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Nitrogen and Phosphorus Uptake Dynamics in Tropical Cerrado Woodland Streams

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Abstract: Pollution abatement through phosphorus and nitrogen retention is a key ecosystem service provided by streams. Human activities have been changing in-stream nutrient concentrations, thereby altering lotic ecosystem functioning, especially in developing countries. We estimated nutrient uptake metrics (ambient uptake length, areal uptake rate, and uptake velocity) for nitrate (NO_3–N), ammonium (NH_4–N), and soluble reactive phosphorus (SRP) in four tropical Cerrado headwater streams during 2017, through whole-stream nutrient addition experiments. According to multiple regression models, ambient SRP concentration was an important explanatory variable of nutrient uptake. Further, best models included ambient NO_3–N and water velocity (for NO_3–N uptake metrics), dissolved oxygen (DO) and canopy cover (for NH_4–N); and DO, discharge, water velocity, and temperature (for SRP). The best kinetic models describing nutrient uptake were efficiency-loss (R^2 from 0.47–0.88) and first-order models (R^2 from 0.60–0.85). NO_3–N, NH_4–N, and SRP uptake in these streams seemed coupled as a result of complex interactions of biotic P limitation, abiotic P cycling processes, and the preferential uptake of NH_4–N among N-forms. Global change effects on these tropical streams, such as temperature increase and nutrient enrichment due to urban and agricultural expansion, may have adverse and partially unpredictable impacts on whole-stream nutrient processing.

Keywords: low-order streams; nutrient retention; self-purification capacity; Tracer Additions for Spiraling Curve Characterization; tropical water bodies

1. Introduction

Streams are biogeochemical active systems that alter the amount and chemical form of nutrients and organic matter transported from their catchments to downstream systems [1,2]. Stream nitrogen (N) and phosphorus (P) retention is considered an important ecosystem service as it controls the export of these nutrients to downstream rivers, lakes, estuaries, and ultimately oceans, mitigating the negative consequences of cultural eutrophication [3,4]. Human activities have considerably altered nutrient availability, mobility, and distribution in freshwater systems [5–7], affecting ecosystem-wide nutrient and organic matter dynamics [8], and thus freshwater ecosystem functioning.

Variability in N and P retention rates and efficiency has been associated with primary production [9–11], hydrology and stream geomorphology [12,13], carbon availability [14–16], and nutrient limitation and availability [11,17–19]. Several studies have assessed how environmental concentration influences the uptake of a particular nutrient [20–23]. Some empirical studies have considered multi-elemental effects and dynamics [24,25], but they are rare in tropical regions (but see [19]). In addition, some authors have explored correlations between N and P uptake in streams including measurements of either nitrate–nitrogen (NO_3–N) [26,27] or ammonium–nitrogen (NH_4–N) [28,29], but rarely both.

Few studies have measured the uptake of soluble reactive phosphorus (SRP), NO_3–N, and NH_4–N together (e.g., [30]), and those suggested that colimitation by N and P can occur in streams [19]. There is increasing evidence of the predominance of colimitation and interactions among multiple elements in both aquatic and terrestrial ecosystems (e.g., [8,31–33]). The assessment of nutrient limitation is important because the dynamics and downstream transport of a limiting nutrient differ from those of a non-limiting one [19]. Understanding the implications of elemental interactions in nutrient retention studies is essential for the management of water quality in streams and downstream ecosystems.

Studies of nutrient dynamics in the tropics are rare, but especially important in developing countries, which experience high rates of population growth and rural-urban migration [34]. In these countries, 90–95% of all sewage, and 70% of industrial waste are still not treated [35]. The lack of sewage treatment and increasing urbanization in catchments can significantly alter in-stream nutrient concentrations [11,36]. Other impacts occurring in tropical regions are related to deforestation of pristine vegetation for pastoral and agricultural uses. These are associated with decreases in stream nutrient concentrations in some tropical catchments, due to the depletion of soil organic matter stocks, but with increases in other catchments, probably due to fertilizer use (see literature review in [37]). Some studies have reported strong correlations between uptake rates and ambient nutrient availability [38–40], while others found no influence of background nutrient concentrations on retention [41], highlighting the need for further research.

Nutrient uptake can be affected by nutrient enrichment in three different ways. First, the biotic community can track nutrient availability, resulting in constant uptake velocity and linear increases in areal uptake rate with increasing nutrient concentration [2,17]. Second, biological processes can be less efficient or saturated under higher ambient concentrations. Saturation or loss of efficiency causes uptake velocity to decline and areal uptake rate to increase in a curvilinear fashion with increasing concentration [11,42,43]. Third, the uptake of a particular nutrient can covary with the relative availability of another nutrient [18,44]. For example, P enrichment can lead to N limitation and stimulate N uptake, and vice-versa.

The aforementioned studies usually examined nutrient enrichment associated with land use gradients (e.g., [18,22,42,45]). Land use can also influence other variables, such as water discharge, light availability, sediment inputs, and ecosystem metabolism, all of which directly or indirectly influence nutrient uptake [46,47]. Here, we investigated a gradient in nutrient concentration across forested, tropical headwater streams. We studied whole-stream NO_3–N, NH_4–N, and SRP uptake across a gradient of ambient stream nutrient concentrations to expand our knowledge about interactions between N and P cycling and how these interactions are affected by nutrient availability in tropical streams. We studied four streams located in the Brazilian Cerrado savanna biome to address the following research questions: (1) How does nutrient uptake vary across a gradient of ambient nutrient availability? (2) How do the absolute and relative availability of one nutrient influence the uptake of the other nutrient? (3) Are there positive or negative relationships between NO_3–N, NH_4–N, and SRP uptake rates? (4) Are nutrient uptake rates higher or lower than those reported for temperate streams in the literature? In general, we hypothesized a higher demand for NH_4–N than for SRP and NO_3–N, because NH_4–N is the N-form preferentially assimilated by biota [45]. More enriched streams were expected to be less retentive than less enriched ones, showing lower areal uptake rates and uptake velocities. Finally, we hypothesized that none of the studied streams would exhibit saturation conditions (i.e., significant fits to the Michaelis–Menten kinetic model) because of the absence of chronical nutrient inputs.

