Variation in stream C, N and P uptake along an altitudinal gradient: a space-for-time analogue to assess potential impacts of climate change

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ABSTRACT

A space-for-time substitution approach was used to evaluate potential effects of climate change on stream nutrient uptake by examining the relationship between stream environmental parameters and carbon (C), nitrogen (N) and phosphorus (P) uptake along an altitudinal gradient. The study was carried out in 14 streams located in the Central Pyrenees (NE Spain) draining calcareous catchments that cover an altitudinal range of 700–2,100 m a.s.l. In these streams, uptake of inorganic (soluble reactive phosphorus (SRP), ammonium and nitrate) and organic (acetate and glycine) nutrients was estimated. Additionally, several physical, chemical and biological parameters were measured. Results showed higher uptake for both SRP, a potentially limiting nutrient in these streams, and glycine, a labile source of dissolved organic N, than for the rest of the nutrients. Uptake of SRP, nitrate, glycine and acetate varied along stream environmental gradients associated with changes in stream hydromorphology, SRP availability and epilithic biomass. However, these gradients did not vary with altitude. These results indicate that climate change effects on stream nutrient uptake are more likely to be driven by indirect effects on hydromorphology and nutrient availability induced by shifts in the precipitation and run-off regime than by direct modifications in the thermal regime.

Key words | carbon, climate change, nitrogen, phosphorus, streams, uptake

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INTRODUCTION

It is now widely accepted that climate change is affecting many ecosystems around the globe and that its impact is rapidly increasing (Walther *et al.* 2002; Oreskes 2004). In southern Europe, recent research has already reported alterations in the phenology of some plants and animals (Peñuelas *et al.* 2002, 2003). Future impacts of climate change are expected to be especially intense, causing marked increases in temperature, decreases in the amount of precipitation and a higher frequency of extreme precipitation events (de Castro *et al.* 2005; Schröter *et al.* 2005).

Stream ecosystems are expected to be influenced by climate change primarily through alterations of the hydrological and thermal regime and indirectly through the alteration of terrestrial ecosystems (Carpenter *et al.* 1992). In southern Europe, generally higher mean temperature and lower mean precipitation will provoke lower base flow, causing many perennial streams to turn into intermittent ones or even disappear (Alvarez-Cobelas *et al.* 2005). In addition, the risk of floods may increase due to a higher frequency and magnitude of extreme precipitation events (Mas-Pla 2005). While many studies have addressed the implications of climate change for stream structural properties (Malmqvist & Rundle 2002), fewer studies have focused on the effects of climate

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change on stream biogeochemical processes (but see Meyer *et al.* 1999).

Headwater streams are important sites for nutrient uptake, the set of processes by which nutrients are stored, transformed and removed from the water column (Alexander *et al.* 2000; Peterson *et al.* 2001). Understanding how climate change affects stream biogeochemical processes is important because these processes are crucial for the maintenance of many of the ecosystem services that streams provide to humans (Palmer *et al.* 2004). In particular, stream nutrient uptake is an ecosystem service per se because it can help mitigate problems associated with nutrient enrichment by reducing nutrient delivery to downstream ecosystems (Peterson *et al.* 2001).

Changes in temperature and hydrologic regime may have large implications for the ability of streams to take up nutrients. Increasing discharge has been shown to decrease nutrient uptake efficiency by reducing the interaction between solutes and biological compartments responsible for nutrient uptake (Valett *et al.* 1996; Butturini & Sabater 1998; Peterson *et al.* 2001). Stream hydromorphology may also influence nutrient uptake through changes in water transient storage (Valett *et al.* 1996; Mulholland *et al.* 1997; Butturini & Sabater 1999); however, this effect is less consistent than the effect of discharge. Changes in temperature and hydrologic regime may also have large implications for many organisms living in the stream, which have narrow thermal and hydrological tolerances (Malmqvist & Rundle 2002). As a consequence, the biological diversity of many of these ecosystems may decline and the rates of metabolic and biogeochemical processes may be altered (Carpenter *et al.* 1992; Alvarez-Cobelas *et al.* 2005).

The effects of climate change on stream nutrient uptake may vary depending on the nutrient considered. Inorganic nutrients such as nitrogen (N) and phosphorus (P) commonly limit primary production in streams (Elwood *et al.* 1981; Borchardt 1996). On the other hand, organic nutrients (dissolved organic carbon DOC and dissolved organic nitrogen DON) are crucial for heterotrophic activity, which dominates ecosystem metabolism in most headwater streams (Battin *et al.* 2008). Nevertheless, few studies have simultaneously addressed the uptake of organic and inorganic nutrients in streams (but see Newbold *et al.* 2006) and the potential implications of climate change on their dynamics.

In this study, a space-for-time substitution approach was used to explore the potential effects of climate change on the ability of streams to uptake multiple nutrients. It was posited that microclimatic changes along an altitudinal gradient within the same climatic region may be representative of shifts induced by future climate change conditions. Thus, the variability in uptake of inorganic (ammonium, nitrate and phosphate) and organic (acetate and glycine) nutrients was examined together with the variability of a set of environmental factors among a series of headwater streams located at different altitudes within the same mountain range. Multivariate analyses were used to explore the relationship between stream nutrient uptake and environmental factors (i.e. hydromorphology, water chemistry and biological characteristics) that varied along this altitudinal gradient.

METHODS

Study sites

This study was conducted in 14 headwater streams located in the Central Pyrenees (river Ebro catchment, NE Spain), covering an altitudinal gradient ranging from 700 to 2,100 m a.s.l. (Table 1). Although the dominant climate in the region is Mediterranean-continental, this altitudinal gradient encompassed a broad range of microclimatic conditions from alpine at the highest altitudes to semi-arid at the lowest. All selected streams drained catchments of similar size, with low human pressure and dominated by calcareous geology. These criteria were set to allow for comparisons among streams that varied mainly in the environmental conditions imposed by the altitudinal gradient.

Within each stream, a riffle-pool dominated reach with a relatively unmodified channel was selected. The length of the reaches ranged from 65 to 144 m, and did not include any tributaries. The streambed of most reaches was composed of a mixture of boulders, cobbles and gravel with patches of sand. Only in the streams at the lowest altitudes (i.e. $<$ 1,000 m a.s.l.) were bedrock slates dominant. Riparian vegetation along the reaches was well preserved and varied among sites following the altitudinal gradient. In the streams at the highest altitude, riparian vegetation was dominated by conifers (*Abies alba*, *Pinus sylvestris*, *Pinus uncinata*), beech

Table 1 | Location of the study streams

Stream	Code	Location	Altitude (m)	Reach length (m)	Riparian canopy
Muntanyó de llacs	LLA	42° 32′ 27″N, 0° 55′ 01″E	2,029	144	Open
Pont de llacs	PON	42° 40′ 42″N, 0° 39′ 13″E	1,923	84	Open
Matasomers	MAT	42° 34′ 13″N, 0° 30′ 42″E	1,743	88	Closed
Muntanyeta	MUN	42° 33′ 11″N, 0° 49′ 58″E	1,701	80	Closed
Puimestre	PUI	42° 34′ 31″N, 0° 32′ 12″E	1,643	103	Semi-closed
Les Paüles	PAU	42° 29′ 05″N, 0° 33′ 07″E	1,487	90	Semi-closed
Barbarruens	BAR	42° 30′ 47″N, 0° 22′ 16″E	1,412	100	Closed
Ramastué	RAM	42° 32′ 39″N, 0° 30′ 09″E	1,399	95	Open
Lliri	LLI	42° 32′ 04″N, 0° 30′ 40″E	1,274	65	Closed
Renanué	REN	42° 29′ 11″N, 0° 31′ 16″E	1,257	110	Closed
Bisaurri	BIS	42° 29′ 56″N, 0° 30′ 34″E	1,146	107	Semi-closed
Urmella	URM	42° 30′ 32″N, 0° 30′ 39″E	1,077	99	Semi-closed
Villas del Turbón	TUR	42° 25′ 23″N, 0° 27′ 52″E	909	77	Semi-closed
Campo	CAM	42° 12′ 16″N, 0° 29′ 37″E	798	104	Open

(*Fagus sylvatica*) and evergreen shrubs such as boxwood (*Buxus sempervirens*) and common juniper (*Juniperus communis*). In the streams located at the lowest altitude, riparian vegetation was dominated by deciduous trees such as alder (*Alnus glutinosa*), silver birch (*Betula pendula*) and common ash (*Fraxinus excelsior*) and by deciduous shrubs such as willows (*Salix spp*.) and common hazel (*Corylus avellana*). The riparian canopy cover was denser in streams located at intermediate altitudes and sparser at both the higher alpine sites and the lower semi-arid sites.

