CL	CL	CL
Medely Branch	Non-Urban	N	N.D.	N.D.	N.D.
		N-Co	97	104	2.19
		P	339	37	0.63
		P-Co	127	119	1.67

Table 8. Ambient Uptake lengths (S_{w-amb}), areal uptake rates (U_{-amb}), and uptake velocities (V_{f-amb}), for experiments that had significant $S_{w-add-int}$ versus $NCS_{-tot-dyn}$ regressions (i.e. $p < 0.05$), and their r^2 values. Dashes represent those experiments that generated negative ambient uptake metrics, which indicates that ambient nutrient uptake was not detected.

Class	Season	Site	Injection	r^2	S_{w-amb} m	U_{-amb} $\mu\text{g m}^{-2} \text{min}^{-1}$	V_{f-amb} mm min^{-1}
Urban	Summer	RD	N	0.81	-	-	-
		RD	N-CO	0.87	-	-	-
		RS	P-CO	0.82	-	-	-
	Winter	RD	P	0.54	64	85	5.52
		RD	P-CO	0.59	52	147	6.73
		BR	P	0.6	100	44	5.26
		BR	P-CO	0.89	36	155	14.75
		RS	N	0.69	185	3162	2.12
		RS	P	0.38	158	46	2.50
	Non-Urban	Summer	BB	N	0.57	295	31
BB			N-CO	0.42	-	-	-
MD			P	0.60	15	224	7.88
Winter		HD	P	0.48	506	10	3.37
		BB	N	0.57	-	-	-
		BB	P	0.61	-	-	-
		MD	N-CO	0.84	51	7	4.21
MD	P-CO	0.50	87	63	2.43		

Figures

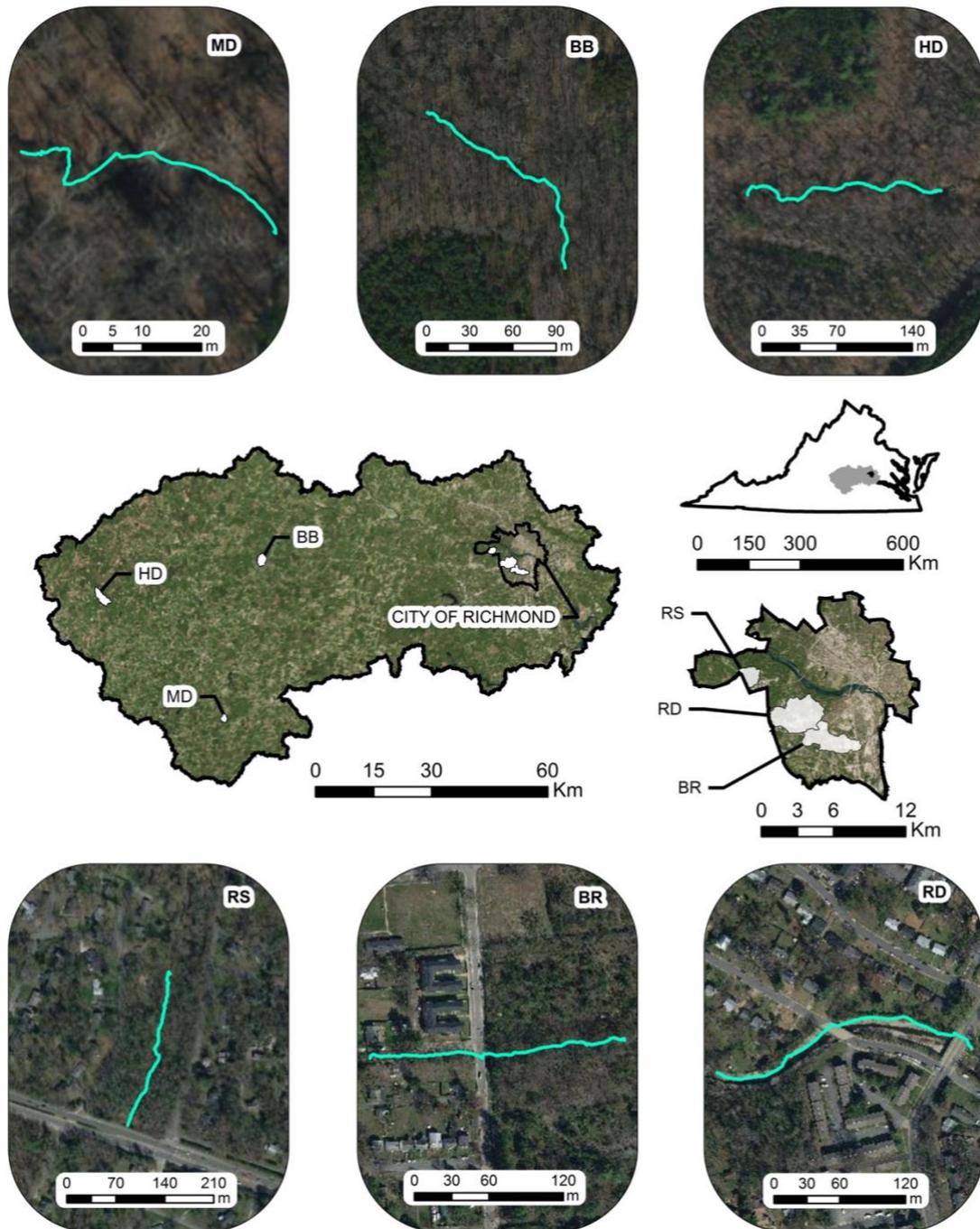


Figure 1. Continuous line shapefiles delineating reaches used for nutrient uptake experiments among urban (i.e. RS, BR, RD) and non-urban (MD, BB, HD) streams, including spatial context for watersheds within Virginia and the City of Richmond. RS= Rattlesnake Creek, BR= Broad Rock Creek, RD= Reedy Creek, MD= Medely Branch, BB= Bonbrook Creek, HD= Holiday Creek.

