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

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ABSTRACT

Quantification of the transient storage zone (A_s) has become critical in stream biogeochemical studies addressed to examine factors controlling nutrient uptake. It is expected that higher A_s may enhance the interaction between nutrients and biota and thus, increase nutrient uptake. However, results from the literature are controversial. We hypothesized that besides of the size of A_s, the intrinsic physical and biological characteristics of stream structures that generate A_s are also relevant for nutrient uptake. We performed 24 additions of phosphate, ammonium, and chloride in 4 reaches of a man-made channel where we introduced 3 types of naturally-colonized substrata packs (mud, sand and cobbles) to modify A_s. We estimated ammonium and phosphate uptake coefficients in both the main channel and A_s using a solute transport model (OTIS-P) and compared the results among reaches with different substrata types. The introduction of substrata packs decreased water velocity and increased A_s similarly among treatments. Nutrient uptake coefficients in the main channel were similar among reaches with different type substrata packs; however, nutrient uptake coefficients measured in A_s differed among them as well as the ratio between ammonium and phosphorus uptake coefficients in A_s, which were 1.6 in reaches with mud packs and 0.02 in reaches with sand or cobble packs. Results obtained in this study suggest that the contribution of A_s in nutrient uptake not only depends on the size of A_s but on the type of materials used to increase A_s, and thus, have biogeochemical implications on restoration projects aimed to modify channel morphology.

Keywords: nutrient cycling; transient storage; nutrient assimilation; ammonium; phosphorus, stream

INTRODUCTION

In recent decades, studies focusing on stream nutrient uptake have gained importance due to 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 (N) inputs to streams from the watershed can be transformed and retained by in-stream processes (Peterson et al., 2001), and denitrification in streams can account for up to 18% of N loss (Mulholland et al., 2008). Mulholland (2004) found that 30% of soluble reactive phosphorus (P) annually entering the stream was retained by in-stream processes. Nevertheless, although there is an increasing understanding of the factors that control variability in stream nutrient uptake response both among streams and within streams over time (Tank et al. 2003; Fellows et al., 2006; Gücker and Pusch, 2006), the influence of hydromorphological factors on stream nutrient uptake still remains unclear. This knowledge is of great relevance not only ecologically, but also for stream management because most of the restoration projects are based on modifications of the channel morphology.

The nutrient uptake and transformation capacity of streams is mostly driven by biological communities, but hydrology and physical features of stream channels can be important factors influencing nutrient cycling at the whole-reach scale (Battin et al., 2008). Therefore, stream nutrient uptake results from the interaction of hydrologic, chemical, and biological retention (Valett et al., 1996; McClain et al., 2003). Hydrologic retention is created by transient storage zones, i.e., stream locations where water moves much slower than the average channel velocity and the residence time is longer than the channel residence time (Bencala and Walters, 1983). Surface-to-subsurface water exchange is 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 within the channel also contribute to 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 greater transient storage may enhance the interaction between nutrients and microbial communities developed in the different stream habitats and thus, increase whole-reach nutrient uptake. For this reason, quantification of transient storage has become a critical issue in biogeochemical studies of stream ecosystems addressed to examine factors controlling spatial or temporal variation of nutrient uptake. However, results from existing studies on the relationship between nutrient uptake and transient storage are controversial. Some studies support a positive association between nutrient uptake and transient storage (Valett et al., 1996; Thomas et al., 2003; Ensign and Doyle, 2005; Ryan et al., 2007). Others report only weak relationships (Lautz and Siegel, 2007; Bukaveckas, 2007) or contrasting findings depending on the nutrient considered. For instance, Hall et al. (2002) found a positive correlation between the relative size of transient storage (A_s/A) and uptake velocity for ammonium (NH_4) but not for phosphate (PO_4). Nonetheless, when we consider studies which compile data from different streams this relation is not as clear. Webster et al. (2003), in a study where they analyzed data collected from 11 streams from different biomes, found no relation between transient storage parameters and NH_4 uptake. Simon et al. (2005) studied the temporal variation of N and P uptake in two New Zealand streams and concluded that 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 transient storage and nutrient uptake. All these studies are mostly based on physical characteristics of the transient storage zones (i.e., A_s/A or water exchange coefficient) to examine relationships with nutrient uptake parameters. However, uptake processes and rates may vary among stream compartments (e.g., pools, algal mats, hyporheic zone) that contribute to transient storage because communities developed in these compartments are distinct (Fisher et al., 1998). Therefore, the biological differentiation among stream compartments may help explain the lack of consistent results on the influence of transient storage as a whole on stream nutrient uptake between existing studies. Based on this reasoning, we hypothesize that

besides the size of transient storage zone, the intrinsic physical and biological features of it have also a relevant role on whole-reach nutrient uptake.

The objective of this study was to examine the influence of physical and biological characteristics of stream structures that generate transient storage on nutrient uptake. To achieve this objective, we experimentally increased the transient storage of a man-made channel by introducing deflectors, which were made of different naturally-colonized substrata types (mud, sand and cobbles). Deflectors differed in physical (i.e., sediment size and hydraulic conductivity) and biological attributes (i.e., biomass of epilithon and fine benthic organic matter, nitrogen assimilation rate). We measured ammonium and phosphate uptake rates in both main channel and transient storage zones using a one-dimensional solute transport model and compared the results among reaches with different substrata types.

METHODS

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 the Santa Fe stream for irrigation. 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 m to avoid a bend in the canal. The natural substratum of the canal was homogeneous and similar among reaches, and it was composed of a 5 – 6 cm layer of sand. Discharge in the canal was regulated by a head gate, which we operated during the experiments to ensure a relatively constant discharge.