Field sampling

Field experiments were conducted in 2005 and 2006 during summer (July–August), a period characterized by base flows and high temperatures. They involved measurements of uptake parameters for dissolved organic and inorganic nutrients, together with descriptors of physical, chemical and biological stream conditions that were expected to vary along the gradient and were potentially important for determining uptake parameters.

Solute additions

Nutrient uptake parameters and hydraulic characteristics were measured in each stream using the short-term constant rate addition technique (Webster & Valett 2006). The nutrients added were ammonium (NH_4, as NH_4Cl), nitrate (NO_3, as KNO_3), soluble reactive phosphorus (SRP, as $Na(H_2PO_4){\cdot}2H_2O$), glycine (as H_2NCH_2COOH) and acetate (as $NaCH_3COO$) in conjunction with chloride (Cl^-, as NaCl) as a conservative tracer. Acetate and glycine were used as simple labile sources of DOC and DON, respectively. The additions of NH_4 and NO_3 were carried out in the summer of 2005, whereas the additions of glycine and acetate were carried out in the summer of 2006. Additions of NH_4 and NO_3, and those of glycine and acetate, were conducted separately on two consecutive days to avoid interferences. The solution of all the additions carried out over the two years also contained SRP. All additions were carried out around midday.

For each addition, a Masterflex (Vernon Hills, IL, USA) peristaltic pump was used to deliver the addition solution to the top of the reach until conductivity reached a plateau (i.e. 1–4 h) at the bottom of the reach. Conductivity was automatically recorded at the bottom of the reach every 5 s using a WTW (Weilheim, Germany) portable conductivity meter connected to a Campbell Scientific data logger. Before each addition, background conductivity was measured and water samples were collected for nutrient chemistry at 5–6 stations along the reach (two replicates per station). Once conductivity reached a plateau, samples were collected at the same stations for plateau concentrations (five replicates per station). Water samples for NH_4, NO_3 and SRP were filtered in the field using Whatman (Kent, UK) GF/F glass fibre filters (0.7 μm pore size), whereas samples for acetate and glycine were filtered using Whatman cellulose membrane filters (0.45 μm pore size). All filtered samples were stored on ice in the field and then refrigerated at 4°C or frozen in the laboratory until analysis.

Environmental parameters

Physical and biological parameters were measured during both sampling periods (2005 and 2006). Wetted width (w, m) and water depth (h, m) were determined on cross-sectional transects evenly spaced along the reach. Samples for standing stocks of coarse benthic organic matter (CBOM, wood and leaves > 1 mm), fine benthic organic matter (FBOM, particles < 1 mm) and epilithic biofilm (hereafter epilithon) were collected at five locations along the reach. Samples for CBOM biomass were collected only when this compartment was present in more than 10% of the channel surface. CBOM material was collected from a squared metal frame (0.02 m^2) that was placed on the sediment surface and stored in plastic bags. FBOM was sampled by sealing an 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 of a known volume was collected and filtered onto Whatman GF/F filters. Epilithon was sampled from five cobbles obtained at random locations along the reach. Five additional cobbles were collected to determine the chlorophyll *a* content of the epilithon. Percentage coverage of CBOM, FBOM and epilithon along the reach was visually estimated at evenly spaced intervals, and values were averaged to obtain a whole-reach estimate.

Whole-stream metabolism was determined in each stream using the open-channel, single-station approach (Bott 2006). Measurements were conducted on both summers on the same dates as the nutrient addition experiments. Dissolved oxygen (DO) concentration and temperature were automatically recorded at the bottom of the study reach at intervals of 5 min during a 24-hour period using a WTW portable oxygen meter. Percent DO saturation was estimated using DO and temperature data together with a standard altitude-air pressure algorithm to correct for site altitude. Mean daily temperature was estimated from the mean of values recorded over the 24-hour period. During the same period, photosynthetically active radiation (PAR) was recorded at a representative reach location every 5 min over a 24-hour period using a Skye (Powys, UK) SKP215 quantum sensor connected to a Campbell Scientific (Logan, UT, USA) data logger. Instantaneous PAR data for the 24-hour period were integrated to calculate daily PAR (mol m^{-2} d^{-1}) at each stream.

Laboratory analyses

Nutrient chemistry

Concentrations of NH_4, NO_3 and SRP in filtered water samples were analyzed following standard colorimetric methods (APHA 1995) using Bran + Luebbe (Norderstedt, Germany) autoanalyzers. Concentration of glycine was analyzed using a fluorescence method for the determination of total amino acids in natural waters (Josefsson *et al.* 1977) on a Shimadzu (Tokyo, Japan) RF-5,000 spectrofluorophotometer. Concentration of acetate was analyzed using ion exclusion chromatography with an HPX-87H organic acid column (APHA 1995).

Standing stocks

Samples of CBOM, FBOM and epilithon were oven-dried (at 60°C for 48 hours), weighed, ashed (at 450°C for 4 hours) and re-weighed to determine biomass, expressed as ash-free dry mass (AFDM, g m^{-2}). To estimate the chlorophyll *a* content (μg cm^{-2}) of epilithon each sample was placed in a vial with a known volume of 90% v/v acetone, left in the refrigerator overnight for pigment extraction and analyzed spectrophotometrically using the methodology described by Jeffrey & Humphrey (1975). The area of each cobble was measured to report epilithon AFDM and chlorophyll *a* content per unit area. Patch-specific values of AFDM and chlorophyll *a* were weighted by the percentage coverage of each patch to estimate reach-weighted values.

Parameter calculations

Hydraulic parameters

Discharge (Q, $\mathrm{L\,s^{-1}}$) was measured using the recorded time-curve conductivity data. Calculation of Q was based on a tracer mass balance approach (Gordon *et al.* 1992). The cross-sectional area of the stream channel (A, $\mathrm{m^2}$) was calculated as $w \times h$.

Data from the conductivity time-curves of 2005 additions were also analyzed using an advection–dispersion model with transient storage and lateral inflow (OTIS; Runkel 1998) to estimate the cross-sectional area of the transient water storage zone (A_s, $\mathrm{m^2}$). The ratio between the cross-section of the transient water storage zone and that of the surface stream channel (A_s/A) was calculated as an estimate of the size of the transient storage zone relative to the size of the free-flowing water.