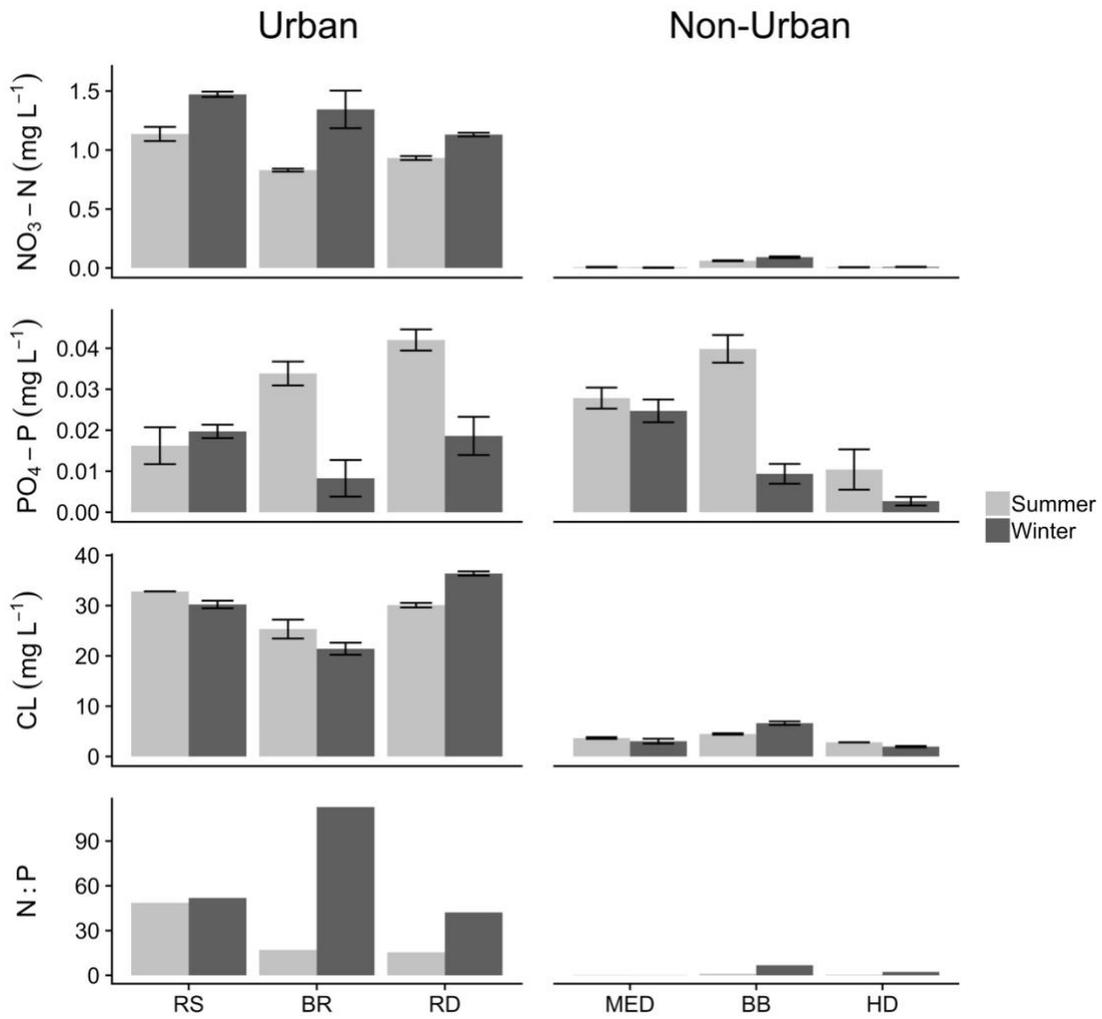


Figure 2. Ambient NO₃-N, PO₄-P, N:P molar ratios, and Cl concentrations with standard errors in urban and non-urban streams across summer and winter experiments.

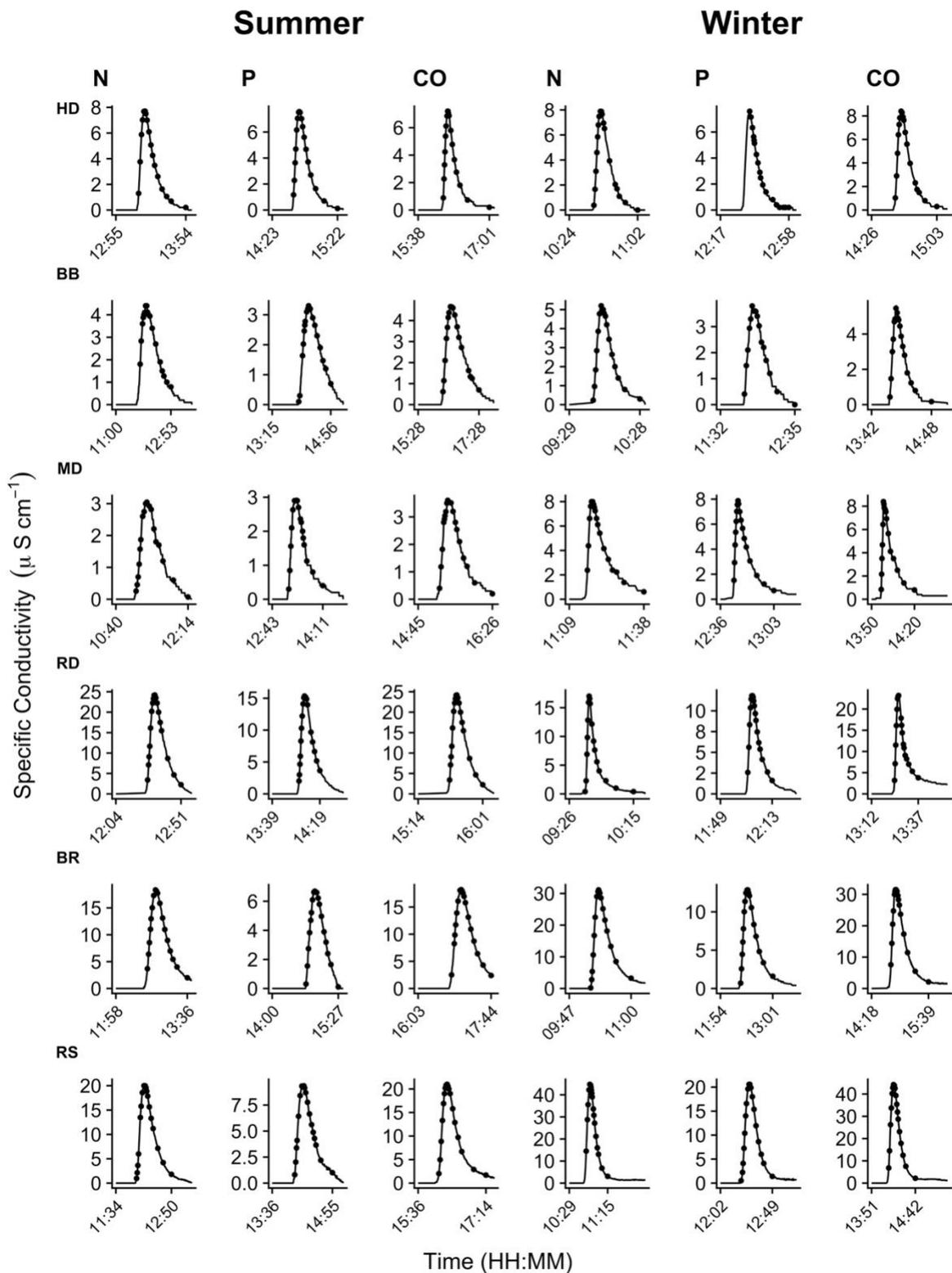


Figure 3. Background corrected specific conductivity breakthrough curves for each experiment across seasons during N, P, and combined (CO) additions of N and P during the summer and winter. The first time represents when the injection occurred, and the second time is when the last sample was taken.

