How do chronic nutrient loading and the duration of nutrient pulses affect nutrient uptake in headwater streams?

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Abstract Our study aimed to analyze the effects of chronic nutrient loading on the capacity of headwater streams to retain phosphorus and ammonium pulses of different duration. For this purpose, we selected nine headwater streams located across a gradient of increasing agricultural land use and eutrophication. In each stream, we performed sequential plateau additions with increasing nutrient concentrations in summer 2015 and instantaneous slug additions in summer 2016 under similar hydrological conditions. We modelled kinetic uptake curves from the slug additions via the Tracer Additions for Spiraling Curve Characterization method and calculated ambient

uptake parameters. Ambient uptake rates generally increased ($1.4\text{--}20.8\ \mu\text{g m}^{-2}\text{ s}^{-1}$ for $\text{NH}_4\text{-N}$ and $0.3\text{--}10.3\ \mu\text{g m}^{-2}\text{ s}^{-1}$ for SRP, respectively), while ambient uptake velocities decreased from oligotrophic to polytrophic streams ($1.8\text{--}14.0\ \text{mm min}^{-1}$ for $\text{NH}_4\text{-N}$ and $1.6\text{--}9.9\ \text{mm min}^{-1}$ for SRP, respectively). However, correlations between ambient uptake parameters and background concentrations were weak. Concentration-dependent uptake rates followed either a linear or a Michaelis–Menten saturation model, regardless of the degree of nutrient loading. Uptake rate curves showed counter-clockwise hysteresis in oligotrophic streams and clockwise hysteresis in streams of higher trophic states, indicating a reduced significance of hyporheic uptake with increasing nutrient loading. Comparisons of slug and plateau additions revealed that oligotrophic streams were most efficient in uptake during short nutrient pulses, while eutrophic streams profited from longer pulse duration. The results indicate that nutrient uptake is increasingly transport-controlled in polluted streams where increased biofilm thickness and clogging of sediments restrict nutrient transport to reactive sites.

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Introduction

In-stream nutrient uptake is a fundamental ecosystem service, which controls nutrient fluxes from catchments to rivers, lakes, and oceans (Mulholland et al. 2008). Numerous studies have shown the importance of headwaters in the retention of nutrients imported from the terrestrial catchment (Peterson et al. 2001; Ensign and Doyle 2006; Weigelhofer 2017). An impairment of this retention capacity results in the nutrient loading and eutrophication of recipient water bodies, deteriorating the water quality and the ecological state there. In-stream nutrient retention largely depends on the uptake capacity of the benthic community, which is determined by the ratio of nutrient demand-to-supply (Ensign and Doyle 2006; Jarvie et al. 2006; Ribot et al. 2013). Chronic nutrient loading affects this ratio in two ways. On the one hand, biotic uptake efficiency is assumed to decrease with increasing nutrient concentrations due to the gradual saturation of the benthic community (Bernot and Dodds 2005; O'Brien et al. 2007; O'Brien and Dodds 2010; Martin et al. 2011). Consequently, concentration-dependent uptake rates often follow a Michaelis–Menten saturation model, which yields maximum uptake rates $U_{\max}$ under saturated conditions (Dodds et al. 2002; O'Brien et al. 2007; Trentman et al. 2015). On the other hand, chronic nutrient loading leads to increased abundances and activities of both benthic algae and bacteria (Bothwell 1989; Mulholland et al. 1990; O'Brien et al. 2007; García et al. 2017), resulting in an increased nutrient demand of the benthic community and a shift of the saturation threshold to higher nutrient concentrations and higher $U_{\max}$ (Niyogi et al. 2004). In-stream nutrient addition experiments have shown that chronically loaded streams often exhibit higher maximum uptake capacities than near-natural streams, while being less efficient in nutrient retention at low nutrient concentrations (e.g. Covino et al. 2012; García et al. 2017).

Chronic nutrient loading may also change the accessibility of potential uptake sites to nutrients. Both biotic and abiotic uptake in the benthic and the hyporheic zone are determined by the degree of nutrient diffusion through biofilms and sediments, which depends on the concentration gradient, the transport distance, and the structure and density of the substrate (Dodds et al. 2002; Stewart 2003). Elevated nutrient concentrations lead to increased biofilm

thickness (Sabater et al. 2011; Ribot et al. 2013), thereby affecting the exchange of water and solutes within the biofilm as well as between the water column and the sediments (Battin et al. 2003; Teissier et al. 2007; Ribot et al. 2013). Furthermore, chronic nutrient loading often coincides with increased siltation (Hancock 2002), which reduces the exchange between the water column and the hyporheic zone and restricts nutrient retention to channel processes (Ensign and Doyle 2005; Bukaveckas 2007; Weigelhofer et al. 2018). While nutrient uptake in eutrophic streams is supposed to be kinetically controlled by the saturation degree of the biofilm (Dodds et al. 2002), restricted diffusion and transport of nutrients to reactive sites may add transport-limitation as driver of uptake processes. Consequently, the duration of nutrient pulses may become a relevant factor for the capacity of chronically loaded streams to retain nutrients (Martin et al. 2011).

Our study aimed to analyze the effects of chronic nutrient loading on the capacity of headwater streams to retain nutrient pulses of different duration. For this purpose, we selected nine headwater streams across a gradient of increasing agricultural land use and eutrophication. We applied two field methods to study nutrient uptake kinetics at the reach-scale: sequential plateau additions with increasing nutrient concentrations in summer 2015 (Payn et al. 2005); and instantaneous slug additions followed by the Tracer Additions for Spiraling Curve Characterization (TASCC) method in summer 2016 (Covino et al. 2010b). Sequential plateau additions expose stream communities to elevated nutrient concentrations over longer periods, but are restricted in the number of concentration levels studied. Slug additions cover a broad range of nutrient concentrations, thereby enabling the modelling of kinetic uptake curves, while the exposure time of the benthos to the nutrient pulse is short (for method comparisons, see also Trentman et al. 2015). We performed the experiments under similar hydrological and environmental conditions to study potential effects of pulse duration on the in-stream nutrient uptake.