To address the objective of this study, we introduced deflectors in the experimental reach. These deflectors measured 25-cm long and 8-cm wide and consisted of nylon mesh bags filled with a single type of substrata. We used 3 substrata types (mud, sand and cobbles) that were introduced in the reaches in discrete packs. Each substrata pack was filled with substrata from the adjacent stream, and thus the substrata packs were naturally colonized. On each sampling date, we placed the substrata packs in three of the selected reaches and the fourth reach was left without any substrata packs 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 in the upstream direction of flow (Fig.1).

Each reach contained a different substrata type. The 4 treatments (i.e., control, cobbles, sand, and mud) rotated between reaches on consecutive sampling dates. A total of 6 sampling dates were completed (i.e., n=6 for each treatment). Therefore, each reach was exposed to all the treatments at least once. 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.

Characterization of substrata packs

Physical features. Dominant grain size of each type of substrata was measured in the laboratory by sieving the content of 5 packs of the same type. Each type of substrata pack was also characterized in terms of maximum water residence time (T_p, h) within the pack (as a surrogate of hydraulic conductivity), To measure T_p, we submerged 4 packs of each substrata type in a container with stream water and NaCl for 1 day for cobble and sand packs, and for 1 week for mud packs. We then placed these packs in the canal 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 data logger (510X Campbell

Scientific, Logan, UT, USA). T_p was calculated as the time needed for the downstream point to reach upstream conductivity values, which indicated that the interstitial tracer had been completely released from the packs.

Biological features. Substrata packs were characterized in terms of total organic matter (OM) content. In addition, we quantified the proportion of OM standing stock associated with the two dominant biotic compartments of the substrata packs. For cobbles and sand substrata packs, we separated the attached biofilm on mineral substrata (hereafter referred to as epilithon) and interstitial loosely associated fraction of OM (hereafter referred to as fine benthic organic matter - FBOM). The C and N content of these two compartments and their N assimilation rate were also measured. For mud substrata packs, the differentiation of the two compartments was not methodologically possible because of the small size of the particles, and thus we associated the OM content only to the FBOM fraction. We used ^{15}N as a tracer to estimate the N assimilation rate of both epilithon and FBOM for each substrata type. A single addition in the canal of a solution of 104.2 mg $^{15}NH_4$ and 104.2 g NaCl mixed in 5 L of ultrapure water, to obtain a water enrichment of 2000‰, was conducted at constant rate during 14 h. The lack of a stable isotope for P prevented us from measuring P assimilation rate. For this addition, we included the 4 reaches together (i.e., the total reach length was 88 m). Substrata packs were arranged as described above; however, substrata types were alternated along the entire reach. The $^{15}NH_4$ addition began at 22:30 and lasted until 12:30 the following day. We collected one 4-L water sample and three substrata packs of each type at 3 locations along the reach (10, 48, and 78 m from the addition point) prior to the $^{15}NH_4$ addition, and at 4 locations (18, 38, 66 and 86 m) just before the addition was stopped. Additional 100 mL water samples were collected at each sampling location for the analysis of nutrient concentrations. Water samples for nutrient concentrations were filtered in the field using Whatman® GF/F fiberglass filters and kept refrigerated until analysis (see details on the section below). The 4-L water samples were processed for ^{15}N content of NH_4^+-N following procedures adapted from Holmes et al. (1998). Samples were filtered through Whatman® GF/F fiberglass filters (0.7 µm pore diameter) and spiked with 300 µL of unlabeled NH_4^+-N to increase NH_4^+-N concentration to the ideal working range for mass-spectrometric measurement. Samples were then amended with 50 g/L of NaCl, 3.0 g/L of MgO, and a Teflon filter packet. The filter packet consisted of a precombusted 1-cm glass-fiber filter (Whatman GFD) spiked with 25 µL of 2.5 molL^{-1} KHSO$_4$ and sealed between two pieces of Teflon plumbing tape. All water samples and substrata packs collected were transported refrigerated to the laboratory for further processing and analysis. Once in the laboratory, we incubated the 4L water samples at 40 °C for two weeks on a shaker table. We then removed the filter packets and the filters were dried in the oven prior to encapsulation in tins for ^{15}N analysis.

The collected substrata packs were brought to the laboratory on ice and processed to estimate the biomass of FBOM and epilithon, and the carbon (C), N and ^{15}N content of these compartments. All sampled substrata packs were weighed and divided into two parts. One part was used to measure the dry weight (DW) and the biomass expressed as ash-free dry mass (AFDM) of the whole pack. The other part was submerged and gently swirled in a known volume of water, and 100 mL of supernatant were filtered through a precombusted Whatman GF/F fiberglass filter. Half of the filter was processed to measure the AFDM of 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 fraction. The biomass of epilithon of cobble and sand substrata packs was measured as the difference between the AFDM of FBOM and that of the whole pack. To measure the ^{15}N signature and the C and N content of the epilithon, the submerged sub-sample was gently elutriated several times until the FBOM was totally removed. A known volume of distilled water was then added to the elutriated substrata and this sample was exposed to ultrasonic waves for 10 minutes. After this time, water was filtered through precombusted Whatman GF/F fiberglass filters. Samples for DW and AFDM measurements were weighed, dried at 60ºC for ≥3 days, weighed again, combusted a 500ºC for 5h, and reweighed. Filters with epilithon and FBOM samples for the analysis of C and N content, and the ^{15}N signature were dried in the oven (60 °C, ≥3 days) and weighed. We cut 1-cm diameter disc from each filter, weighed it on a Mettler-Toledo (Greifensee, Swizertland) MX5 microbalance, and encapsulated in tins for analysis.