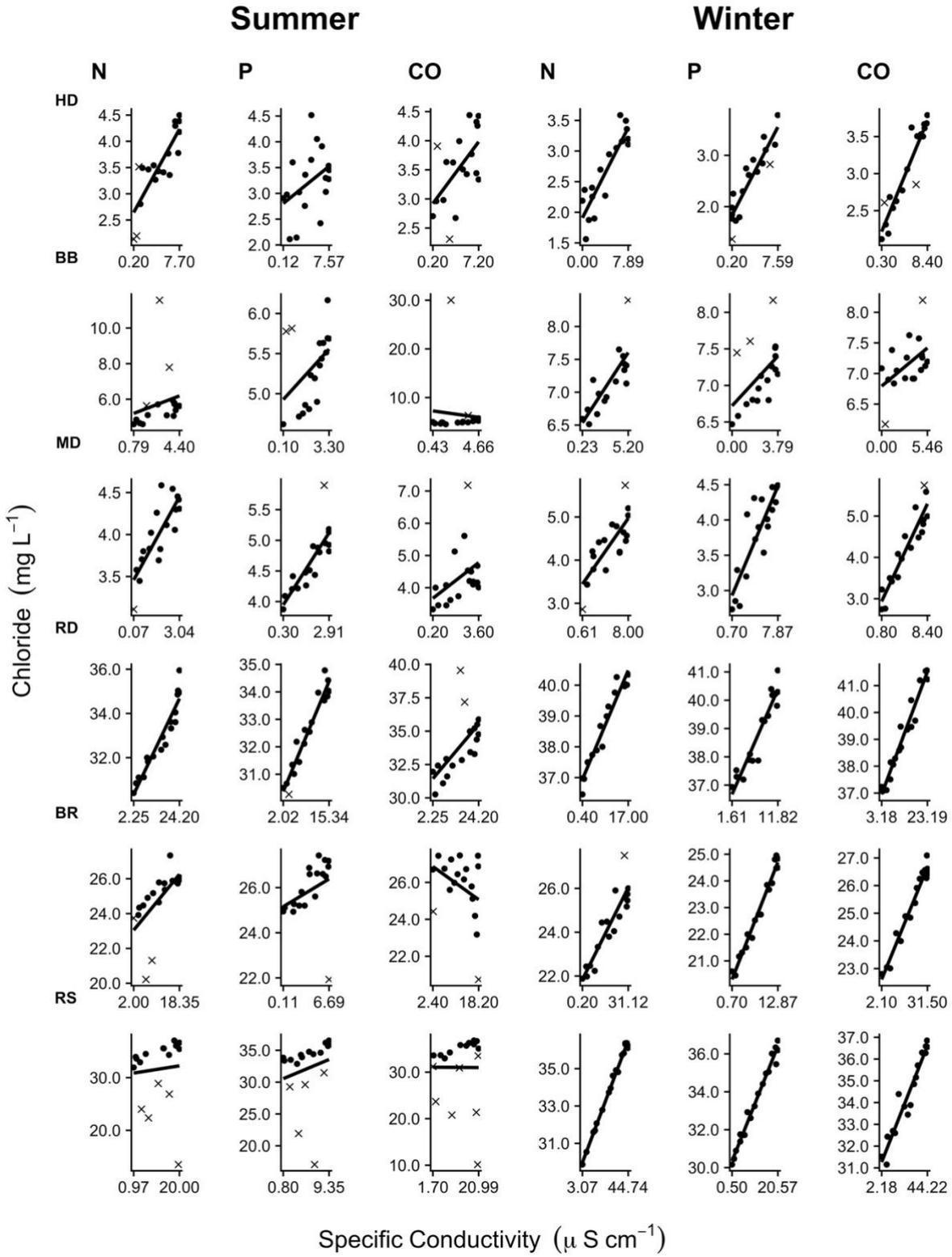


Figure 4. Linear models between background corrected specific conductivity (Figure 3) and grab sample chloride concentrations for N, P, and combined (CO) additions of N and P during the summer and winter. R² and P-values are included in Supplementary Table 1.

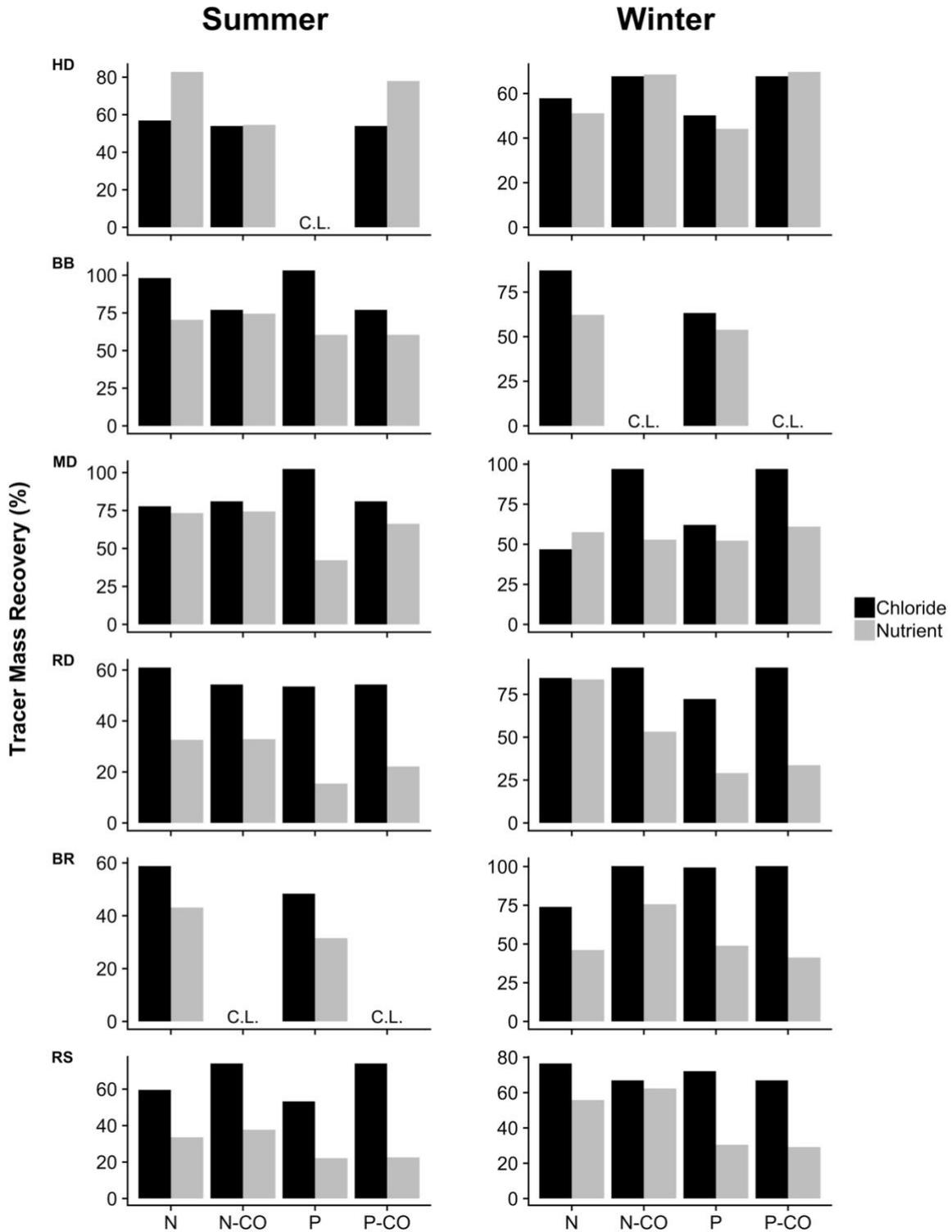


Figure 5. Tracer mass recovery of chloride and the added nutrient as a percentage of the mass of tracer added for N during single additions (N) of N, N during the co-conditions of N and P (N-CO), P during single additions of P (P), and P during the co-conditions of N and P (P-CO). C.L. indicates that there was no significant relationship between specific conductivity and chloride concentrations.

