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Report on results from the stream variability in retention of multiple nutrients and metabolism along an altitudinal gradient in the Central Pyrenees (Spain)

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INTRODUCTION

Stream nutrient dynamics are influenced by both biotic and abiotic processes occurring in the watershed as well as within the stream. While factors operating at the watershed scale may determine stream nutrient availability and its regime, factors operating within the stream influence the coupling between nutrient transport and in-stream processing and retention. In turn, in-stream biogeochemical processes can be highly influenced by factors operating at both the catchment (e.g., climate, geology and vegetation) and the stream (e.g., morphology and nutrient loading) scales. Many studies evaluating stream biogeochemical responses have focused on headwater streams given their importance in processing and retaining nutrients (Alexander et al. 2000, Peterson et al. 2001) and their metabolic relevance, especially in organic matter processing (Grimm and Fisher 1984, Jones et al. 1995, Fischer et al. 2002). The influence of single or multiple environmental factors on nutrient retention and stream metabolism has commonly been assessed by examining relationships between the variability in factors and processes measured among streams or within streams over time. Based on this approach, discharge or specific discharge (velocity*depth) has been shown to account for a large fraction of the variation in nutrient retention efficiency among and within streams (Valett et al. 1996, Butturini and Sabater 1998, Peterson et al. 2001). Stream nutrient retention efficiency decreases as discharge increases. Stream geomorphology and hydraulics may also influence nutrient uptake through the influence of these characteristics on water and solute transient storage, which increases residence time and the interaction between solutes and microorganisms (Valett et al. 1996, Mulholland et al. 1997; Butturini and Sabater 1999). However, published results on this topic are less consistent than those for the effect of discharge. On the other hand, biological activity (i.e., metabolism) and its potential capacity for nutrient uptake can be influenced by light availability, which is modulated by the existence of riparian canopy or sediment loads and turbidity of stream water (Feminella et al. 1989, Young and Huryn 1996). In addition, other studies have found significant relationships between stream metabolism and catchment vegetation (Minshall 1978, Minshall et al. 1983,

Lamberti and Steinman 1997, Mulholland et al.2001) and a great number of studies evidence the relevance of temperature on ecosystem respiration (Bott et al. 1985, Sinsabaugh 1997, Uehlinger et al. 2000).

Variation of these key factors among streams is mostly imposed by their climatic and physiographic setting; and thus, it is expected that some spatial patterns on biogeochemical responses emerge when examining their variation among bioclimatic distinct regions. For instance, several authors have compared metabolism between tropical and temperate streams (Dudgeon 1983, Talling and Lemoalle 1998, Ortiz-Zayas 2005). Other studies have used an inter-biome perspective as an approach to determine fundamental controls on ecosystem processes like ammonium uptake (Webster et al. 2003) and steam metabolism (Mulholland et al. 2001). This information is relevant within the context of current climate change to help forecast the sensibility of stream biogeochemical responses to future changes and the potential direction of the change. However, current knowledge is still restricted to few studies mostly done at large spatial scales that difficult the realistic extrapolation of space-for-time substitution.

This study aims to examine patterns in stream biogeochemical responses along an altitudinal gradient; and thus, associated to differences in microclimatic conditions. Among-stream changes in environmental factors like temperature, precipitation regime, light, riparian vegetation, and geomorphology are associated to the altitudinal position of each stream along the gradient. The study was done in the Central region of the Pyrenees (N of Spain), which exhibits marked altitudinal gradients. The region is subjected to a Mediterranean-continental climate, but the altitudinal gradient determines dramatic changes in microclimatic conditions over relatively short geographical distances. Gradual changes along the gradient within the same climatic region may be more representative of shifts induced by future climate change conditions. The Mediterranean regions are often rugged with a marked change in elevation and, therefore, also in climatic conditions along relatively short horizontal distances. Consequently, headwaters of some streams may be in

high elevation areas where the climate is too wet and cold to be Mediterranean (Gasith & Resh 1999).

Most of the multifactorial studies addressed to stream biogeochemical responses tend to focus on a single functional parameter (e.g., Webster et al. 2003) or on two of them (e.g., Martí and Sabater 1996). We haven't found in the literature any study that performed a study of variability of multiple functional responses. Nutrient retention in lotic ecosystems is a function of synergistic effects resulting from the interaction of hydrologic, chemical, and biological properties (Valett et al. 1996), but also can be influenced by the relative availability of different solutes. Biotic components of stream ecosystems influence retention by generating, immobilizing, transforming, or removing biologically active solutes (Martí and Sabater 1996, Mulholland et al. 2005) through ecosystem metabolism. Therefore, some authors have focused on the analysis of the interaction between nutrient retention and ecosystem metabolism (Mulholland and Marzolf 1997). Finally, while most of the studies have focused on retention efficiency of inorganic nutrients (Webster et al. 2003, Hall and Tank 2003), very few studies have examined retention efficiency of organic forms of nutrients; despite the marked heterotrophic character of most headwater streams.

In this study we evaluated the variation in retention of multiple nutrients (acetate, glycine, nitrate, ammonium and phosphate) and stream metabolism among 14 headwater streams located along an altitudinal gradient ranging from 700 to 2100 m a.s.l. in the Central Pyrenees (North of Spain). We also examined how changes in physical, chemical and ecological factors associated to the altitudinal gradient influenced spatial variability in the biogeochemical responses (nutrient retention and metabolism) among the streams by using a multiparametric approach.

METHODS

Study sites

The present study was conducted in l'Ebro catchment located in the NE of Spain. The area of the catchment is 85.362 Km^2. It is the more extensive catchment of the Spain (17.3 % of Spain territory). Within this catchment, 14 streams were selected for this study in the Central Pyrenees region. The streams were located along an altitudinal gradient ranging from 700 to 2100 m a.s.l. (Table 1). Although the dominant climate of the region is Mediterranean-continental, this altitudinal gradient encompasses a broad range of micro-climatic conditions from alpine at the highest altitude to semi-arid at the lowest. Criteria for selection of the streams were that (1) they drained catchments with similar geology, in particular we selected streams draining catchments with calcareous geology, (2) the streams drained catchments with low human pressure (i.e., pristine streams), (3) they were similar in size (i.e., stream order and catchment size). This criteria was set to allow comparison of biogeochemical responses among sites and basically emphasize variability subjected to differences in environmental conditions imposed by the altitudinal gradient.

In each stream, we selected a reach where all the measurements were conducted. Stream reaches ranged from 64 to 144 m (Table 1). Details of each stream are provided in table 1. Most of the reaches were riffle-pool dominated. Susbtrata type was basically composed of boulder, cobbles, and gravel with patches of sand, with the exception of the streams at the lowest elevation where bedrock slates were dominant. Riparian vegetation along the reaches was well preserved and varied among sites according to altitudinal changes in vegetational stages. In the streams at the highest elevation riparian vegetation was dominated by Abies alba, *Pinus sylvestis*, *Pinus uncinata* and *Fagus sylvatics* with a important presence of *Buxus sempervirens* and *Juniperus comunis*. Streams located at the lower elevation, riparian vegetation was dominated by *Alnus glutnosa*, *Betula pendula*, *Salix sp.*, *Fraxinus excelsior* and *Corylus avellana* and a great presence of *Buxus sempervirens*. Canopy cover

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was denser in the streams at intermediate elevations, while it was less relevant at the higher alpine sites and at the lower semi-arid sites.

Physical, chemical and biological parameters (described below) were measured in all stream reaches once in summer 2005 and again once in summer 2006. In addition, in summer 2005 we conducted experiments to measure NH4 and NO3 retention parameters. In summer 2006, we measure retention parameters for glycine, acetate and SRP and daily rates of metabolism.

| 798m a,s,l, | 1487m a,s,l, | 1643m a,s,l, | 2029m a,s,l, |

Measurement of physical and chemical parameters

Basic physical measurements were made at each stream reach including water temperature, stream width (at 20-m intervals along the reach) and stream depth (5–10 measurements at each transect where width was measured). We also recorded photosynthetically active radiation (PAR) at 5-min intervals over 24 h using a quantum sensor (LiCor SKP215 Quantum sensor) connected with a data logger (Campbell Scientific®), which was located on the stream bank at one representative location. Instantaneous PAR data for the 24 h period was integrated to calculate daily PAR at each site.