2. Methods

We carried this study out in four low-order Cerrado streams (discharge less than 20 L·s^{-1}) located in São Carlos and Brotas (São Paulo, Southeastern Brazil; Table 1; Figure 1), and in each of them, we selected a representative ~100-m long stream reach. The Cerrado biome (Brazilian woodland savannah) is the second-largest South American biome, rich in springs and low-order lotic networks, which contribute to 8 of the 12 large Brazilian river basins [48]. The sampling sites Espraiado (ESP) and Broa (BRO) were located in relatively preserved basins with extensive and dense riparian vegetation, natural substrate, and restricted access. Canchim (CAN) was located in the EMBRAPA Pecuária Sudeste experimental farm, presenting a preserved riparian zone and natural substrata. Mineirinho tributary (TBM) had an urban drainage basin with fragmented riparian vegetation and advanced erosive features; however, it did not receive domestic or industrial effluents and its headwaters were preserved. The studied streams differed in water depth (from 0.04 to 0.48 m), and were relatively narrow with wetted widths ranging from 0.50 to 1.20 m.

Table 1. Characterization of the streams used in this study with geographic coordinate, drainage area, and general reach characterization.

Stream (Site Code)	Geographic Coordinates	Drainage Area (km^2)	General Characteristics
Broa (BRO)	22°11′40.93″ S 47°53′55.78″ W	2.68	Natural vegetation, forested and dense riparian zone, natural substrate, located upstream from a natural wetland
Canchim (CAN)	21°57′54.69″ S 47°50′38.02″ W	1.17	Natural vegetation, forested and dense riparian zone, natural substrate, located next to the spring
Espraiado (ESP)	21°58′46.75″ S 47°52′23.11″ W	2.49	Natural vegetation, forested and dense riparian zone, meandric channel, natural substrate
Mineirinho tributary (TBM)	22°00′12.78″ S 47°55′40.82″ W	0.82	Relatively degraded vegetation, natural substrate, presence of erosive features and pluvial runoff

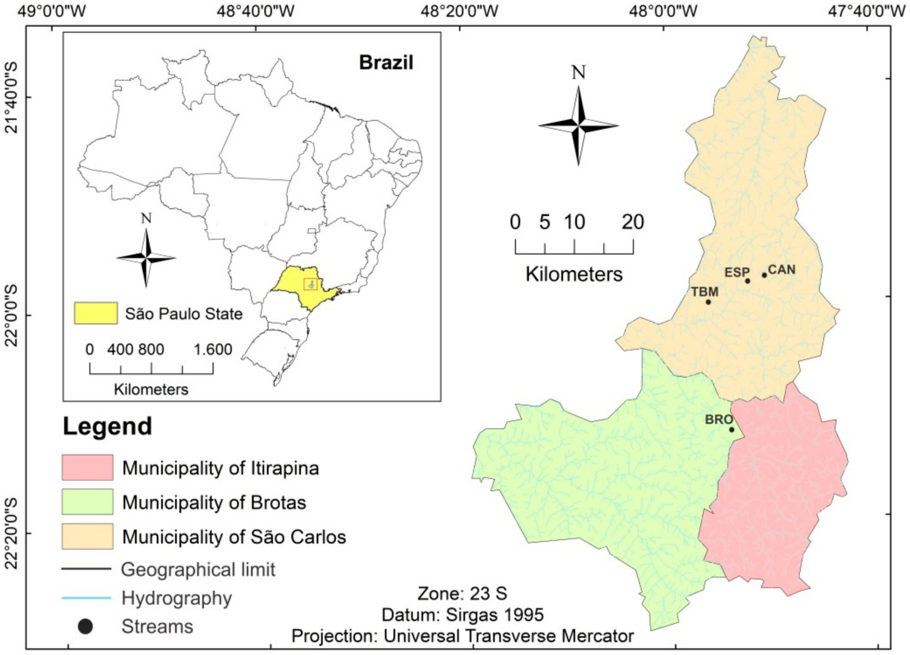


Figure 1. Sampling locations BRO (Broa), CAN (Canchim), ESP (Espraiado), and TBM (Mineirinho River tributary) in southeast Brazil.

We ran four sets of nutrient additions over the year 2017 (January, April, July, and October), encompassing wet and dry seasons. All addition experiments were carried out under base-flow conditions. We used the tracer additions for spiraling curve characterization (TASCC) approach described in the literature [49] to estimate uptake metrics from pulsed nutrient additions. This method allows determining total ($_{\text{tot-dyn}}$), added ($_{\text{add-dyn}}$), and ambient ($_{\text{amb}}$) uptake metrics. We calculated the uptake metrics uptake length (S_w), areal uptake rate (U), and uptake velocity (V_f) for different N and P forms according to the nutrient spiraling concept [2].

To all stream reaches, we simultaneously added NO_3–N as $NaNO_3$, NH_4–N as NH_4Cl, and SRP as K_2HPO_4 as bioavailable reactive tracers to characterize nutrient dynamics, and chloride (Cl^-) as NaCl as a conservative tracer to characterize stream hydrodynamics. The added mass of conservative tracer was calculated prior to each experiment to increase in-stream electrical conductivity (EC) at measurable, but moderate levels (i.e., 5–10-fold of background EC), while the added mass of nutrients was calculated to raise instream concentrations to up to 2–5-fold of background concentrations [49]. The ratio of NH_4–N to NO_3–N in nutrient additions ranged from 0.25 to 0.62. We dissolved all salts in a 5 L bucket with stream water and then poured the solution carefully into the stream at the top of the experimental reach over one minute (i.e., as a slug). Electric conductivity was measured at 10 s intervals over the experiment using a multiparameter probe at the downstream end of the reach (Model HI 9829, HANNA Instruments, Woonsocket, RI, USA). At this station, we took water samples over the full pulse, with sampling frequency ranging from 15 s to 5 min as a function of EC rate of rise or decline, resulting in 21–26 samples per experiment, in order to obtain a well-characterized breakthrough curve. Immediately before the additions, we collected three water samples to determine nutrient background (i.e., ambient) concentrations. Water samples were filtered immediately upon collection (GF/C Glass Microfiber Membranes, 0.45 µm, Whatman International, Kent, UK) and frozen at −18 °C until analysis. All water samples were analyzed within a maximum of two weeks after collection.