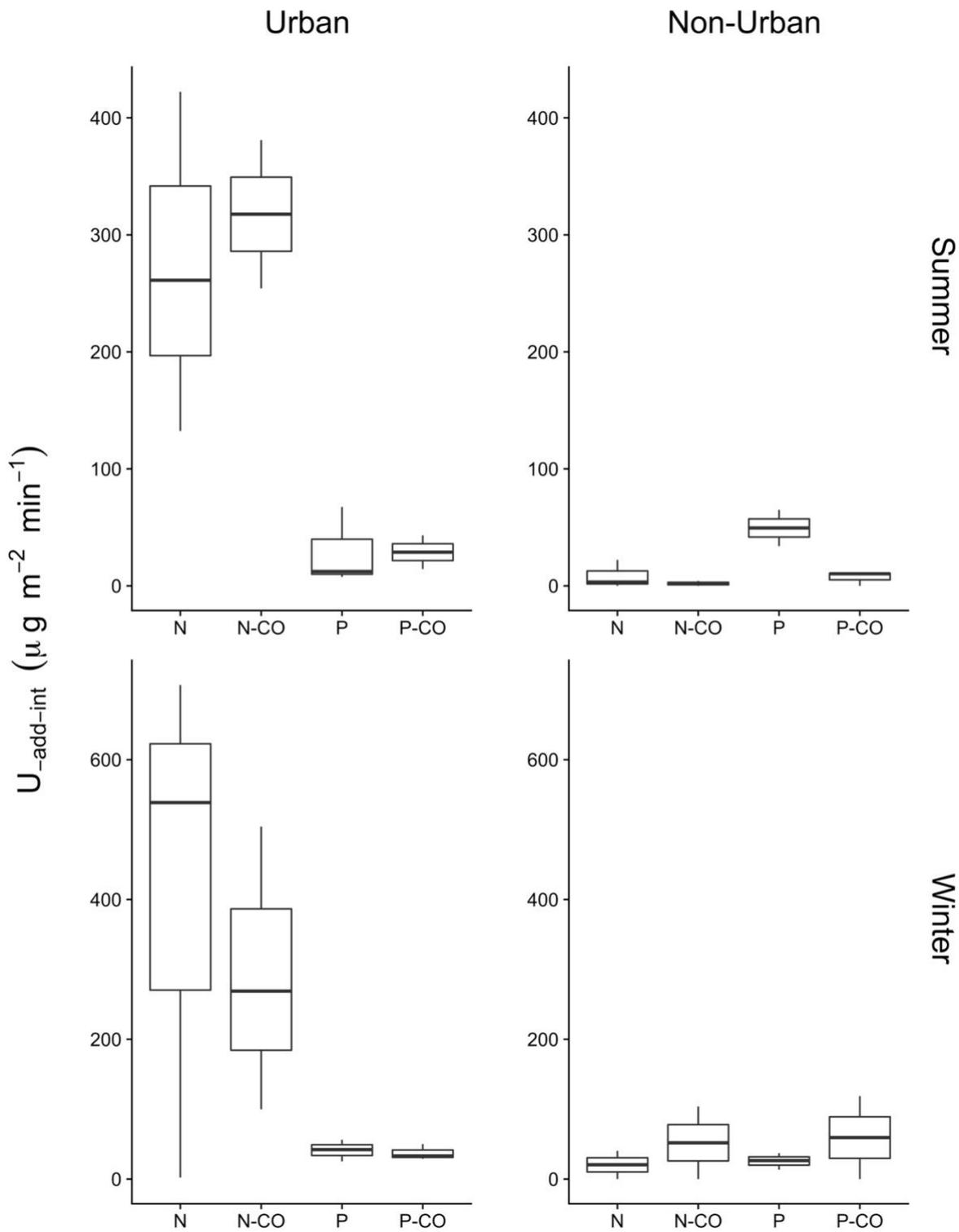


Figure 6. Areal uptake rates ($U_{\text{-add-int}}$) calculated using the breakthrough curve integrated approach, for single additions of N, -additions of N with P (N-CO), single additions of P, and co-additions of P with N (P-CO).

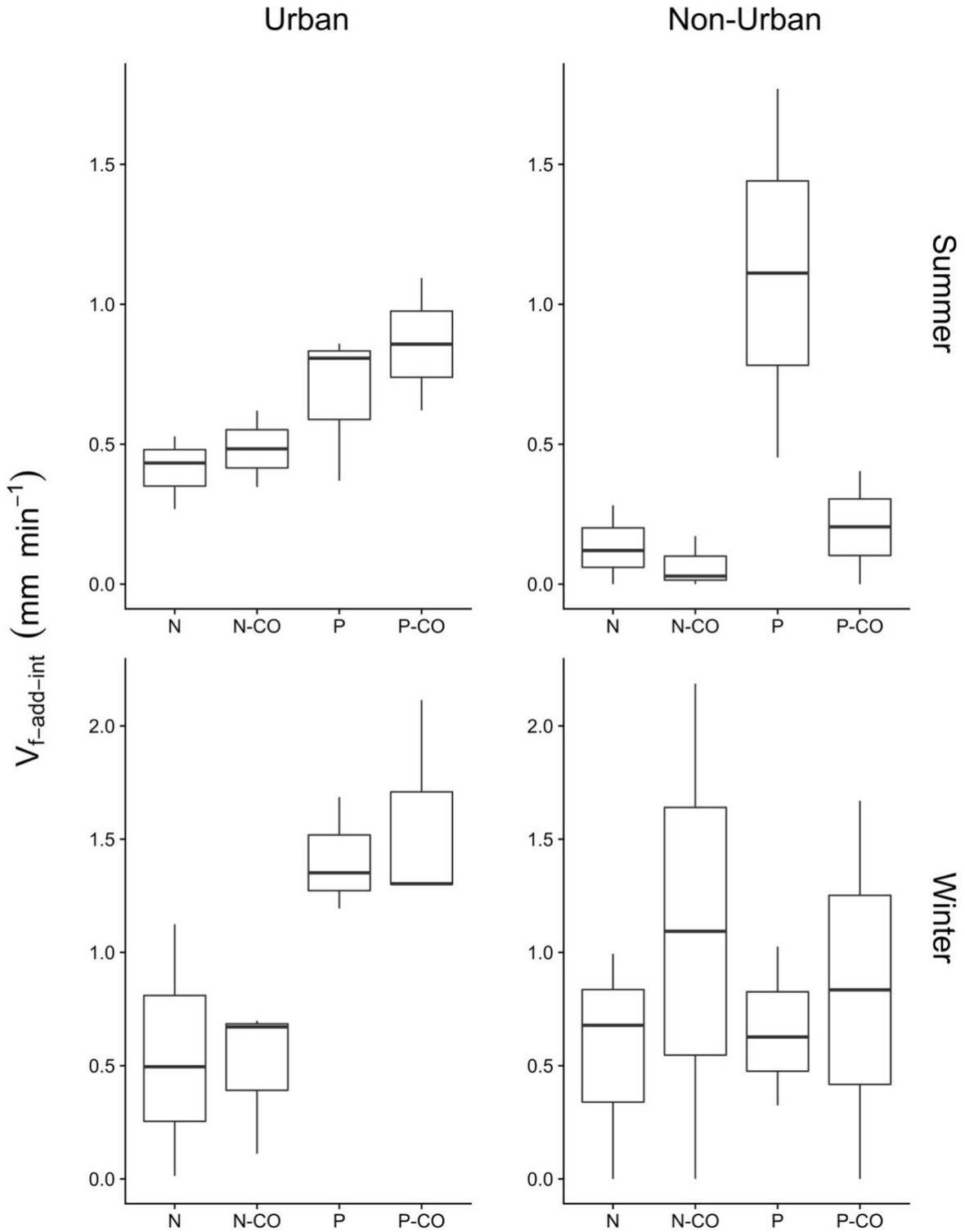


Figure 7. Uptake velocities ($V_{f-add-int}$) of added nutrients calculated using the breakthrough curve integrated approach, for N during single additions (N) of N, N during the co-additions of N and P (N-CO), P during single additions of P (P), and P during the co-addition of N and P (P-CO).