Hydrological and hydraulic characteristics, such as discharge, average velocity and water transient storage, were determined using short-term conservative (i.e., NaCl) solute addition experiments. A known concentration of the conservative solute was released at the head of the reach at a constant rate using a peristaltic pump for a period of 1–4 h, and conductivity was automatically measured at 5-s intervals using a conductivity meter (WTW®,

model LF 340) connected to a data logger (Campbell Scientific®) placed at the bottom of the reach. Discharge (Q, L s^{-1}) and average water velocity (u, m s^{-1}) were measured using the time-curve conductivity data recorded at the bottom of the reach. Calculation of u was done by dividing the reach length by the time needed to increase the conductivity one half of the plateau (i.e., nominal travel time). Calculation of Q was based on a tracer mass balance approach.

Data from the conductivity time-curve were also analysed using the OTIS model (Runkel 2007), which is an advection-dispersion model with transient storage and lateral inflow parameters. With this model we estimated two parameters: a) the transient water storage zone (A_s, m^2) and b) the water exchange coefficient (k_1, s^{-1}) from the free flowing water to the transient storage zone. The section of the stream channel (A) was calculated by dividing Q by u and was used to obtain the ratio between the cross section of the transient water storage zone and that of the surface stream channel (A_s: A).

At each site, water samples were collected for ambient concentrations of ammonium (NH4-N), nitrate (NO·-N) and soluble reactive phosphorus (SRP). Water samples were filtered in the field using Whatman® GF/F fiberglass filters (0.7 µm pore diameter) and refrigerated until analysis. NH_4-N, NO_3-N and SRP concentrations were analyzed following standard colorimetric methods (APHA 1998) using Bran+Luebbe® autoanalyzers (TRAACS for NO_3-N and SRP, and Technicon for NH_4-N).

Measurement of biological parameters

We measured biomass (measured as ash-free dry mass, AFDM g/m^2) of epilithon, fine benthic organic matter (FBOM, particles <1 mm) and coarse benthic organic matter (CBOM, wood and leaves>1 mm) in each study reach on both sampling periods. Samples of epilithon, FBOM and CBOM were collected at 5 locations along each reach. Samples for CBOM biomass were collected only when this compartment accounted for more than 10% of the channel surface. CBOM was collected using a metal square (0.02 m^2) that was placed on the sediment surface. CBOM material within the metal square was sorted into wood and leaves, which were placed in two different plastic bags. In the laboratory,

CBOM samples were dried, weighed, and ashed (at 450 º C for 4 h) and re-weighed to determine AFDM (as the difference between dry and ashed weight). FBOM was sampled at five locations by sealing a open cylinder (0,05 m^2) on the stream bottom and gently mixing the sediment up to 5 cm depth and recording the total water volume. A subsample with a known volume of the water was collected and filtered onto a Whatman® GF/F fiberglass filters (0.7 µm pore diameter). AFDM of FBOM retained in the filter was measured as described above for CBOM. Samples for epilithon biomass were obtained from 5 cobbles obtained at random locations along each reach. Cobbles were placed in plastic bags and brought to the laboratory where they were dried, weighted, and ashed (at 450 º C for 4 h) and re-weighted for estimation of AFDM. The area of each cobble was measured to report AFDM per unit area. Additional cobbles (5) were also collected along the reach to determine chlorophyll *a* of the epilithon. In the field, each sample was placed in a vial with 90% acetone. Vials were brought to the laboratory and placed in the refrigerator overnight for pigment extraction and analysed spectrophotometrically for chlorophyll *a*, using the methodology described by Jeffrey and Humphrey (1975). Filamentous algae and bryophytes, when present, were sampled by scraping or coring material from known areas of substratum with 100% coverage of filamentous algae or bryophytes. Biomass (AFDM) of these biologic compartments was measured following the same procedure as described above for CBOM.

Percentage coverage of each biological compartment along the reach was visually estimated at 20-m intervals, and values were averaged to have a whole-reach estimate. Patch-specific values of biomass and chlorophyll a were weighted by the percentage coverage of each patch to estimate reach-weighted values. We used reach-weighted values in the data analysis. These values provide more integrative information at the reach scale than patch-specific and are more appropriate when examining relationships with biogeochemical responses, which come from whole-reach measurements.

Nutrient retention parameters

In each stream, we measured retention of multiple nutrients (phosphate, ammonium, nitrate, acetate and glycine) using the short-term nutrient addition technique (Webster and Ehrman 1996) experiments using chloride as a conservative tracer to measure the uptake efficiency in 14 streams from summer 2005-summer 2006. During summer, we performed these injections early in the morning to avoid daily discharge decreases from evapotranspiration. Reach length ranged from 65 to 144 m. At each study stream, we conducted the additions of SRP, NH_4-N and NO_3-N in summer 2005 and the additions of SRP, glycine and acetate in summer 2006. Additions of NH_4-N and NO_3-N and those of glycine and acetate were conducted on two consecutive days to avoid interferences among nutrient responses. Reagents for the selected nutrients plus sodium chloride were dissolved in stream water and this solution was injected to the stream using a peristaltic pump fed with batteries to keep the addition flow constant. After collecting water samples at 5/6 sites along each reach for ambient nutrient concentrations, we started the addition. An additional set of water samples were collected at the sampling sites when the conservative tracer concentration was constant through time at the downstream end of the reach (i.e., the addition reached plateau conditions). Conductivity was measured at the sampling stations both before the addition and once it reached plateau. Water samples for ammonium, phosphate, and nitrate were filtered in situ using glass-microfibre filters (Wathman GF/F) and kept frozen until analysis. Water samples for acetate and glycine were filtered with cellulose acetate membrane filters with a 0.45 µm diameter and kept frozen until analysis. Concentrations of ammonium, nitrate and phosphate were analyzed with a standard colorimetric methods (APHA 1998) using Bran+Luebbe® autoanalyzers (TRAACS for NO_3-N and SRP, and Technicon for NH_4-N). Concentration of glycine was analyzed using a fluorescence method for the determination of total amino acids in natural waters (Josefsson et al, 1977). Concentration of acetate was analyzed using Ion exclusion chromatography with an HPX-87H organic acid column.

Retention of nutrients along the reach is characterized using 3 different metrics: (i) uptake length (S_w, m), the average distance an ion travels in the water column before it is removed or transformed by either biological or physical processes (Newbold et al, 1981); (ii) uptake velocity (V_f, mm s^{-1}), also known as mass transfer coefficient, which is the velocity at which one nutrient moves from the water column to the stream substratum; and (iii) uptake rate (U, mg m^{-2} day^{-1}), the mass flux of nutrient from the water column to the stream substratum per unit area. In this study, we have used nutrient uptake length and nutrient uptake velocity as a nutrient retention metrics.

We calculated S_w for each nutrient as the negative inverse of the slope (K) of the line relating the natural log of nutrient flux to distance downstream:

$$\text{Ln } N_x = \ln N_0 - kx \qquad\qquad S_w = 1/K$$

Where N_0 and N_x are nutrient concentration at the addition site (0 m) and x m downstream from the addition site and k is the per-meter uptake rate (Newbold et al. 1981).

Because stream depth and velocity strongly influence uptake length (Hall et al. 2002), we calculated a nutrient uptake velocity. It was calculated as stream velocity (v) multiplied by mean stream depth (d) divided by uptake length:

$$V_f = v \, d/S_w$$

Metabolism

Whole-stream rates of gross primary production (GPP) and ecosystem respiration (R) were determined using the open-channel, single station diurnal dissolved oxygen change technique (Owens 1974; Bott 1996). Metabolism parameters were measured during summer 2006. At each stream, the measurement of whole-stream metabolism was done on the same day we conducted the nutrient addition experiments and at the same reach. At each stream, we placed an oxygen meter at the top and at the bottom of the reach and measurements of water temperature and dissolved oxygen (DO) were automatically recorded at 5 minute intervals for 24 h. Exchange of dissolved oxygen with the atmosphere was calculated based on the average oxygen saturation deficit or excess within the study reach and the reaeration rate determined from the nighttime regression method (Young & Huryn 1998). Calculation of reaeration coefficient is based on the following equation:

$$dO/dt = R + kD$$

where R is the respiration rate, k is the coefficient of reaeration and D is the oxygen deficit. A PAR intensity of 2 μmol quanta m^{-2} s^{-1} was used to differentiate the photoperiod from darkness. Rates of net dissolved oxygen change due to metabolism (net metabolism) were determined as the difference between successive 5-min measurements corrected for air-water oxygen exchange between measurements and average water depth. Nighttime R was calculated as the sum of the net metabolism measurements during the night. Daytime R was determined by interpolating between respiration rates measured 1 h before dawn and 1 h after dusk. Total daily R was the sum of nighttime and daytime R over 24 h periods. Daily GPP was the sum of the differences between the interpolated daytime respiration rates and the observed net metabolism.