Stream water variables (i.e., pH, EC, temperature, and dissolved oxygen) were measured using the multiparameter probe (HANNA HI 9829, HANNA Instruments, Woonsocket, RI, US). All nutrient concentrations were determined via colorimetry using a Hach DR 4000V spectrophotometer (Hach Environmental, Loveland, CO, USA). The method used for NH_4–N was based on the literature [50], modified for a 7 mL sample volume, and the ones used for SRP and NO_3–N followed the literature [51]. Dissolved organic carbon (DOC) was analyzed using a Shimadzu SSM 5000 TOC analyzer (Shimadzu Corp., Norcross, GA, USA) the combustion method.

We also estimated the canopy cover percentage in each stream reach using a concave densitometer (Forestry Suppliers Inc., Jackson, MS, USA) following the literature [52,53]. Dilution gauging was used to measure stream discharge at the downstream ends of each experimental reach [54]. Air temperature and total precipitation were obtained from a nearby meteorological station belonging to the Brazilian National Institute of Meteorology (INMET, www.inmet.gov.br), located in São Carlos (code: 83726, latitude −21′96°, longitude −47′86°, altitude 856 m).

Differences among ambient nutrient concentrations across streams were tested using a Kruskal–Wallis test due to the non-normality of data. Spearman rank correlation was initially used to explore general correlations between uptake metrics and in-stream variables (i.e., physical, chemical, and hydraulic variables). We performed general regression analyses using uptake metrics as dependent variables, and as independent variables, those selected as important uptake drivers based on literature research (e.g., [17,30,55–57]). Among the selected variables, we excluded those that were autocorrelated (i.e., through Spearman rank tests). The remaining set of variables was then submitted to backward stepwise selection to obtain the multiple linear regression models that best described relationships between uptake metrics and environmental variables. We considered data from all streams and samplings together in regression models; therefore, the generated models incorporated typical spatial and seasonal variability of the study region, which we considered to be representative of tropical

Cerrado woodlands. All data were previously ln(x + 1) transformed, in order to achieve normally distribution, as a prerequisite of linear regression models.

We also adjusted our experimental data to three kinetic models to evaluate the uptake dynamic as function of nutrient concentration. Here, we used the TASCC total dynamics metrics (i.e., total areal uptake rate as $U_{tot\text{-}dyn}$, and total dynamic nutrient concentration as $C_{tot\text{-}dyn}$ [49]). The Michaelis–Menten kinetic model (M–M) represents saturation in uptake when nutrient availability greatly exceeds uptake via biological processes [18]. The efficiency-loss (ELS) kinetic model suggests that nutrient uptake is less efficient at high concentrations, even if saturation is not reached [20]. The first-order kinetic model (FTO) assumes that there is no saturation of uptake rate, and that the relationship between uptake rate and nutrient concentration is positive and linear. This model is often assumed to represent nutrient uptake in pristine, nutrient-limited settings.

Linear regression ($p < 0.05$) was used to determine the regression fit with the ELS and FTO models. Dependent and independent variables were ln(x + 1) transformed to satisfy the normality of residuals assumption of this method and reduce the effects of extreme values. Fits to the M–M model were processed using untransformed data [20]. Saturation was considered if there was a significant adjustment to the M–M model and the calculated K_m (i.e., the half-saturation constant) was within the range of experimental nutrient concentrations. To fit the data to all three models, we used least squares regressions with the Levenberg–Marquardt algorithm. We conducted statistical analyses using Statistica 10 (Statsoft, Tulsa, OK, USA) and fits to kinetics models and graphical representation were performed with Origin 2017 (OriginLab Corp., Northampton, MA, USA).

3. Results

3.1. General Characterization of the Streams

Total annual precipitation between December 2016 and November 2017 was 1042 mm. Monthly precipitation varied between 0 and 178 mm, with 72% of total rainfall concentrated between December 2016 and May 2017. Stream discharge varied between 2.0 and 19.2 $L \cdot s^{-1}$ considering all sites and sampling dates (Table 2). Water velocities ranged from 0.04 to 0.60 $m \cdot s^{-1}$. Ranges for EC were 4–32 $\mu S \cdot cm^{-1}$, for water temperature 15–23 °C, for pH 4.4–6.5, and for dissolved oxygen 5.4–8.6 $mg \cdot L^{-1}$. The riparian zones were dense in all sites with canopy cover varying between 77% and 98%, with ESP as the most forested site (92–98%).

Table 2. Ambient physical and chemical characteristics of studied streams. DOC (dissolved organic carbon), DO (dissolved oxygen), EC (electric conductivity), T (water temperature), Q (stream discharge), v (water velocity), H (depth), w (wetted width), and CC (percentage of canopy cover).

Site	DOC ($mg \cdot L^{-1}$)	DO ($mg \cdot L^{-1}$)	pH	EC ($\mu S \cdot cm^{-1}$)	T (°C)	Q ($L \cdot s^{-1}$)	v ($m \cdot s^{-1}$)	H (cm)	w (m)	CC (%)
BRO	1.3–3.8	6.9–7.9	4.4–5.3	4–6	16.5–21.6	7.5–11.2	0.10–0.30	4–16	0.6–1.0	90–98
ESP	1.4–3.3	5.5–7.4	4.9–5.6	11–19	15–20.6	5.0–19.2	0.06–0.26	5–48	0.5–0.6	93–98
TBM	1.2–2.4	7.1–8.3	5.8–6.1	19–28	17.5–22.8	3.4–6.9	0.10–0.60	1–5	1.0–1.2	77–91
CAN	1.6–2.7	7.5–8.5	5.9–6.5	21–32	15–20.5	2.0–4.0	0.04–0.25	1–8	0.9–1.1	92–97

Background dissolved organic carbon concentration did not differ significantly among streams ($p = 0.94$) and was always lower than 4.0 $mg \cdot L^{-1}$ (Table 2). Concentrations of NO_3–N and NH_4–N (Table 3) also did not differ significantly among streams ($p = 0.47$ and $p = 0.57$, respectively), with annual medians of the four studied streams ranging from 37–457 $\mu g \cdot L^{-1}$ for NO_3–N, and 5–36 $\mu g \cdot L^{-1}$ for NH_4–N. However, there were significant differences in SRP concentrations among streams ($p < 0.005$), with annual medians ranging between 1 and 23 $\mu g \cdot L^{-1}$. Accordingly, we classified our streams using background SRP concentrations to facilitate the visualization of SRP limitation effects on nutrient uptake. Therefore, all graphs and tables are presented in the following order of SRP concentration (Table 3): BRO < ESP < TBM < CAN.