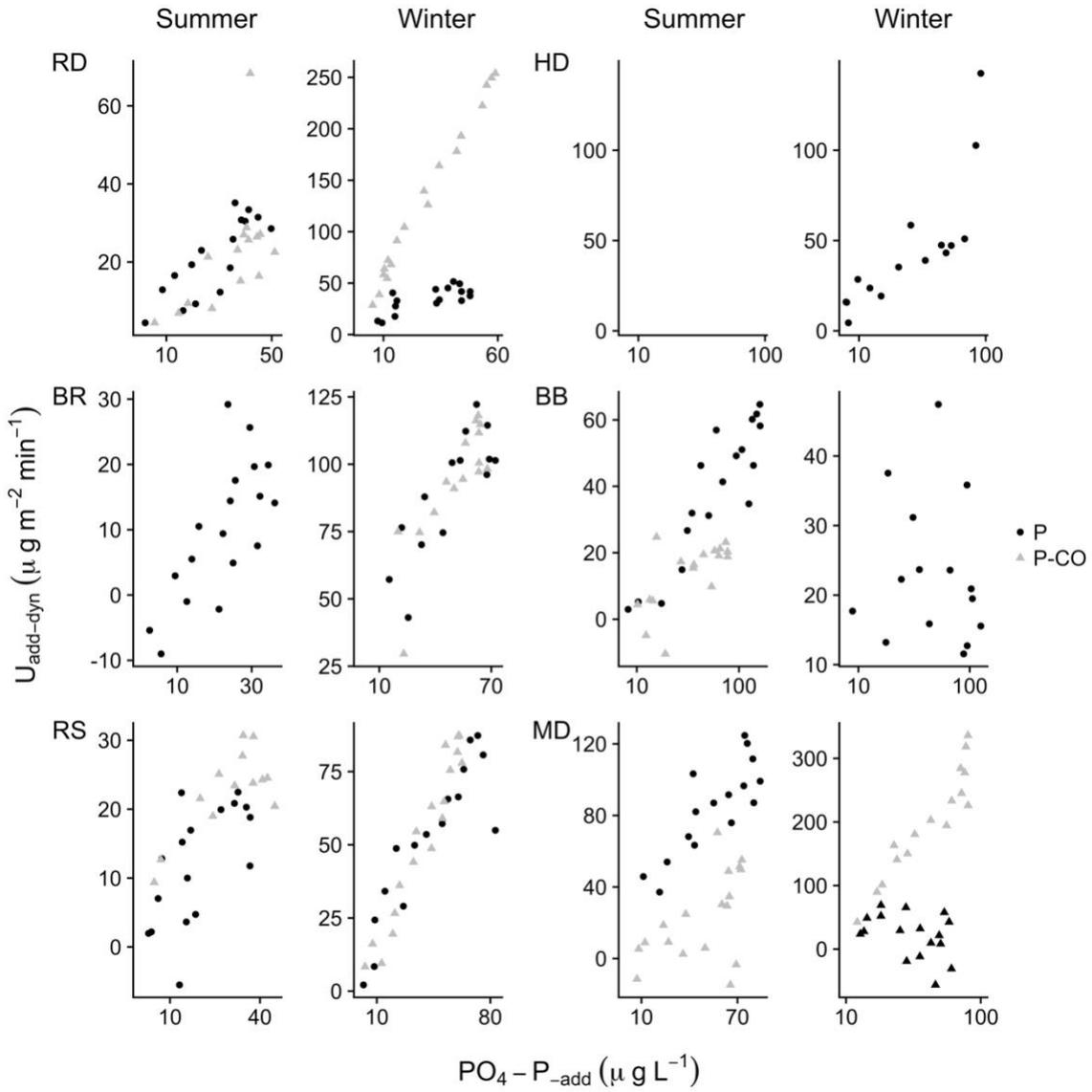


Figure 8. Relationships between areal uptake rates of the added nutrient and added nutrient concentrations for phosphorus injection experiments.

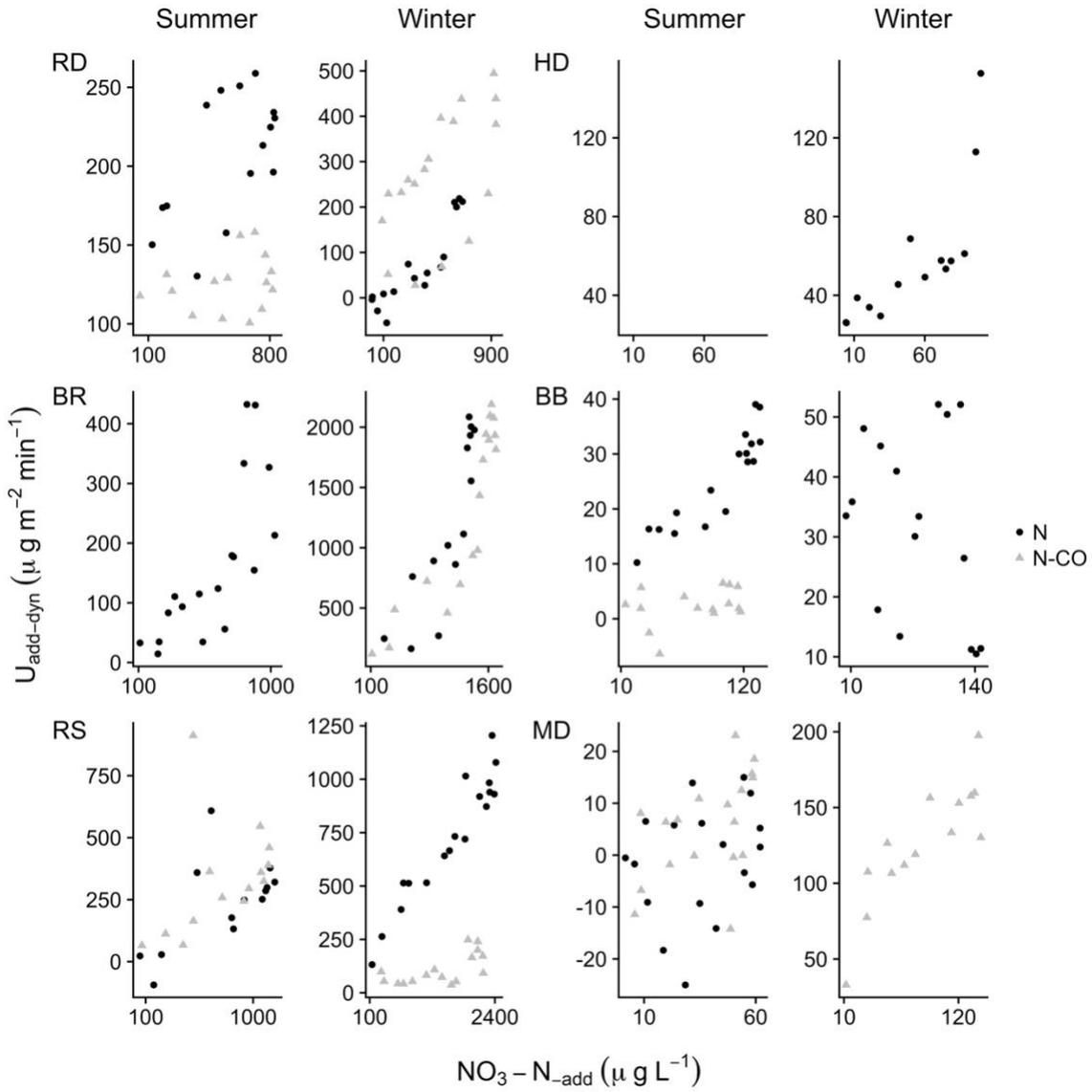


Figure 9. Relationships between areal uptake rates of the added nutrient and added nutrient concentrations for nitrogen injection experiments.