Data Analysis

A multivariate analysis was used to examine which variables (physical, chemical and ecological) explain the largest proportion of the variability in nutrient retention responses among streams. We used a Principal Component Analysis (PCA) to reduce the number of independent variables into three new variables or factors that were a combination of original variables and explained the most variability among streams. A PCA was performed using the variables measured both first and second summer. In this analysis were included physical, chemical and biological variables. A pairwise value treatment was used to test differences in measured variables between years and variables values were standardized. The statistical program used to perform the analysis was STATGRAPHICS plus 5.1.

Redundancy analysis (RDA) was performed on a correlation matrix and is a form of direct gradient analysis. In a first step in RDA, the entire set of 26 environmental variables was tested to determine the significance of individual variables using a Monte Carlo permutation test (with 999 unrestricted permutations). Variables that were not significantly correlated with nutrient uptake variables or that were found to co-vary with other environmental variables were removed (n= 20) from the data set. The remaining variables were grouped into three subsets to yield ecologically interpretable variance components as follows: (1) variables describing the hydromorphology of the reach (H), (2) variables that described the water chemistry (Q), and (3) ecological variables. Variation partitioning analysis technique was used in this RDA analysis. This technique has been previously described by Borcard and others (1992) and hence we will not go into detail here. In brief, the procedure allows for the variance in the explanatory data set to be partitioned into different variables components through the use of covariables (i.e., variables whose influence is partially out of the analysis).

The total variance explained and the unique contributions of each subset and their joint effects were obtained by the following: (1) RDA was run with all three subsets as environmental variables and no covariables to obtain a measure of the total variance, (2) partial RDA was run with one of the three

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subsets as environmental variables and no covariables, and (3) partial RDA was run with one of the three subsets as environmental variables constrained by the remaining two groups as covariables and reverse. The third step was repeated three times and each subset was treated as environmental variables constrained by the remaining subsets as covariables. This procedure resulted in four runs of RDA for each subset combination or a total of 13 runs of RDA were done for the full set of analyses. With three subsets of environmental data, the total variation of nutrient uptake was then partitioned into seven components including covariance terms. The variation explained by these subsets is substracted from the total variation (1.0 in case of RDA) to obtain the unexplained variation. Redundancy analyses and partial RDA were done using CANOCO version 4.5 for Windows.

RESULTS

Variation in physical characteristics

Physical and hydraulic characteristics didn't show statically significant differences between years (Pair t-test, $p>0.05$). Thus, we used the average values between the two years for the characterization of the study streams in terms of physical parameters. Water temperature varied among the streams following the altitudinal gradient ($r^2 = 0.73$, $p< 0.05$). Temperature ranged from 9 ºC in Muntanyó de llacs (the stream at the highest elevation) to over 22 ºC in Campo (Table 1). Among-stream variation in daily PAR depended on the particular weather conditions on each sampling date as well as on the riparian vegetation coverage at each site. PAR was low in heavily forested streams (1.46 and 9.36 mol $m^2 d^{-1}$ in Muntanyeta and Matasomers, respectively) and very high at both ends of the altitudinal gradient (36.58 and 42.16 mol $m^2 d^{-1}$ in Muntanyó de llacs and Villas del Turbón, respectively), where the riparian vegetation was scarce due to the alpine and semi-arid conditions (Table 1). Discharge ranged from 1.5 to 32 L/s, though most of the streams were at the lower end of this range (median 6.7 L/s). The 14 streams ranged in width from 0.97 to 3.3 m. All streams were very shallow with average depth ranging from 0.02 m in lowest streams to 0.07 m in highest stream. Water velocity was the hydraulic

parameter with highest variability (based on CV) and ranged from 0.03 to 0.23 m/s.

Water dispersion coefficients determined from hydraulic simulations using data from solute injections done in summer 2005, ranged from 0.03 to 0.45 with a median of 0.097 $m^2 s^{-1}$. Water exchange coefficient between free-flowing water and transient storage zone (α) ranged from 0.00001(Les Paüles) to 0.12 (Pont de llacs). The size of the transient storage zone (A_s) ranged from 0.001 to 0.035 m^2. To standardise transient storage to stream size A_s was divided by surface water cross-sectional area and reported as As/A. The values ranged from 0.07 to 0.442.

Variation in chemical characteristics

Chemical characteristics showed statically significant differences between years (Pair t-test, $p<0.05$). It could be consequence of difference analysis techniques were used between years. Concentration of SRP was very low in all streams and ranged from 0.4 to 8.2 µg P L^{-1} in summer 2005 and from 4 to 7.4 µg P L^{-1} in summer 2006. NH_4-N concentration was generally low and ranged from 0.001 to 0.05 mg N L^{-1} in summer 2005 and from 0.005 mg N L^{-1} to 0.02 mg N L^{-1} in summer of 2006. Concentration of NO_3-N was the most variable of the rest inorganic nutrients and values ranged from 0.01 to 0.8 mg N L^{-1} (Table 2)

Variation in biological characteristics

Statistically significant differences between the two years were found for epilithic AFDM values. However, the other biological parameters considered didn't show any statistically significant difference between years. Thus, results for these parameters are reported as the average value for the two years, except for epilithic biomass where values for the two summers are reported separately.

There was great variation in the presence and abundance of algae and bryophytes among streams. Filamentous algae were present only in few streams, and therefore have not been included as a biological parameter in the

multivariate analysis. Epilithic biomass varied from 40 to 447.6 g AFDM m^{-2} in summer 2005 and from 48 to 400.5 g AFDM m^{-2} in summer 2006. Epilithic chlorophyll was expressed in mg/ m^2 and varied from 1345.94 mg/m2 to 9044.95 mg/m2.

The FBOM standing stock ranged from 14.6 g AFDM m^{-2} to 212.4 g AFDM m^{-2} .Leaves were abundant in forest streams like Matasomers, Barbarruens, Bisaurri and Lliri (752.4, 509.7, 434.3 and 1390.4 g m^{-2} , successively), but other streams had few or any leaves because of few or distant riparian trees or they were meadow steams like Campo, Villas del Turbón, Ramastué (Table 3)

Physical, chemical and biological variation along the environmental gradient

The first two factors of the Principal Component Analysis (PCA) including physical, chemical and biological, variables explained 25.2 and 16.8 % of the total variability among streams, respectively, using data from summer 2005 and summer 2006. Factor 1 was explained by water temperature, depth, nitrate concentration and N: P ratio. Water temperature, nitrate concentration and N: P ratio had a positive weight and depth had a negative depth (Figure 1). Factor 2 was explained by wetted width, discharge, conductivity and Epilithon AFDM. Wetted width and discharge had a positive weight and conductivity and epilithon AFDM had a negative weight. And, finally, Factor 3 explained the 12.4 % of the total variability among streams. It was explained, principally, by daily PAR, width, and exchange coefficient w-TS (K_1), FBOM AFDM and Epilithon chlorophyll. Exchange coefficient w-TS and epilithon chlorophyll had a negative weight and the others variables had a positive weight.

A Paired T-tested was performed to analyse if there ware significant differences in the principals factors that explained the most variability among streams between the two years. We found that there was not a significance differences between years for scores of factor 1, 2 and 3. Thus, we could consider that the environmental and biological variables which ordered the streams in the space were similar between the two years. Linear regression