Encapsulated filters of both water and substrata pack samples were analyzed in the Stable Isotope Facility at the University of California-Davis (Davis, CA, USA). The content (as a percent of dry mass) of N and C and the stable isotope ratios of N were measured by continuous flow isotope ratio mass spectrometry (20-20 mass spectrometer; PDZEuropa, Northwich, UK) after sample combustion at 1000ºC in an on-line elemental analyzer (PDZ-Europa ANCA-GSL).

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:

$$\delta^{15}N = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] \times 1000 \qquad (1)$$

To calculate the N assimilation rates, we used $\delta^{15}N$ values from the ^{15}N tracer addition. We first estimated the amount of ^{15}N in each compartment ($^{15}N_{compartment}$, µg ^{15}N pack^{-1}) within the pack of each substrata type using the following equation:

$$^{15}N_{comp} = B_{comp} \times \left(\frac{\%N}{100}\right) \times (MF_i - MF_b) \qquad (2)$$

where B_{comp} is the biomass of either epilithon or FBOM compartment in the pack (µg AFDM pack^{-1}), %N is percentage of N of the compartment, MF is the molar fraction of ^{15}N in the compartment at plateau conditions (MF_i) and at background conditions (MF_b). Molar fractions were calculated as:

$$MF = \frac{^{15}N}{^{15}N + ^{14}N} = \frac{\left(\frac{\delta^{15}N}{1000} + 1\right) * 0.0036765}{1 + \left[\left(\frac{\delta^{15}N}{1000} + 1\right) * 0.0036765\right]} \qquad (3)$$

We then estimated N assimilation rate of each compartment (U_{comp}, µg N h^{-1} pack^{-1}) using the equation adapted from von Schiller et al. (2007):

$$U_{comp} = \frac{^{15}N_{comp}}{t_{add} \times \frac{^{15}N_{flux}}{N_{flux}}} \qquad (4)$$

where t_{add} is the duration of the ^{15}N addition (h), $^{15}N_{flux}$ is the NH_4^+-^{15}N flux at plateau conditions (µg ^{15}N s^{-1}) and N_{flux} is the total NH_4^+-N flux (µg N s^{-1}). We also calculated the N assimilation efficiency of each biotic compartment as U_{comp} divided by B_{comp}. N assimilation rate of the pack was calculated as the weighted sum of U for each compartment within the pack.

Measurements of whole reach transient storage and nutrient uptake

Solute addition experiments. To quantify the effect of the introduction of substrata packs on transient water storage and nutrient uptake at the whole reach scale, we conducted 24 short-term additions at constant rate of a conservative tracer (Cl$^-$ as NaCl) together with NH_4^+-N (as NH_4Cl) and PO_4^{3-}-P (as $Na(H_2PO_4) \cdot 2H_2O$) using a Masterflex (Vernon Hills,

Illinois, USA) L/S battery-powered peristaltic pump. Solutes were injected at the thalweg of the channel in a constrained site, which was created with cobbles to ensure rapid mixing of the injected solution with stream water. Before the experiments began, we conducted an addition only with the conservative tracer to estimate the reach length needed for the injected solution to completely mix across the channel. Several transect measurements of EC across the channel close to the injection site indicated that the solution was completely mixed 5 m downstream of this site. Therefore, in the following solute additions, the injection site was located 5 m upstream of the 20-m experimental reaches. On each sampling date, we conducted 4 solute additions; one at each of the 20-m reaches that were exposed either to a different substrata pack type or left as control. Additions started at the most downstream reach and moved consecutively upstream. Duration of each addition 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 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 the analysis of NH_4^+-N and soluble reactive phosphorus (SRP) concentration were taken at the bottom of the reach from the beginning of the addition until EC returned to background levels after the addition was stopped. In each addition, sample frequency was accommodated to changes in EC, but in general samples were collected at 10-second intervals during the rise and the fall of the curve and at 30-second intervals during 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 NH_4^+-N and SRP were analyzed following standard colorimetric methods (APHA, 1998) using a Bran+Luebbe TRAACS 2000 Autoanalyzer (Norderstedt, Germany). Background concentrations of SRP and NH_4^+-N were low throughout the study and the average (± SEM) was 4.2 ± 0.4 µg P L^{-1} and 9.4 ± 0.8 µg N L^{-1}, respectively. Additions increased EC by 34 ± 2 µS cm^{-1}, SRP concentration by 67.7 ± 6.2 µg P L^{-1}, and NH_4^+-N concentration by 55.6 ± 4.8 µg N L^{-1}. In addition, for each addition, we measured the 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 and nutrient uptake 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 Q was based on a tracer mass balance approach (Gordon, 1992). Water velocity was calculated by dividing the reach length by the time needed to increase the EC by one half of the plateau (i.e., T_n nominal travel time, s). Nutrient uptake parameters and hydraulic parameters were estimated using a one-dimensional transient storage model that accounts for advection, dispersion, dilution due to lateral water inflow, exchange with transient storage zones, and first-order uptake in the main channel and in the transient storage zone (OTIS; Runkel, 1998). The model relies on the solution to two equations (equations 5 and 6) that simulates the change in solute concentration over time in the main channel and in the transient storage zone assuming a first-order mass transfer process between the two compartments:

$$\frac{\partial C}{\partial t} = -\frac{Q}{A}\frac{\partial C}{\partial x} + \frac{1}{A}\frac{\partial}{\partial x}\left(AD\frac{\partial C}{\partial x}\right) + \frac{q_L}{A}\left(C_L - C\right) + \alpha\left(C_s - C\right) - \lambda C \qquad (5)$$

$$\frac{\partial C_s}{\partial t} = \alpha\frac{A}{A_s}\left(C - C_s\right) - \lambda_s C_s \qquad (6)$$

where C is the solute concentration in the main channel (ML^{-3}), C_s is the solute concentration in the transient storage zone (ML^{-3}), t is time (T), A is the cross-sectional area of the main channel (L^2), x is the reach length (L), D is the dispersion coefficient (L^2T^{-1}), q_L is the lateral inflow rate on a per length basis ($L^3T^{-1}L^{-1}$), C_L is the solute concentration of the lateral inflow (ML^{-3}), α is the exchange coefficient between the main channel and the transient storage zone (T^{-1}), λ (T^{-1}) is the

uptake coefficient of a non-conservative solute in the main channel, and λ_s (T^{-1}) is the uptake coefficient of a non-conservative solute in the transient storage zone.

Hydraulic parameters (i.e., D, A, A_s, and α) were estimated using data from the conservative tracer in the OTIS model and setting λ and λ_s to zero in the simulations. Based on these parameters, we calculated the Damkölher values (DaI) for each addition to determine whether the length of the reach was suitable for measuring transient storage parameters (Wagner and Harvey, 1997). Values obtained ranged between 1 and 5, and therefore hydraulic parameters estimated with the OTIS model were considered reliable if we assume a single timescale of exchange. We also calculated the fraction of the median travel time attributable to transient storage (F_{med}^{200}, Runkel, 2002) for a standardized length of 200 m using the following equation:

$$F_{med}^{200} = \left[1 - e^{-L(\alpha/v)}\right]\frac{A_s}{A + A_s} \tag{7}$$

where L is reach length.

Nutrient uptake parameters (i.e., λ and λ_s) were estimated using data from nutrient concentrations in the OTIS model and using the hydraulic parameters estimated from the conservative tracer data. 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. Additionally, to check the performance of the model describing the variation in nutrient concentrations, we calculated the root-mean square error (RMSE) between the observed data and the simulated data from the model. RMSE was calculated using the estimated λ and λ_s, and also considering λ and λ_s to be zero. RMSE from the two approaches were compared using a Wilcoxon test for non-parametric samples to test if the use of estimated λ and λ_s improved significantly the fitting of the model. We also calculated the total uptake coefficient (k, s^{-1}) for each nutrient as a descriptor of nutrient uptake at a whole reach scale using the following equation based on Runkel's (2007) "Case II-IV" transport:

$$k = \lambda + \frac{\alpha\lambda_s A_s}{\alpha A + \lambda_s A_s} \tag{8}$$

In addition, we estimated the total percentage of the added nutrient that was taken up in the reach as well as the proportion of it associated with the main channel and the transient storage zone. To estimate these percentages, we calculated the mass of nutrient retrieved from the additions at the endpoint of the reach based on OTIS simulations under the following scenarios: (A) conservative transport (λ and λ_s=0), (B) non-conservative transport in both the channel and the transient storage zone (λ and λ_s>0), (C) main channel uptake only (λ>0 and λ_s=0), and (D) storage zone uptake only (λ=0 and λ_s>0). For each simulation, the mass of nutrient retrieved was calculated as the integrated area of each curve. The percentage of nutrient uptake in the main channel (%uptake$_c$) and in the storage zone (%uptake$_{ts}$) was then calculated as:

$$\%uptake_c = \frac{mass_{simA} - mass_{simC}}{mass_{simA} - mass_{simB}} \times 100 \tag{9}$$

$$\%uptake_{ts} = \frac{mass_{simA} - mass_{simD}}{mass_{simA} - mass_{simB}} \times 100 \tag{10}$$

The total percentage of nutrient taken up in the reach was calculated as the sum of the percentages estimated in both the main channel and the transient storage after adjusting them proportionately to obtain a sum equal to 100% (Runkel, 2007).

Statistical analysis

To compare hydraulic and nutrient uptake parameters between control reaches and reaches with substrata packs, and also among substrata pack types, we ran 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 control reaches vs. reaches with substrata packs. 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 nutrient uptake) were log-transformed and others (i.e., λ and λ_s) inverse log-transformed prior to analysis to meet assumptions of normality. We conducted analysis of variance (one-way ANOVA) to compare ^{15}N uptake rates among substrata packs. Multiple comparisons were made among treatments following a *post-hoc* Tukey HSD procedure in all significant ANOVA tests. Finally, we examined the relationships between transient storage parameters (A_s, α, and F_{med}^{200}) and nutrient uptake parameters for SRP and NH_4^+-N (λ and λ_s, relative proportion of uptake coefficient to total uptake coefficient (λ_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).