Regarding the influence of chronic nutrient loading on the streams' uptake capacities, we hypothesized that eutrophic streams will show higher maximum uptake rates $U_{\max}$ and half-saturation constants K_m (concentration, at which the uptake rate is half of $U_{\max}$) than oligotrophic streams due to an overall

higher uptake potential (Covino et al. 2012; Ribot et al. 2013). However, we also expected a decrease in uptake efficiencies with increasing nutrient loading, shown by decreased ambient uptake velocities in impacted streams (Martin et al. 2011; Covino et al. 2012).

Regarding the effects of pulse duration, we hypothesized that oligotrophic streams will show a decrease in the uptake efficiency with increasing exposure time, as thinner biofilms may become saturated soon. Consequently, uptake rates *U* and uptake velocities *V_f* should be lower during plateau additions than during slug additions at the same concentration levels. In contrast, we expected eutrophic streams to profit from longer pulse duration because of transport-limitations in thicker biofilms and clogged sediments. Thus, *U* and *V_f* should be higher during plateau additions than during slug additions at the same concentration levels. At last, we expected to observe signs of nutrient saturation in streams exposed to particularly high chronic nutrient loading (polytrophic streams) during both slug and plateau additions.

Materials and methods

Study sites

We selected nine headwater streams in Austria located along a land use gradient, which ranged from extensive forests in the south to fertilized pastures in the middle and intensive grain farming with regular tillage in the north (Table 1, Online resource 1; Weigelhofer et al. 2018). With increasing percentage of agriculture in the catchment, the study streams showed an increasing trophic index (Rott et al. 1999) according to the Austrian Water Quality Assessment by the Federal Government of Lower Austria (Table 1) as well as increasing concentrations of dissolved inorganic nutrients and organic carbon in the water column (Table 2). We selected 300-m long homogenous study reaches without visible surface water inflow for the nutrient addition experiments. The study reaches were characterized by meandering stream courses, heterogeneous channel and bank structures (pools, riffles, debris dams), and a riparian forest of at least 5 m width on both banks. Hence, stream channels were mostly shaded during summer. Sediments were dominated by small gravel with increasing percentage of fine

Table 1 Characteristics of the study sites, showing the trophic state, average nutrient and DOC concentrations in the water (*n* = 10), benthic chlorophyll-*a* concentrations (Chl-*a*; *n* = 15), discharge (*Q*), depth-to-width ratios (*d:w*), and current velocities (*v*) in summer 2015 and 2016 (means for each year; *n* = 2–3 for hydromorphology)

Streams	ID	Coordinates	% arable land	Trophic state ^a	SRP g L ⁻¹	NH ₄ -N (μg L ⁻¹)	NO ₃ -N (mg L ⁻¹)	DOC (mg L ⁻¹)	Chl- <i>a</i> (μg cm ⁻²)	<i>Q</i> (L s ⁻¹)	<i>v</i> (m s ⁻¹)
Kothbergbach	Kb	47°52'49"N 14°59'24"E	10	1.2 Oligotrophic	4/4	4/7	1.0/1.1	1.3/1.5	1.2/1.3	120/148	0.25/0.22
Kleine Erlauf	Er	47°56'02"N 15°01'38"E	46	1.4 Oligotrophic	7/8	12/10	1.2/1.1	1.8/1.6	1.9/1.7	100/121	0.22/0.24
Grestenbach	Gr	47°58'55"N 15°00'01"E	54	1.4 Oligotrophic	39/31	12/19	1.3/1.2	1.7/1.7	1.7/1.4	42/58	0.19/0.19
Zettelbach	Zb	48°05'05"N 15°21'59"E	50	1.8 Mesotrophic	20/30	25/35	1.6/1.5	1.8/2.0	1.6/1.5	93/70	0.20/0.18
Gamingbach	Ga	47°54'32"N 15°05'26"E	8	2 Meso-eutrophic	15/18	45/23	0.8/1.1	1.5/1.0	8.7/7.5	63/78	0.22/0.26
Schweinsbach	Sb	48°06'39"N 15°19'08"E	83 ^b	2.7 Eutrophic	70/78	25/28	3.0/3.3	2.5/2.0	3.8/3.6	91/86	0.18/0.19
Sternung	Si	48°06'50"N 15°25'50"E	74 ^b	3.1 Eu-polytrophic	151/144	152/145	5.6/3.6	3.3/2.4	3.9/4.2	38/44	0.09/0.12
Russbach	Ru	48°26'37"N 16°26'52"E	70 ^b	3.6 Polytrophic	460/272	180/130	4.0/6.8	5.3/2.7	9.6/11.6	69/75	0.20/0.16
Göllersbach	Go	48°35'55"N 16°05'27"E	92 ^b	3.4 Polytrophic	410/390	232/220	2.3/3.5	6.4/5.7	6.8/5.9	70/66	0.11/0.12

^aAccording to Rott et al. (1999); Data from the Austrian water quality assessment by the Federal Government of Lower Austria in 2003–2012

^bMore than 80% of the arable land used for intensive grain farming with regular tillage

