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Influence of transient storage on stream nutrient uptake based on substrata manipulation

Alba Argerich
Universitat de Barcelona, Barcelona, Spain

Eugènia Martí
Limnology group, Centre d’Estudis Avançats de Blanes (CSIC), Blanes, Spain

Francesc Sabater
Universitat de Barcelona, Barcelona, Spain

Roy Haggerty
Oregon State University, Corvallis, Oregon, USA

Miquel Ribot
Limnology group, Centre d’Estudis Avançats de Blanes (CSIC), Blanes, Spain

Alba Argerich, Dept. d’Ecologia, Universitat de Barcelona, Diagonal 645, Barcelona 08028, Spain. (alba@ceab.csic.es)

Eugènia Martí, Centre d’Estudis Avançats de Blanes, Consejo Superior de Investigaciones Científicas, Accés a la Cala St. Francesc 14, Blanes 17300, Girona, Spain. (eugenia@ceab.csic.es)

Francesc Sabater, Dept. d’Ecologia, Universitat de Barcelona, Diagonal 645, Barcelona 08028, Spain. (fsabater@ub.edu)

Roy Haggerty, Dept. of Geosciences, 104 Wilkinson Hall, Oregon State University, Corvallis, OR, USA 97331-5506. (haggertr@geo.oregonstate.edu)

Miquel Ribot, Centre d’Estudis Avançats de Blanes, Consejo Superior de Investigaciones Científicas, Accés a la Cala St. Francesc 14, Blanes 17300, Girona, Spain. (mribot@ceab.csic.es)
Abstract

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Keywords: water transient storage, stream nutrient uptake, phosphate, ammonium, nitrogen assimilation.
1. Introduction

In the last decades, studies focusing on stream nutrient uptake have gained importance due to the global concern about eutrophication of freshwater ecosystems and nutrient delivery to coastal ecosystems [Alexander et al., 2000]. Many studies have demonstrated the functional relevance of streams and rivers in modulating the concentrations and the forms of nutrients exported downstream. Up to 50-75% of nitrogen load of a watershed can be reduced by in-stream processes [Peterson et al., 2001], and denitrification in streams can account for up to 18% on average of N loss [Mulholland et al., 2008]. In relation to stream contribution to phosphorus retention, Mulholland [2004] found that 30% of the soluble reactive phosphorus entering the stream was annually removed by in-stream processes. Nevertheless, we still need to increase our understanding on the factors that control the variability in stream nutrient uptake response both among streams and within streams over time.

The biogeochemical processing capacity of streams and rivers is mostly mediated by the biological communities developed in these ecosystems, but hydrology and physical features of stream channels modulate the biological response through modifying their opportunity [Battin et al., 2008]. Stream nutrient retention results from the interaction of hydrologic, chemical, and biological retention [Valett et al., 1996; McClain et al., 2003]. Higher hydrologic retention is expected to increase nutrient retention through increases in water residence time and sediment-water interaction that enhance the interaction between nutrients and stream biota. Hydrologic retention is influenced by both stream hydrology and channel morphology that creates zones of water transient storage. While the influence of stream discharge on nutrient uptake is widely documented, results on the influence of transient water storage on nutrient uptake are less consistent. Several studies have shown that nutrient uptake efficiency decreases as stream discharge increases, with the most plausible explanation being the decrease in the ratio between the stream bioreactive surface and the water volume [Hall et al., 2002; Marti et al., 2004; Argerich et al., 2008]. Stream transient water storage occurs where water moves at much slower velocity than the average [Bencala and Walters, 1983]. Surface-to-subsurface water exchange is widely thought to be a major contributor to transient storage; and thus it is usually associated with the hyporheic zone [Triska et al., 1989; Morrice et al., 1997; Butturini and Sabater, 1999]. However, surface structures determining the physiographic nature of the channel morphology also contribute to enlarge transient storage. For instance, pools, eddies, backwaters and debris dams, but also leaf packs and woody debris, increase water residence time along a stream reach [Lautz et al., 2006; Bukaveckas, 2007; Roberts et al., 2007; Argerich et al., 2008]. It is expected that higher transient storage may enhance interaction between nutrients and microbial communities developed in the different stream habitats and thus, increase nutrient uptake. For this reason, quantification of transient storage has become a critical issue in biogeochemical studies of stream ecosystems addressed to examine spatial or temporal patterns of nutrient uptake. However, while some studies support the expected relationship between nutrient uptake and transient storage [Valett et al., 1996; Ensign and Doyle, 2005], others indicate a lack of consistent patterns. Webster et al. [2003] and Simon et al. [2005] found that transient storage did not explain temporal variation of nutrient uptake.
velocity nor nutrient uptake rate. Hall et al. [2002] found a weak relation between ammonium uptake velocity and $A_r/A$ and no relation with phosphate. Bukaveckas [2007] reported an ambiguous relationship between transient storage and N and P retention efficiencies. Lautz and Siegel [2007] found a modest correlation between NO$_3$ retention efficiency and transient storage in the Red Creek watershed, Wyoming. Thomas et al. [2003] showed that transient storage accounted for less than half of NO$_3$ retention measured by $^{15}$N in a small headwater stream in North Carolina. Retention processes associated with different stream compartments contributing to transient storage (e.g., pools, algal mats or hyporheic zone) likely differ because communities developed in these habitats are distinct. This may help to explain the lack of consistent patterns between transient storage parameters and nutrient uptake responses. Based on this, we hypothesize that besides the size of transient storage, physical and biotic features of it can be a key factor controlling nutrient uptake. The objective of this study is to experimentally test this hypothesis. With this purpose, we combined the nutrient spiraling approach with hydraulic transport modeling to examine the effects of introducing different types of substrata into the channel both differing in physical and biological characteristics on phosphate and ammonium retention.