Nutrient uptake parameters

Using data from the solute additions, two metrics of uptake for each nutrient (NH_4, SRP, NO_3, glycine and acetate) and stream were calculated: uptake length (S_w, m) and uptake velocity (V_f, mm $\mathrm{min^{-1}}$). S_w, the average distance travelled by a nutrient molecule before it is removed from the water column (Newbold *et al.* 1981) was calculated as the negative inverse of the slope of the regression between the ln-transformed and background-corrected nutrient:conductivity ratio and the distance downstream from the addition point. S_w is an indicator of the nutrient uptake efficiency at the reach scale (Webster & Valett 2006). Short S_w values indicate higher retention efficiency. This metric was converted to V_f, calculated as the stream-specific discharge (i.e. Q/w) divided by S_w. V_f describes the velocity by which a nutrient molecule is removed from the water column and is an indicator of nutrient demand (Hall *et al.* 2002).

Metabolism parameters

Gross primary production (GPP, g O_2 $\mathrm{m^{-2}}$ $\mathrm{day^{-1}}$) and ecosystem respiration (ER, g O_2 $\mathrm{m^{-2}}$ $\mathrm{day^{-1}}$) were estimated by integrating the DO measurements at a single station during the 24-hour period following the method of Bott (2006). Reaeration coefficients and respiration at night were determined based on DO change rates and DO deficits using the night-time regression method (Young & Huryn 1996). Respiration at night was extrapolated to 24 hours to estimate daily rates of ER. A PAR intensity of 2 μmol quanta $\mathrm{m^{-2}}$ $\mathrm{s^{-1}}$ was used to differentiate the photoperiod from darkness. Daily rates of GPP were computed by integrating the difference between the measured net DO change rate (corrected by the reaeration flux) and the extrapolated day-time respiration rate. GPP and ER were multiplied by the mean reach depth to obtain areal estimates, which allow for the comparison among streams of different size.

Data analysis

A multivariate approach was used to examine among-stream variation in terms of physical, chemical and biological characteristics and to determine to what extent this variation was related to the altitudinal gradient. A principal components analysis (PCA) was used to reduce the total number of variables to a few components, which are linear combinations of the original variables. PCA components are linearly independent and are ordered by the amount of among-stream variance that they capture. Due to the statistical constraints of PCA imposed by the availability of cases ($n = 14$), 13 variables were selected out of the total number of environmental parameters measured (Tables 2 and 3). Reduction of variables was based on the combination of some parameters; specifically, w and h was combined as the w:h ratio, NO_3 and NH_4 concentrations were added as dissolved inorganic N (DIN) and the FBOM and CBOM biomass were added as the total biomass in the detrital compartment.

For each variable included in the PCA, values for the two years were averaged prior to the analysis except for transient storage parameters and chlorophyll *a*, which were only available for 2005. Variables were ln-transformed, standardized and a correlation matrix was used for the PCA. The weight of a variable on a PCA component was considered significant when its loading was > 0.7 and marginally significant when loadings were > 0.6. Results from the PCA allowed us to examine which variables contributed most to the variation among streams in the database, as well as their correlation structure. The scores of the first three components of the PCA were used as

Table 2 | Physical and chemical characteristics of the study streams. Values for each stream are the mean of the two sampling years. The table also includes the mean, the standard error of the mean (SE) and the coefficient of variation (CV) for all streams together

Code	Discharge ($L\ s^{-1}$)	Width (m)	Depth (m)	A_s/A	PAR ($mol\ m^{-2}\ d^{-1}$)	Temperature (°C)	Conductivity ($\mu S\ cm^{-1}$)	NO_3 ($\mu g\ N\ L^{-1}$)	NH_4 ($\mu g\ N\ L^{-1}$)	SRP ($\mu g\ P\ L^{-1}$)
LLA	25.0	3.3	0.07	0.06	42.0	8.9	67	146	15	6
PON	9.8	1.7	0.06	0.09	9.7	9.9	45	30	12	6
MAT	2.9	1.8	0.04	0.30	7.5	10.4	307	11	25	4
MUN	14.8	2.2	0.03	0.13	1.8	10.7	210	111	18	5
PUI	10.1	1.9	0.03	0.11	17.9	11.7	202	89	4	5
PAU	7.9	1.8	0.05	0.24	16.2	14.3	494	129	12	4
BAR	8.2	3.3	0.06	0.19	28.4	10.1	270	210	18	3
RAM	2.3	1.0	0.03	0.22	33.1	16.4	429	64	18	5
LLI	6.7	1.5	0.04	0.44	19.0	13.7	364	23	33	4
REN	3.3	1.6	0.04	0.12	25.9	13.4	681	35	17	4
BIS	7.2	1.5	0.05	0.01	21.0	15.2	811	20	28	5
URM	13.5	2.6	0.06	0.02	32.4	13.6	307	153	13	6
TUR	3.8	1.9	0.02	0.02	27.9	17.9	378	509	13	5
CAM	7.1	1.4	0.02	0.30	48.4	22.6	340	890	14	3
Mean	8.8	2.0	0.04	0.16	23.7	13.5	350	173	17	5
SE	1.6	0.2	0.004	0.03	3.5	1.0	56	65	2	0.3
CV (%)	68.2	34.7	36.4	79.4	55.1	27.7	60	140	44	22

uncorrelated dependent variables in a simple linear regression analyses to examine the relationship between stream environmental characteristics and altitude.

The among-stream variation in terms of uptake of the different nutrients and how this variation was related to the stream characteristics as described by the PCA components were examined. All estimated S_w values reported in this study are from statistically significant regressions of nutrient-addition data. A paired *t*-test analysis was used to compare S_w and V_f values for SRP from 2005 and 2006. This analysis revealed no significant differences between years; thus, the average S_w and V_f values for SRP was used for further comparison with the uptake parameters for the other nutrients, which were measured only once.

Because S_w is strongly dependent on discharge, the study focused on V_f which provides a more appropriate variable for comparison across streams of different size (Webster & Valett 2006). Differences in S_w and V_f among nutrients were tested using a one-way analysis of variance (ANOVA) with nutrient as a factor. Tukey Honestly Significant Differences (HSD) tests were used for *post hoc* pairwise comparisons. Correlations among V_f for all nutrients were explored using Pearson correlation analysis. Finally, for each of the nutrients, the influence of stream environmental characteristics on the among-stream variation in uptake was examined. To do so, a simple linear regression analysis of V_f values against the scores of the first three components of the PCA were used.

Results from statistical analyses were considered significant if $p < 0.05$. Statistical analyses were carried out with Statistica 6.0 (Statsoft, OK, USA).

RESULTS

Among-stream variation in environmental characteristics

The characteristics of the study streams in terms of physical, chemical and biological parameters during the experiments are summarized in Tables 2 and 3. Discharge was low (i.e. $< 10\ L\ s^{-1}$) in most streams and representative of

Table 3 | Standing stocks and metabolism characteristics of the study streams. Values for each stream are the mean of the two sampling years. The table also includes the mean, the standard error of the mean (SE) and the coefficient of variation (CV) for all streams together