Table 3. Ambient uptake length ($S_{w\text{-}amb}$; m), areal uptake rate (U_{amb}), uptake velocity ($V_{f\text{-}amb}$; $mm \cdot min^{-1}$), and nutrient concentration (C_{amb}; $\mu g \cdot L^{-1}$) during nutrient additions in four tropical streams for each nutrient form expressed as annual medians (minimum-maximum values). SRP—soluble reactive phosphorus.

Site	**NH_4–N**				**NO_3–N**				**SRP**			
	$S_{w\text{-}amb}$ (m)	**U_{amb} ($\mu g \cdot m^{-2} \cdot min^{-1}$)**	**$V_{f\text{-}amb}$ ($mm \cdot min^{-1}$)**	**C_{amb} ($\mu g \cdot L^{-1}$)**	**$S_{w\text{-}amb}$ (m)**	**U_{amb} ($\mu g \cdot m^{-2} \cdot min^{-1}$)**	**$V_{f\text{-}amb}$ ($mm \cdot min^{-1}$)**	**C_{amb} ($\mu g \cdot L^{-1}$)**	**$S_{w\text{-}amb}$ (m)**	**U_{amb} ($\mu g \cdot m^{-2} \cdot min^{-1}$)**	**$V_{f\text{-}amb}$ ($mm \cdot min^{-1}$)**	**C_{amb} ($\mu g \cdot L^{-1}$)**
All sites	70 (32–253)	75 (5–593)	7.5 (0.8–68)	6 (5–36)	150 (7–418)	593 (56–46,373)	2.4 (0.5–123)	315 (37–457)	144 (55–559)	35 (9–149)	4.3 (0.5–17)	13 (1–23)
BRO	44 (32–59)	251 (121–593)	24.3 (16–68)	8 (5–24)	63 (8–418)	8829 (714–46,373)	32.0 (2–123)	357 (177–382)	64 (55–105)	53 (10–79)	16.0 (7–17)	3 (1–5)
ESP	90 (57–152)	112 (41–274)	9 (7–25)	8 (5–36)	249 (139–313)	1131 (667–2038)	4.0 (3.6–5.6)	322 (183–407)	138 (42–154)	87 (60–149)	8.0 (7–12)	11 (8–13)
TBM	116 (62–172)	13 (10–20)	3 (1.6–3)	6 (5–6.7)	194 (95–351)	264 (56–519)	1.3 (1–3)	142 (37–392)	283 (156–560)	12 (9–15)	1.0 (0.6–1)	13 (8–15)
CAN	193 (35–253)	8 (76–96)	0.9 (0.7–6)	10 (5–12)	132 (131–373)	313 (155–328)	1.0 (0.5–1.6)	316 (202–457)	201 (77–391)	24 (10–36)	1.0 (0.5–1.5)	21 (18–23)

3.2. Nutrient Uptake Metrics and Kinetics

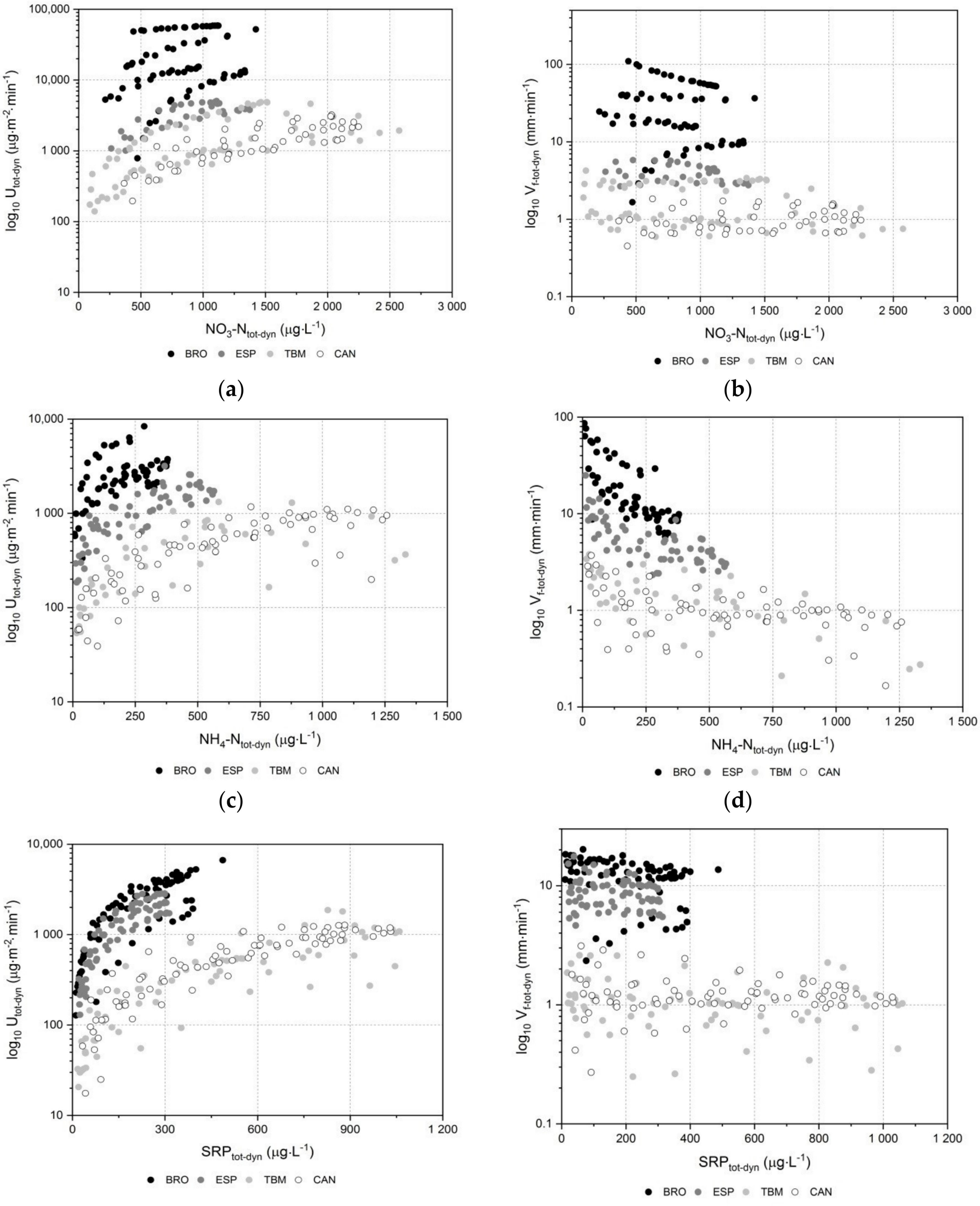
Among streams, annual median uptake lengths ranged from 44–193 m for NH_4–N, 63–249 m for NO_3–N, and 64–283 m for SRP (Table 3). Annual median areal uptake rates ranged from 8–251 $\mu g \cdot m^{-2} \cdot min^{-1}$ among streams for NH_4–N, 264–8829 $\mu g \cdot m^{-2} \cdot min^{-1}$ for NO_3–N, and 12–87 $\mu g \cdot m^{-2} \cdot min^{-1}$ for SRP. Annual median uptake velocities ranged from 0.9–24.3, 1.0–32.0, and 1.0–16.0 $mm \cdot min^{-1}$ for NH_4–N, NO_3–N, and SRP, respectively (Table 3).