Table 2 Uptake parameters of the study streams, showing ambient uptake lengths (Sw_{amb}), rates (U_{amb}), and velocities (Vf_{amb}) for both plateau and slug additions, the best-fitted model for kinetic uptake curves (including r^2), and the type of hysteresis (asc/desc = ascending/descending limb)

Streams	Plateau addition				Slug addition				Model	r ²	U _{max} (μg m ⁻² s ⁻¹)	K _m (μg L ⁻¹)	Hysteresis
	SW _{amb_plat} (m)	U _{amb_plat} (μg m ⁻² s ⁻¹)	Vf _{amb_plat} (mm min ⁻¹)	r ²	SW _{amb_sl} (m)	U _{amb_sl} (μg m ⁻² s ⁻¹)	Vf _{amb_sl} (mm min ⁻¹)						
NH ₄ -N													
Kb	132	2.4	14	0.99	192	1.6	14.1	Linear	0.98	–	–	asc < desc	
Er	200	1.4	7.8	0.95	386	1.4	8.0	MM	0.92	46	209	asc < desc	
Gr	75	4.0	12.6	0.98	303	2.7	5.5	Linear	0.95	–	–	asc < desc	
Zb	43	13.4	44.6	0.73	189	6.2	10.7	MM	0.79	57	254	no	
Ga	213	2.9	5.3	0.26	281	1.8	5.4	MM	0.26	20.5	101	asc > desc	
Sb	nd	–	–	–	292	2.0	4.8	MM	0.33	21	135	asc >> desc	
Si	195	9	4	0.82	138	20.8	8.2	MM	0.43	111	68	asc >> desc	
Ru	>1500	–	–	ns	777	4.5	2.1	Linear	0.76	–	–	asc > desc	
Go	>3000	–	–	ns	892	6.7	1.8	Linear	0.35	–	–	asc >> desc	
SRP													
Kb	92	3.4	20.2	0.98	550	0.31	4.9	Linear	0.88	–	–	asc < desc	
Er	207	1.0	7.6	0.98	313	1.3	9.9	MM	0.6	30	143	asc < desc	
Gr	–	–	–	ns	1795	0.5	0.9	linear	0.94	–	–	asc < desc	
Zb	–	–	–	ns	371	2.7	5.5	MM	0.98	431	5005	no	
Ga	198	0.2	5.6	0.89	455	0.9	3.3	MM	0.94	67	1182	no	
Sb	nd	–	–	–	307	6.0	4.6	MM	0.81	63	696	asc >> desc	
Si	790	2.2	1.0	0.34	293	9.4	3.9	Linear	0.87	–	–	asc >> desc	
Ru	> 3000	–	–	ns	884	8.3	1.8	Linear	0.96	–	–	no	
Go	> 3000	–	–	ns	1021	10.3	1.6	Linear	0.63	–	–	asc >> desc	

In the case of Michaelis–Menten curves, maximum uptake rates (U_{max}) and half saturation constants (K_m) are given

ns not significant, nd not determined

sediments in the agricultural streams (Weigelhofer et al. 2018).

Experimental design

In summer 2015, we conducted three sequential plateau additions with increasing concentrations of soluble reactive phosphorus (SRP; as $\text{Na}(\text{H}_2\text{PO}_4) \cdot 2\text{H}_2\text{O}$) and $\text{NH}_4\text{-N}$ (as NH_4Cl) in each study reach (Stream Solute Workshop 1990; Weigelhofer et al. 2013). Sodium chloride (NaCl) was used as conservative tracer to determine plateau conditions and correct for groundwater inflow. Sampling sites were evenly distributed at 20 m intervals between 100 and 300 m downstream of the injection point to ensure complete mixing of the solutes during sampling. Prior to the additions, water samples were taken at each site to determine nutrient background concentrations. SRP and $\text{NH}_4\text{-N}$ were injected at constant rate via a peristaltic pump at the head of each reach for approximately 1 h. Electrical conductivity was recorded at the bottom of each reach at 10 s intervals using a Hach Lange HQ40d conductivity meter. About 15 min after plateau conditions were reached, water samples were collected at each sampling site in a downstream direction to follow the nutrient pulse. The addition was turned off and the next addition started after conductivity had reached background concentrations again (10–20 min after stopping the injection). In general, the experiments started around 10 a.m. and lasted until 2 p.m.. The water samples were filtered with 0.7 μm pre-combusted GFF filters in the field, filled into pre-combusted glass vials, and stored in a dark cooler. After each experiment, we measured discharge and channel morphology at the top, in the middle, and at the bottom of each reach. Furthermore, we took 15 randomly selected stones from each study reach to determine benthic chlorophyll-a concentrations.

In summer 2016, we performed instantaneous slug additions of SRP, $\text{NH}_4\text{-N}$, and Cl in each of the nine streams. Nutrients and chloride were dissolved in 10 L of stream water and were released at the head of the reach as a single pulse. Prior to the release, a sample was taken from the carboy to determine initial nutrient and chloride concentrations. We measured conductivity in real-time (Hach Lange HQ40d conductivity meter) and collected grab (water) samples across the breakthrough curve (BTC) at the bottom of the reach

until conductivity returned to ambient levels ($n = 20\text{--}30$ samples per release). Stream discharge was determined both at the head and the bottom of each reach. In addition, 15 stones were taken for determination of benthic chlorophyll-a concentrations. Water samples were processed in the same way as for the plateau additions.