Code	CBOM biomass (g AFDM m^{-2})	FBOM biomass (g AFDM m^{-2})	Epilithon biomass (g AFDM m^{-2})	Epilithon chlophyll a (μg cm^{-2})	GPP (g O_2 m^{-2} d^{-1})	ER (g O_2 m^{-2} d^{-1})
LLA	0.0	47.6	130.9	4.3	0.61	11.86
PON	0.0	6.7	133.1	10.0	0.14	0.50
MAT	83.8	20.7	79.0	4.1	0.02	0.22
MUN	27.5	36.7	16.2	0.3	0.03	3.05
PUI	26.6	15.1	80.5	1.5	0.002	0.18
PAU	18.6	88.0	130.5	3.3	1.09	2.51
BAR	49.5	88.3	24.9	3.0	0.01	0.13
RAM	0.0	6.5	129.9	1.6	0.01	0.50
LLI	127.5	23.3	126.6	5.0	0.10	3.09
REN	28.6	116.3	53.1	1.4	0.24	0.79
BIS	65.9	50.9	103.5	3.0	0.72	2.24
URM	0.0	4.8	86.9	1.2	0.35	0.45
TUR	0.0	27.2	87.2	2.4	1.79	1.53
CAM	0.0	17.0	34.6	1.3	0.36	2.03
Mean	30.6	39.2	86.9	3.0	0.4	2.1
SE	10.4	9.4	11.1	0.6	0.1	0.8
CV (%)	127.1	89.6	47.8	80.1	132.5	145.2

summer conditions. Streams were very shallow (i.e. mean depth = 0.04 m) and relatively narrow ranging from 1 to 3 m in width. Water transient storage (A_s/A) was the hydraulic variable showing the largest variability among streams (i.e. coefficient of variation (CV) = 79%). Values of A_s/A varied two orders of magnitude among streams ranging from 0.007 to 0.442, and tended to be larger in the streams located at intermediate altitudes. Stream water temperatures gradually increased from the highest to the lowest location, spanning a range of 13.8°C between the two extremes of the altitudinal gradient. Daily PAR varied considerably among streams ranging from 1.8 to 48.4 mol m^{-2} d^{-1} (CV = 55.1%). PAR values tended to follow the altitudinal pattern observed for water temperature, with the exception of the high value recorded at the highest altitude stream.

Water conductivity was low ($< 100\ \mu S\ cm^{-1}$) in the two streams at the highest elevations and moderate to high (i.e. 200 to 800 $\mu S\ cm^{-1}$) in the rest of streams. Concentrations of SRP and NH_4 were consistently low among streams regardless of site location and averaged 4.7 $\mu g\ P\ L^{-1}$ and 17.1 $\mu g\ N\ L^{-1}$, respectively. In contrast, NO_3 concentration showed a dramatic variability among streams (CV = 140%), ranging from 11 to 890 $\mu g\ N\ L^{-1}$. In streams with high DIN concentration, NO_3 was the dominant N form, whereas in streams with low DIN concentration both NO_3 and NH_4 had a similar contribution. The DIN:SRP molar ratio varied from 15 to 630, indicating potential P limitation in most streams. Background concentrations of acetate and glycine were all below detection limits.

Among-stream variability in FBOM (CV = 90%) and CBOM (CV = 127%) standing stocks was larger than the variability in epilithon standing stocks (CV = 50%). Biomass in the CBOM compartment was negligible in streams lacking riparian canopy cover and ranged from 18.6 to 127.5 g AFDM m^{-2} in streams flanked by riparian trees. FBOM was present in all streams, but standing stocks varied by two orders of magnitude among streams, ranging from 4.8 to 116.3 g AFDM m^{-2}.

Biomass of the epilithic compartment was relatively high and ranged from 16.2 to 133.1 g AFDM m^{-2}. Epilithic chlorophyll *a* was relatively low but showed considerable variability (CV = 83.3%) among streams, ranging from 0.3 to 10 $\mu g\,cm^{-2}$. None of these parameters followed a clear pattern along the altitudinal gradient. However, the biomass of detrital compartments (FBOM and CBOM) accounted for a larger proportion of the total standing stocks in streams with dense canopy cover, which were mostly located at intermediate altitudes. Variability among streams in the daily rates of GPP and ER was high (CV = 132.5% and 145.2%, respectively). Rates of GPP were generally low, ranging from almost nothing to $1.8\,g\,O_2\,m^{-2}\,d^{-1}$. Rates of ER were relatively high and ranged from 0.13 to $11.9\,g\,O_2\,m^{-2}\,d^{-1}$. Rates of ER exceeded those of GPP in all streams.

The first three components of the PCA accounted for 63.3% of total variance among streams. The first component of the PCA explained 24.6% of the variance (Figure 1). The variables with significant loadings on this component were epilithon biomass (0.86), $w{:}h$ ratio (−0.80) and chlorophyll *a* (0.73). The concentration of SRP had a marginally significant loading on this first PCA component (0.66). The second component of the PCA explained 20.5% of the total variance (Figure 1). The variables with significant loadings on this component were water temperature (0.83), PAR (0.70) and GPP (0.89). Finally, the third component of the PCA explained 18.2% of the total variance (Figure 1). Only Q had a significant loading on this component (−0.79). Results from linear regression analysis showed that only the second component of the PCA was significantly related to stream altitude (Figure 2).

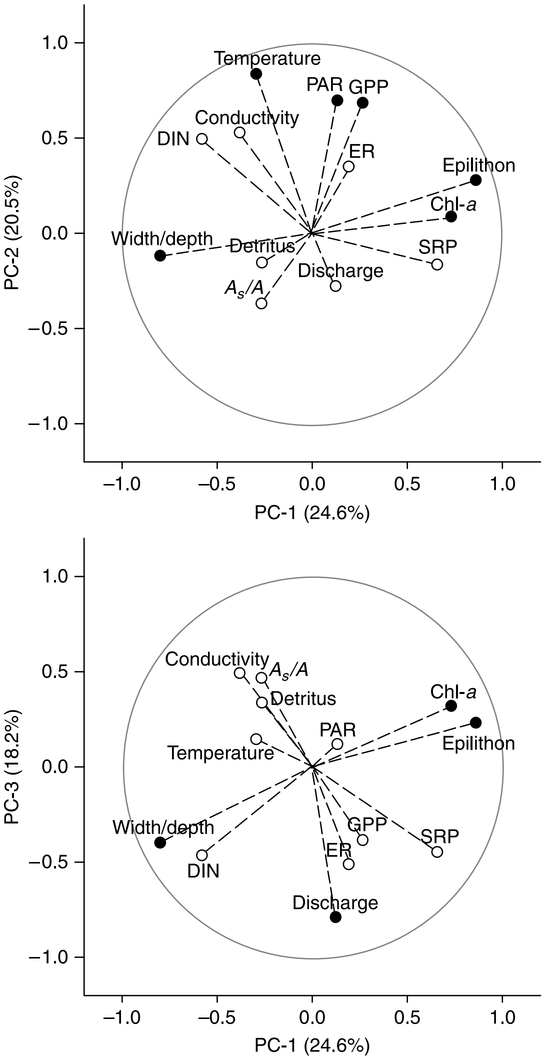

Figure 1 | Principal components analysis (PCA) of selected stream variables. The percent values on each axis represent the amount of variance explained by each PCA component (PC). Closed symbols denote significant variables (loading > 0.7). See Tables 2 and 3 for a more detailed description of the variables included in the PCA. DIN = $NO_3 + NH_4$, Detritus = FBOM + CBOM biomass.

Among-stream variation in nutrient uptake

Results for nutrient uptake metrics (S_w and V_f) from all the streams are summarized in Table 4. S_w for all nutrients was in the range of tens to hundreds of metres. Streams showed lowest S_w variability, expressed in terms of CV, for SRP and glycine and highest variability for acetate. Results from a one-way ANOVA indicated significant differences in S_w among nutrients. On average, S_w for SRP was 3.6, 4.2 and 6.6 times significantly shorter than S_w for NH_4, NO_3 and acetate, respectively (Table 4). S_w for glycine was not significantly different from S_w for the other nutrients (Table 4).