RESULTS

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, maximum water residence time within the pack (T_p), organic matter content, the relative proportion of epilithon and FBOM, and the C:N ratio of these biotic components (Table 1). Dominant grain size ranged from <1 mm in mud packs to a few cm in cobble packs. T_p followed a negative relationship with grain size. Mud packs had the finest grain size and the highest water residence time inside the pack (i.e., >half day). Water residence time in the sand packs was in the range of few hours and in the cobble packs was in the range of minutes (Table 1). Regarding biological features, organic matter content in mud packs was 10.5 times and 1.6 times higher than in cobble and sand packs, respectively. Almost all organic matter content was attributable to epilithon in cobble (98.3%) and in sand packs (86.6%). In mud packs the relative contribution of the two components could not be evaluated due to methodological constrains that prevented the quantification of the epilithon compartment in substrata with small grain size. Thus, we associated all the organic matter content present in mud packs to the FBOM fraction (Table 1). The C:N ratio was slightly higher in epilithon than in FBOM, and sand packs had higher C:N ratios than cobble and mud packs (Table 1).

Assimilation rates of N estimated from the ^{15}N tracer addition were significantly different among types of substrata packs (one-way ANOVA, $F_{(2,23)}=4.164$, $p=0.03$, Fig. 2a). Cobbles showed the highest N assimilation rate (0.95 ± 0.34 µg N h^{-1}) followed by mud packs (0.57 ± 0.14 µg N h^{-1}), and by sand packs (0.15 ± 0.02 µg N h^{-1}). Most of N uptake in cobbles and sand packs was attributable to the epilithon compartment. In mud packs, we attributed all N uptake to the FBOM compartment. In addition, biotic compartments differed in N assimilation efficiency (i.e., N assimilation rate per unit of AFDM; Fig. 2b). N assimilation efficiency of FBOM was 3.5 ± 0.5 and 3.7 ± 1.2 times higher than that of epilithon in cobble packs and in sand packs, respectively. N assimilation efficiency was highest in cobble packs, intermediate in sand packs and lowest in mud packs.

Effects of substrata packs at the reach scale

Hydraulic parameters. Discharge varied slightly among sampling dates and did not significantly differ between control reaches and reaches with different types of substrata packs. The average ($\pm$SEM) discharge for the entire study was

4.5±0.5 L s^{-1} (n=24). Values of v ranged between 0.08 and 0.30 m s^{-1} and were on average 0.8 times lower in reaches with substrata packs than in control reaches (Table 2). Dispersion was low in all the experiments (< 0.06 m^{2}s^{-1}, Table 2) and was similar among reaches with different treatments. The rest of the hydraulic parameters (i.e., A$_s$, α, and F_{med}^{200}) increased after introducing substrata packs. Values of A$_s$ were 2.3 times higher in reaches with substrata packs than in control reaches (one-way ANOVA, F$_{(1,17)}$=28.16, p<0.001), with no significant differences among different types of substrata packs (Fig. 3a). Values of α ranged between 0.8 x10^{-3} s^{-1} and 5.0 x10^{-3} s^{-1} and were higher in reaches with substrata packs, especially of sand and mud, than in control reaches (one-way ANOVA, F$_{(1,17)}$= 12.51, p<0.001, Fig. 3b). F_{med}^{200} was on average 2.4 times higher in reaches with substrata packs than in control reaches (one-way ANOVA, F$_{(1,17)}$=11.77, p<0.001); greatest differences were observed between control reaches and reaches with mud packs (Fig 3c).

Nutrient uptake parameters. Values of k for phosphate ranged from 0.3 x10^{-3} s^{-1} to 6.2 x10^{-3} s^{-1}, and did not significantly differ between control reaches and reaches with different types of substrata packs (Table 3 and Fig. 4a). This k range resulted in an average of 20.8±2.9 % of the phosphate added being taken up in the reaches. Values of λ for phosphate were close to k for this nutrient (Table 3). No significant differences in λ for phosphate were found either between control reaches and reaches with substrata packs or among reaches with different substrata pack types. On average, values of λ for phosphate resulted in 92.0±3.5 % of total phosphate uptake occurring in the main channel. Values of λ$_s$ for phosphate ranged between values close to zero and 7.9 x10^{-3} s^{-1}. In contrast to λ, λ$_s$ for phosphate significantly differed among reaches (one-way ANOVA, F$_{(1,17)}$= 4.49, p=0.02, Table 3 and Fig. 4c). λ$_s$ for phosphate was lowest in control and sand-pack reaches and highest in reaches with mud packs (Fig. 4c). Phosphate uptake occurring in the transient storage accounted between 0.05 and 16.8 % of the percentage of total uptake in the control reaches and in reaches with mud packs, respectively. Phosphate uptake coefficients, when added to the model to explain variation in nutrient concentration among time, provided a better approximation to observed values, i.e., RMSE obtained with estimated lambda values was significantly lower than RMSE obtained with lambdas equal to zero (Wilcoxon test for RMSE paired samples, Z= -3.06, p=0.00; Table 3). The ratio between λ$_s$ and k for phosphate was positively related to the transient storage size (λ$_s$/k= 5.29x10^{-13}e$^{0.71A_s}$, R^2=0.37, p=0.00; Fig. 5a), the water exchange coefficient (λ$_s$/k= 5.1 x10^{-10}α$^{13.16}$, R^2=0.36, p=0.00), and F_{med}^{200} (λ$_s$/k= 7.11 x10^{-10}e$^{0.12Fmed}$, R^2=0.23, p=0.02).