2. Methods

2.1. Experimental setting

The study was conducted during Autumn 2006 in a man-made canal located at the village of Gualba, 60 km north of Barcelona (latitude 41.7332°N, longitude 2.5034°E, northeastern Iberian Peninsula). This canal diverts water from Santa Fe stream for irrigation purposes. The canal is oriented NW to SE and meanders 2 km parallel to the stream with an average slope of 0.0051. The canal is excavated in the soil, with a width of 61±6 cm (mean±standard deviation) and a water depth of 6.7±1.4 cm during the study period. Along the canal, we selected 4 adjacent straight reaches of 20 m each. Between the bottom of reach 2 and the beginning of reach 3 we left 8 meters to avoid a bend in the canal. Natural substratum was homogeneous and comparable among reaches, and it was composed of a 5-6 cm layer of fine-grained sand. Discharge in the canal was regulated by a gate, which we operated during the experiments to ensure a relatively constant discharge.

To address the objective of this study, we used 3 substrata types (mud, sand and cobbles) that were introduced in the reaches in discrete packs. Each substrata pack was 25 cm long and 8 cm wide and consisted of nylon mesh bags filled with a single type of substrata. All the substrata needed to create the packs were collected from the adjacent stream, and thus were naturally colonized. On each sampling date, we placed the substrata packs in three of the 20-m reaches and the fourth reach was left without any substrata to serve as a control. Along each reach, we distributed 40 substrata packs of a single type at 50 cm intervals and with an orientation of 45º relative to the channel margin. Each reach contained a different substrata type. The 4 treatments (i.e., control, cobbles, sand, and mud) rotated among reaches on consecutive sampling dates. A total of 6 sampling dates were done (i.e.,
Substrata packs were removed from the reaches and left in the stream channel between sampling dates. The whole experiment was completed between October 23 and November 29. During this period weather conditions were relatively stable and free of precipitation.

2.2. Characterization of substrata packs

Physical features. Hydraulic conductivity for each substrata pack was estimated based on the water residence time of a conservative tracer within the pack ($T_p$, h). For this we submerged 4 packs of each substrata type on a container with stream water with NaCl during 1 day for packs of cobbles and sand and during 1 week for packs of mud. We then placed these packs in the canal following the same distribution described above, and we recorded electrical conductivity (EC) upstream and 3 meters downstream of the packs with a conductivity meter (LF 340 WTW, Weilheim, Germany) connected to a datalogger (510X Campbell Scientific, Logan, UT, USA). We measured water residence time within the pack as the time needed for downstream point to recover to upstream conductivity values, which indicated that all the interstitial tracer had been released from each substrata type. Water residence time within the packs varied from minutes for cobble packs, to 2.3 hours for sand packs, and more than 16 hours for mud packs. Dominant grain size of each type of substrata packs was in the laboratory by manual sieving of a sample coming from the mixture of the content of 5 packs of the same type.

Biological features. Characterization of assimilation rate of each biotic compartment of the substrata packs was assessed by a single addition of a solution of 104.2 mg $^{15}$NH$_4$ and 104.2 g NaCl mixed in 5 L of ultrapure water during 14 h to obtain an enrichment of 2000‰. The lack of a stable isotope for P did not allowed to test the substrata types and biological compartments for this element. To conduct this addition we included all the 4 reaches together; and thus, the total reach length considered was 88 m. Substrata packs were arranged as in the previous additions; however, substrata types were alternated along the entire reach. The $^{15}$NH$_4$ addition began at 22:30 and continued until 12:30 of the following day. Prior to the $^{15}$NH$_4$ addition, we collected water samples and 3 substrata packs of each type at 10, 48, and 78 m from the addition point to measure background levels of $^{15}$N of both the NH$_4^+$-N in water and the N in the biomass of each pack. Just before the addition was stopped, we collected 3 substrata packs of each type at 18, 38, 66 and 86 m and 4-L water samples at -5, 20, 40, 48, 68, 88 m from the addition point. Those 4-L samples, after being filtered through Whatman® GF/F fiberglass filters (0.7 µm pore diameter), were spiked with 300 µL of unlabeled NH$_4^+$-N to increase NH$_4^+$-N concentration to the ideal working range for mass-spectrometric measurement. Additionally, we added 0.5 g of MgO to reduce all dissolved NH$_4^+$ to NH$_3$, and a filter packet to absorb all the NH$_3$ diffused into the headspace of the sealed bottle consisting of a precombusted 1-cm glass-fiber filter (Whatman GFD) sealed between two Teflon filters and to which we added 25 ml of 2.5 molL$^{-1}$ KHSO$_4$. We placed the filter packet on the surface of the 4-L sample, and we capped the bottle tightly after placing Parafilm over the bottle mouth. An additional 100 mL sample was collected at each sampling site for the analysis of nutrient concentrations. Water samples for nutrient
concentrations were also filtered in the field using Whatman® GF/F fiberglass filters and together with
the substrata packs collected and the 4-L samples bottles were transported refrigerated to the
laboratory.

To measure mass of fine benthic organic matter (FBOM) and epilithic content of each type of
substrata pack, we weighed all sampled substrata packs and divided them into two parts. One part
was used to measure mass of the pack (dry weight (DW), and ash-free dry mass (AFDM)), and the
other part was submerged in a known volume of water, agitated, and 100 mL of water was filtered
through a precombusted Whatman GF/F fiberglass filter. Half of the filter was processed to measure
FBOM in the pack and the other half was used to measure the content of C and N and the $^{15}$N
signature of the FBOM. Epilithic biomass of each substrata pack was measured as the difference
between the content in FBOM and the total ash-free dry mass of the pack. To measure $^{15}$N
assimilation rates at the epilithic compartment we submerged the elutriated substrata in a known
volume of water and we exposed it to ultrasonic waves for 10 minutes. After this time, water was
filtered through precombusted Whatman GF/F fiberglass filters and the filter was dried in the oven
and stored for later analysis of C and N content, and $^{15}$N signature of the epilithon developed on the
surface of the substrata. We did not measure epilithic parameters with the mud packs because it was
impossible to elutriate de sediments due to the small size of the particles forming this type of packs.
Samples for biomass measurements (i.e. part of a substrata pack and filters from FBOM of the packs)
were weighed, dried at 60ºC for ≥3 days, weighed again, combusted a 500ºC for 5h, and reweighed
for calculation of dry mass and ash fry dry mass.