Spearman rank correlations used for parameter selection are available as Supplementary Material (Table S1). For multiple regression models, we used U_{amb} and $V_{f\text{-}amb}$ as the dependent variables rather than $S_{w\text{-}amb}$. Uptake length is sensitive to stream discharge, that is, hydrologic transport, and accordingly, we found only weak relationships between $S_{w\text{-}amb}$ and other environmental variables. Therefore, we considered U_{amb} and $V_{f\text{-}amb}$ to be more representative of biogeochemical nutrient demand. All multiple linear regression models were significant ($p < 0.05$, adjusted R^2 0.48 to 0.89; Table 4). In these multiple regressions, there was a negative influence of SRP concentrations on N uptake rates and velocity. NO_3–N concentration had a positive effect on NO_3–N uptake rate. Furthermore, SRP concentrations had a negative influence on SRP uptake velocity, while NH_4–N concentrations did not affect any nutrient uptake metrics. More forested streams appeared to be more NH_4–N retentive than more open-canopy ones, as we found a strong positive relationship between the percentage of canopy cover and NH_4–N uptake rate. DO concentrations had a negative effect on NH_4–N uptake, while DO, water temperature, and velocity had negative effects on SRP retention.

Table 4. Multiple linear regressions of ambient uptake metrics (U_{amb} and $V_{f\text{-}amb}$) as function of environmental variables. All data used were ln-transformed [ln(x + 1)].

Dependent Variable	Independent Variable	B	SE	*p*-Value	Adjusted R^2
U_{amb} NO_3–N ($\mu g \cdot m^{-2} \cdot min^{-1}$)	Intercept	2.20	3.50	0.54	0.482
	SRP_{amb}	−1.33	0.52	2×10^2	
	NO_{3amb}	1.14	0.57	3×10^2	
U_{amb} NH_4–N ($\mu g \cdot m^{-2} \cdot min^{-1}$)	Intercept	−12.25	13.15	0.37	0.824
	SRP_{amb}	−1.64	0.24	3×10^5	
	DO	−4.68	1.52	1×10^2	
	%CC	6.61	2.65	3×10^2	
U_{amb} SRP ($\mu g \cdot m^{-2} \cdot min^{-1}$)	Intercept	24.28	4.20	1×10^4	0.816
	DO	−4.60	1.13	2×10^3	
	Q	0.47	0.21	4×10^2	
	V	−3.08	0.99	1×10^2	
	T	−3.85	0.92	1×10^3	
$V_{f\text{-}amb}$ NO_3–N ($mm \cdot min^{-1}$)	Intercept	5.31	0.97	2×10^4	0.508
	SRP_{amb}	−1.12	0.36	1×10^2	
	v	−5.31	2.20	3×10^2	
$V_{f\text{-}amb}$ NH_4–N ($mm \cdot min^{-1}$)	Intercept	13.03	3.00	1×10^3	0.731
	SRP_{amb}	−1.35	0.22	8×10^4	
	DO	−3.74	1.39	2×10^2	
$V_{f\text{-}amb}$ SRP ($mm \cdot min^{-1}$)	Intercept	21.34	3.10	3×10^5	0.889
	SRP_{amb}	−1.05	0.12	2×10^6	
	DO	−3.71	0.84	1×10^3	
	T	−2.97	0.73	2×10^3	
	v	−2.77	0.79	4×10^3	

For all streams, there was a better fit of total dynamic uptake rate ($U_{tot\text{-}dyn}$) with total dynamic nutrient concentration ($C_{tot\text{-}dyn}$) than with total dynamic uptake velocity ($V_{f\text{-}tot\text{-}dyn}$) (Figure 2, Supplementary Tables S2–S4). For uptake rate (U) kinetics (Table 5), there was no significant fit to the M–M model, suggesting no saturation of uptake rates. In general, the relationship between U and nutrient concentration did not appear to follow a specific model across streams. For U, site BRO had a better fit to the ELS model for all nutrients (R^2 = 0.12, 0.47, and 0.79 for NO_3–N, NH_4–N, and SRP, respectively) than to M–M and FTO models. Site ESP showed a better fit to the ELS model for NO_3–N U (R^2 = 0.70) and SRP U (R^2 = 0.88), while NH_4–N U followed the FTO model (R^2 = 0.80). Site TBM followed the FTO model for NO_3–N U and SRP U (R^2 = 0.60 and R^2 = 0.83, respectively), while for NH_4–N U, the ELS model showed the best fit (R^2 = 0.68). The FTO model showed the best fit in site CAN for NH_4–N and SRP U (R^2 = 0.70 and R^2 = 0.85, respectively), while there was a better fit to the ELS model for NO_3–N U (R^2 = 0.73) in CAN.