Values of k for ammonium ranged from 1.1 x10^{-3} s^{-1} to 8.7 x10^{-3} s^{-1} and did not significantly differ between control reaches and reaches with different types of substrata packs (Table 3, Fig. 4b). This k range resulted in an average of 37.9±3.2 % of the ammonium added being taken up in the reaches. Similar to phosphate, values of λ for ammonium were close to k for this nutrient (Table 3). No significant differences in λ for ammonium were found between control reaches and reaches with substrata packs or among reaches with different substrata types. Ammonium λ values accounted for 96.1±2.4% of total uptake for this nutrient, which indicates that most of the ammonium uptake occurred in the main channel. Values of λ$_s$ for ammonium ranged from values close to zero to 16.5 x10^{-3} s^{-1}, and there were significant differences among the different reaches (one-way ANOVA, F$_{(1,17)}$= 7.25, p=0.00). Reaches with mud packs showed the highest λ$_s$ for ammonium (Fig. 4d). While ammonium uptake occurring in the transient storage accounted for 13 % of total ammonium uptake in reaches with mud packs, this percentage was negligible in the rest of the reaches. Ammonium uptake coefficients, when added to the model to explain variation in nutrient concentration among time, provided a better approximation to observed values, i.e., RMSE obtained with estimated lambda values was significantly lower than RMSE obtained with lambdas equal to zero (Wilcoxon test for RMSE paired samples, Z= -3.71, p= 0.00; Table 3). The ratio between λ$_s$ and k for ammonium was positively related to the transient storage size (λ$_s$/k= 7.6x 10^{8}e$^{0.29As}$, R^2=0.26, p=0.01; Fig. 5b), but not to the water exchange coefficient nor to F_{med}^{200}.

DISCUSSION

The objective of this study was to examine the influence of transient storage on nutrient uptake by considering not only the physical features (i.e., size of A$_s$ or hydrologic exchange with main channel) but also the biological characteristics

(i.e., biomass of epilithon and fine benthic organic matter, C:N ratios) associated with the transient storage generated by the introduction of different substrata packs to the streambed.

As expected, the introduction of substrata packs into the channel doubled the size of transient storage (both A_s and A_s/A) and increased water exchange with the main channel (α). This increase was similar among the substrata packs considered regardless of differences in their hydraulic conductivity. The percentage of contribution of transient storage to the overall water median travel time (F_{med}^{200}) along the reaches also increased as a result of the introduction of substrata packs, especially when mud packs were introduced in the reaches. However, the increases in transient storage size did not result in changes in total uptake of ammonium and phosphate at the whole reach scale compared to control reaches for any of the treatments considered. These results are in contrast with those found in previous studies in which an increase in transient storage similar to that in our study resulted in concomitant increases in nutrient uptake. For instance, Ensign and Doyle (2005) increased A_s/A and F_{med}^{200} by 1.2 times by experimentally introducing baffles into the channel and observed a significant increase in both phosphate and ammonium uptake velocity. Similarly, Roberts et al. (2007) found a significant increase in ammonium uptake when A_s/A and F_{med}^{200} was doubled by introducing coarse woody debris into 4 stream channels. Lack of effects of changes in transient storage on nutrient uptake found in our study could in part be attributed to the scale of the experimental approach. Our study was conducted in small and relatively short channels (i.e., 20m long by 60 cm wide) with relatively small-size background transient storage (i.e., based on the control treatment), in comparison to the reach scale dimensions considered in the studies cited above. The amount of transient storage induced by the introduction of substrata packs easily doubled the initial amount of A_s, but it was still small in comparison to the size of A_s observed in the experiments cited above.

Nevertheless, despite total nutrient uptake was similar among treatments, results indicate that treatments differed in nutrient uptake occurring in the transient storage zone (i.e., in λ_s). In general, λ_s were greater in reaches with mud packs followed by reaches with cobbles and sand packs. We also observed differences in the relative dominance of nutrient uptake in transient storage compared to main channel among types of substrata packs. Reaches with mud substrata packs had higher nutrient uptake in the transient storage than in the main channel in comparison to the other substrata types. In addition, the ratio between λ_s-NH$_4$ and λ_s-PO$_4$ differed among treatments. In mud packs, average λ_s-NH$_4$ was 1.6 times higher than average λ_s-PO$_4$, while in sand and cobble packs this ratio was less than 0.02. Since all treatments presented similar A_s, we suggest that the differences in nutrient uptake responses observed among treatments are explained by differences in N:P requirements of the biologic communities developed on and into each type of substrata packs. For instance, mud packs have higher organic matter content and higher water residence time which probably reduced oxygen concentration in the pack in comparison to other treatments. These conditions would promote the development of different types of biotic communities in mud packs than in sand or cobbles. Additionally, based on direct observations, while algae were present in the epilithon compartment of cobble and sand packs, they were not obvious in mud packs, which suggested that microbial communities in these packs were mostly heterotrophic or chemoautotrophic.

Results from the ^{15}N addition evidenced that FBOM was more efficient in assimilating ^{15}N than epilithon. Similar results were observed by Mulholland et al. (2000) and Tank et al. (2000) who examined N assimilation in several stream compartments. Cobble packs presented higher ^{15}N assimilation rate than sand packs, while mud packs did not show significant differences to the rest of the treatments. However, the higher N assimilation rate observed in cobbles was not reflected on higher total uptake at the whole-reach scale. Data from the short-term additions showed higher rates of ammonium uptake at the transient storage zone of reaches with mud packs. This apparent disparity in results is explained by the use of two different approaches to quantify nutrient uptake. While the ^{15}N approach quantifies the amount of N uptake due to biological uptake within each substrata pack, the short-term solute additions at whole-reach scale measure the N uptake resulting from the combined effects of hydrological, biological, and chemical retention (Valett et al., 1996; McClain et al., 2003). The combination of results from the two approaches shows that while cobble packs presented the

highest biological N uptake, mud packs created more hydrological and/or chemical retention; and thus, at a reach scale, mud packs were the substrata type resulting in a more effective N uptake, at least in the transient storage zone.