Nutrient concentrations (SRP, N-NH_4 , N-NO_3 , and N-NO_2) were analyzed in the lab using a continuous flow analyzer (CFA, Systema Analytical Technology). Concentrations of dissolved organic carbon (DOC) were determined with a Sievers*900 portable TOC-Analyzer. For the determination of benthic chlorophyll-a concentrations, approximately 25 cm^2 of biofilm were scrapped off each stone surface, suspended in 5 mL MilliQ water and homogenized with a Polytron-mixer (PT 1600E). The chlorophyll-a was extracted in 10 ml cold 90% acetone at 4 °C in the dark overnight. After centrifugation (2500 rpm 20 min), the chlorophyll-a and pheophytin contents were determined with a Hitachi Fluorescence Spectrophotometer F-7000 (APHA 2005).

Kinetic models for in-stream nutrient uptake

Nutrient uptake is usually expressed via three inter-related parameters (Stream Solute Workshop 1990; Dodds et al. 2002; Ensign and Doyle 2006; Trentman et al. 2015). The uptake length S_w represents the average travel distance of a nutrient molecule in the water column before removal. The areal uptake rate U is the amount of nutrients removed per area of stream bottom and unit time. The uptake velocity V_f is the velocity with which nutrients move through the water column to the benthos. As V_f is standardized against hydro-morphology, it is assumed to reflect the nutrient demand of the benthic community.

The following four models are most frequently used to describe the functional relationship between nutrient concentrations C and uptake rates U (O'Brien et al. 2007; Ribot et al. 2013; Trentman et al. 2015). The linear model assumes a linear increase in U with increasing C , while V_f is expected to be independent of nutrient loads (Dodds et al. 2002; O'Brien et al. 2007; Trentman et al. 2015). The Michaelis–Menten saturation model describes a hyperbolic relation between U and C according to the equation

$$U = C * U_{\max} / [K_m + C], \quad (1)$$

where $U_{\max}$ is the maximum uptake rate and K_m is the half saturation constant. Thus, U is expected to saturate if the nutrient supply exceeds the demand, while V_f should decrease hyperbolically with C (Dodds et al. 2002; O'Brien et al. 2007; Trentman et al. 2015). Although Michaelis–Menten uptake kinetics were originally developed for individual cells or cell cultures, several studies have shown a good model fit for whole-stream uptake of nutrient pulses (O'Brien and Dodds 2010; Covino et al. 2012; Arce et al. 2014). The Efficiency Loss model predicts a non-linear increase of U with increasing C following a power relationship with the equation

$$U = k \cdot C^m, \quad (2)$$

where k and m are constants and $m < 1$ (O'Brien et al. 2007). Thus, while U increases with nutrient availability, the uptake efficiency relative to the concentration decreases. In a few cases, a fourth model, referred to as biostimulation model, was observed, in which V_f increased and Sw decreased with increasing nutrient concentrations (Covino et al. 2012; Diemer et al. 2015; Rodríguez-Cardona et al. 2016). The authors ascribed this pattern to above-average adsorption or uptake rates, as e.g. occurs in the course of phosphorus luxury uptake.

Calculation of uptake parameters

We calculated in situ nutrient uptake parameters $Sw_{\exp}$, $U_{\exp}$, and $V_{f_{\exp}}$ from plateau additions via the longitudinal decline of the added nutrients (Stream Solute Workshop 1990). In short, nutrient concentrations during plateau conditions were corrected for ambient concentrations and dilution and were regressed against distance to yield exponential uptake curves. We took the negative inverse of the regression slopes as $Sw_{\exp}$ and calculated $U_{\exp}$ and $V_{f_{\exp}}$ from $Sw_{\exp}$, water velocity, water depth, and nutrient concentrations as described in the Stream Solute Workshop (1990; for details, see Online resource 2). In addition, we used $Sw_{\exp}$ of the three consecutive additions to calculate ambient uptake lengths $Sw_{\text{amb\_plat}}$ according to Payn et al. (2005). For this purpose, $Sw_{\exp}$ were plotted against the added nutrient concentrations, and the linear regression curve was extrapolated to negative ambient nutrient concentrations.

From $Sw_{\text{amb\_plat}}$, we calculated ambient uptake rates $U_{\text{amb\_plat}}$ and ambient uptake velocities $V_{f_{\text{amb\_plat}}}$ in the same way as $U_{\exp}$ and $V_{f_{\exp}}$.

Data of the slug additions were used to estimate ambient and kinetic nutrient spiraling metrics based on the TASCC approach developed by Covino et al. (2010a, b). In this approach, dynamic uptake lengths Sw_{dyn} are determined for each grab sample along the BTC by plotting the background corrected and $\ln$ -transformed nutrient:Cl ratios against the distance and calculating Sw_{dyn} from the inverse of the linear regression slopes analog to the calculations for plateau additions. Dynamic areal uptake rates U_{dyn} and uptake velocities $V_{f_{\text{dyn}}}$ are calculated from Sw_{dyn} , discharge, stream width, and nutrient concentrations as described by Covino et al. (2010a, b; Online resource 2). We determined ambient uptake lengths from slug additions ($Sw_{\text{amb\_sl}}$) by plotting the Sw_{dyn} of the individual grab samples against the respective nutrient concentrations of the BTC and calculating $Sw_{\text{amb\_sl}}$ at ambient nutrient concentrations from the linear regression models. From $Sw_{\text{amb\_sl}}$ estimates, we calculated ambient areal uptake rates ($U_{\text{amb\_sl}}$) and ambient uptake velocities ($V_{f_{\text{amb\_sl}}}$) similar to the calculations of the dynamic uptake rates and velocities. In order to obtain kinetic uptake curves, we calculated total nutrient uptake (U_{tot} , $V_{f_{\text{tot}}}$) by adding ambient to dynamic uptake parameters for each grab sample (for details, see Covino et al. 2010a, b). We plotted U_{tot} and $V_{f_{\text{tot}}}$ data against nutrient concentrations for each grab sample along the BTC and fitted linear, hyperbolic, and power functions to these relationships via least-squares regression (SigmaPlot version 13.0). In the case of valid Michaelis–Menten models for U_{tot} ($p < 0.05$), we determined both the maximum uptake rate $U_{\max}$ and the half saturation constant K_m for this slug addition.