Content of $^{15}$N in NH$_4$-N dissolved in water was analyzed from 4-L filtered water samples. Once in the
laboratory we incubated the bottles at 40ºC for two weeks on a shaker table, we removed the filter
packets and dried them in a desiccator prior to $^{15}$N analysis. Filters with samples for the analysis of the
content of C and N, and the $^{15}$N signature (i.e., epilithic and FBOM samples from the substrata packs,
and filter packets from water samples), were dried in the oven (60ºC, ≥3 days) and weighed. We cut
1 cm-diameter disc from the filter, weighed it on a Mettler-Toledo (Greifensee, Switzerland) MX5
microbalance, and encapsulated in tins. Samples were sent to the Stable Isotope Facility at the
University of California-Davis (Davis, CA, USA) where they were combusted to CO$_2$ and N$_2$ at 1000ºC
in an on-line elemental analyzer (PDZ-Europa ANCA-GSL). Contents of $^{15}$N and $^{14}$N were measured by
continuous flow isotope ratio mass spectrometry (20-20 mass spectrometer; PDZ-Europa, Northwich,
UK).

Results of $^{15}$N were given in $\delta^{15}$N values (units of ‰) as an expression of the $^{15}$N:$^{14}$N ratio in the
sample ($R_{sample}$) relative to the $^{15}$N:$^{14}$N ratio in air used as the standard ($R_{standard}$=0.003663) according
to the following equation:

$$\delta^{15}N = \left[ \frac{R_{sample}}{R_{standard}} - 1 \right] \times 1000$$ (1)
Assimilation rates from 15N addition. We used δ15N values to measure NH₄⁺-N uptake rates at different biological compartments (i.e., epilithon and FBOM) at each type of substrata pack. We first estimated the amount of 15N in each compartment of the different types of substrata packs using the following equation:

\[
^{15}N_{\text{compartmen}} (\mu g^{15}N \cdot \text{pack}^{-1}) = B_{\text{compartmen}} \times \left(\frac{\%N}{100}\right) \times (\text{MF}_i - \text{MF}_b) 
\]  

(9)

where \(^{15}N_{\text{compartmen}}\) is the amount of 15N tracer in each compartment (\(\mu g\) \(^{15}N \text{ pack}^{-1}\)), \(B_{\text{compartmen}}\) is the biomass of the compartment (\(\mu g\) AFDM \cdot \text{pack}^{-1}\)), %N is its percentage of N, MF is the molar fraction of 15N in the compartment at plateau conditions (MF\(_i\)) and at background conditions (MF\(_b\)). Molar fractions were calculated as:

\[
\text{MF} = \frac{^{15}N}{^{15}N + ^{14}N} \left(\frac{\delta^{15}N}{1000} + 1\right) * 0.0036765 
\]  

(10)

We then estimated assimilation rate of each compartment using the equation adapted from von Schiller et al. [2007]:

\[
U_{\text{compartmen}} (\mu g\text{ Nh}^{-1}) = \frac{^{15}N_{\text{compartmen}}}{t_{\text{add}} \times ^{15}N_{\text{flux}}/N_{\text{flux}}} 
\]  

(11)

where \(^{15}N_{\text{compartmen}}\) is the amount of 15N tracer obtained in equation 10 (\(\mu g\) \(^{15}N\text{ pack}^{-1}\)), \(t_{\text{add}}\) is the duration of the 15N addition (h), \(^{15}N_{\text{flux}}\) is the \(^{15}N\text{NH}_4^+\text{-N} \text{ flux at plateau conditions} (\mu g \text{ Nh}^{-1})\) and \(N_{\text{flux}}\) is the total \(\text{NH}_4^+\text{-N} \text{ flux} (\mu g \text{ Nh}^{-1})\). We also calculated the assimilation efficiency of each biotic compartment as the assimilation rate divided by the specific biomass of each compartment (\(B_{\text{compartmen}}\)). Assimilation rate of the pack was calculated as the weighed sum of the assimilation rate at each compartment forming the pack.

2.3. Solute addition experiments

To quantify transient water storage and nutrient uptake, we conducted short-term additions of a conservative tracer (Cl⁻ as NaCl) together with NH₄⁺-N (as NH₄Cl) and PO₄³⁻-P (as Na(H₂PO₄)·2H₂O) in each reach and on each sampling date. On each date, additions started at the most downstream reach and moved consecutively to the nearest upstream reach. Duration of the additions varied between 20 and 30 minutes, based on the time needed for the solution to reach plateau conditions at the bottom of the reach. We automatically recorded EC at 10-s intervals at the head and at the bottom of the reach using conductivity meters (LF 340 WTW, Weilheim, Germany) connected to data
loggers (510X Campbell Scientific, Logan, UT, USA). Water samples for nutrient analysis were taken at the bottom of the reach from the beginning of the addition until EC returned to background levels once the addition was stopped. In each addition, sample frequency was accommodated to changes in EC, but in general we collected samples every 10 seconds during the rise and the fall of the curve and every 30 seconds at plateau. Samples were collected using 10 mL acid-washed syringes, filtered in situ using Whatman® GF/F fiberglass filters (0.7 µm pore diameter), and kept refrigerated until analysis. Concentrations of NH₄⁺-N and SRP were analyzed following standard colorimetric methods [APHA, 1998] using Bran+Luebbe (Norderstedt, Germany) TRAACS 2000 Autoanalyzer.