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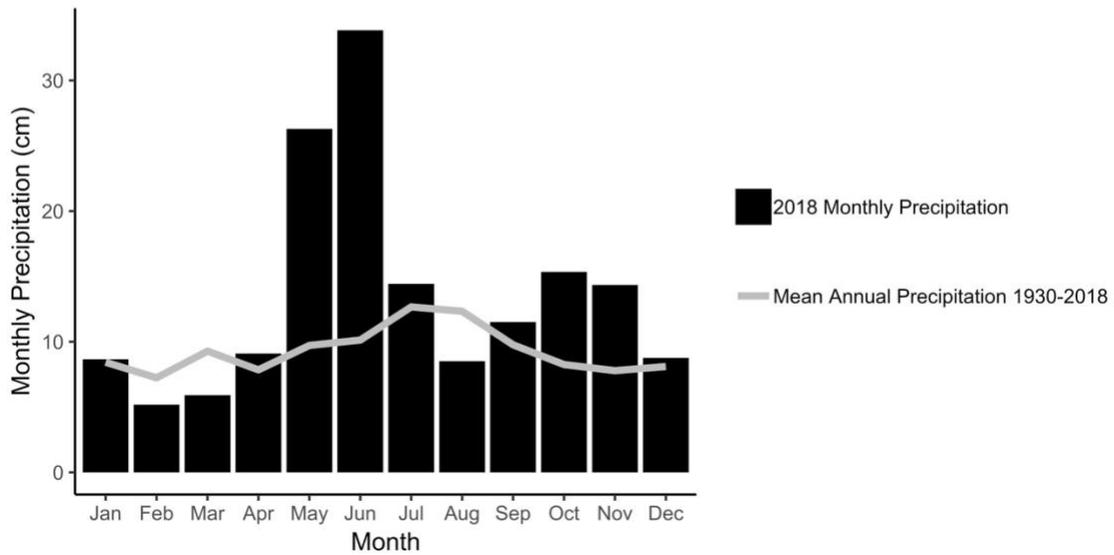
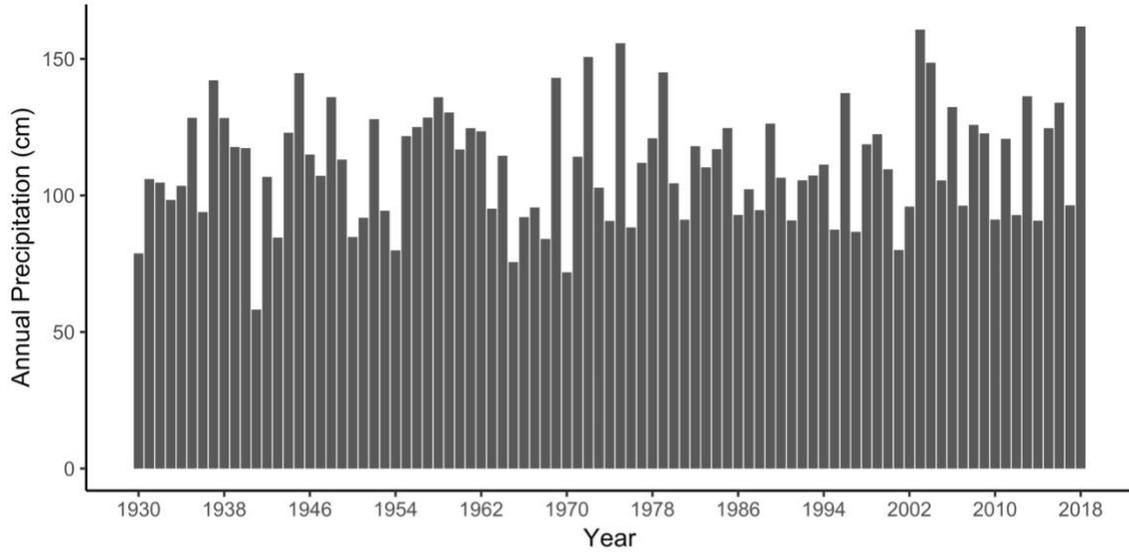
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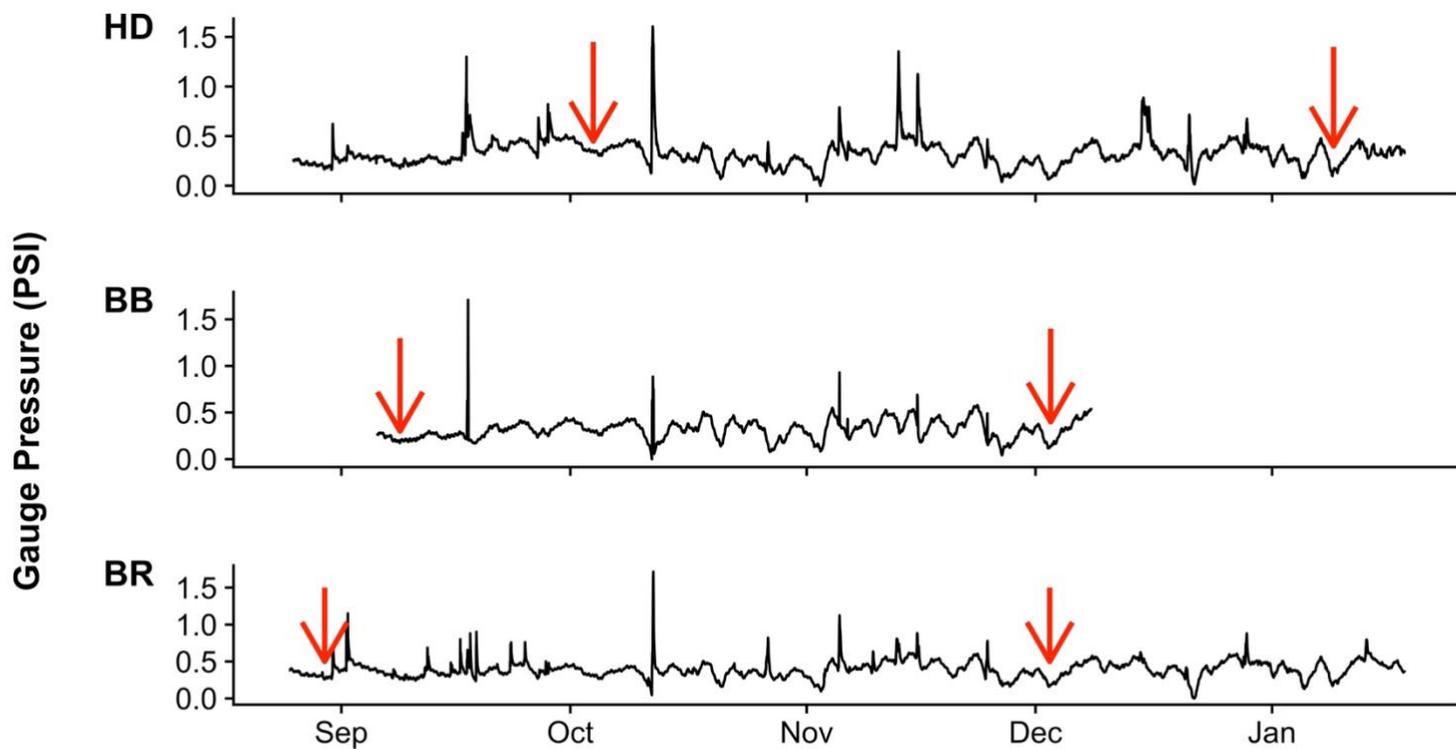
Supplementary Materials

Supplementary Table 1. P- and r^2 values for regressions between observed conductivity and measured chloride concentrations for single additions of N and P and co-additions of N and P (CO) during the summer and winter.