(**a**) (**b**)

(**c**) (**d**)

(**e**) (**f**)

Figure 2. Relationships between $U_{tot\text{-}dyn}$, $V_{f\text{-}ot\text{-}dyn}$, and $C_{tot\text{-}dyn}$ for NO_3–N, NH_4–N, and SRP during the period from January to October 2017. (**a**) $U_{tot\text{-}dyn}$ versus $C_{tot\text{-}dyn}$ for NO_3–N; (**b**) $V_{f\text{-}ot\text{-}dyn}$ versus $C_{tot\text{-}dyn}$ for NO_3–N; (**c**) $U_{tot\text{-}dyn}$ versus $C_{tot\text{-}dyn}$ for NH_4–N; (**d**) $V_{f\text{-}ot\text{-}dyn}$ versus $C_{tot\text{-}dyn}$ for NH_4–N; (**e**) $U_{tot\text{-}dyn}$ versus $C_{tot\text{-}dyn}$ for SRP; (**f**) $V_{f\text{-}ot\text{-}dyn}$ versus $C_{tot\text{-}dyn}$ for SRP.

Table 5. Kinetic model adjustments between $V_{ft\text{-}tot\text{-}dyn}$ versus $C_{tot\text{-}dyn}$ and $U_{tot\text{-}dyn}$ versus $C_{tot\text{-}dyn}$ for each stream considering all data collected from January to October 2017. In any case, R^2 is represented. ELS: efficiency-loss; FTO: first-order; M–M: Michaelis–Menten.

Stream	**Best Kinetic Model (R^2) for $V_{ft\text{-}tot\text{-}dyn}$ versus $N_{tot\text{-}dyn}$**		
	NO_3–N	**NH_4–N**	**SRP**
BRO	-	M-M (0.653)	-
ESP	FTO (0.649)	ELS (0.658)	-
TBM	-	FTO (0.453)	-
CAN	–	ELS (0.364)	-
Stream	**Best Kinetic Model (R^2) for $U_{tot\text{-}dyn}$ versus $N_{tot\text{-}dyn}$**		
	NO_3–N	NH_4–N	SRP
BRO	-	ELS (0.465)	ELS (0.787)
ESP	ELS (0.697)	FTO (0.795)	ELS (0.881)
TBM	FTO (0.596)	ELS (0.679)	FTO (0.831)
CAN	ELS (0.733)	FTO (0.702)	FTO (0.854)

- $R^2 < 0.200$; – $R^2 < 0.200$; and slope > 1.

4. Discussion

Forested heterotrophic headwater streams, such as the streams we studied [58], typically have a considerable nutrient retention capacity (i.e., short $S_{w\text{-}amb}$ and high U_{amb} and $V_{f\text{-}amb}$ [10,14]). Several studies have also reported substantial NO_3–N, NH_4–N, and SRP retention in pristine tropical headwater streams [40,41,57,59]. The authors of [41] attributed high rates of NH_4–N uptake in tropical headwater streams to microbial activity, which is probably energy-limited because decomposition of terrestrial leaf litter is rapid, dissolved organic C is strongly retained by mineral soils, and light availability limits primary production in these streams. Further, the authors of [57] highlighted the importance of structural complexity, which increases residence time of solutes and available instream surface area, both important variables for aquatic microbial biofilms and thus for ammonium uptake in tropical Cerrado savanna streams. High nutrient uptake in tropical streams appears to be related to these processes rather than to water column nutrient concentrations, or just high temperature increasing microbial metabolism. In general, the tropical Cerrado woodland stream sites studied here corresponded well with this pattern; nutrient uptake lengths were short and uptake velocities and rates for all nutrient forms were high compared with literature data from temperate streams, especially for N forms. However, the metrics reported here were similar to those reported from other tropical streams of similar size (Table 6; representing a literature review of peer-reviewed articles on nutrient spiraling in tropical streams published prior to July 2018 and available at Web of Science and Science Direct). For a comparison with temperate systems, we used data from a literature review [14], which presented uptake metrics from 52 published studies, mostly of them carried out in temperate zones.

Table 6. Compilation of NO_3–N, NH_4–N, and SRP uptake results from natural streams sites located in different regions. The values are shown as range of metrics (min–max) when available; otherwise, the mean values are represented. Data from Ensign and Doyle [14] are uptake metrics from first-order streams.

Nutrient	Stream	Location	$S_{w\text{-amb}}$ (m)	U_{amb} ($\mu g \cdot m^{-2} \cdot min^{-1}$)	$V_{f\text{-amb}}$ ($mm \cdot min^{-1}$)	Reference
NO_3–N	–	Temperate	101–478	5.8–19.1	0.8–4.2	[14]
	Barra Pequena	Tropical	467–499	94	–	[19]
	Q Bisley		1192	16	1.92	[59]
	–		315–8480	–	0.13–0.94	[40]
	BRO		6 (8–418)	8829 (714–46,373)	32 (2–123)	This study
	ESP		249 (139–313)	1131 (667–2038)	4 (3.6–5.6)	
	TBM		194 (95–351)	264 (56–519)	1.3 (1–3)	
	CAN		132 (131–373)	313 (155–328)	1 (0.5–1.6)	
NH_4–N	–	Temperate	23–275	5.3–52.6	2.5–17.0	[14]
	Barra Pequena	Tropical	138–501	5.45–14.2	–	[19]
	Q. Bisley		15–26	33–42	87.3	[59]
	R. Cipó		–	1000	–	[13]
	–		–	–	0.3–8.5	[41]
	BRO		44 (32–59)	251 (121–593)	24.3 (16–68)	This study
	ESP		90 (57–152)	112 (41–274)	9 (7–25)	
	TBM		116 (62–172)	13 (10–20)	3 (1.6–3)	
	CAN		193 (35–253)	8 (76–96)	0.9 (0.7–6)	
SRP	–	Temperate	24–161	3.4–15.7	1.5–6.6	[14]
	Barra Pequena	Tropical	461–1065	6.5–63	–	[19]
	Carapa		–	950–1150	–	[60]
	BRO		64 (55–105)	53 (10–79)	16 (7–17)	This study
	ESP		138 (42–154)	86 (60–149)	8 (7–12)	
	TBM		283 (156–560)	12 (9–15)	1 (0.6–1)	
	CAN		201 (77–391)	24 (10–36)	1 (0.5–1.5)	