analyses between the altitude at which each stream was located and the scores of the 3 first factors of the PCA showed that scores of Factor 1 were significantly related with altitude ($p < 0.05$, $r^2 = 66.9\%$,) (Figure 2). However, we did not find any statistically significant relationship with scores of Factor 2 and 3.
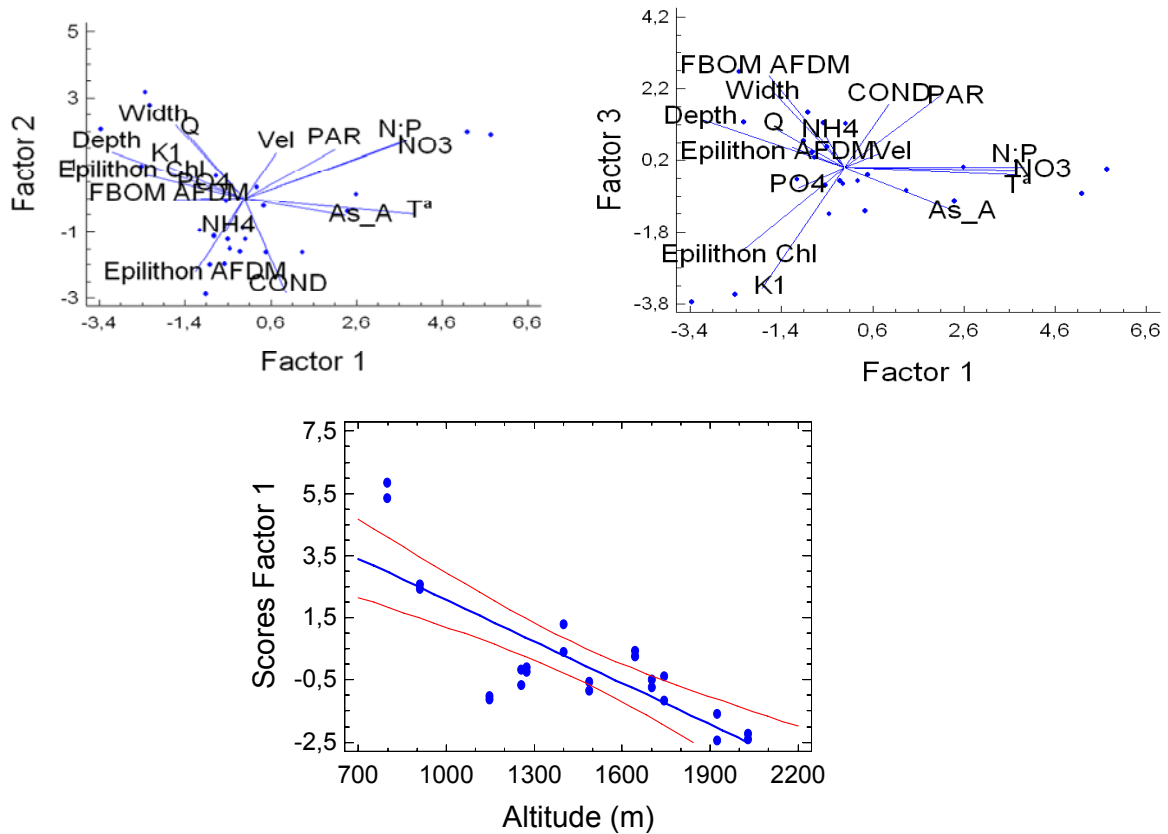

Figure 1 Biplot of component weights in the three first Principal factors of PCA and the relationship between the scores of the first principal factor and the altitude.

Variation in Ecosystem metabolism

Rates of GPP ranged from 0.002 to 1.78 g O_2 m^{-2} day^{-1}. Rates of ER exceeded GPP rates in all streams (i.e., NEP was negative in all streams) and ranged from 0.56 g O_2 m^{-2} day^{-1} to 9.04 g O_2 m^{-2} day^1. There was substantially higher variability among stream in GPP than in R (i.e., CV were 64.7 and 130.8 % for ER and GPP, respectively). The ratio between GPP and ER was <1 for all streams (Table 3).

The daily rate of ER was significantly correlated with discharge (Pearson correlation, r= 0.46, p<0.1). ER did not show any significant correlation with

the rest of the physical, chemical and biological parameters considered. Daily rates of GPP were not significantly correlated with any of the physical, chemical anb biological parameters considered. However, using a multivariate regression analysis, we found that 94.8 % of the variation in GPP among the streams could be explained by a model that included nitrate concentration, epilithic biomass and FBOM biomass.

Variability of retention of multiple nutrients

The average uptake length was low in all nutrients with a value minor than 600 m in all cases. But, the efficiency in nutrient uptake was different and it depended on the nutrient added. We found that all streams were more efficient (i.e., shorter uptake lengths) in retaining phosphate and glycine than the other nutrients. S_w for phosphate and glycine averaged 81.02 and 123.1 m, respectively, among streams (Table 2). The nutrient with the longer (i.e., lower efficiency) average uptake length was acetate (532.9 m).

Uptake length for acetate ranged from 42.2 m to 1428.6 m. The range in ammonium uptake length was similar to that for acetate (from 55.9 m to 625 m). On the other hand, uptake length for phosphate ranged from 29.3 m to 270.3 m and uptake length for glycine ranged from 43.9 m to 217.4 m. In the case of nitrate, uptake length ranged from 59.2 m to 400m. However we couldn't compare this variability with the others because of we had some problems with the analysis technique and we only could analyse 6 streams. We found only significant relationships between uptake length for acetate and uptake length for ammonium (Figure 2; r = 0.72, p<0.05).
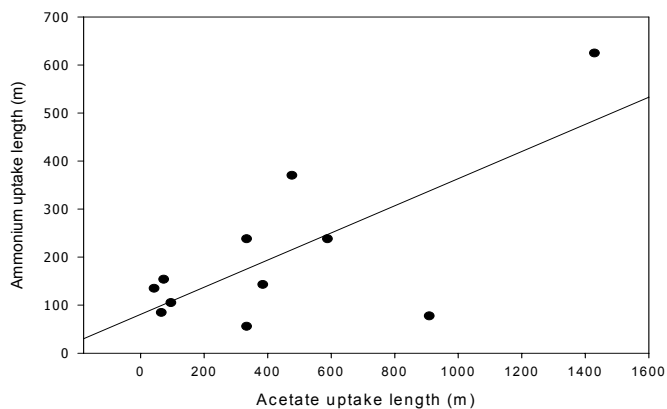

Figure 2 Relationship between acetate uptake length and ammonium uptake length.

Therefore, we found that stream sensibility to response was different across streams. So, we found a high spatial variability across streams in uptake response depend on the nutrient added.
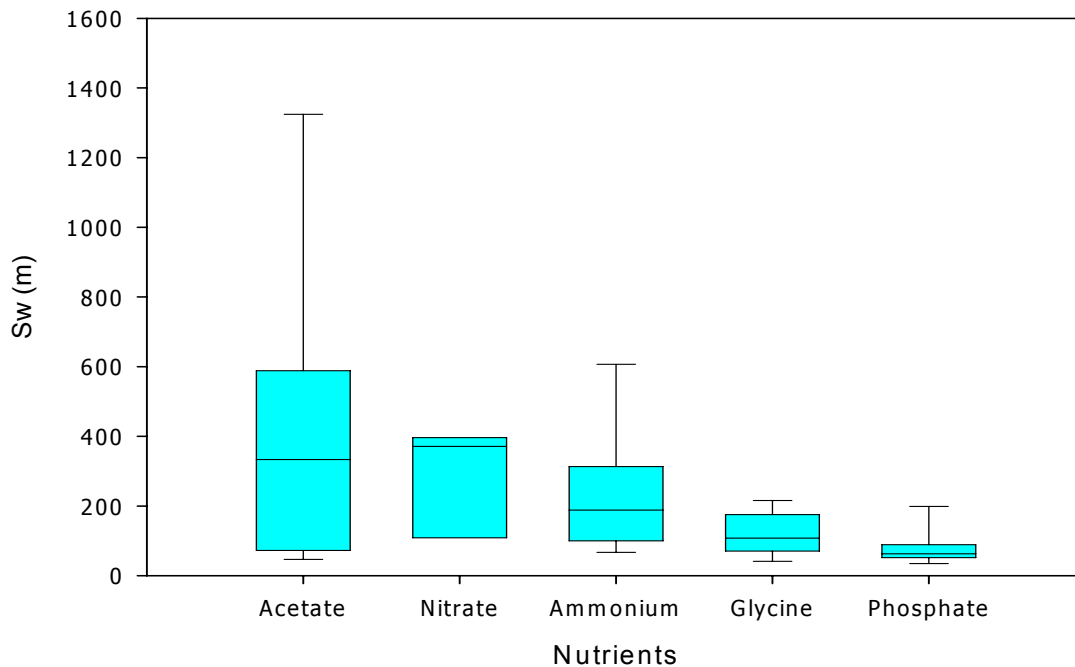

Figure 3 Variability in uptake length of all add nutrients. Each Box Plot shows the mean line and the 5th/95th percentile.

We found that variability in the response was greater in the case of acetate and ammonium. In case of glycine and phosphate, all streams responded with a great efficiently and the variability in the functional response was low (Table 2). Thus, the CV for uptake length of acetate was 98.6% and the CV for uptake length of ammonium and nitrate were 75,5 % and 75,8 % , respectively. On the other hand, the CV for uptake length of phosphate and glycine were 39,2 % and 51,02 %, respectively.

On the other hand, uptake lengths were compared among nutrients using one-way ANOVA (nutrient as a factor) and we found significant differences between nutrients (one-way ANOVA; F = 5.91, p = 0.0016). We didn't use nitrate values in this analysis because of the few values we had. Therefore, two homogenous groups were identified. In the first group there

was ammonium, glycine and phosphate and in the second group there was the acetate. Thus, acetate mean was significantly different from the others.