Considering all nutrients and streams together, values of V_f spanned 3 orders of magnitude, ranging from 0.08 to $20.8\,mm\,min^{-1}$. Variability among streams in V_f, expressed in terms of CV, was larger than that in S_w. SRP was the nutrient showing the lowest variability in V_f among streams. As with S_w, results from a one-way ANOVA indicated significant differences in V_f among nutrients. On average, V_f for SRP was 2.5 and 3.0 times significantly higher than V_f for acetate and NH_4, respectively (Table 4). V_f for NO_3 and glycine were not significantly different from V_f for the other nutrients. Variation in V_f for SRP, acetate and glycine was

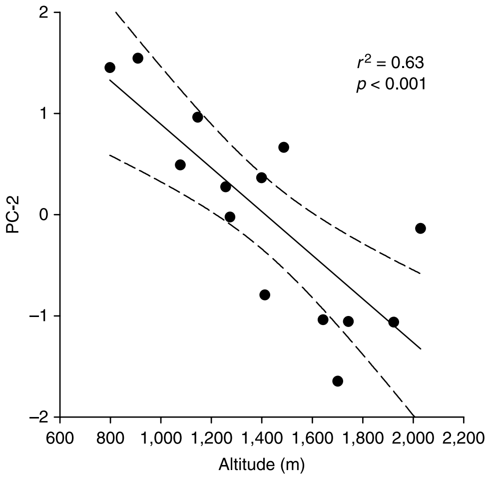

Figure 2 | Linear regression between site altitude and the scores of the second component of the PCA (PC-2), which indicates a gradient of temperature, PAR and GPP. Points correspond to the study streams. Dashed lines represent 95% confidence regression bands.

positively correlated (Figure 3). V_f for glycine was also positively correlated with V_f for NO_3 (Figure 3).

Results from linear regression analysis between V_f for each nutrient and the components of the PCA only showed significant relationships with the first and third PCA components. Variation in V_f for SRP, acetate and glycine was positively related to the first PCA component (Figure 4). Variation in V_f for glycine and NO_3 was negatively related to the third PCA component (Figure 4).

DISCUSSION

The space-for-time substitution approach has been proposed as an alternative to long-term studies to forecast future changes in ecosystem structure and function (Pickett 1989). The present study used this approach to evaluate potential effects of climate change on stream nutrient uptake by examining the relationship between stream environmental parameters and C, N and P uptake along an altitudinal gradient. Each stream was considered a reflection of local climatic conditions and used in a space for time substitution approach as a potential scenario that could shift the altitudinal gradient upwards or downwards under future climate change conditions.

Table 4 | Nutrient uptake parameters for the study streams. The table also includes the mean, the standard error of the mean (SE) and the coefficient of variation (CV) for all streams together. Different letters in brackets next to the mean values indicate significant differences ($p < 0.05$) among nutrients resulting from Tukey post-hoc tests after one-way ANOVAs for the two uptake parameters separately. Empty cells denote lack of data due to analytical problems

	Uptake length, S_w (m)					Uptake velocity, V_f (mm min^{-1})				
Code	NO_3	NH_4	SRP	Acetate	Glycine	NO_3	NH_4	SRP	Acetate	Glycine
LLA	–	105	75	95	39	–	4.2	10.0	8.5	20.8
PON	–	222	79	–	44	–	2.1	14.9	–	11.6
MAT	–	78	54	909	104	–	0.9	1.4	0.1	0.7
MUN	–	238	172	333	217	–	0.4	1.4	0.3	0.5
PUI	400	56	63	333	208	1.5	10.6	8.9	1.6	2.6
PAU	385	625	53	1,429	130	0.3	0.2	2.5	0.1	1.0
BAR	–	143	58	385	100	–	1.3	3.0	0.4	1.5
RAM	–	588	52	–	116	–	0.1	2.0	–	1.3
LLI	–	154	38	72	89	–	1.8	11.3	5.0	4.0
REN	385	85	44	65	213	0.2	1.1	1.9	1.0	0.3
BIS	357	135	36	42	143	0.7	1.7	7.3	6.6	1.9
URM	62	370	86	476	108	5.7	1.0	6.9	1.4	6.3
TUR	–	294	40	–	–	–	0.6	4.2	–	–
CAM	59	238	65	588	52	6.2	1.5	6.6	0.4	4.9
Mean	274.5(b)	238.0(b)	65.4(a)	429.8(b)	120.3(ab)	2.4(ab)	2.0(b)	5.9(a)	2.3(b)	4.4(ab)
SE	67.9	48.0	9.2	127.8	17.0	1.1	0.7	1.1	0.9	1.6
CV (%)	60.6	75.5	52.4	98.7	51.0	113.3	136.0	72.2	128.2	132.1

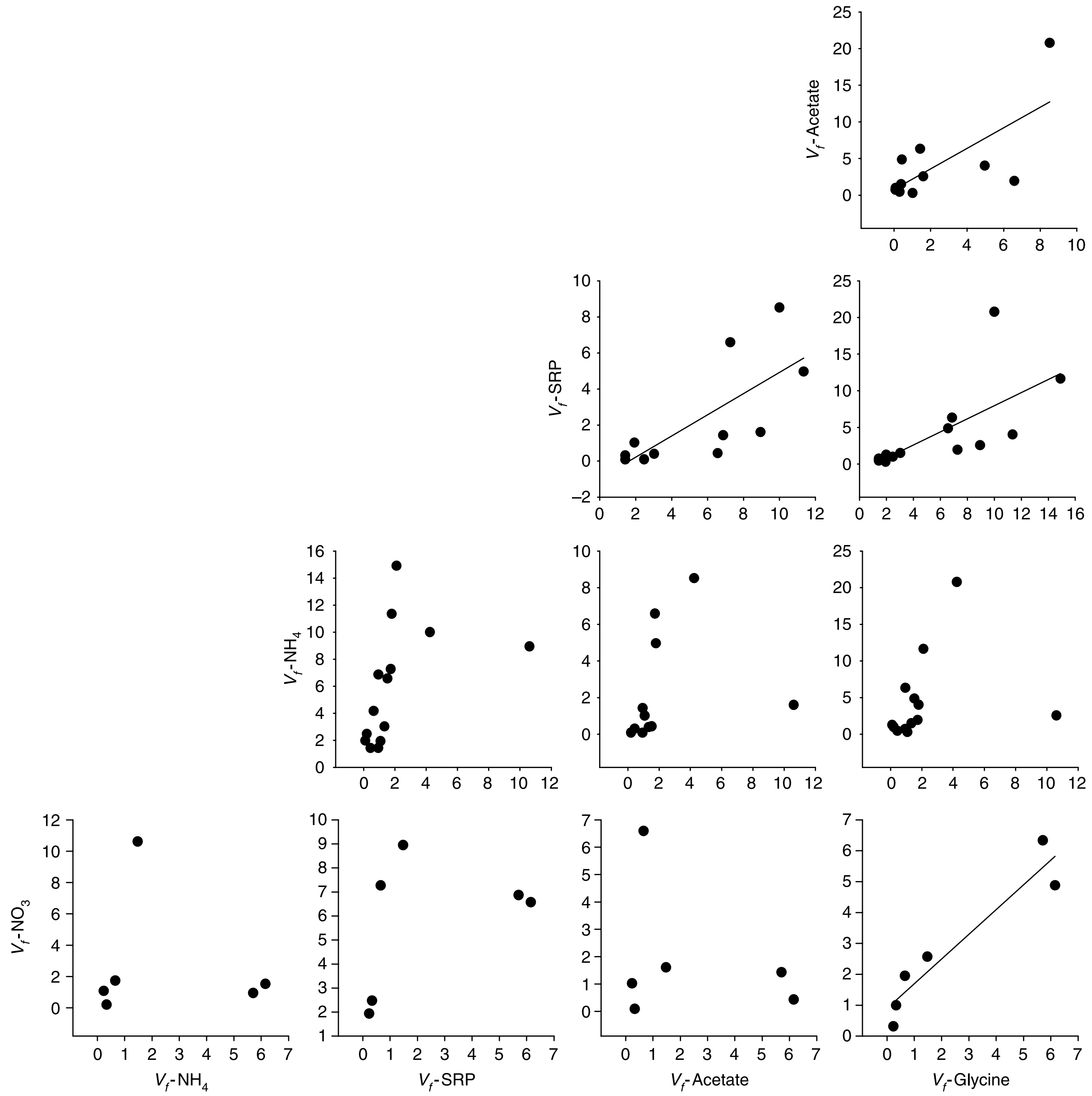

Figure 3 | Pearson correlations among the uptake velocities (V_f) of nitrate (NO$_3$), ammonium (NH$_4$), soluble reactive phosphorus (SRP), acetate and glycine. Points correspond to the study streams. Significant ($p < 0.05$) correlations are marked with a straight line.