On average, additions increased background EC by 34±2 µScm⁻¹, soluble reactive phosphorus (SRP) by 67.7±6.2 µg N P⁻¹, and ammonium (NH₄⁺-N) concentrations by 55.6±4.8 µg N L⁻¹ above ambient concentrations. Background concentrations of SRP and NH₄⁺-N were low throughout the study (4.2±0.4 µg P L⁻¹ and 9.4±0.8 µg N L⁻¹). Average background DIN:P molar ratio was 78.9±6.6. On each sampling date and at each reach, we measured wet width and water depth (at 10, 30, 50, 70, and 90% of the wet width) across transects done every 5 meters along the reach. Slope of the streambed was measured at each reach following the hydrostatic levelling method [Gordon, 1992].

Estimation of hydraulic parameters. Discharge (Q, L s⁻¹) and average water velocity (v, m s⁻¹) were measured using the time-curve EC data recorded at the bottom of the reach. Calculation of v was done by dividing the reach length by the time needed to increase the EC one half of the plateau (i.e., Tn nominal travel time, s). Calculation of Q was based on a tracer mass balance approach.

Transient water storage cross section (As, cm²), exchange coefficient between the main channel and the transient storage zone (α, s⁻¹), and cross-sectional area of the main channel (A, cm²) were estimated from EC breakthrough curves using the One-dimensional Transport model with Inflow and Storage (OTIS; Runkel [1998]). This model of downstream solute transport is based on a transient storage model presented by Bencala and Walters [1983]. It accounts for advection, dispersion, dilution due to lateral water inflow, and exchange with transient storage zones (Fig.1). The model relies on the solution to two equations (equation 2 and 3) that calculate the change in solute concentration over time in the main channel and in the transient storage zone:

$$\frac{\partial C}{\partial t} = -\frac{Q}{A} \frac{\partial C}{\partial x} + \frac{1}{\frac{A}{A_s}} \left( AD \frac{\partial C}{\partial x} + q \frac{A}{A_s} (C_L - C) + \alpha (C_s - C) \right) \quad (2)$$

$$\frac{\partial C_s}{\partial t} = \alpha \frac{A}{A_s} (C - C_s) \quad (3)$$

where A is the main channel cross-sectional area (L²), A_s is the storage zone cross-sectional area (L²), C is the main channel solute concentration (ML⁻³), C_s is the storage zone solute concentration (ML⁻³), C_L is the lateral inflow solute concentration (ML⁻³), D is the dispersion coefficient (L²T⁻¹), Q is
discharge \((L^3T^{-1})\), \(q_L\) is the lateral inflow rate on a per length basis \((L^3T^{-1}L^{-1})\), \(t\) is time \((T)\), \(x\) is distance \((L)\), and \(\alpha\) is the storage zone exchange coefficient \((T^{-1})\).

Damköhler values \((\text{Da}_1)\) were calculated with data from each addition to determine whether the length of the reach was suitable for measuring transient storage \([\text{Wagner and Harvey, 1997}]\). Values obtained ranged between 1 and 5, and therefore hydraulic parameters estimated with the OTIS model were considered reliable. The fraction of the median travel time attributable to transient storage \((F_{\text{med}}^{200}, \text{Runkel}[2002])\) was calculated for a standardized length of 200 m using the following equation:

\[
F_{\text{med}}^{200} = \frac{1 - e^{-L(\alpha/\nu)}}{A_\alpha + A_s} \tag{4}
\]

where \(L\) is reach length.

**Estimation of nutrient uptake parameters.** Nutrient uptake coefficients for the main channel \((\lambda, T^{-1})\) and for the transient storage zone \((\lambda_s, s^{-1})\) were estimated using the OTIS model which can account also for first-order uptake at the main channel and at the transient storage zone by adding \((-\lambda C)\) and \((-\lambda_s C_s)\) to equations 2 and 3. We used OTIS with the previously estimated hydraulic parameters to perform simulations of nonconservative solutes \((i.e., \lambda > 0\) and \(\lambda_s > 0)\) using the observed nutrient concentrations \([\text{Runkel, 2007}]\). With those estimated parameters as initial conditions we then run OTIS-P, a modified version of OTIS that allows the automated parameter estimation using nonlinear regression techniques to obtain better adjustments to the observed data-set. Total uptake coefficients \((k, s^{-1})\) for each nutrient where then calculated as:

\[
k = \lambda + \frac{\alpha \lambda_s A_s}{\alpha A + \lambda_s A_s} \tag{5}
\]

To estimate the mass of nutrient retrieved at the bottom of the reach under each scenario, we used the obtained uptake coefficients to perform 4 simulations: (A) conservative transport \((\lambda\) and \(\lambda_s=0)\), (B) nonconservative transport \((\lambda\) and \(\lambda_s>0)\), (C) main channel uptake only \((\lambda>0\) and \(\lambda_s=0)\), and (D) storage zone uptake only \((\lambda=0\) and \(\lambda_s>0)\). Using the results from the simulations, we integrated the area of each curve following equation 6.

\[
\text{mass} = \int_0^t Q(C - C_b) dt \tag{6}
\]

where \(C\) is the simulated nutrient concentration, \(C_b\) is the background nutrient concentration, \(Q\) is stream discharge, and \(t\) is duration of the addition. The percentage of nutrient uptake at the main channel and at the storage zone was then calculated as:
\[
\%\text{uptake}_{\text{main channel}} = \frac{\text{main channel uptake}}{\text{total uptake}} = \frac{\text{mass}_{\text{simA}} - \text{mass}_{\text{simC}}}{\text{mass}_{\text{simA}} - \text{mass}_{\text{simB}}} \times 100
\]

(7)

\[
\%\text{uptake}_{\text{transient storage}} = \frac{\text{transient storage uptake}}{\text{total uptake}} = \frac{\text{mass}_{\text{simA}} - \text{mass}_{\text{simD}}}{\text{mass}_{\text{simA}} - \text{mass}_{\text{simB}}} \times 100
\]