We estimated water velocities (m s^{-1}) integrated over the entire reach from the BTC of the slug additions by dividing reach length by the time at which (a) conductivity increased (maximum velocity), (b) conductivity reached its maximum (average velocity), and (c) conductivity was back at background (minimum velocity). For the plateau additions, reach length was divided by the time at which (a) half of the maximum conductivity was reached for average velocity, and (b) the plateau was reached for minimum velocity (Gordon et al. 2004).

Statistics

We checked all data for normal distribution and homogeneity of variance with Kolmogorov–Smirnov, Shapiro–Wilks, and Levene tests. Variables were $\log(x + 1)$ -transformed to meet the assumption of normality whenever necessary. We used paired t-tests to detect differences in average hydrology (discharge, water velocity), morphology (stream width, depth), and nutrient and DOC concentrations between summer 2015 and 2016. We performed Pearsons or Spearman rank correlation tests to determine the relationship between mean hydro-morphology, water chemistry, chlorophyll-a concentrations, and ambient uptake metrics. Results at $p < 0.05$ were considered significant. All statistical analyses were performed in IBM SPSS Statistics 24.0 (IBM Corporation 2016).

Results

Water chemistry and hydro-morphology

Paired t-tests revealed no significant differences in average discharge, current velocities, stream width, channel depth, and nutrient and DOC concentrations between summer 2015 and 2016 ($p < 0.05$, $n = 18$ per parameter). Thus, data from both years were merged for subsequent correlation analyses. We observed significant positive correlations among mean SRP, $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, and DOC concentrations and the trophic state of the streams (Pearson, $p < 0.04$, $n = 18$). Average concentrations of SRP and $\text{NH}_4\text{-N}$ increased from a few Micrograms per liter in the oligotrophic streams to $> 400 \mu\text{g L}^{-1}$ SRP and $> 150 \mu\text{g L}^{-1}$ $\text{NH}_4\text{-N}$ in the polytrophic streams (Table 1). Average $\text{NO}_3\text{-N}$ and DOC concentrations were between 1 mg L^{-1} in oligo/mesotrophic streams and $3\text{--}7 \text{ mg L}^{-1}$ in the eu/polytrophic streams. Mean chlorophyll-a concentrations ranged from about 1 to $12 \mu\text{g cm}^{-2}$ and correlated positively with SRP (Spearman, $p < 0.004$, $n = 18$), $\text{NH}_4\text{-N}$ ($p < 0.001$), $\text{NO}_3\text{-N}$ ($p < 0.05$), and the trophic state ($p < 0.001$).

Impacted streams showed slightly lower mean discharges and current velocities and increased depth-to-width ratios than near-pristine streams (Table 1). This resulted in significant negative correlations between discharge and $\text{NH}_4\text{-N}$ and SRP concentrations (Pearson, $p < 0.01$, $n = 18$). Mean

current velocities correlated negatively with average nutrient and DOC concentrations ($p < 0.01$), while depth-to-width ratios correlated positively with nutrient concentrations ($p < 0.05$). Average discharge ranged from 40 to 130 L s^{-1} . Mean reach-integrated current velocities were between 0.2 and 0.25 cm s^{-1} , with values around 0.1 cm s^{-1} in the two polytrophic streams Si and Go. Depth-to-width ratios were between 0.3 and 0.6 for most streams, with the exception of Go, where the slightly incised profile resulted in a depth-to width ratio of 0.1.

Ammonium and SRP uptake kinetics

Uptake rates for $\text{NH}_4\text{-N}$ and SRP across the BTC either followed a hyperbolic curve of the Michaelis–Menten type or showed a positive linear relation with nutrient concentrations (Table 2, Figs. 1, 2). However, in three of the nine MM-models, estimated K_m values were so high that the models were essentially linear at realistic in-stream nutrient concentrations ($< 500 \mu\text{g L}^{-1}$). Therefore, it was not possible to identify any patterns of U_{max} or K_m across all streams. However, in the case of $\text{NH}_4\text{-N}$, K_m generally decreased with increasing trophic state, showing the lowest values in the eu-polytrophic stream Si (Table 2, Fig. 1). There, K_m was less than half the average $\text{NH}_4\text{-N}$ background concentrations, indicating saturation. In contrast, U_{max} was highest in Si, while the (oligo-)mesotrophic streams showed intermediate and the (meso-)eutrophic streams the lowest maximum uptake rates. We observed linear $\text{NH}_4\text{-N}$ uptake curves in two of the oligotrophic streams as well as in the two polytrophic streams, whereby slopes decreased with increasing trophic state (Fig. 1). SRP uptake rates showed linear models in five of the nine streams (Table 2). We could not observe any patterns in slope, U_{max} or K_m across streams regarding SRP uptake (Fig. 2).