This work is one of the few that discriminates whole-reach nutrient uptake occurring in the transient storage zone to that occurring in the main channel (but see McKnight et al., 2004; Runkel, 2007). Conventionally, it has been thought that zones of transient storage are biogeochemical "hotspots" and that they are responsible for an important part of nutrient transformation and uptake. In this study, the experimentally generated transient storage zones were not contributing significantly to the whole-reach total nutrient uptake, but they contributed differentially to nutrient uptake within these zones. In addition, we found a good relation between the relative contribution of nutrient uptake in the transient storage zone to total uptake and the size of transient storage zone (A_s). We suggest that the lack of patterns observed in the literature when relating nutrient uptake and transient storage could be caused by an underestimation of the particular role played by different physical and biological features of structures contributing to transient storage on nutrient uptake. In most of the existing studies, specific mechanisms within transient storage are not identified and 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 contribute to differentiated nutrient uptake responses. New tendencies in stream restoration mostly focus on restoring stream channel morphology and, ultimately the functioning of these ecosystems. Methodologies developed to achieve this objective include the use of diverse in-channel structures to increase stream habitat heterogeneity. Results obtained in this study suggest that the type of structures introduced in the channel would enhance different biotic communities, and thus will influence not only overall nutrient uptake but also stream biogeochemical processes.

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FIGURE CAPTIONS

Figure 1. View of the channel with substrata packs. White arrow indicates flow direction.

Figure 2. (a) Nitrogen assimilation rates and (b) assimilation efficiencies of each type of substrata packs calculated from ^{15}N addition. Different colors represent the contribution of each compartment of the pack to N uptake. 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) size of the transient storage (A_s), (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 total uptake coefficient (a and b) and uptake coefficient in the transient storage zone (c and d) for phosphorus and ammonium. Error bars represent SE. Significant differences were found for λ_s-PO_4 and λ_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 in the transient storage zone to the total uptake coefficient for (a) phosphate and (b) ammonium.

Table 1. Characterization of each type of substrata pack based on the dominant grain size, maximum residence time of a conservative tracer within the pack (T_p), total organic matter content in the pack (AFDM), contribution of epilithon and fine benthic organic matter (FBOM) to total AFDM in the pack (%), and C:N mass ratio of epilithon and FBOM. Data reported are mean values ± standard error from 15 measurements done with each type of substrata pack. "nm" not measurable.

		Cobbles	Sand	Mud
	grain size	5-10 cm	1-3 mm	< 1 mm
	T_p (h)	0.2	2.3	>16.3
	AFDM (g)	5.4 ± 0.9	8.4 ± 0.4	56.6 ± 2.9
epilithon	%	98.3 ± 0.3	86.6 ± 2.4	nm
	C:N	9.0 ± 0.4	11.7 ± 0.3	nm
FBOM	%	1.7 ± 0.3	13.4 ± 2.4	100.0 ± 0.0
	C:N	8.0 ± 0.2	11.5 ± 0.2	11.1 ± 0.1

Table 2. Discharge (Q), average water velocity (v) and estimated transient storage model parameters (A_s: size of the transient storage zone, α: water exchange coefficient between the main channel and the transient storage zone, and F_{med}: fraction of the median travel time attributable to transient storage) for each experimental treatment. Data reported are mean values $\pm$ standard error from experiments performed on 6 different dates (n=6).

	Control	Cobbles	Sand	Mud
Q (Ls^{-1})	4.84 ± 1.08	3.95 ± 0.91	4.55 ± 1.21	4.49 ± 0.98
v (ms^{-1})	0.21 ± 0.02	0.15 ± 0.02	0.17 ± 0.03	0.18 ± 0.02
D (m^2s^{-1})	0.05 ± 0.01	0.04 ± 0.02	0.06 ± 0.02	0.06 ± 0.02
A_s (cm^2)	13.0 ± 2.3	29.2 ± 4.3	28.2 ± 3.2	32.0 ± 1.9
α $(x10^{-3}\ s^{-1})$	1.5 ± 0.4	2.7 ± 0.4	3.5 ± 0.5	3.1 ± 0.4
F_{med} (%)	4.5 ± 1.2	10.2 ± 1.4	10.9 ± 2.2	12.0 ± 1.9