It was anticipated that the variability in environmental factors imposed by the altitudinal gradient would influence stream biogeochemical processes. Thus, consistent patterns in nutrient uptake response along the altitudinal gradient were expected. These patterns would point to potential effects of climate change on nutrient uptake. Overall, results did not support the predictions, indicating that under similar broad-scale catchment conditions (i.e. geology and regional climate) both stream environmental characteristics and nutrient uptake responses tend to be controlled by fine-scale factors that are site specific and independent of altitudinal location.

Despite the broad altitudinal range encompassed by the study streams, the highest, percentage of spatial environmental variability was associated with factors that did not vary according to the altitudinal gradient. As indicated by

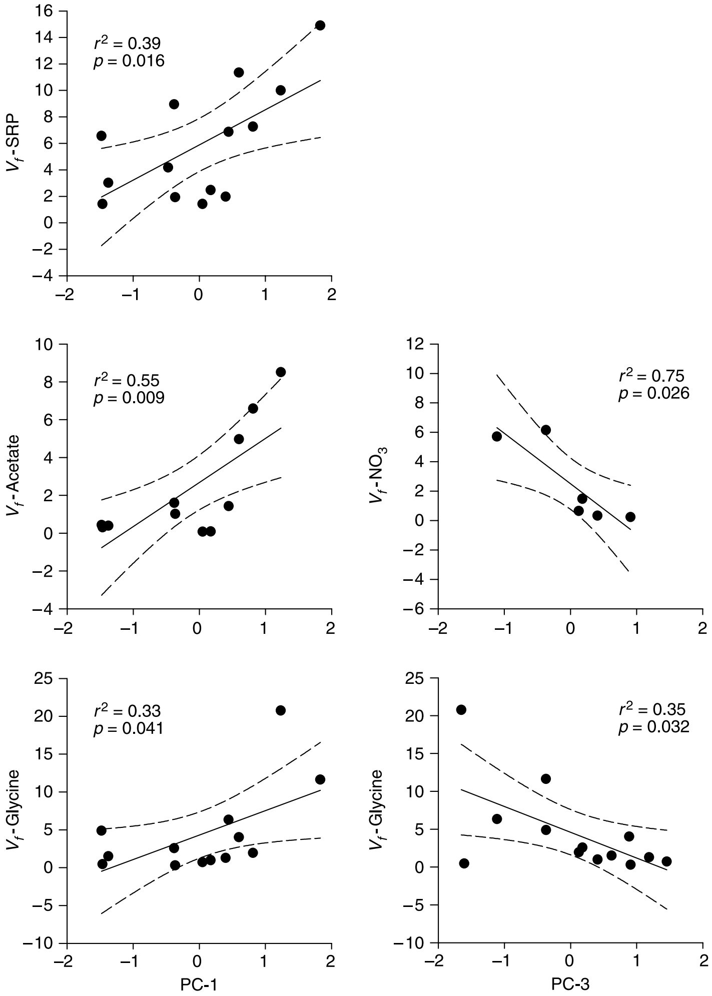

Figure 4 | (Left) Linear regression between the scores of the first component of the PCA (PC-1), which indicates a gradient of hydromorphological and biological changes, and demand for soluble reactive phosphorus (V_f-SRP), acetate (V_f-Acetate) and glycine (V_f-Glycine). (Right) Linear regression between the scores of the third component of the PCA (PC-3), which indicates a gradient of discharge changes, and demand for nitrate (V_f-NO_3) and glycine (V_f-Glycine). Points correspond to the study streams. Dashed lines represent 95% confidence regression bands.

the first PCA component, major environmental differences among streams were driven by stream hydromorphologic conditions (i.e. *w:h* ratio), SRP availability and epilithic biofilms (i.e. chlorophyll *a* and biomass). Regardless of their altitudinal location, the more constrained streams tended to have higher epilithic biomass and SRP concentration than the less constrained streams. A plausible explanation for this result is that under oligotrophic conditions, such as those found in these streams, more constrained channels result in faster currents that may stimulate growth and maintenance of epilithon (Stevenson 1996). Similarly to the first PCA component, the third component did not vary with altitude. Discharge, which was generally low but varied considerably among reaches, was the only variable associated with this component.

Only variables associated with the second PCA component (temperature, light and gross primary production) varied with the altitudinal gradient. Streams at lower altitudes tended to be warmer, less shaded and more productive. The strong coupling between light and primary production has been demonstrated both within and across streams in previous studies (Young & Huryn 1996; Mulholland *et al.* 2001; Roberts *et al.* 2007). Fewer studies have reported a relationship between water temperature and primary production (de Nicola 1996; Morin *et al.* 1999). Instead, water temperature has been commonly reported as a key factor driving ecosystem respiration (Bott *et al.* 1985; Uehlinger 2000; Acuña *et al.* 2008). Surprisingly, neither ecosystem respiration nor standing stocks of detrital compartments were variables significantly contributing to among-stream variability. Nevertheless, all streams were dominated by heterotrophic metabolism (i.e. GPP $<$ ER), which is characteristic of headwater streams (Battin *et al.* 2008).

Streams showed considerable variability (i.e. high coefficient of variation) in nutrient uptake response. However, streams were highly efficient in retaining all added nutrients, as indicated by S_w values in the range of the few hundreds of metres. This is in agreement with findings from previous studies in headwater streams (Martí & Sabater 1996; Peterson *et al.* 2001; von Schiller *et al.* 2008), which indicate the biogeochemical relevance of these ecosystems (Alexander *et al.* 2000; Lowe & Likens 2005). Despite this general trend, differences in uptake response among nutrients were found.

Variability among streams was consistently lower for SRP uptake than for the rest of nutrients. In addition, streams showed higher uptake efficiency and demand for glycine and SRP than for the rest of the nutrients. High uptake of glycine, which is the smallest of the amino acids commonly found in proteins (Nelson & Cox 2005), was expected because it is easily incorporated in microbial metabolic processes (Kirchman 2003). Low variability and high uptake of SRP could be explained by the low SRP concentrations and DIN:P ratios >16, which suggest potential P limitation in these streams and therefore high biological demand for this element. In addition, the stream chemistry derived from the calcareous geology of the catchments enhances chemical uptake of SRP through the co-precipitation with calcium carbonate (Reddy *et al.* 1999), increasing overall uptake of this element. This was also suggested by Martí & Sabater (1996) who found higher SRP uptake efficiency in a stream draining a calcareous catchment than in one draining a siliceous catchment. Demand for SRP was correlated with demand for the organic nutrients (i.e. glycine and acetate), but not with demand for inorganic N forms. Organic nutrients are primarily taken up by heterotrophic organisms (Kaplan & Bott 1983; Allan 1995). This result therefore indicates heterotrophic control of SRP uptake and corroborates P-limitation of heterotrophic activity, which dominates metabolism in the study streams. Streams with highest glycine demand also showed highest demand for NO_3; however, a reasonable explanation for this relationship was not found. The lack of correlation between demand for NH_4 and that for the rest of nutrients suggests that uptake of this N source was additionally driven by alternative dissimilatory processes such as nitrification.