Uptake rate curves of both nutrients usually showed distinct hysteresis (Table 2, Fig. 3a, Online resource 3), whereby oligotrophic streams tended to exhibit counter-clockwise hysteresis (Kb, Er, and Gr), while streams with higher trophic states showed clockwise hysteresis. Regarding the other uptake parameters, Sw increased and V_f decreased (Online resource 4) with increasing nutrient concentrations in the case of MM-models. In the case of linear models, Sw and V_f did not show any relation with concentrations. We also

Fig. 1 $\text{NH}_4\text{-N}$ uptake rates U versus $\text{NH}_4\text{-N}$ concentrations during the experiments. Lines represent modelled kinetic uptake rates from the break-through curves of the slug additions. Symbols represent in situ uptake rates during plateau additions with increasing concentrations. Upper graph: oligotrophic (black) and meso- to eutrophic (grey) streams. Lower graph: eu-polytrophic and polytrophic streams

observed one case of bio-stimulation in the stream Gr, where Sw decreased and Vf increased with increasing SRP concentrations across the BTC.

When comparing kinetic uptake curves of the slug additions with the uptake rates of the plateau additions, we observed different patterns depending on the degree of nutrient loading (Figs. 1, 2). In streams with ambient nutrient concentrations $< 20 \mu\text{g L}^{-1}$ (Kb, Er, and Gr for $\text{NH}_4\text{-N}$), slug additions yielded distinctly higher uptake rates (U_{dyn}) than plateau additions (U_{ex}) at the same nutrient concentrations. At ambient concentrations of $20\text{--}50 \mu\text{g L}^{-1}$ (Zb, Ga, Gr for SRP, Sb for $\text{NH}_4\text{-N}$), U_{ex} were either higher or similar to U_{dyn} , while above an average of $70 \mu\text{g L}^{-1}$, U_{ex} were again distinctly lower than U_{dyn} . Similar patterns were observed for experimental uptake velocities (Online resource 4). Across streams, moderately

impacted streams showed higher U_{ex} and Vf_{ex} than slightly and heavily impacted streams at comparable concentrations.

Ambient ammonium and SRP uptake patterns across streams

Consecutive uptake lengths of plateau additions (Sw_{ex}) did not always follow a linear curve when plotted against plateau concentrations. This rendered the calculation of ambient uptake parameters impossible for some of the plateau additions (Table 2). In three cases, Sw_{ex} was highest during the second addition (Ga for $\text{NH}_4\text{-N}$, Gr and Zb for SRP), in one case it was lowest (Si for SRP), resulting in either low r^2 values or non-significant regression curves. In Sb, we observed decreasing Sw_{ex} with increasing added

Fig. 2 SRP uptake rates U versus SRP concentrations during the experiments. Lines represent modelled kinetic uptake rates from the break-through curves of the slug additions. Symbols represent in situ uptake rates during plateau additions with increasing concentrations. Upper graph: oligotrophic (black) and meso- to eutrophic (grey) streams. Lower graph: eu-polytrophic and polytrophic streams

nutrient concentrations for both $\text{NH}_4\text{-N}$ and SRP (biostimulation). Furthermore, uptake during plateau conditions was so low in the two highly polluted streams Ru and Go, that none of the plateau additions yielded significant uptake lengths Sw_{ex} .

Estimates of ambient uptake parameters differed depending on the assessment method (slug or plateau additions) and the nutrient loading of the streams (Table 2). In the cases of counter-clockwise hysteresis (oligotrophic streams), $\text{Sw}_{\text{amb\_sl}}$ calculated from slug additions were generally larger than $\text{Sw}_{\text{amb\_plat}}$ calculated from plateau additions. Concurrently, $U_{\text{amb\_sl}}$ and $V_{\text{f\_amb\_sl}}$ were lower than $U_{\text{amb\_plat}}$ and $V_{\text{f\_amb\_plat}}$. In the cases of clockwise hysteresis, we usually observed the reverse pattern with shorter Sw_{amb} and higher U_{amb} and $V_{\text{f\_amb}}$ calculated from slug additions

than those from plateau additions. Ambient uptake parameters determined from slug additions were also affected by hysteresis (Fig. 3b). Depending on the direction and magnitude of the hysteresis, $U_{\text{amb\_sl}}$ and $V_{\text{f\_amb\_sl}}$ calculated from the ascending part of the curve were either lower (counter-clockwise hysteresis) or higher (clockwise hysteresis) than those calculated from the descending part.

In most streams, ambient uptake lengths were larger and ambient uptake rates and uptake velocities were lower for SRP than for $\text{NH}_4\text{-N}$ (Table 2). Considering the differences in ambient uptake parameters between plateau and slug additions, we decided to take only data from slug additions for the correlation analyses. In general, $\text{Sw}_{\text{amb\_sl}}$ and $U_{\text{amb\_sl}}$ increased, while $V_{\text{f\_amb\_sl}}$ decreased with increasing ambient

Fig. 3 a: Examples of NH_4-N uptake rates U versus NH_4-N concentrations across the break-through curves of the slug additions showing counter-clockwise (left graph; Gr = Grestenbach) and clockwise hysteresis (right graph; Go = Göllersbach). b: Hysteresis patterns of NH_4-N uptake curves showing ambient

uptake velocities (V_f) calculated from the whole curve (black bars), the ascending part of the curve (white bars), and the descending part of the curve (grey bars) for each stream. Arrows indicate clockwise (down) and counter-clockwise (up) hysteresis

concentrations of the respective nutrient across all streams (Table 2). However, variability among streams was high and relationships often showed non-linear behavior, thus resulting in non-significant correlations. We observed positive correlations between U_{amb_sl} of both nutrients and concentrations of SRP (Spearman, $p < 0.005$, $n = 9$), NH_4-N ($p < 0.001$), NO_3-N ($p < 0.032$), and DOC ($p < 0.004$). In addition, U_{amb_sl} correlated negatively with mean current velocity ($p < 0.01$) and discharge ($p = 0.002$; only NH_4-N). Except significant negative correlations between NH_4 and $N\ V_{f_{amb\_sl}}$ and ambient SRP concentrations ($p = 0.014$) as well as chlorophyll-a concentrations

($p = 0.016$), we observed no other correlations between $V_{f_{amb}}$ and Sw_{amb} and any of the hydro-morphological or water quality parameters.