On the other hand, uptake velocity indicates nutrient uptake independent of the effect of discharge. It can be viewed as the rate or velocity at which uptake processes associated with the stream bottom remove one nutrient from the overlying water column. It could be understand like an indicator of nutrient demand. We found that acetate was the add nutrient with an average uptake velocity value lowest (V_f = 0.000025 m/s). And phosphate and glycine were the nutrients with an average uptake velocity highest (0.00007 and 0.00006 m/s, respectively). Finally, ammonium average uptake velocity had a value of 0.000035 m/s. The mean value of nutrient uptake velocities was compared using a One-Way ANOVA (nutrient as a factor). However, we didn't find significance differences between nutrients (One-Way ANOVA, F= 2.35, P= 0.0839). But, we applied a multiple comparison to determine which means were significantly different from which others. And, we found only a statically significance difference between acetate mean value and phosphate mean value.

On the other hand, acetate uptake velocities were in a small range of 0.000001 to 0.0001 m/s. Ammonium uptake velocity ranged from 0.000003 to 0.00013 m/s and glycine and phosphate uptake velocity ranged from 0.00001 to 0.00021 m/s and 0.00001 to 0.0002 m/s, respectively. In the case of nitrate uptake velocity ranged from 0.00000323 to 0.00078 m/s. Thus, nitrate was the nutrient with a range widest. But, this result it is not very significance because we only analysed nitrate uptake from seven streams. Thus, when nutrient uptake length was corrected by the effect of discharge, we found that variability in the response was greater in the case of phosphate and glycine (different from uptake length variability).

We found a positive significance relationship between acetate uptake velocity and discharge, phosphate concentration (measured in summer 2005) and epilithon chlorophyll (r^2=29.9 %, p=0.06; r^2= 30.2 %, p=0.06; r^2= 25.6%, p=0.09, respectively). There was a significance positive relationship between ammonium uptake velocity and discharge, velocity, depth and wetted width (r^2 = 41.5%, p=0.01; r^2 = 43.7%, p= 0.01; r^2= 32.6%, p= 0.03 and r^2 = 22.3%,

p= 0.087; respectively). A positive significance relationship we found between glycine uptake velocity and dispersion, phosphate concentration (measured in summer 2005), epilithon chlorophyll and discharge (lineal regression; $r^2=$ 52.3%, p= 0.0035; $r^2=$ 44.4%, p= 0.009; $r^2=$ 25.6%, p= 0.09; $r^2=$ 23.23%, p= 0.08, respectively). Besides, there was a negative significance relationship between glycine uptake velocity and conductivity and phosphate concentration measured in summer 2006 (lineal regression; $r^2=$ 33.14%, p= 0.03; $r^2=$ 24.8%, p= 0.069, respectively). Finally, we found a significance positive relationship between phosphate uptake velocity and discharge, depth, dispersion and phosphate concentration measured in summer 2005 (lineal regression; $r^2=$ 46.43%, p= 0.007; $r^2=$ 31.3%, p= 0.03; $r^2=$ 28.9%, p= 0.047; $r^2=$ 22.8%, p= 0.08).

Acetate uptake velocity was statically correlated with phosphate uptake velocity and glycine uptake velocity (r= 0.77, P= 0.003; r= 0.65, P= 0.02, respectively).

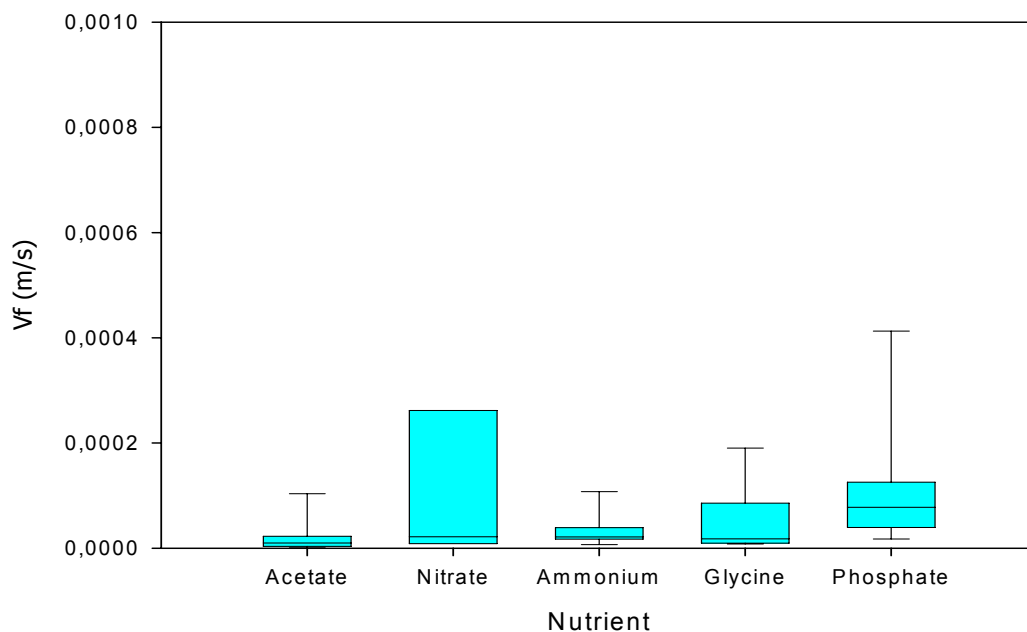**Figure 4** Variability in uptake velocity of all add nutrients. Each Box Plot shows the mean line and the 5th/95th percentile.

Sources of variation in nutrient uptake

Variance decomposition using redundancy analysis showed that all independent variables combined explained all total variation in stream nutrient uptake. The largest proportion of variance was explained by the interaction between all three set factors.

Nutrient uptake (V_f) was more influenced by hydromorphological factors than either by chemical and ecological factors. However, the unique variance explained by hydromorphological, chemical and ecological variables was low (<10%). In brief, the unique variance explained by hydromorphological, chemical and ecological variables was 5%, 3.9% and 2.2%, successively. Hydromorphological and ecological factors (H&E) were better predictors of nutrient uptake than hydromorphological and chemical (HQ) or chemical and ecological (QE) factors. The strongest interaction was found between hidromorfological variables and ecological variables and explained 51.8 % of the variance in nutrient uptake. The relation between hidromorfological variable and chemical variables was much weaker. The H&Q interaction explained 7.8 % of the variance in nutrient uptake. The amount of variance explained by the interaction between chemical and ecological variables was 11%.

Ordination of nutrient uptake and environmental variables showed that the primary RDA axis explained the 58.8 % of total variance in nutrient retention and the second RDA axis explained the 27.7 % of total variability. Thus, the two first axes explained the 86.5 54 of total variability. Eight out of 26 selected explanatory variables were significant (discharge, water velocity, dispersion coefficient, exchange coefficient w-TS (K_1), A_s/A, nitrate concentration, FBOM biomass and ecosystem respiration; $p < 0.1$). Throughout a correlation matrix obtained in the RDA analyses we found that discharge, dispersion coefficient and epilithon chlorophyll were positively correlated with axis 1 and exchange coefficient w-TS (K1) and FBOM biomass were correlated with axis 2 (positively in case of K1 and negatively in case of FBOM).

The RDA diagram showed that ammonium uptake velocity, acetate uptake velocity and phosphate uptake velocity were positively correlated. Besides, we found that acetate, ammonium and phosphate demand were

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related to discharge, water velocity and ecosystem respiration and glycine demand was related to transient storage parameters (like K_1 and dispersion coefficient). Thus, as you can see in the diagram, all streams were ordinate along the space described by two first axes. For example, Muntanyó de Llac was located in lower right quadrant because of it was the stream with highest phosphate demand, with highest ER value and with highest discharge and Muntanyeta and Matasomers were located in the higher left quadrant because of they were the streams with dispersion coefficient lowest.