In contrast to what it was expected, uptake for all nutrients except NH_4 varied along stream environmental gradients that were not related to altitude. Variation in demand for SRP, glycine and acetate was related to changes in hydromorphologic features, SRP availability and the biomass of epilithic biofilms. This result indicates that in more constrained streams higher flow velocities together with oligotrophic conditions may not only stimulate growth and maintenance of epilithon, but also increase its biological nutrient demand. Previous studies have demonstrated the influence of hydromorphology (Valett *et al.* 1996; Gücker & Boechat 2004), nutrient availability (Dodds *et al.* 2002; Newbold *et al.* 2006) and epilithic biomass (Mulholland *et al.* 1994) on stream nutrient uptake.

Demand for glycine and NO_3 increased with stream discharge. This was unexpected because converting S_w to V_f is expected to remove the influence of stream size (Webster & Valett 2006). Given that discharge was relatively low in all streams, this finding suggests stimulation of nutrient uptake at higher flow. Nevertheless, the data do not allow further testing of this hypothesis. Together, these results indicate no significant effects of factors associated with altitudinal gradient which would be expected to respond mostly to increases in temperature driven by climate change. However, factors found to influence nutrient uptake, although subjected to local conditions, can ultimately be modified by interactions between both changes in temperature and precipitation regime, which will have consequences for stream nutrient uptake.

CONCLUSIONS

Climate change is expected to alter both the thermal and hydrologic regime of stream ecosystems. This can have direct or indirect consequences on stream communities and processes. Results from this study indicate that stream C, N and P uptake is susceptible to these effects through complex interactions among different environmental factors that operate at a local scale. The lack of relationships between nutrient uptake and the environmental gradient associated with altitude, and the presence of relationships with environmental gradients other than altitude, suggest that climate change may not affect stream nutrient uptake directly through changes in temperature, but rather indirectly through changes in stream hydromorphology and nutrient availability. It is expected that predicted changes in the precipitation and evapotranspiration regime in this region (Schröter *et al.* 2005) will modify stream hydrology, channel structure and nutrient run-off. Results from this study indicate that these changes will affect stream nutrient uptake, and that this effect will vary depending on the nutrient considered.

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REFERENCES

Acuña, V., Wolf, A., Uehlinger, U. & Tockner, K. 2008 Temperature dependence of stream benthic respiration in an Alpine river network under global warming. *Freshw. Biol.* **53**, 2076–2088.

Alexander, R. B., Smith, R. A. & Schwarz, G. E. 2000 Effect of stream channel size on the delivery of nitrogen to the Gulf of Mexico. *Nature* **403**, 758–761.

Allan, J. D. 1995 *Stream Ecology: Structure and Function of Running Waters*. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Alvarez-Cobelas, M., Catalan, J. & García de Jalón, D. 2005 Impactos sobre los ecosistemas acuáticos continentales. In: Moreno, J. M. (ed.) *A Preliminary General Assessment of the Impacts in Spain due to the Effects of Climate Change*. Ministerio de Medio Ambiente, Madrid, Spain, pp. 113–146.

APHA 1995 *Standard Methods for the Examination of Water and Wastewater*, 19th edition. American Public Health Association, Washington, USA.

Battin, T. J., Kaplan, L. A., Findlay, S., Hopkinson, C. S., Martí, E., Packman, A. I., Newbold, J. D. & Sabater, F. 2008 Biophysical controls on organic carbon fluxes in fluvial networks. *Nat. Geosci.* **1**, 95–100.

Borchardt, M. A. 1996 Nutrients. In: Stevenson, R. J., Bothwell, M. L. & Lowe, R. L. (eds) *Algal Ecology*. Academic Press, San Diego, USA, pp. 150–181.

Bott, T. L. 2006 Primary productivity and community respiration. In: Hauer, F. R. & Lamberti, G. A. (eds) *Methods in Stream Ecology*. Academic Press, San Diego, USA, pp. 663–690.

Bott, T. L., Brock, J. T., Dunn, C. S., Naiman, R. J., Ovink, R. W. & Petersen, R. C. 1985 Benthic community metabolism in four temperate stream systems: an inter-biome comparison and evaluation of the river continuum concept. *Hydrobiologia* **123**, 3–45.

Butturini, A. & Sabater, F. 1998 Ammonium and phosphate retention in a Mediterranean stream: hydrological versus temperature control. *Can. j. Fish. Aquat. Sci.* **55**, 1938–1945.

Butturini, A. & Sabater, F. 1999 Importance of transient storage zones for ammonium and phosphate retention in a sandy-bottom Mediterranean stream. *Freshw. Biol.* **41**, 593–603.

Carpenter, S. R., Fisher, S. G., Grimm, N. B. & Kitchell, J. F. 1992 Global Change and freshwater ecosystems. *Annu. Rev. Ecol. Syst.* **23**, 119–139.

de Castro, M., Martín-Vide, J. & Alonso, S. 2005 The climate of Spain: past, present and scenarios for the 21st century. In: Moreno, J. M. (ed.) *A Preliminary General Assessment of the Impacts in Spain due to the effects of climate change*. Ministerio de Medio Ambiente, Madrid, Spain, pp. 1–62.

de Nicola, D. M. 1996 Periphyton responses to temperature at different ecological levels. In: Stevenson, R. J., Bothwell, M. L. & Lowe, R. L. (eds) *Algal Ecology*. Academic Press, San Diego, USA, pp. 150–181.

Dodds, K. D., López, A. L., Bowden, W. D., Gregory, S. V., Grimm, N. B., Hamilton, S. K., Hershey, A. E., Martí, E., McDowell, W. H., Meyer, J. L., Morral, D., Mulholland, P. J., Peterson, B. J., Tank, J. L., Valett, H. M., Webster, J. R. & Wollheim, W. M. 2002 N uptake as a function of concentration in streams. *J. North Am. Benthol. Soc.* **21**, 206–220.

Gordon, N. D., McMahon, T. A. & Finlayson, B. L. 1992 *Stream Hydrology: an Introduction for Ecologists*, 1st edition. John Wiley and Sons, West Sussex, UK.

Gücker, B. & Boechat, I. G. 2004 Stream morphology controls ammonium retention in tropical headwaters. *Ecology* **85**, 2818–2827.

Elwood, J. W., Newbold, J. D., Trimble, A. F. & Stark, R. W. 1981 Limiting role of phosphorus in a woodland stream ecosystem: effects of P enrichment on leaf decomposition and primary producers. *Ecology* **62**, 146–158.

Hall, R. O., Bernhardt, E. S. & Likens, G. E. 2002 Relating nutrient uptake with transient storage in forested mountain streams. *Limnol. Oceanogr.* **47**, 255–265.

Jeffrey, S. W. & Humphrey, G. F. 1975 New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae, and natural phytoplankton. *Biochem. Physiol. Pflanz.* **167**, 191–194.

Josefsson, B., Lindroth, P. & Östling, G. 1977 An automated fluorescence method for the determination of total aminoacids in natural waters. *Anal. Chim. Acta* **89**, 21–28.

Kaplan, L. A. & Bott, T. L. 1983 Microbial heterotrophic utilization of dissolved organic matter in a piedmont stream. *Freshw. Biol.* **13**, 363–377.

Kirchman, D. L. 2003 The contribution of monomers and other low-molecular weight compounds to the flux of dissolved organic material in aquatic ecosystems. In: Findlay, E. G. & Sinsabaugh, R. L. (eds) *Aquatic Ecosystems. Interactivity of Dissolved Organic Matter*. Academic Press, San Diego, USA, pp. 218–241.