Discussion

How does chronic nutrient loading affect the nutrient uptake capacities of headwater streams?

O'Brien and Dodds (2010) defined chronic loading as long-term increases in ambient nutrient and DOC

concentration above reference conditions (see also e.g., García et al. 2017). Chronic loading may lead to adaptations of the benthic community in both structures (e.g. biomass, community composition; Niyogi et al. 2004; Ribot et al. 2013) and processes (e.g. mineralization, nitrification, etc.; Dodds et al. 2002; Sabater et al. 2011; Rodríguez-Cardona et al. 2016; Weigelhofer et al. 2018), affecting in-stream nutrient uptake significantly (Niyogi et al. 2004; O'Brien and Dodds 2010). Nutrient saturation curves from both a field study and a flume experiment yielded flatter slopes, but higher maximum uptake rates in nutrient-rich than in nutrient-poor systems (Covino et al. 2012; Ribot et al. 2013). These results suggest that streams exposed to chronic loading are less efficient in nutrient uptake than pristine systems at low nutrient concentrations, but possess a higher retention capacity at elevated concentrations (Covino et al. 2012). Contrary to our expectations, though, we did not find a clear relationship between the degree of nutrient loading and the uptake capacities of our study streams. The kinetic uptake models from the BTC curves were highly variable and did not show any consistent patterns of $U_{\max}$ or K_m across streams in our study. In fact, the majority of uptake curves exhibited linear behavior at realistic concentrations, an observation also made in other studies (Dodds et al. 2002; O'Brien and Dodds 2010; Diemer et al. 2015; García et al. 2017). According to Dodds et al. (2002), nutrient uptake curves tend to be linear if nutrient uptake is limited by mass transport, i.e. if diffusion rather than saturation is the main driver for the uptake process. Consequently, linear uptake curves are expected to occur mainly at low nutrient concentrations (Dodds et al. 2002; Earl et al. 2006). However, in some studies, streams exhibited linear relationships between uptake rates and nutrient concentrations up to highly nutrient-enriched conditions, showing no signs of saturation at all (Dodds et al. 2002; O'Brien and Dodds 2010). Likewise, we observed linear uptake models in both the least and the highest impacted streams. Nutrient uptake in stream ecosystems is controlled by several factors, such as mass transfer, biotic uptake, abiotic adsorption, and various dissimilatory processes, all of which may exhibit different forms of concentration-dependent uptake curves (Sanford and Crawford 2000; Dodds et al. 2002; O'Brien et al. 2007; Trentman et al. 2015). Consequently, linear relationships between uptake rates and nutrient

concentrations in slightly and heavily impacted streams may result from different mechanisms controlling nutrient uptake.

Both, ambient uptake lengths and uptake rates of our impacted streams were usually in the upper range of other studies and, thus, representative of nutrient-enriched systems (Macrae et al. 2003; Haggard et al. 2005; Ensign and Doyle 2006; Gücker and Pusch 2006; Newcomer Johnson et al. 2016). As expected, ambient uptake rates generally increased with nutrient loading, while ambient uptake velocities decreased, indicating a decreasing nutrient demand with increased nutrient supply (Covino et al. 2010b; Martin et al. 2011; Covino et al. 2012; Sheibley et al. 2014). However, the variability among our streams was high and our concentration-dependent uptake curves did not follow any clear patterns across streams. Reasons for this lack of uptake patterns across streams are (1) differences in biomass and/or hydromorphology among stream systems (Ensign and Doyle 2006; Covino et al. 2010a; Diemer et al. 2015); (2) other factors than nutrient concentrations influencing biotic uptake, such as e.g., light or temperature (Earl et al. 2006; Arce et al. 2014; Rodríguez-Cardona et al. 2016); (3) different responses of communities to nutrient pulses in pristine and impacted streams (O'Brien et al. 2007; García et al. 2017); (4) interactions among nutrients and/or dissolved organic matter (Earl et al. 2006; Gibson et al. 2015). Especially if streams cover a wide range of concentrations, as was the case in our study, synergistic and antagonistic effects between influencing factors and different response curves may lead to non-linear, multifactorial uptake curves.

How does the duration of nutrient pulses affect the nutrient uptake in streams across a gradient of nutrient loading?

According to our expectations, oligo-mesotrophic streams showed distinctly higher uptake rates and velocities during slug than during plateau additions at comparable nutrient concentrations. This suggests a fast response of reactive sites to sudden nutrient pulses in an otherwise nutrient-depleted system, followed by a similarly fast saturation of the (usually) thin biofilm communities. The high initial uptake rates may result from mechanisms, which support the short-term storage of nutrients in the stream, such as e.g., luxury