Table 4 Calculation of explanatory power of each component in the variance partitioning model

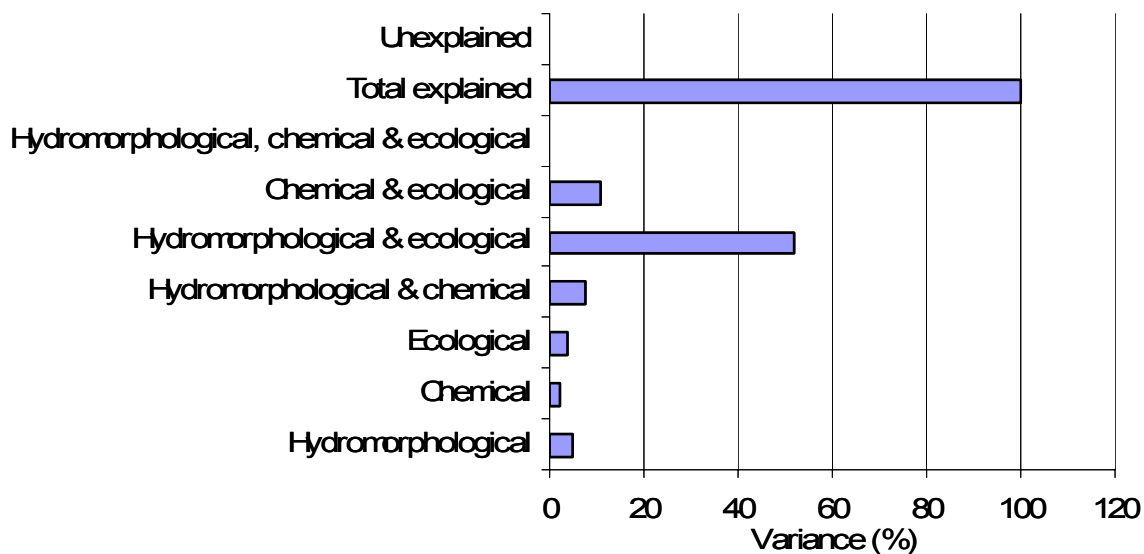| Variation explained by factors	Abbreviation	λ	Variance (%)
Hydromorphological	H	0,05	5
Chemical	Q	0,022	2,2
Ecological	E	0,039	3,9
Hydromorphological & chemical	H&Q	0,078	7,8
Hydromorphological & ecological	H&E	0,518	51,8
Chemical & ecological	Q&E	0,11	11
Hydromorphological, chemical & ecological	HQE	0	0
Total explained	TotX	1	100
Unexplained	UX	0	0
Total variance	TotV	1	100

Figure 5 Sources of variation in stream nutrient retention, Colums labels indicate the variation (%) in acetate, ammonium, glycine and phosphate uptake demand for by each subset and their combination,
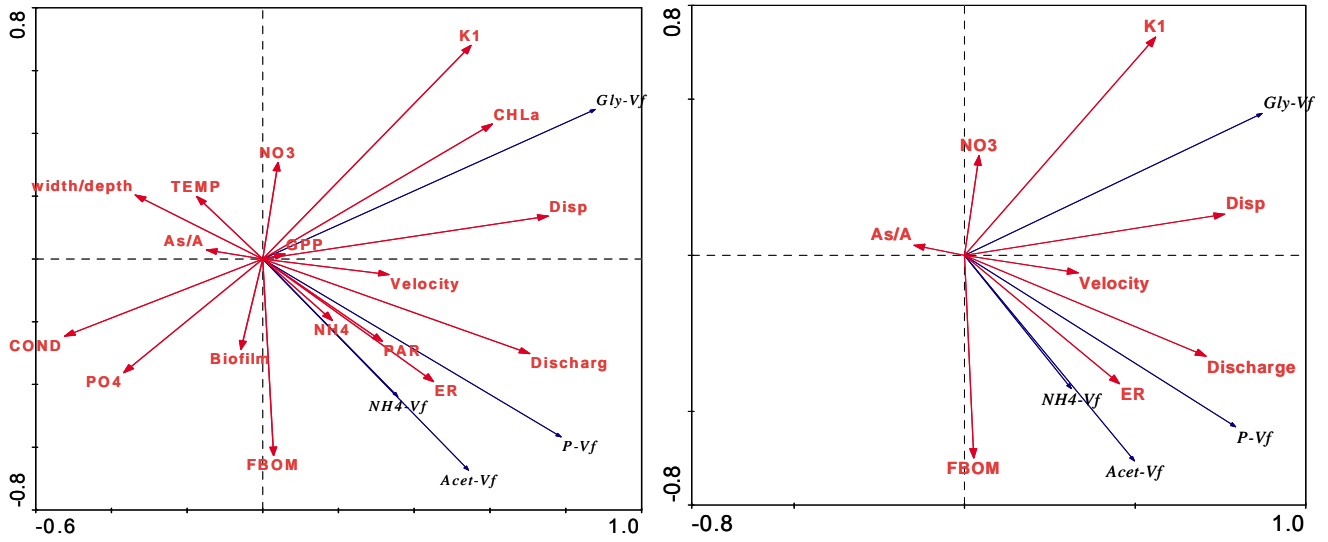
Figure 6 Results from RDA, a direct gradient technique that maximizes covariance between environmental and biological variables (nutrient V_f). In the first diagram there are all environmental variables that we have use in RDA analysis and in the second diagram there are only represented the significance variables.

Table 1 List of streams and physical, chemical and biological characteristics at or near the time of nutrient additions measurements. length were measured directly.

Stream	Altitud (m)	Temperature (ºC)	Daily PAR mmol m-2d -1	Reach length (m)	Riparian canopy	Velocity (m/s)	Depth (cm)
Muntanyó de llacs (42 ° 32' 27"N, 0º 55' 01"W)	2029	8,87	41977,04	144	Open	0,13	0,07
Pont de llacs	1923	9,95	9682,14	84	Open	0,12	0,06
Matasomers (42º 34' 13"N, 0º 30' 42"W)	1743	10,37	7498,48	88	Closed	0,04	0,04
Muntanyeta	1701	10,67	1786,34	80	Closed	0,10	0,03
Puimestre (42º 34' 31"N, 0º 32' 12"W)	1643	11,73	17873,58	103	Semi-closed	0,09	0,03
Les Paüles (42º29'05"N, 0º33'07"W)	1487	14,32	16239,34	90	Semi-closed	0,10	0,05
Barbarruens (42º30'47"N, 0º22'16"W)	1412	10,14	28382,87	100	Closed	0,04	0,06
Ramastué (42º32'39"N, 0º30'09"W)	1399	16,36	33128,70	95	Open	0,21	0,03
Lliri (42º32'04"N, 0º30'40"W)	1274	13,74	18977,85	65	Closed	0,12	0,04
Renanué (42º29'11"N, 0º31'16"W)	1257	13,44	25939,39	110	Closed	0,06	0,04
Bisaurri (42º29'56"N, 0º30'34"W)	1146	15,21	21035,82	107	Semi-closed	0,05	0,05
Urmella (42º30'32"N, 0º30'39"W)	1077	13,59	32392,01	99	Semi-closed	0,40	0,06
Villas del Turbón (42º25'23"N, 0º27'52"W)	909	17,95	27900,88	77	Semi-closed	0,05	0,02
Campo (42º12'16"N, 0º29'37"W)	798	22,64	48389,67	104	Open	0,18	0,02

Table 1 (continued)

Stream	Dispersion Coefficient (D) m² s-1	Transient Storage Zone (As) m²	Exchange Coefficient w-TS K_1, s-1
Muntanyó de llacs (42 ° 32' 27"N, 0º 55' 01"W)	0,42	0,0099	0,0003
Pont de llacs	0,45	0,005	0,12
Matasomers (42º 34' 13"N, 0º 30' 42"W)	0,08	0,019	0,0003
Muntanyeta	0,08	0,035	0,00028
Puimestre (42º 34' 31"N, 0º 32' 12"W)	nm	nm	nm
Les Paüles (42º29'05"N, 0º33'07"W)	0,03	0,011	0,00001
Barbarruens (42º30'47"N, 0º22'16"W)	0,07	0,032	0,00018
Ramastué (42º32'39"N, 0º30'09"W)	0,33	0,004	0,00055
Lliri (42º32'04"N, 0º30'40"W)	0,07	0,032	0,00018
Renanué (42º29'11"N, 0º31'16"W)	0,095	0,005	0,0015
Bisaurri (42º29'56"N, 0º30'34"W)	0,18	0,001	0,0002
Urmella (42º30'32"N, 0º30'39"W)	0,2	0,0015	0,0015
Villas del Turbón (42º25'23"N, 0º27'52"W)	nm	nm	nm
Campo (42º12'16"N, 0º29'37"W)	0,1	0,009	0,0015