Lowe, W. H. & Likens, G. E. 2005 Moving headwater streams to the head of the class. *Bioscience* **55**, 196–197.

Malmqvist, B. & Rundle, S. 2002 Threats to the running water ecosystems of the world. *Environ. Conserv.* **29**, 134–153.

Martí, E. & Sabater, F. 1996 High variability in temporal and spatial nutrient retention in Mediterranean streams. *Ecology* **77**, 854–869.

Mas-Pla, J. 2005 Recursos hídrics, dinàmica hidrològica i canvi climàtic. In: Queralt, A. & Rodó, J. (eds) *Informe sobre el canvi climàtic a Catalunya*. Generalitat de Catalunya, Barcelona, Spain, pp. 489–516.

Meyer, J. L., Sale, M. J., Mulholland, P. J. & Poff, N. L. 1999 Impacts of climate change on aquatic ecosystem functioning and health. *J. Am. Water Resour. Assoc.* **35**, 1373–1386.

Morin, A., Lamoureux, W. & Busnarda, J. 1999 Empirical models predicting primary productivity from chlorophyll a and water temperature for stream periphyton and lake and ocean phytoplankton. *J. North Am. Benthol. Soc.* **18**, 299–307.

Mulholland, P. J., Steinman, A. D., Marzolf, E. R., Hart, D. R. & DeAngelis, D. L. 1994 Effect of periphyton biomass on hydraulic characteristics and nutrient cycling in streams. *Oecologia* **98**, 40–47.

Mulholland, P. J., Marzolf, E. R., Webster, J. R., Hart, D. R. & Hendricks, S. P. 1997 Evidence that hyporheic zones increase heterotrophic metabolism and phosphorus uptake in forest streams. *Limnol. Oceanogr.* **42**, 443–451.

Mulholland, P. J., Fellows, C. S., Tank, J. L., Grimm, N. B., Webster, J. R., Hamilton, S. K., Martí, E., Ashkenas, E., Bowden, W. B., Dodds, W. K., Mcdowell, W. H., Paul, M. J. & Peterson, B. J. 2001 Inter-biome comparison of factors controlling stream metabolism. *Freshw. Biol.* **46**, 1503–1517.

Newbold, J. D., Elwood, J. W., O'Neill, R. V. & van Winkle, W. 1981 Measuring nutrient spiralling in streams. *Can. J. Fish. Aquat. Sci.* **38**, 860–863.

Newbold, J. D., Bott, T. L., Kaplan, L. A., Dow, C. L., Jackson, J. K., Aufdenkampe, A. K., Martin, L. A., Van Horn, D. J. & d. Long, A. A. 2006 Uptake of nutrients and organic C in streams in New York City drinking-water-supply watersheds. *J. North Am. Benthol. Soc.* **25**, 998–1017.

Nelson, D. L. & Cox, M. M. 2005 *Lehninger Principles of Biochemistry*, 4th edition. W.H. Freeman and Company, New York, USA.

Oreskes, N. 2004 The scientific consensus on climate change. *Science* **306**, 1686.

Palmer, M., Bernhardt, E., Chornesky, E., Collins, S., Dobson, A., Duke, C., Gold, B., Jacobson, R., Kingsland, S., Kranz, R., Mappin, M., Martinez, M., Micheli, F., Morse, J., Pace, M., Pascual, M., Palumbi, S., Reichman, O. J., Simons, A., Townsend, A. & Turner, M. 2004 Ecology for a crowded planet. *Science* **304**, 1251–1252.

Peñuelas, J. & Boada, M. 2003 A global change-induced biome shift in the Montseny mountains (NE Spain). *Glob. Change Biol.* **9**, 131–140.

Peñuelas, J., Fililla, I. & Comas, P. 2002 Changed plant and animal life cycles from 1952 to 2000 in the Mediterranean region. *Glob. Change Biol.* **8**, 531–544.

Peterson, B. J., Wollheim, W. M., Mulholland, P. J., Webster, J. R., Meyer, J. L., Tank, J. L., Martí, E., Bowden, W. D., Valett, H. M., Hershey, A. E., McDowell, W. H., Dodds, K. D., Hamilton, S. K., Gregory, S. V. & Morrall, D. 2001 Control of nitrogen export from watersheds by headwater streams. *Science* **292**, 86–90.

Pickett, S. T. A. 1989 Space-for-time substitution as an alternative to long-term studies. In: Likens, G. E. (ed.) *Long-Term Studies in Ecology: Approaches and Alternatives*. Springer-Verlag, New York, USA.

Reddy, K. R., Kadlec, R. H., Flaig, E. & Gale, P. M. 1999 Phosphorus retention in streams and wetlands: a review. *Crit. Rev. Environ. Sci. Technol.* **29**, 83–146.

Roberts, B. J., Mulholland, P. J. & Hill, W. R. 2007 Multiple scales of temporal variability in ecosystem metabolism rates: results from 2 years of continuous monitoring in a forested headwater stream. *Ecosystems* **10**, 588–606.

Runkel, R. L. 1998 *One Dimensional Transport with Inflow and Storage (OTIS): A Solute Transport Model for Streams and Rivers*. Water Resources Investigations Report 98–4018.US Geological Survey, Denver, CO, USA.

Schröter, D., Cramer, W., Leemans, R., Prentice, I. C., Araújo, M. B., Arnell, N. W., Bondeau, A., Bugmann, H., Carter, T. R., Gracia, C. A., de la Vega-Leinert, A. C., Erhard, M., Ewert, F., Glendining, M., House, J. I., Kankaanpää, S., Klein, R. J. T., Lavorel, S., Lindner, Ma., Metzger, M. J., Meyer, J., Mitchell, T. D., Reginster, I., Rounsevell, M., Sabaté, S., Sitch, S., Smith, B., Smith, J., Smith, P., Sykes, M. T., Thonicke, K., Thuiller, W., Tuck, G., Zaehle, S. & Zierl, B. 2005 Ecosystem service supply and vulnerability to global change in Europe. *Science* **310**, 1333–1337.

Stevenson, R. J. 1996 The stimulation and drag of current. In: Stevenson, R. J., Bothwell, M. L. & Lowe, R. L. (eds) *Algal Ecology*. Academic Press, San Diego, USA, pp. 321–340.

Uehlinger, U. 2000 Resistance and resilience of ecosystem metabolism in a flood-prone river system. *Freshw. Biol.* **45**, 319–332.

Valett, H. M., Morrice, J. A., Dahm, C. N. & Campana, M. E. 1996 Parent lithology, surface-groundwater exchange, and nitrate retention in headwater streams. *Limnol. Oceanogr.* **41**, 333–345.

von Schiller, D., Martí, E., Riera, J. L., Ribot, M., Argerich, A., Fonollà, P. & Sabater, F. 2008 Inter-annual, annual and seasonal variation of P and N retention in a perennial and an intermittent stream. *Ecosystems* **11**, 670–687.

Walther, G. R., Post, E., Convey, P., Menzel, A., Parmesank, C., Beebee, T., J, C., Fromentin, J. M., Guldberg, O. H. & Bairlein, F. 2002 Ecological responses to recent climate change. *Nature* **416**, 389–395.

Webster, J. R. & Valett, H. M. 2006 Solute dynamics. In: Hauer, F. R. & Lamberti, G. A. (eds) *Methods in Stream Ecology*. Academic Press, San Diego, USA, pp. 169–185.

Young, R. G. & Huryn, A. D. 1996 Interannual variation in discharge controls ecosystem metabolism along a grassland river continuum. *Can. J. Fish. Aquat. Sci.* **53**, 2199–2211.

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