P uptake or adsorption (Reddy et al. 1999). In contrast, our meso-eutrophic streams were more efficient in nutrient uptake when the nutrient pulse lasted longer. Chronically loaded streams are usually characterized by increased biofilm biomass (Niyogi et al. 2004; Sabater et al. 2011; this study). However, an increase in biomass does not necessarily lead to an increase in the areal extend of the reactive sites, but may rather result in the vertical stratification of potential uptake sites on the sediment surface (Battin et al. 2003). As nutrient diffusion through biofilms depends on nutrient residence times, microorganisms in deeper biofilm layers should profit from longer pulse duration (Battin et al. 2003; Teissier et al. 2007; Ribot et al. 2013). Equally, the chance for hyporheic uptake increases the longer the nutrient-enriched condition exists. This is especially important for nutrient loaded streams, where the hyporheic water exchange may be restricted due to sediment clogging (Macrae et al. 2003; Ensign and Doyle 2005; Weigelhofer et al. 2013; Sheibley et al. 2014). Surprisingly, the most striking differences between slug and plateau additions occurred in our polytrophic streams. There, uptake lengths derived from plateau additions were so high that we considered nutrient uptake insignificant. In contrast, slug additions showed uptake of both nutrients up to the peak of the pulse. Again, these differences may derive from a reduced uptake capacity of the biofilm community, which becomes more relevant the longer the pulse lasts. In addition, prolonged nutrient enrichment may stimulate mineralization of organic matter especially in systems adapted to nutrient pulses, thereby counteracting uptake (Dodds et al. 2002; Suberkropp et al. 2010).

We are naturally aware that the comparison of different in-stream nutrient addition methods rely on comparable environmental conditions during the experiments. Although hydro-morphology and water quality did not reveal significant differences between our two sampling periods, overall stream conditions were surely not identical and may eventually have caused the above described differences in uptake patterns between slug and plateau additions. Besides, the number of study streams is too low actually to draw conclusions about the effects of pulse duration on the various mechanisms controlling in-stream nutrient uptake in nutrient-low and nutrient-rich systems. Nevertheless, the results of our study indicate that time may play a role in the uptake of nutrient pulses, as

different transport and uptake mechanisms may occur both simultaneously and consecutively during the pulse depending on the degree of nutrient loading. Further and more systematic research is needed to clarify the effects of time on the uptake of nutrient pulses in both pristine and chronically loaded systems.

What may hysteresis patterns reveal about nutrient uptake in chronically loaded streams?

Several authors have observed hysteresis in kinetic uptake curves similar to our observations (Gibson et al. 2015; Rodríguez-Cardona et al. 2016; Koenig et al. 2017). Hysteresis is assumed to reflect the significance of in-channel processes versus transient storage (hyporheic) processes for nutrient uptake, represented by the rising and the falling limb of the uptake curve, respectively (Gibson et al. 2015; Rodríguez-Cardona et al. 2016). As hyporheic processes depend on longer nutrient residence times, a dominance of hyporheic uptake should, thus, result in counter-clockwise hysteresis, yielding higher uptake rates in the falling limb (Gibson et al. 2015). In contrast, clockwise hysteresis indicates an initially high uptake of nutrients directly at the water–sediment interface, followed by a slower and less efficient uptake in other, possibly transport-controlled stream compartments. Koenig et al. (2017), for example, found clockwise hysteresis patterns in streams where hydrological models indicated a dominance of in-channel uptake over hyporheic processes. In our study, hysteresis changed from counter-clockwise in the oligotrophic streams to clockwise in the higher impacted streams. This would mean that the hyporheic zone becomes less important for in-stream nutrient uptake as the degree of nutrient loading increases. Again, the low number of cases does not allow for generalization of this pattern. However, a study on phosphorus adsorption yielded increased fine sediment accumulations in our moderately to highly impacted streams, indicating clogging of the hyporheic zone there (Weigelhofer et al. 2018). Other studies confirm the assumption that hyporheic uptake is less important in chronically loaded (agricultural) streams (Macrae et al. 2003; Ensign and Doyle 2005; Sheibley et al. 2014). Macrae et al. (2003), for example, measured the lowest SRP retention rates in pools where the interaction between the water column and the sediments was reduced by fine sediments.

Further investigations are needed across different stream systems to understand the mechanisms behind clockwise and counter-clockwise hysteresis occurring in nutrient uptake curves.

Conclusions

Chronic nutrient loading affects the capacity of headwater streams to retain nutrients imported from the terrestrial catchment. Our study indicates that these effects may reach far beyond a mere saturation of the affected community. Comparisons of slug and plateau additions revealed that oligotrophic streams were most efficient in nutrient uptake during short nutrient pulses, while eutrophic streams profited from longer pulse duration. These results suggest that increased biofilm thickness and clogging of the sediments restrict nutrient transport to reactive sites in nutrient loaded systems, thereby adding transport-limitation at the micro-scale as driver for nutrient uptake in these streams. In accordance with these findings, hysteresis patterns of concentration-dependent uptake rates indicated a decreasing significance of hyporheic uptake processes in streams exposed to chronic nutrient loading.

Understanding the multiple effects of nutrient loading on the various uptake and retention processes in headwater streams is vital for a sustainable management of these systems. Interactions among biological, physico-chemical, and hydrological retention processes as well as differences in short- and long-term responses to nutrient enrichment may create both antagonistic and synergistic effects of nutrient loading on nutrient uptake dynamics. Increased algal and microbial biomass, for example, increases the capability of streams to retain nutrients biologically, but it may also restrict the physical access of nutrients to potential uptake sites. Fine sediments from the agricultural catchment, in turn, possess enhanced adsorption capacities, but may reduce the hyporheic water exchange, thereby restricting nutrient uptake to channel processes. Like others, our study underlines the importance of applying different field (and laboratory) methods to identify the main mechanisms responsible for nutrient retention in streams exposed to nutrient loading. We want to encourage further and more systematic research of time-dependent interactions among the different biological, physico-chemical, and

hydrological retention processes to increase our understanding of the consequences of chronic nutrient loading for nutrient uptake dynamics in streams.

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Compliance with ethical standards

Conflict of interest :The authors declare that they have no conflict of interest.

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