Low SRP concentrations and predominantly high ambient molar DIN:SRP ratios (annual median of 24 to 279 across streams) would predict short uptake lengths and high uptake velocities for SRP compared with DIN forms. The authors of [61] suggested P limitation in streams at molar DIN:SRP ratios higher than 20, and consequently, a stoichiometric dominance of P uptake over N uptake. However, NH_4–N presented the shortest $S_{w\text{-amb}}$ and the greatest $V_{f\text{-amb}}$ in our study, indicating high retention and demand for NH_4–N. NO_3–N had the longest $S_{w\text{-amb}}$ among all nutrient forms, indicating lowest retention relative to hydrologic transport of this nutrient form, and SRP had the lowest $V_{f\text{-amb}}$, suggesting lowest demand.

Above a molar Redfield ratio of N:P of 16:1 [62], P is expected to limit algal growth (N is in excess), and below this ratio there is an N deficit (P is in excess). The authors of [58] presented molar TN:TP ratios of 44 to 195 in the stream water for the same reaches we studied here, which would suggest P limitation. The lack of a relationship between SRP uptake metrics and DIN concentrations further supports P limitation as the increased availability of N should not facilitate SRP uptake if phosphorus is the primary limiting nutrient. Surprisingly, the slope of the relationship of N uptake rates and velocities, as well as U_{DIN}:U_{SRP} ratios, with SRP concentrations was persistently negative in our study. We hypothesize that this negative relationship may have been caused by the rather special P geochemistry of the studied region (see discussion in subsequent paragraphs), that is, anaerobic sediment conditions stimulating P release, but at the same time negatively affecting aerobic assimilatory N uptake. In conclusion, the rather complex relationships between NO_3–N, NH_4–N, and SRP uptake in our streams appear to be due to the interplay of biotic P limitation, abiotic P cycling processes, and the preferential uptake of NH_4–N among N-forms.

While inorganic N uptake appeared to be driven mostly by biotic process in our study, because it was dominated by NO_3-uptake (i.e., higher areal NO_3–N than NH_4–N uptake rates), that does not present relevant abiotic uptake processes, SRP uptake might be related to abiotic processes. The relative importance of biotic and abiotic mechanisms for P retention can vary depending on P loading rates and in-stream conditions. At low levels of P loading, biotic uptake exceeds sorption [63], while under elevated P inputs, sorption can become a predominant mechanism depending on stream geochemistry [64]. Experimental whole-stream SRP additions and laboratory assays based on sediment sorption isotherms have shown that abiotic factors can dominate P retention in streams [60,65,66].

Abiotic P uptake occurs through sorption, which includes both adsorption to surfaces of cationic minerals and precipitation with electrolytes [67,68], and could be high in small streams, in which the sediment surface area to water volume ratio is high and contact time of stream water with sediments is long [69]. Fe and Al complexed with organic matter can be responsible for P sorption [68], however, we did not find relationships between SRP uptakes and dissolved organic C. In sediments dominated by Fe minerals, such as those present in Cerrado streams [70], reduction of soluble ferrous oxyhydroxide compounds results in the formation of P sorption sites. This reduction is the result of facultative organisms using ferric iron as an electron acceptor during their metabolic process in absence of oxygen. On the other hand, a reduced sediment surface layer can allow for considerable PO_4^{3-} release from Fe(III) oxide, whereas an oxidized sediment surface represents an efficient geochemical barrier for sediment P release [71].

Phosphorus uptake can be primarily governed by temperature, which can be attributed to biological mechanisms [72]. Therefore, if biotic uptake was relevant in our streams, the relationship with temperature should have been positive, because increases in thermal energy stimulate biotic P uptake [73]. However, we found a negative effect of temperature on P uptake, suggesting lower P uptake at high temperatures. We also found a negative slope in the correlations between SRP U_{amb} ($B = -4.60$), as well as $V_{f\text{-}amb}$ ($B = -3.71$), and DO. The negative effects of both higher temperature and DO concentration on P uptake seem contradictory, as low DO saturation occurs at higher temperature. As both higher streamwater DO and temperature should stimulate aerobic biotic P uptake in the advective zone, these negative relationships may also point to the importance of sediment processes, that is, geochemical SRP sorption and SRP release processes [71]. In conclusion, our data suggested that abiotic P sorption may be a relevant process in our streams.

Among inorganic N-forms, the demand for NH_4–N was high, while the mass removal of NO_3–N was greater; NH_4–N $V_{f\text{-}amb}$ was higher than NO_3–N $V_{f\text{-}amb}$, but NO_3–N U_{amb} was much greater than NH_4–N U_{amb}. The persistently high NH_4–N $V_{f\text{-}amb}$ suggested preferential assimilation of NH_4–N by aquatic biota (bacteria, fungi, and algae) over NO_3–N, which is consistent with studies carried out in the literature [74–76]. In general, these authors observed that consumers track water column NH_4–N more closely than water column NO_3–N, as a result of the lower energy-demand associated with the assimilation of NH_4–N than with that of NO_3–N.

Despite the lower demand for NO_3–N relative to supply, as represented by its uptake velocities, the utilization of NO_3–N was considerable. Areal uptake rates (U) for NO_3–N were up to 80 times greater than U_{amb} for NH_4–N. This high U_{amb} showed that NO_3–N is an important source of N in these systems. Some studies [23,42] have related this condition to the generally greater background concentrations of NO_3–N than those of NH_4–N. For instance, the authors of [15] observed that NO_3–N concentrations 10- to 1000-fold greater than NH_4–N reduced NH_4–N retention to down to three times in streams in New Hampshire, USA. Similarly, the N demand of microorganisms was primarily satisfied by NO_3–N as a result of its greater availability, that is, 95% of the total DIN concentrations across our streams.