(8)

### 2.4. Statistical analysis

To compare hydraulic and nutrient uptake parameters between control reaches and reaches with substrata packs, and also among substrata pack treatments we run one-way ANOVA with randomized block design. As a block we considered addition date and as a factor we considered type of substrata pack and treated vs. control reaches. We used the Shapiro-Wilk test to examine the normality of the variables and the Levene’s statistic to test the homogeneity of variances. Some variables (i.e., k, and % of retention) were log-transformed and others inverse log-transformed (i.e., λ and λ\text{s}) prior to analysis in order to meet assumptions of normality. We conducted analysis of variance (one-way ANOVA) to compare \textsuperscript{15}N uptake rates among substrata packs. Multiple comparisons were made among treatments following post-hoc Tukey HSD procedure in all significant ANOVA tests. Finally, we examined the relationships between transient storage parameters (A\text{s}, α, and F\text{med}) and nutrient uptake parameters for SRP and NH\textsubscript{4}\textsuperscript{+}-N (λ and λ\text{s}, relative proportion of uptake coefficient to total uptake coefficient (λ\text{s}/k) and percentage of nutrient uptake) by using regression analysis. The significance level for the tests was p=0.05. All statistical analyses were done using SPSS for Windows (version 12.0, SPSS Inc., Chicago).

### 3. Results

#### 3.1. Physical and biological characterization of substrata packs

The packs of the different substrata types were comparable in size and volume; however, they differed in grain size, hydraulic conductivity, organic matter content, size of different biologic compartments, and C:N content. Dominant grain size of each type of substrata packs varied between <1 mm at mud packs to few cm at cobble packs. Hydraulic conductivity followed an inverse relation with grain size. Mud packs presented the finest grain size and the higher water residence time inside the pack, followed by sand packs and cobble packs [Table 1]. Regarding biological features, organic matter content at mud packs was 10.5 times higher than at cobble packs and 1.6 times higher at sand packs. Almost all organic content was attributable to epilithon at cobble packs (98.3%) and at sand packs (86.6%) while mud packs were mainly composed by FBOM [Table 1].

Elemental composition of each biotic compartment also differed between them and among substrata packs. C:N ratio was higher at epilithon than at FBOM, and sand packs presented the highest C:N ratios among treatments. Both biotic compartments differed in \textsuperscript{15}N assimilation. Assimilation rates of


\[ ^{15}\text{N} \text{ were significantly different among types of substrata packs (one-way ANOVA, } F_{(2,23)}=4.164, \ p=0.03, \text{ Fig. 2A)}. \text{ Cobbles showed the highest N assimilation rate (0.95±0.34 µg N h}^{-1}) \text{ followed by mud packs (0.57±0.14 µg N h}^{-1}), \text{ and by sand packs (0.15±0.02 µg N h}^{-1}). \text{ Regarding biotic compartments, } ^{15}\text{N assimilation efficiency was 3.5±0.5 more efficient at FBOM compartment than at epilithic compartment at cobbles and 3.7±1.2 times and sand packs (Fig. 2A). Assimilation efficiency was highest at cobbles packs, intermediate at sand packs and lowest at mud packs.}

### 3.2. Effects of substrata packs at reach scale

**Comparison of hydraulic parameters.** Discharge was on average 4.5±0.5 L s}^{-1} (n=24, Table 2) and did not significantly differ between treatments, although it slightly varied among sampling dates. Average \( \nu \) ranged between 0.08 and 0.30 m s}^{-1} and was lower in the reaches with substrata packs than in the control reaches. Water transient storage parameters (i.e., \( A_\nu \), \( \alpha \), and \( F_{\text{med}}^{200} \)) increased after introducing substrata packs. Values of \( A_\nu \) increased by 2.3 times at reaches with substrata packs (one-way ANOVA, \( F_{(1,17)}=28.16, p=0.00 \)) and this increase was similar among treatments (Fig. 3A). Values of \( \alpha \) ranged between 0.8 x10}^{-3} s}^{-1} and 5.0 x10}^{-3} s}^{-1} and also increased at treated reaches (from 1.5±0.4 x10}^{-3} s}^{-1} to 3.1±0.3 x10}^{-3} s}^{-1}; one-way ANOVA, \( F_{(1,17)}=12.51, p=0.00, \text{ Fig. 3B). The fraction of median travel time attributable to transient storage (\( F_{\text{med}}^{200}, \% \)) was on average 2.4 times higher at reaches with substrata packs that at control reaches (one-way ANOVA, \( F_{(1,17)}=11.77, p=0.00 \)); greatest differences were observed between control reaches and reaches with mud packs (Fig 3C).

**Comparison of nutrient uptake.** Total phosphate uptake coefficient (k) ranged between 0.3 x10}^{-3} s}^{-1} and 6.2 x10}^{-3} s}^{-1}, it was highest at reaches with sand packs and lowest at reaches with mud packs (Fig. 4A). The average value considering all treatments was 2.1±0.4 x10}^{-3} s}^{-1} which represents a percentage of overall retention of 20.8±2.9% (Table 3). As almost all the phosphate retention occurred at the main channel (i.e., 92.0±3.5 %), \( \lambda \) also ranged between 0.3 x10}^{-3} s}^{-1} and 6.2 x10}^{-3} s}^{-1} and it followed the same pattern showed for k (Table 3). No significant differences were found between control and treated reaches or among treatments for uptake occurring at the main channel zone. Uptake coefficients at the transient storage zone ranged between 1.0 x10}^{-17} s}^{-1} and 7.9 x10}^{-3} s}^{-1}, lowest average values were found at control and sand reaches and highest at reaches with mud packs (Fig. 4C). Percentage of retention at the transient storage zone accounted for 8.0±3.5% of the total phosphate retention but this fraction differed among treatments. Significant differences were found for uptake coefficients at the transient storage zone (one-way ANOVA, \( F_{(1,17)}=4.49, p=0.02 \) being greater between reaches with mud packs and control reaches. Relative contribution of uptake at the transient storage zone to total uptake (\( \lambda_{\nu}/k \)) was positively related to water transient storage size (\( \lambda_{\nu}/k= 5.29e^{-13}0.71As, R^2=0.37, p=0.00; \text{ Fig. 5A), to water exchange coefficient (} \lambda_{\nu}/k= 5.1e^{-10}0.13-16, R^2=0.36, p=0.00), and to \( F_{\text{med}}^{200} (\lambda_{\nu}/k= 7.11e^{-10}0.12F_{\text{med}}, R^2=0.23, p=0.02). \)