Table 2 Nutrient concentrations and nutrient dynamics in the studied streams

Stream	PO_4-P mg/L	NH_4-N mg/L	NO_3-N mg/L	Ammonium uptake length (Sw, m)	Ammonium uptake velocity (Vf, m/s)	Phosphate uptake length (Sw, m)	Phosphate uptake velocity (Vf, m/s)
Muntanyó de llacs (42 ° 32' 27"N, 0° 55' 01"W)	0,006	0,015	0,146	105,26	0,000085	75,27	0,00021
Pont de llacs	0,006	0,012	0,030	222,22	0,000019	96,73	0,00008
Matasomers (42° 34' 13"N, 0° 30' 42"W)	0,004	0,025	0,017	77,52	0,000034	54,26	0,00002
Muntanyeta	0,005	0,018	0,116	238,10	0,000013	270,27	0,00001
Puimestre (42° 34' 31"N, 0° 32' 12"W)	0,005	0,004	0,102	55,87	0,000029	127,46	0,00006
Les Paüles (42°29'05"N, 0°33'07"W)	0,004	0,012	0,024	625,00	0,000019	52,82	0,00004
Barbarruens (42°30'47"N, 0°22'16"W)	0,003	0,018	0,146	142,86	0,000021	58,45	0,00003
Ramastué (42°32'39"N, 0°30'09"W)	0,005	0,018	0,015	588,24	0,000011	69,93	0,00007
Lliri (42°32'04"N, 0°30'40"W)	0,004	0,033	0,023	153,85	0,000018	60,42	0,00012
Renanué (42°29'11"N, 0°31'16"W)	0,004	0,017	0,027	84,75	0,000055	48,73	0,00007
Bisaurri (42°29'56"N, 0°30'34"W)	0,005	0,028	0,016	135,14	0,000027	29,36	0,00004
Urmella (42°30'32"N, 0°30'39"W)	0,006	0,013	0,126	370,37	0,000130	86,37	0,00007
Villas del Turbón (42°25'23"N, 0°27'52"W)	0,005	0,013	0,450	294,12	0,000003	39,59	0,00004
Campo (42°12'16"N, 0°29'37"W)	0,003	0,007	0,807	238,10	0,000022	64,69	0,00005

Table 3 Metabolic and organic parameters of the study sites. Epilithon, FBOM and leaves were measured during

Stream	Temperature (°C)	Riparian canopy	Gross primary production ($gO_2\,m^{-2}\,day^{-1}$)	Ecosystem respiration ($gO_2\,m^{-2}\,day^{-1}$)	P:R ratio	Epilithic biomass (gAFDM m
Muntanyó de llacs (42 ° 32' 27"N, 0° 55' 01"W)	8,87	Open	0,620	9,040	0,069	182,659
Pont de llacs	9,95	Semi-closed	0,137	3,126	0,044	170,682
Matasomers (42° 34' 13"N, 0° 30' 42"W)	10,37	Closed	0,024	0,795	0,030	233,477
Muntanyeta	10,67	Closed	0,027	3,570	0,008	72,764
Puimestre (42° 34' 31"N, 0° 32' 12"W)	11,73	Closed	0,002	1,546	0,001	163,350
Les Paüles (42°29'05"N, 0°33'07"W)	14,32	Semi-closed	1,080	5,980	0,181	322,965
Barbarruens (42°30'47"N, 0°22'16"W)	10,14	Closed	0,009	0,562	0,016	107,896
Ramastué (42°32'39"N, 0°30'09"W)	16,36	Open	0,007	3,171	0,002	280,835
Lliri (42°32'04"N, 0°30'40"W)	13,74	Closed	0,104	3,093	0,034	235,745
Renanué (42°29'11"N, 0°31'16"W)	13,44	Closed	0,239	1,875	0,127	192,918
Bisaurri (42°29'56"N, 0°30'34"W)	15,21	Closed	0,970	7,510	0,129	400,528
Urmella (42°30'32"N, 0°30'39"W)	13,59	Semi-closed	0,348	4,678	0,074	nm
Villas del Turbón (42°25'23"N, 0°27'52"W)	17,95	Open	1,780	3,720	0,479	223,258
Campo (42°12'16"N, 0°29'37"W)	22,64	Open	0,329	3,900	0,084	39,667

DISCUSSION

Variability in functional responses

We anticipated that variability in environmental factors imposed by the altitudinal gradient will influence in-stream in biogeochemical responses. In addition, we hypothesize that if factors with greater influence on nutrient retention would follow a marked altitudinal gradient pattern, we should also see a clear spatial pattern in biogeochemical responses. Our study takes a multifactorial approach to examine the biogeochemical variability among the streams.

Our results indicate that the study streams were arranged along the gradient according to some environmental parameters (such as water temperature, light, nutrient concentration, morphohydraulic parameters) that, like we expected, have been shown to influence in-stream biogeochemical responses. Results from the PCA indicated that these parameters were associated to the first PCA factor, and thus, explained the maximum variance among streams. This suggests that the maximum variance among streams in terms of environmental conditions was driven by changes imposed by the altitudinal gradient. Parameters having a significant weight on first PCA factor were temperature, water depth, nitrate concentration and N: P ratio. Water temperature and stream depth tended to decrease and nitrate concentration and the N:P ratio tended to increase from streams located at the higher altitude to those located at the lower altitude. Other parameters having a high component weight in factors 2 and 3 of the PCA were not related with altitude and, therefore, were more subjected to local characteristics of each site.

Our data indicated that variability in biogeochemical responses among streams differed depending on the functional parameter considered. This agrees with previous studies (e.g., Martí and Sabater 1996). Functional parameters with high variability, as expressed by the coefficient of variation, reflect higher sensibility (i.e., are more susceptible) to environmental changes along the altitudinal gradient than those parameters with lower variability. In this context, our results show that GPP and glycine uptake velocity are the biogeochemical responses more susceptible to the environmental variation

along the altitudinal gradient. In fact, based on the RDA results, these two parameters are related with environmental parameters showing a higher CV (i.e., nitrate concentration, K_1 and dispersion coefficient).Therefore, if variability in environmental factors related to these functional responses are high we would expect a high variability in glycine uptake velocity and GPP as well. On the other hand, the functional parameter that was less sensible to changes in environmental factors was the daily rate of ER. The contrast between GPP and ER variability supports previous findings from an interbiome comparison (Mulholland *et al.* 2001). In that study they also found that ER was greater than GPP in most of the studied streams, as we have found in our study. This highlights the consistent heterotrophic nature of headwater streams and suggests that GPP may be influenced by local particularities that may expand the variability range above that of ER. In fact, several studies have suggested that temperature is the best single predictor of ER in streams (Bott et al. 1985; Sinsabaugh 1997; Uehlinger et al. 2000), which tends to change gradually across biomes or along altitudinal gradients. Nevertheless, this is surprising considering that in our environmental gradient temperature is the environmental factor with highest weight in the first PCA factor (i.e., the factor that explains the maximum variance among streams), and thus, we expected to find higher variability in ER than in GPP.

GPP values measured in this study (0.002 to 1.78 g O_2 m^{-2} day^{-1}) fall in the lower range of values reported for other small pristine streams in an interbiome comparison by Mulholland *et al.* (2001), but are similar to those measured in a small Mediterranean stream (0.05 to 1.9 g O_2 m^{-2} day^{-1}, Acuña et al. 2004). In a study of several stream located in the same region in Wyoming, Hall and Tank (2003) found a range of values of GPP rates lower than range that we have found, despite the higher values were bracketed within those found in the present study. Ecosystem respiration dominated whole-stream metabolism in all our streams as indicated by P: R ratios <1 in all streams. Therefore, all study sites were heterotrophic ecosystems, similar to what has been found by other authors in their studies. The values of ER falls within the

range of values reported for other small streams (Young and Huryn 1996; Mulholland *et al.* 2001).

We also found that retention of organic forms of nutrients (i.e., acetate and glycine) showed a higher variability among streams than retention of inorganic nutrient forms (ammonium and phosphate). Because this is the first study considering retention of multiple nutrients, including retention of organic forms, we cannot ensure if this is a common trend among streams. High variability in retention of organic forms could be influenced by the dominant heterotrophic activity within the study streams that may result in a broader array of rates associated to organic matter utilization. However, it has been impossible to compare nutrient retention metrics of organic nutrients added in this study with other values or ranges because we have not found other published studies in the literature on this issue.