The relationships between stream nutrient uptake and its concentrations have received much attention in recent studies that also used pulse nutrient additions for kinetic analysis [16,27,47,77]. These authors highlighted that the amount of data provided by the TASCC method is useful for kinetic modelling. However, pulsed additions do not represent ambient uptake from a whole-stream perspective, because they do not reflect stable-state transport conditions, but rather transient experimental conditions, with rising and falling limbs of breakthrough curves representing different transport processes [2].

We did not observe saturation conditions, that is, no significant fit to the M–M model was found across streams, which was expected because there was no evidence of chronical nutrient enrichment. In some cases, the lack of saturation suggests the existence of a mass transfer component, high-saturation sorption kinetics, or even the occurrence of dissimilatory processes such as nitrification and denitrification that may only saturate at very high concentrations [17]. Our study did not address the specific processes that dominate nutrient uptake, but demonstrated the dominance of biotic nitrate uptake and suggested the importance of abiotic SRP uptake. First-order responses to experimental nutrient enrichment or seasonal or spatial variation in nutrient concentration are commonly observed in streams with low to moderate nutrient concentrations [43], but efficiency loss and especially saturation are the typical responses of streams with chronic nutrient inputs. High streamwater nutrient concentrations due to agricultural and urban land use have been reported for tropical regions [37]. Across 35 highly urbanized tropical watersheds, NO_3–N and NH_4–N concentrations as high as 0.41 and 4.42 $mg \cdot L^{-1}$ have been reported [36]. Similar studies in other tropical streams with chronic nutrient loading would advance our understanding about the potential occurrence of nutrient saturation in tropical streams. Further, more nutrient uptake data is required to understand uptake as function of concentration across a wide variety of tropical streams and rivers, including assessments of abiotic versus biotic uptake, and limitation by mass transfer.

In our study, the uptake of different nutrients and nutrient forms appeared to be tightly associated. All nutrient uptake velocities were positively and strongly correlated with each other, which could suggest the occurrence of co-limitation in our streams [30]. The role of co-limitation in nutrient utilization by microorganisms has been widely investigated in recent studies [19,28,29,78], but had been rarely investigated in tropical stream ecology (but see [19]). As nutrient limitation is an important driver of nutrient uptake [79–81], future investigations should aim at more detailed assessments on how anthropogenic impacts on tropical streams are related to their nutrient limitation and co-limitation conditions. Finally, the relatively high uptake rates observed in the studied tropical Cerrado woodland streams highlight the importance of preservation of these headwater streams for the management of ecosystem functioning and services of Cerrado, and potentially other tropical catchments.

5. Conclusions

Here, we evaluated the variability in whole-stream uptake of three important nutrient forms, which is, SRP, NH_4–N, and NO_3–N, in relatively undisturbed, tropical woodland streams, and found relatively high and coupled uptake rates of these nutrients. Relationships between NO_3–N, NH_4–N, and SRP uptake in these streams seemed to be due to rather complex interactions of biotic P limitation, abiotic P cycling processes, and the preferential uptake of NH_4–N among N-forms. Drivers of nutrient uptake were partially nutrient form-specific and included unexpected effects, such as negative temperature dependencies of SRP uptake and negative relationships between the uptake of all nutrient forms and SRP concentration. Thus, global change effects on these tropical streams, such as temperature increases due to climate warming and decreased shading as a result of riparian clear-cutting, as well as nutrient enrichment due to urban and agricultural expansion, may have adverse and partially unpredictable impacts on whole-stream nutrient processing, and thus catchment biogeochemistry. Future studies should investigate the mechanisms of nutrient spiraling in tropical streams, such as abiotic versus biotic uptake processes, mass transfer limitation, and nutrient limitation and co-limitation, and how human activities influence them.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4441/10/8/1080/s1, Table S1: Spearman's correlation coefficients between ambient uptake metrics ($S_{w\text{-amb}}$, U_{amb}, and $V_{f\text{-amb}}$), nutrient concentrations and ratios, and in-stream physical and chemical variables. Significant correlations are highlighted in bold ($p < 0.05$), Table S2: Statistical parameters of Michaelis–Menten models used to evaluate the relationship between total dynamic uptake rate ($U_{tot\text{-}dyn}$) or total dynamic uptake velocity ($V_{f\text{-}tot\text{-}dyn}$), and total dynamic nutrient concentration ($C_{tot\text{-}dyn}$) for both streams. For the Michaelis–Menten model, the maximum uptake rate (U_{max}; $\mu g \cdot m^{-2} \cdot min^{-1}$) and the half saturation constant (K_m; $\mu g \cdot L^{-1}$) are shown. The adjusted R^2 and *p*-value of fits are reported in brackets, Table S3: Statistical parameters of the efficiency-loss model ($U = aN^b$) used to evaluate the fit between the relationship between total dynamic uptake rate ($U_{tot\text{-}dyn}$), total dynamic uptake velocity ($V_{f\text{-}tot\text{-}dyn}$), and total dynamic nutrient concentration ($C_{tot\text{-}dyn}$) for both streams. For the efficiency-loss model, *a* represents the intercept and *b* is the exponent. The adjusted R^2 and p-value of the fits are also reported in brackets, Table S4: Statistical parameters of the first-order model ($U = a + bN$) used to evaluate the fit between the relationship between total dynamic uptake rate ($U_{tot\text{-}dyn}$), total dynamic uptake velocity ($V_{f\text{-}tot\text{-}dyn}$), and total dynamic nutrient concentration ($C_{tot\text{-}dyn}$) for both streams. For the efficiency-loss model, *a* represents the intercept and *b* is the slope. The adjusted R^2 and *p*-value of the fits are also reported in brackets.

Author Contributions: N.R.F and D.G.F.C. designed the framework of the study, conducted fieldwork and analyzed data. N.R.F. coordinated analysis of dissolved nutrients of the samples, and also the modelling of nutrient uptake metrics. I.G.B. and B.G. revised statistical analysis. All authors discussed the results and commented on the manuscript.

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