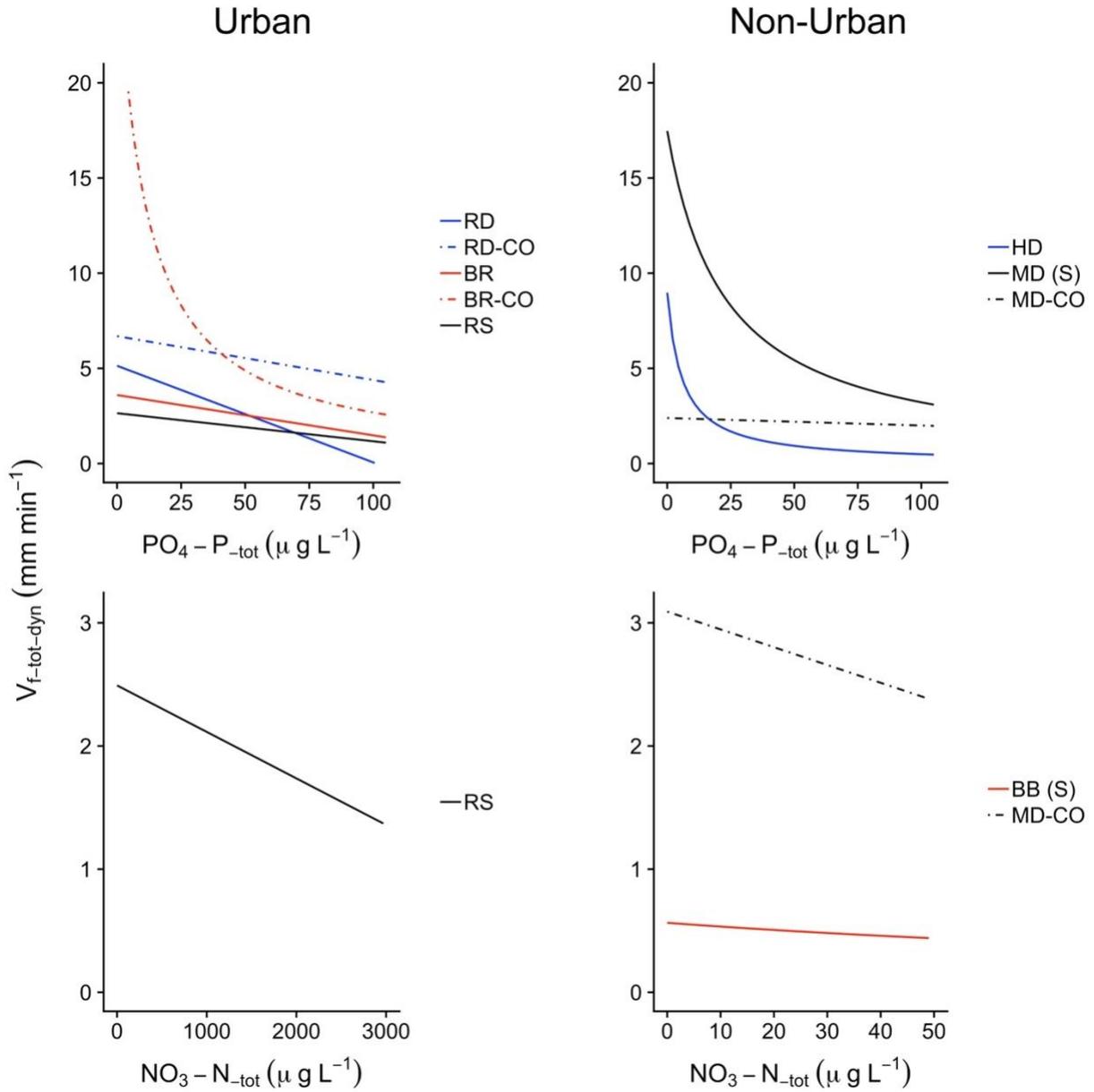
Site	Injection	p - value Summer	r² Summer	p -value Winter	r² Winter
Reedy Creek	N	0.00	0.90	0.00	0.94
	P	0.00	0.93	0.00	0.91
	CO	0.00	0.77	0.00	0.95
Broad Rock Creek	N	0.00	0.64	0.00	0.88
	P	0.00	0.74	0.00	0.98
	CO	0.10	0.18	0.00	0.97
Rattlesnake Creek	N	0.00	0.77	0.00	0.99
	P	0.00	0.79	0.00	0.98
	CO	0.00	0.74	0.00	0.94
Holiday Creek	N	0.00	0.67	0.00	0.80
	P	0.11	0.15	0.00	0.87
	CO	0.00	0.47	0.00	0.94
Bonbrook Creek	N	0.00	0.67	0.00	0.80
	P	0.00	0.75	0.00	0.78
	CO	0.00	0.63	0.28	0.08
Medely Branch	N	0.00	0.63	0.00	0.53
	P	0.00	0.85	0.00	0.73
	CO	0.04	0.23	0.00	0.87



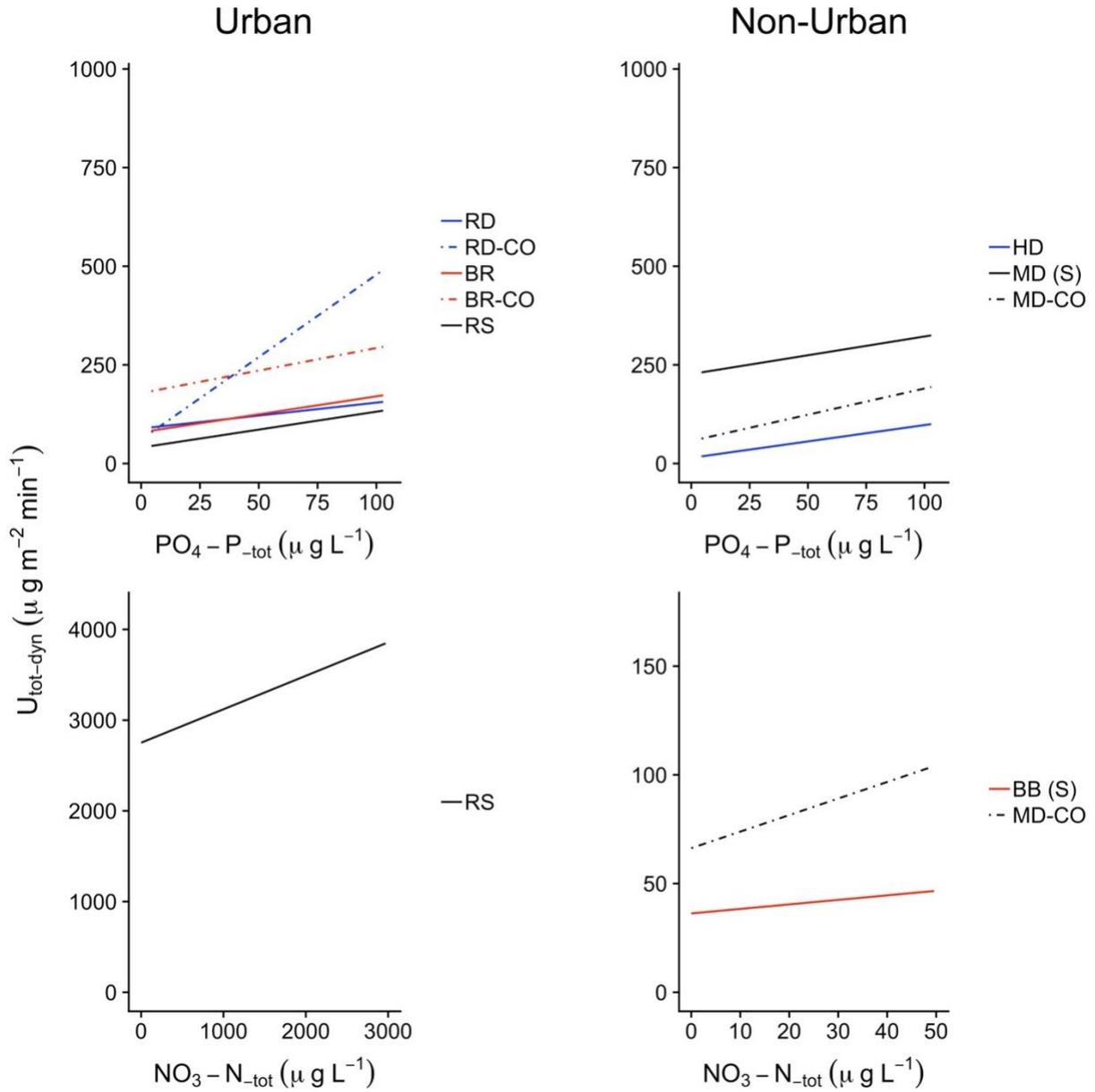
Supplementary Figure 1. Cumulative annual precipitation (top) from 1930 to 2018, and cumulative monthly precipitation throughout 2018 (bottom) with a line demonstrating the mean annual monthly precipitation taken from 1930-2018. Data were retrieved from the National Weather Service and are specific to the Richmond Metropolitan Area.