Table 3. Phosphate and ammonium uptake coefficients and percentage of uptake for each experimental treatment. k = total uptake coefficient, λ = uptake coefficient in the main channel, λ_s = uptake coefficient in the transient storage zone. The table also reports the root-mean square error (RMSE) between the observed nutrient data and the data estimated from the model when considering the estimated λ and λ_s (RMSE1), and when considering λ and λ_s to be zero (RMSE2) Data reported are mean values ± standard error from experiments performed on 6 different dates (n=6).
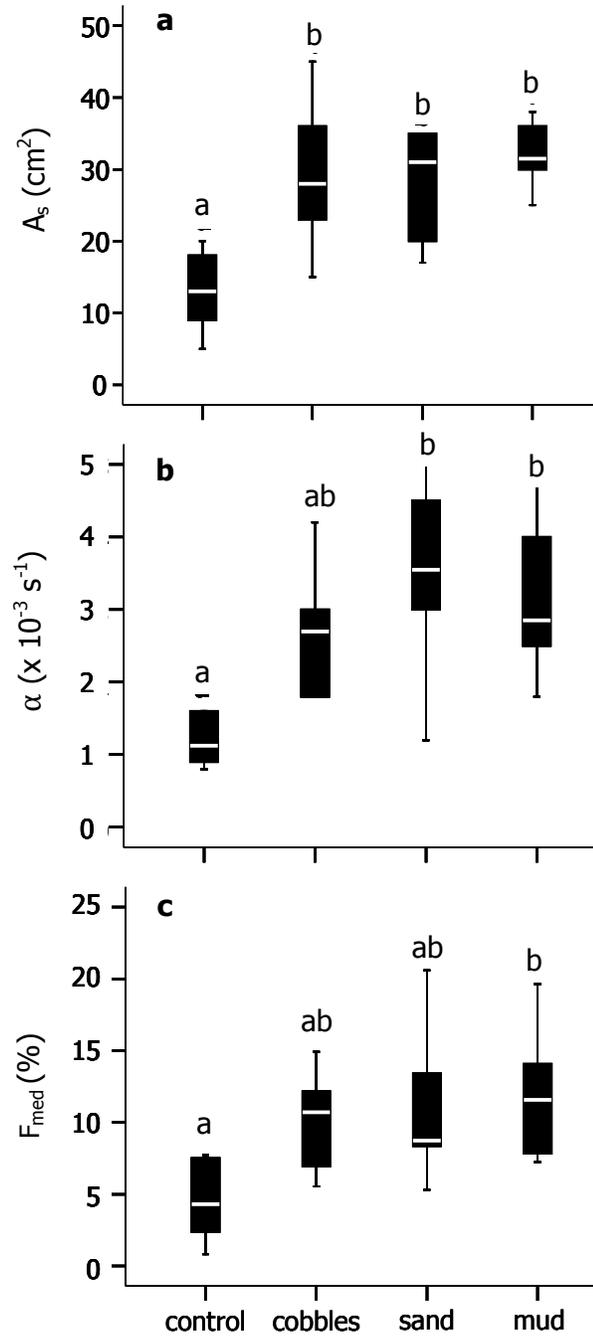
	Control	Cobbles	Sand	Mud
Phosphate				
k ($\times 10^{-3}$ s^{-1})	2.1 ± 1.0	2.2 ± 0.8	2.4 ± 0.8	1.6 ± 0.3
% uptake	17.5 ± 7.4	22.8 ± 5.5	24.9 ± 7.2	17.8 ± 3.6
λ ($\times 10^{-3}$ s^{-1})	2.1 ± 1.0	2.1 ± 0.8	2.4 ± 0.8	1.4 ± 0.3
λ_s ($\times 10^{-6}$ s^{-1})	0.3 ± 0.2	1327.6 ± 1010.6	60.1 ± 44.8	2827.4 ± 1584.2
RMSE1	0.0016 ± 0.0011	0.0012 ± 0.0005	0.0037 ± 0.0030	0.0007 ± 0.0001
RMSE2	0.0040 ± 0.0027	0.0069 ± 0.0024	0.0072 ± 0.0049	0.0036 ± 0.0011
Ammonium				
k ($\times 10^{-3}$ s^{-1})	4.9 ± 1.2	3.5 ± 0.7	4.4 ± 0.8	4.2 ± 0.5
% uptake	34.5 ± 6.3	36.2 ± 6.4	42.0 ± 8.2	38.9 ± 6.0
λ ($\times 10^{-3}$ s^{-1})	4.9 ± 1.2	3.5 ± 0.7	4.4 ± 0.8	3.7 ± 0.6
λ_s ($\times 10^{-6}$ s^{-1})	0.2 ± 0.1	1.3 ± 0.7	1.1 ± 0.6	4458.9 ± 2614.7
RMSE1	0.0038 ± 0.0016	0.0016 ± 0.0006	0.0053 ± 0.0038	0.0013 ± 0.0006
RMSE2	0.0121 ± 0.0056	0.0154 ± 0.0068	0.0247 ± 0.0177	0.0148 ± 0.0075

Figure 1
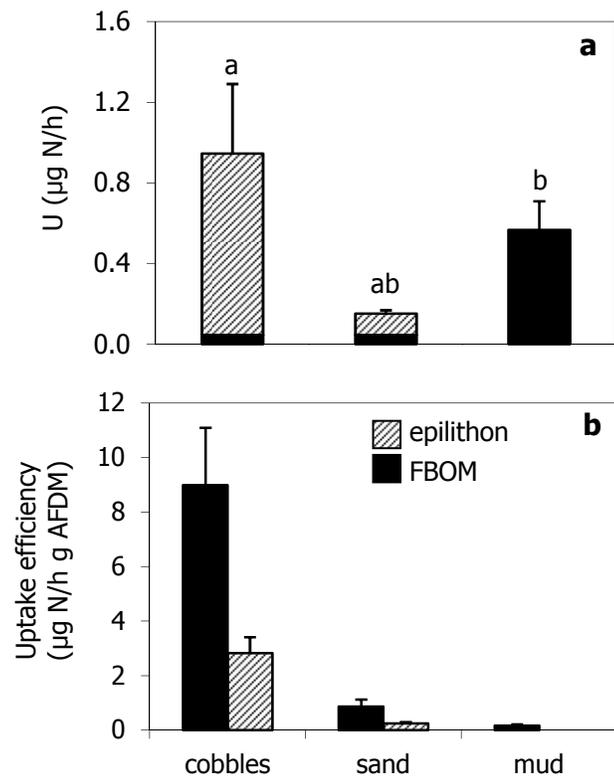
Figure 2

Figure 3

Figure 4

Figure 5