Total ammonium uptake coefficient (k) ranged between 1.1 x10}^{-3} s}^{-1} and 8.7 x10}^{-3} s}^{-1}, it was highest at control reaches and lowest at reaches with cobble packs (Fig. 4B). The average value considering
all treatments was \(4.2 \pm 0.4 \times 10^3\) s\(^{-1}\) which represents a percentage of overall retention of \(37.9 \pm 3.2\%\) (Table 3). As almost all the ammonium retention occurred at the main channel (i.e., \(96.1 \pm 2.4\%\)), \(\lambda\) also ranged between \(1.1 \times 10^3\) s\(^{-1}\) and \(8.7 \times 10^3\) s\(^{-1}\) and it followed the same pattern showed for \(k\) (Table 3). No significant differences were found between control and treated reaches or among treatments for uptake occurring at the main channel zone. Uptake coefficients at the transient storage zone ranged between \(2.0 \times 10^{-14}\) s\(^{-1}\) and \(16.5 \times 10^{-3}\) s\(^{-1}\), lowest average values were found at control reaches and highest at reaches with mud packs [Fig. 4D]. Percentage of retention at the transient storage zone accounted for \(3.9 \pm 2.4\%\) of the total ammonium retention but this fraction differed among treatments. Significant differences were found for uptake coefficients at the transient storage zone (one-way ANOVA, \(F_{(1,17)}= 7.25, p=0.00\)). Greatest differences were observed between mud packs and the rest of the treatments. Relative contribution of uptake at the transient storage zone to total uptake was positively related to water transient storage size \(\lambda_s/k = 7.6 \times 0.29^{0.29\theta}, R^2=0.26, p=0.01;\) [Fig. 5B], but not to water exchange coefficient or to \(F_{\text{med}}\).

4. Discussion

The objective of this study was to test the hypothesis that differences in nutrient uptake when introducing “flow obstacles” into the streambed are not only attributable to the modification of the size of water transient storage but also to the physical and biological characteristics of the structure creating this water transient storage zone.

The introduction of substrata packs into the channel created a similar amount of \(A_s\) using structures differing in their properties. Those differences among types of substrata packs were mainly physical (e.g. different hydraulic conductivity) but also biotic (e.g. different size of two biologic compartments: epilithon and FBOM). The introduction of substrata packs also increased \(A_s/A\) ratio, almost homogeneously among treatments, by 2.0. To avoid possible misinterpretations of hydrological results due to changing discharge we studied the evolution of \(F_{\text{med}}\) which takes in consideration variations in water velocity. \(F_{\text{med}}\) gives the degree of participation (in %) of water transient storage to the overall water median travel time. Results confirmed that introduction of substrata packs increased the contribution of the transient storage zone on the overall water travel time by 2.4 and that mud packs and control reaches presented the greatest differences in terms of transient storage probably due to the fact that mud packs presented the lowest hydraulic conductivity [Runkel, 2002].

Others have studied the effects of introducing flow-obstacles on water transient storage and nutrient uptake parameters with contrasting results. Ensign and Doyle [2005] introduced baffles into a channel and increased \(A_s/A\) and \(F_{\text{med}}\) 1.2 times and in this case nutrient uptake, expressed as uptake velocity, increased several times for both phosphate and ammonium. Similarly, Roberts et al. [2007] increased...
A$_w$/A ratio up to 2.1 times and $F_{200}^{med}$ 1.8 times after introducing coarse woody debris into 4 stream channels and ammonium uptake also increased. Contrary to the results found by above authors, the fact of increasing A$_w$/A and $F_{med}$ in our study, was not reflected in an increase in the overall reach nutrient uptake at treated reaches but only in the retention occurring at the transient storage zone.

The relative contribution of nutrient uptake at the transient storage zone to total uptake was positively related to the size of transient storage zone. Nutrient uptake increased with increasing transient storage in several studies. Lautz and Siegel [2003] constructed a regression model relating transient storage parameters and measures of stream flow to nitrate uptake length. The model explained almost half of the variability in nitrate uptake. Ensign and Doyle [2005] find a significant increase in phosphate and ammonium uptake after adding baffles into the channel. Argerich et al. [2008] also found that an increase in A$_w$ due to leaf litter accumulation resulted in an increase in phosphate and ammonium uptake velocity. Ryan et al. [2007] found a positive relation between K-PO$_4$ and A$_w$/A in two streams from an urbanizing watershed. When we consider studies which compile data from different streams this relation is not as clear. Hall et al. [2002] found a weak relation between ammonium uptake velocity and A$_w$/A and no relation with phosphate. In this case, in a part of the streams studied, phosphate removal was controlled by chemical sorption to sediments and biological uptake was relatively unimportant. Webster et al. [2003], in a study where they analyzed data collected from 11 streams from different biomes, did not found any relation between transient storage parameters and ammonium retention. Simon et al. [2005] studied the temporal variation of N and P uptake in two New Zealand streams and concluded that water transient storage was not explaining variability in uptake rates. Ensign and Doyle [2006], after analyzing data from 52 published papers found little evidence of a cause-effect relation between water transient storage and nutrient uptake. The authors attributed this lack of patterns to the fact that the transient storage data reviewed represented generic measurements in which specific mechanisms of storage were not identified.