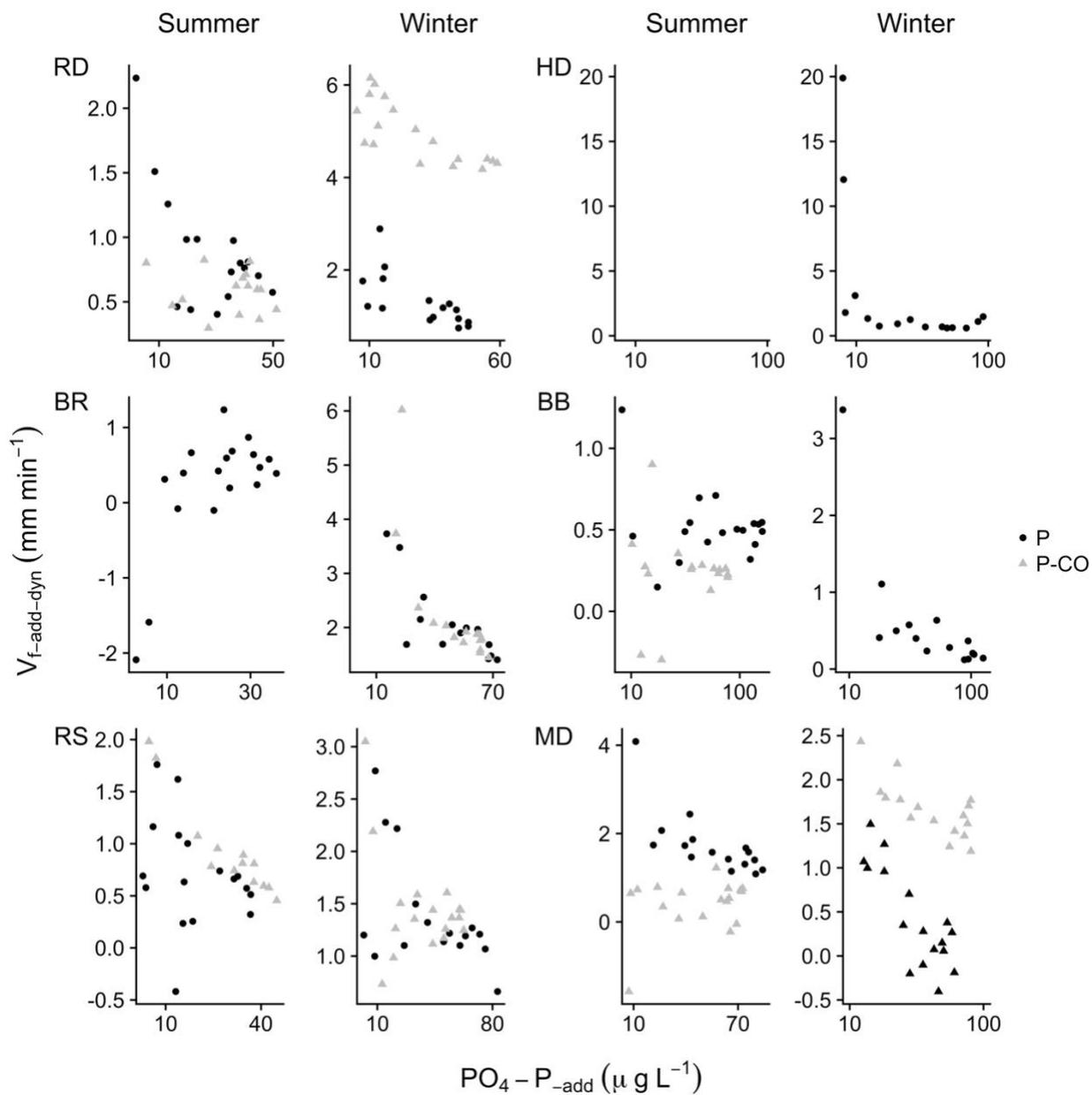
Supplementary Figure 2. Gauge pressure, based on site-relative minima, to reflect discharge conditions across Holiday Creek (HD), Bonbrook Creek (BB), and Broad Rock Creek (BR). Red arrows represent the dates where sampling occurred at each site.



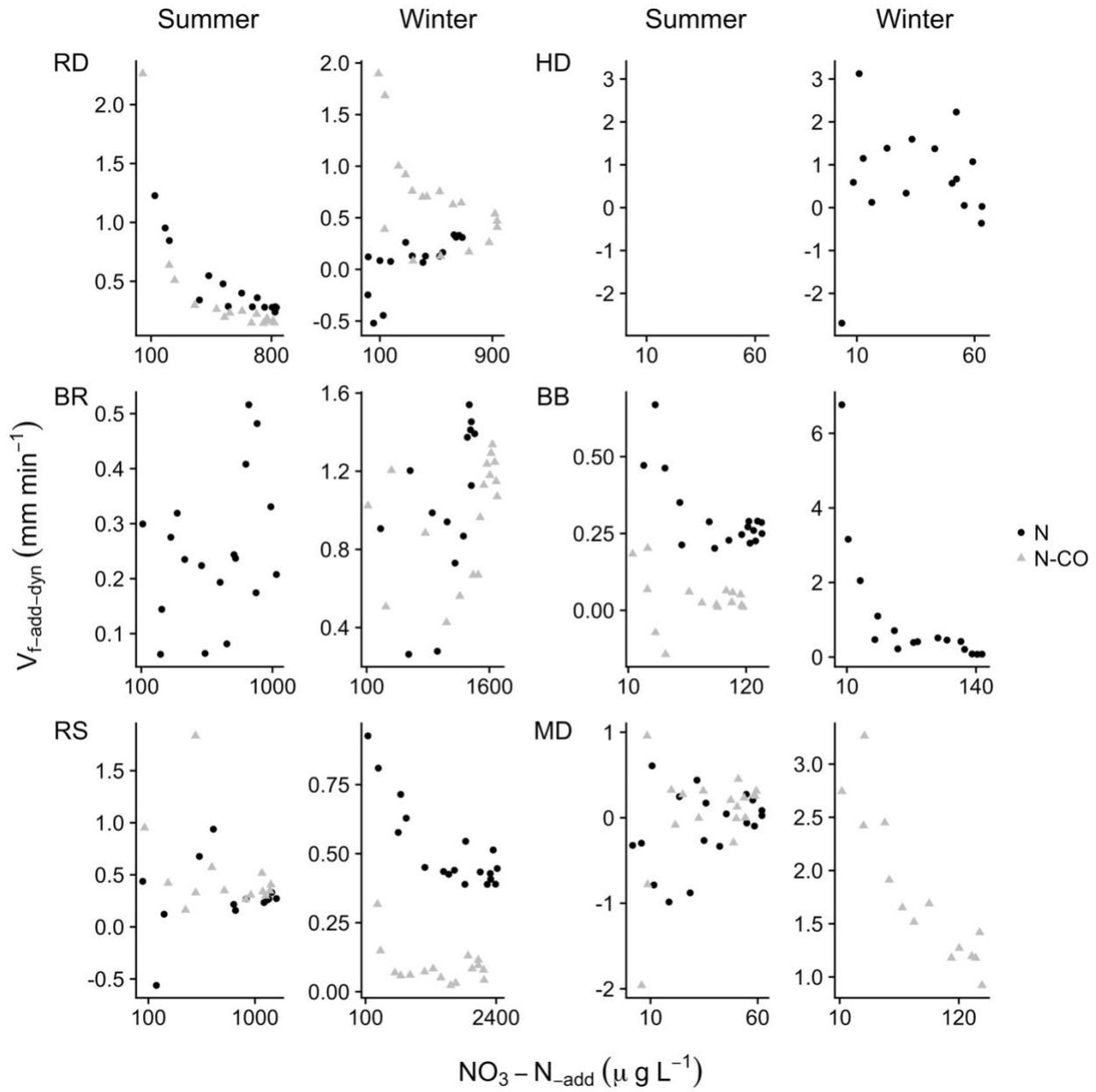
Supplementary Figure 3. Relationships between total uptake velocities and nutrient concentrations for all injection experiments with positive ambient uptake metrics. Dashed lines represent non-urban streams and regular lines are indicative of urban streams. All experiments are from winter injections unless abbreviated with a (S).



Supplementary Figure 4. Total dynamic areal uptake rates for injection experiments with positive ambient uptake metrics. Dashed lines represent non-urban streams and regular lines are indicative of urban streams. All experiments are from winter injections unless abbreviated with a (S).



Supplementary Figure 5. Relationships between uptake velocities of the added nutrient and added nutrient concentrations for phosphorus injection experiments.



Supplementary Figure 6. Relationships between uptake velocities of the added nutrient and added nutrient concentrations for nitrogen injection experiments.