Variability of NH_4-N and SRP demand was similar. This contrast with other studies that reported lower variability for SRP (CV = 43%) demand than for NH_4 (70%) demand (Hall *et al.* 2002) among forested mountain streams. Finally, in addition to the lower variability in retention of inorganic forms among the study streams, we have found that uptake length of these forms was relatively short, especially for SRP. This indicates high efficiency for the retention of these nutrients. Our retention values are comparable to values reported in other studies. This way, Martí and Sabater (1996) found short ammonium and phosphate uptake lengths values very similar in amount as ours when they were studying the temporal and special variation in nutrient retention in two second order Mediterranean streams that differ in watershed lithology, soil type and vegetation. Nevertheless, Webster et al. (2003) found a range of ammonium uptake length values from short to high values. In particular, a high efficiency in phosphate retention compared to that for the rest of the nutrients considered suggests that phosphate could be a potentially limiting nutrient in these streams. In fact, SRP concentration was very low regardless of stream location along the altitudinal gradient. This is a common characteristic of streams draining catchments with calcareous geology. Previous studies have shown high retention efficiency of SRP in streams draining

calcareous catchments (Martí and Sabater 1996), which agree with our present results. In these streams, retention of P is likely to be enhanced by chemical precipitation of this element on top of retention driven by biological activity. Therefore, variability in phosphate demand is lower than in other nutrients because all streams are potentially limited by phosphate.
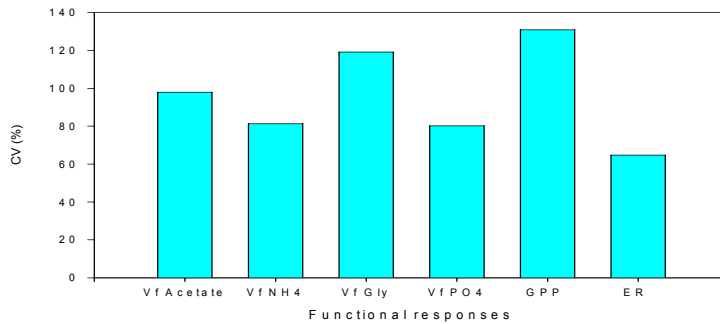

Figure 7 Coefficient of variability of all functional responses studied (nutrient retention and ecosystem metabolism)

Factors influencing nutrient uptake in streams

Nutrient retention in lotic ecosystems is a function of synergistic effects resulting from the interaction of hydrologic, chemical, and biological properties (Valett et al. 1996). Hydrologic retention occurs when water enters flow paths moving slower than the advective velocity of the main channel, resulting in increased water residence times and higher chance of nutrient utilization by microorganisms. In subsurface regions, residence times depend primarily on the hydraulic gradient and hydraulic conductivity (K) of the sediments. Steeper hydraulic gradients and higher K will promote stream-groundwater exchange that results in hydrologic retention and increased residence time for water (Runkel 2007; Harvey et al. 2003; Butturini and Sabater 1999). Biotic components of stream ecosystems influence nutrient retention by generating, immobilizing, transforming, or removing biologically active solutes (Martí and Sabater 1996; Mulholland et al. 2005). Comparably, the processes of sorption, flocculation, and precipitation result in chemical retention by retarding solute

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transport (Morrice et al. 1997; Meyer 1979). All dissolved solutes are influenced by the hydraulic retention of water. Depending on the solute of interest, chemical and biological processes may be minor or major determinants of solute retention (Martí and Sabater 1996). Therefore, total ecosystem retention is the product of multiple process rates (chemical and biological) and water residence time (Valett *et al*. 1996). The interplay of these factors may change over the altitudinal gradient which may enhance or obscure patterns of biogeochemical responses along it. For this reason we took a multifactorial analysis approach to examine patterns and relationships along the gradient.
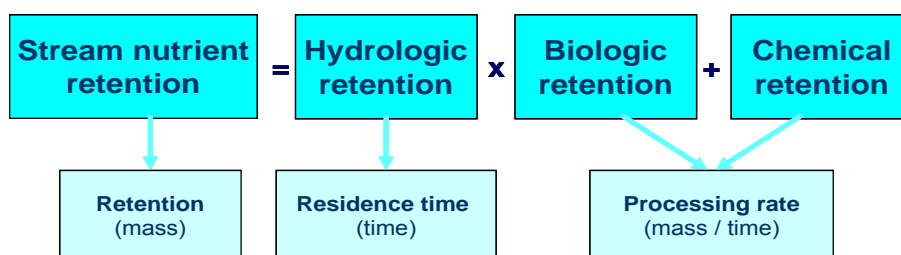

Fig. adapted from Valett et al. 1996

Figure 8 Figure adapted from Valett *et al*. 1996 which illustrate the synergetic effect in nutrient retention of hydrologic, chemical and biologic factors.

Based on the conceptual framework stated above (Figure 8), we tried to elucidate which explanatory variables (i.e., independent parameters) grouped into hydromorphological, chemical and ecological categorical sets accounted for a greater fraction of the among-stream variability in retention of multiple nutrients. We used a redundant analysis (RDA) as an exploratory and summary statistical technique to elucidate categories of factors that may influence nutrient retention variation along the gradient. We did not find any relationship between nutrient retention and environmental factors when we used nutrient uptake length values. However, when this parameter was corrected by the specific discharge (i.e., velocity*depth) some relationships with environmental parameters emerged. This highlights the predominat role of hydrology on in-stream nutrient retention as noticed in prevous studies (Valett *et al*. 1996; Butturini and Sabater 1998, Peterson *et al* 2001). To eliminate dominance of this factor, we considered nutrient demand as the dependent functional variable

in the RDA analysis. Results from this analysis show that variability in nutrient retention among the study streams is mostly explained by the interplay between hydromorphological and ecological factors. Stream geomorphology and hydraulics can influence nutrient uptake through their influence on water and solute transient storage, which increases residence time and the interaction between solutes and microorganisms (Valett *et al*. 1996, Mulholland *et al*. 1997, Butturini and Sabater 1999). Other authors have found a relationship between transient storage and nutrient uptake. For instance, results from Mulholland *et al.* (1997) suggest that nutrient cycling (uptake and remineralization) is intensified in streams with larger transient storage zones. Others studies performed in this line found that model simulations predict that increasing the surface water exchange rate (K_1) decreased nutrient uptake length and that increasing K_1/K_2 will also decreases nutrient uptake length (Mulholland and DeAngelis 2000). Hall *et al*. (2002) found a positive relationship between ammonium V_f and $K_1/K_2.$ An example of the importance of biological processes in steam nutrient retention is shown in experimental leaf-litter exclusion in Coweeta stream where nutrient uptake decreased after leaf-litter was excluded (Webster et al. 2000).

With more detail, results from RDA show a positive relationship between ammonium uptake (V_f), acetate uptake and phosphate uptake. Additional evidence for this relationship is from acetate addition on W6 stream that stimulated bacterial productivity and increased Vf of (Bernhardt and Likens 2002) A positive relationship of these parameters with discharge, velocity, ecosystem respiration, daily PAR and NH_4-N concentration is evidenced. Webster *et al.* (2003) found similar results when they analysed which factors affected NH_4-N uptake in an inter-biome study, in which they reported a positive relationship between Vf of NH_4-N and ecosystem respiration. On the other hand, Mulholland *et al*. (1997) used two streams that varied greatly in ER and showed that the high ER stream had an increased demand for phosphorus. On the other hand, glycine uptake velocity was related with transient storage parameters, like dispersion and exchange coefficient (K_1). This result suggests that glycine is an organic nutrient that plays a role in heterotrophic metabolism.

But we can't understand the relation between epilithon chlorophyll and glycine demand because epilithon chlorophyll is an indirect measure of autotrophic biomass and glycine is an organic nutrient that play a role in heterotrophic metabolism. Findlay and Sinsabaugh (2003) found in their study that addition on ammonium and amino acids caused substantial regeneration of nitrate-N along mesocosmos flowpaths. Similarly, Bernhardt *et al.* (2002) report that nitrification in some streams of the Hubbard Brook Valley (White Mountains, New Hampshire, USA). If organic N is rapidly returned to the overlying water as nitrate, then retention of these compounds is short-term and could have some effect in autotrophic biomass in term of provide availability nitrate.

In conclusion, our study emphasizes the susceptibility of freshwater ecosystems to variability in environmental factors that play an important role at the moment to explain the variability in biogeochemical responses. Our results show that GPP and glycine uptake velocity are the biogeochemical responses more susceptible to the environmental variation along the altitudinal gradient and we also found that retention of organic forms of nutrients (i.e., acetate and glycine) showed a higher variability among streams than retention of inorganic nutrient forms (ammonium and phosphate). High variability in retention of organic forms could be influenced by the dominant heterotrophic activity within the study streams that may result in a broader array of rates associated to organic matter utilization. In fact, we have found that variability in nutrient retention among the study streams is mostly explained by the interplay between hydromorphological and ecological factors (like discharge, velocity, ecosystem respiration, and daily PAR and NH_4-N concentration).

Acknowledgments

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