Nevertheless, results were different between nutrients. While the relative contribution of ammonium uptake at the transient storage zone to total uptake was only weakly related to transient storage size, phosphate uptake was positively related to A$_w$, α and $F_{200}^{med}$. Those results point the idea of different mechanisms governing ammonium and phosphate retention processes. Simon et al. [2005] also found differences in NH$_4$ and PO$_4$ retention patterns when examining temporal variation of nutrient uptake in 2 streams. Butturini and Sabater [1998] compared factors controlling phosphate and ammonium retention at low discharges, and suggested that biotic control may have played a larger role in NH$_4$ uptake than in PO$_4$ uptake. Ammonium in streams can be removed by biotic uptake (assimilation), nitrification and by sorption to the sediments [Peterson et al., 2001]. Phosphorus retention mechanisms in streams include biologic uptake, chemical precipitation and sorption to the sediments [Reddy et al., 1999]. We are not sure about the role that adsorption played in the observed patterns, but this physico-chemical process is expected to influence phosphate more than upon ammonium.
However, we know that sorption was not a significant source of variation between sampling dates or reaches because the experimental design controlled for these factors.

This work is one of the fewer that discriminates whole reach nutrient uptake occurring at the transient storage zone than those occurring at main channel (but see McKnight et al. [2004] and Runkel [2007]). Traditionally it has been though that zones of water transient storage are biogeochemical “hot spots” and that they are responsible of an important part of nutrient transformation. In our case, transient storage zones were not contributing significantly to the whole reach nutrient uptake but were the ones making the difference among treatments. In general, $\lambda_s$ were greater at mud packs, followed by cobbles and sand packs. Additionally, the ratio between average uptake coefficient of ammonium and phosphate changed among treatments. At mud packs, average $\lambda_s$-$\text{NH}_4$ was 1.6 times higher than average $\lambda_s$-$\text{PO}_4$, while at sand packs this ratio was 0.02 and at cobble packs it was 0.001.

Differences observed among treatments can be explained by differences in biologic communities with differing N:P requirements developed on and into the substrata packs. For instance, mud packs have high organic matter content in comparison to the other types of packs. This, in addition to the low hydraulic conductivity and long residence time of mud probably reduced oxygen concentration in the pack. Those characteristics would promote the development of different types of biotic communities in mud packs than in sand or cobbles. C:N content also differed among treatments, sand packs presented the highest C:N ratio followed by mud packs and cobble packs. Additionally, biotic compartments composition presented differences among packs. Mud packs were exclusively composed by FBOM while epilithon dominated at cobble and, in minor measure, at sand packs.

Results from the short-term addition evidence higher percentage of ammonium removed at reaches with sand packs, followed by reaches with mud packs and reaches with cobble packs. Results of the $^{15}\text{NH}_4$ addition indicate that packs made of cobbles have the highest accumulation of $^{15}\text{N}$ and also the highest N uptake efficiencies. The first approach quantifies the whole reach nutrient removal, with the second approach we can identify the type of pack and the biotic compartment more active assimilating N. The epilithon compartment was the one into which more N assimilated per gram of organic matter; similar results were observed by Mulholland et al. [2000] and Tank et al. [2000] who examined N assimilation in several streams compartments. From all types of substrata packs, cobble packs were the ones with a more developed epilithon which explains the high N assimilation rates found. The bacterial component of epilithon has been demonstrated to be an important and very active part of food webs and additionally, to contribute significantly to the transient storage zone [Battin et al., 2003].

In summary, our study showed that although not increasing the overall reach nutrient uptake, introduction of packs of different substrata provoked different responses on nutrient uptake at the transient storage zone. Those responses were related to the extent of phosphate and ammonium retention but also to the relative importance of nitrogen and phosphate uptake coefficients.
Several authors have explored the cause-effect relation between water transient storage and nutrient uptake with contrasting results. We found a positive relationship between transient storage size and the relative contribution of nutrient uptake at the transient storage zone to total uptake. After our results, we suggest that this lack of patterns could be caused by a misunderstanding of the role that physical and biological features of structures involved in water transient storage play in nutrient retention. In most of the existing literature, specific mechanisms of storage are not identified and water transient storage is considered to contribute homogeneously in nutrient uptake processes. Results from this study confirm the hypothesis that nutrient uptake depends not only on the size of transient storage, but also on the physical and biotic features of structures involved in it and that differences among those features will generate differentiated nutrient uptake responses.

New tendencies in stream restoration focus on restoring stream functionality. Methodologies developed to achieve this objective include the introduction of flow obstacles to promote stream habitat heterogeneity. Results presented in this study suggest that the nature of the structure introduced in the channel would affect not only the overall amount of nutrient uptake but the mechanisms of nutrient uptake affecting the final nutrient balance.
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References


Figure captions

Figure 1. Conceptual model of the transient storage and the main channel compartment. Figure adapted from Runkel (1998).

Figure 2. (A) Nitrogen assimilation rates and (B) assimilation efficiencies of each type of substrata packs calculated from 15N addition. Different colors represent the contribution of each compartment of the pack to N retention. Error bars represent total standard error. Different lower case letters denote significantly different groups based on post hoc Tukey HSD test.

Figure 3. Boxplots of hydraulic parameters for each treatment (n=6 for each treatment), (A) As, (B) exchange coefficient between the main channel and the transient storage area (α), and (C) fraction of median travel time due to transient storage for a standardized length of 200 m ($F_{med}^{200}$). Plots show median (horizontal bars), interquartile ranges (rectangles), and ranges. Different lower case letters denote significantly different groups based on post hoc Tukey HSD test.

Figure 4. Average values of (A and C) phosphate uptake coefficients and (B and D) ammonium uptake coefficients. Error bars represent SE. Significant differences were found for $\lambda_s$-PO$_4$ and $\lambda_s$-NH$_4$ among treatments. Different lower case letters denote significantly different groups based on post hoc Tukey HSD test.

Figure 5. Relationship between transient storage and the relative influence of uptake coefficient at the transient storage zone to the total uptake coefficient for (A) phosphate and (B) ammonium.
Table 1. Substrata pack properties. Dominant grain size, water residence time inside the pack (T_p, h), organic matter content (g AFDM), % of weight (in AFDM) for each biotic compartment inside the pack, and C:N mass ratio for each biotic compartment and for each type of substrata pack (n=15 for each treatment). “nm” not measurable.

<table>
<thead>
<tr>
<th></th>
<th>cobbles</th>
<th>sand</th>
<th>mud</th>
</tr>
</thead>
<tbody>
<tr>
<td>grain size</td>
<td>5-10 cm</td>
<td>1-3 mm</td>
<td>&lt; 1 mm</td>
</tr>
<tr>
<td>T_p (h)</td>
<td>0.2</td>
<td>2.3</td>
<td>&gt;16.3</td>
</tr>
<tr>
<td>g AFDM</td>
<td>5.4 ± 0.9</td>
<td>8.4 ± 0.4</td>
<td>56.6 ± 2.9</td>
</tr>
<tr>
<td>epilithon %</td>
<td>98.3 ± 0.3</td>
<td>86.6 ± 2.4</td>
<td>nm</td>
</tr>
<tr>
<td>C:N</td>
<td>9.0 ± 0.4</td>
<td>11.7 ± 0.3</td>
<td>nm</td>
</tr>
<tr>
<td>FBOM %</td>
<td>1.7 ± 0.3</td>
<td>13.4 ± 2.4</td>
<td>100.0 ± 0.0</td>
</tr>
<tr>
<td>C:N</td>
<td>8.0 ± 0.2</td>
<td>11.5 ± 0.2</td>
<td>11.1 ± 0.1</td>
</tr>
</tbody>
</table>

Table 2. Estimated transient storage model parameters, data reported are mean values ± standard error of experiments performed on 6 different dates (n=6).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>control</th>
<th>cobbles</th>
<th>sand</th>
<th>mud</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q (Ls⁻¹)</td>
<td>4.84 ± 1.08</td>
<td>3.95 ± 0.91</td>
<td>4.55 ± 1.21</td>
<td>4.49 ± 0.98</td>
</tr>
<tr>
<td>v (ms⁻¹)</td>
<td>0.21 ± 0.02</td>
<td>0.15 ± 0.02</td>
<td>0.17 ± 0.03</td>
<td>0.18 ± 0.02</td>
</tr>
<tr>
<td>Aₛ (cm²)</td>
<td>13.0 ± 2.3</td>
<td>29.2 ± 4.3</td>
<td>28.2 ± 3.2</td>
<td>32.0 ± 1.9</td>
</tr>
<tr>
<td>α (x10⁻³ s⁻¹)</td>
<td>1.5 ± 0.4</td>
<td>2.7 ± 0.4</td>
<td>3.5 ± 0.5</td>
<td>3.1 ± 0.4</td>
</tr>
<tr>
<td>F_med (%)</td>
<td>4.5 ± 1.2</td>
<td>10.2 ± 1.4</td>
<td>10.9 ± 2.2</td>
<td>12.0 ± 1.9</td>
</tr>
</tbody>
</table>

Table 3. Nutrient uptake coefficients and percentage of nutrient uptake at each injection experiment. Data reported are mean values ± standard error of experiments performed on 6 different dates (n=6).

<table>
<thead>
<tr>
<th></th>
<th>control</th>
<th>cobbles</th>
<th>sand</th>
<th>mud</th>
</tr>
</thead>
<tbody>
<tr>
<td>phosphate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>k (x10⁻³ s⁻¹)</td>
<td>2.1 ± 1.0</td>
<td>2.2 ± 0.8</td>
<td>2.4 ± 0.8</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>% retention</td>
<td>17.5 ± 7.4</td>
<td>22.8 ± 5.5</td>
<td>24.9 ± 7.2</td>
<td>17.8 ± 3.6</td>
</tr>
<tr>
<td>λ (x10⁻³ s⁻¹)</td>
<td>2.1 ± 1.0</td>
<td>2.1 ± 0.8</td>
<td>2.4 ± 0.8</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>λₑ (x10⁻⁶ s⁻¹)</td>
<td>0.3 ± 0.2</td>
<td>1327.6 ± 1010.6</td>
<td>60.1 ± 44.8</td>
<td>2827.4 ± 1584.2</td>
</tr>
<tr>
<td>ammonium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>k (x10⁻³ s⁻¹)</td>
<td>4.9 ± 1.2</td>
<td>3.5 ± 0.7</td>
<td>4.4 ± 0.8</td>
<td>4.2 ± 0.5</td>
</tr>
<tr>
<td>% retention</td>
<td>34.5 ± 6.3</td>
<td>36.2 ± 6.4</td>
<td>42.0 ± 8.2</td>
<td>38.9 ± 6.0</td>
</tr>
<tr>
<td>λ (x10⁻³ s⁻¹)</td>
<td>4.9 ± 1.2</td>
<td>3.5 ± 0.7</td>
<td>4.4 ± 0.8</td>
<td>3.7 ± 0.6</td>
</tr>
<tr>
<td>λₑ (x10⁻⁶ s⁻¹)</td>
<td>0.2 ± 0.1</td>
<td>1.3 ± 0.7</td>
<td>1.1 ± 0.6</td>
<td>4458.9 ± 2614.7</td>
</tr>
</tbody>
</table>
One-dimensional Model with Inflow and Storage

Adapted from Runkel (1998)
Figure 2
Figure 3
Figure 4
Figure 5