Green light: gross primary production influences seasonal stream N export by controlling fine-scale N dynamics

Anna Lupon,[1,3] Eugènia Martí,[2] Francesc Sabater,[1] and Susana Bernal[1,2]

[1]*Department d'Ecologia, Facultat de Biologia, Universitat de Barcelona, Av. Diagonal 643, 08028, Barcelona, Spain*
[2]*Integrative Freshwater Ecology Group, Center for Advanced Studies of Blanes (CEAB-CSIC), Accés a la Cala Sant Francesc 14, 17300, Blanes, Girona, Spain*

Abstract. Monitoring nutrient concentrations at fine-scale temporal resolution contributes to a better understanding of nutrient cycling in stream ecosystems. However, the mechanisms underlying fine-scale nutrient dynamics and its implications for budget catchment fluxes are still poorly understood. To gain understanding of patterns and controls of fine-scale stream nitrogen (N) dynamics and to assess how they affect hydrological N fluxes, we explored diel variation in stream nitrate (NO_3^-) concentration along a headwater stream with increasing riparian area and channel width. At the downstream site, the highest day–night variations occurred in early spring, when stream NO_3^- concentrations were 13% higher at night than at daytime. Such day–night variations were strongly related to daily light inputs ($R^2 = 0.74$) and gross primary production (GPP; $R^2 = 0.74$), and they showed an excellent fit with day–night NO_3^- variations predicted from GPP ($R^2 = 0.85$). These results suggest that diel fluctuations in stream NO_3^- concentration were mainly driven by photoautotrophic N uptake. Terrestrial influences were discarded because no simultaneous diel variations in stream discharge, riparian groundwater level, or riparian solute concentration were observed. In contrast to the downstream site, no diel variations in NO_3^- concentration occurred at the upstream site, likely because water temperature was colder (10°C vs. 12°C) and light availability was lower (4 vs. 9 $mol \cdot m^{-2} \cdot d^{-1}$). Although daily GPP was between 10- and 100-fold lower than daily respiration, photoautotrophic N uptake contributed to a 10% reduction in spring NO_3^- loads at the downstream site. Our study clearly shows that the activity of photoautotrophs can substantially change over time and along the stream continuum in response to key environmental drivers such as light and temperature, and further, that its capacity to regulate diel and seasonal N fluxes can be important even in low-productivity streams.

Key words: diel stream nitrate variation; headwater forested streams; nitrate uptake; metabolism; Montseny Natural Park; nitrate concentration; photoautotrophic activity; Spain.

INTRODUCTION

Human activity has doubled the availability of bioreactive nitrogen (N) worldwide, which compromises the function and biodiversity of terrestrial and freshwater ecosystems, as well as soil and water quality (Schlesinger 2009, Sutton et al. 2011). Nonetheless, biological activity can transform and retain a substantial amount of N inputs, and thus, reduce the pervasive effects of excessive N in ecosystems (Bernhardt et al. 2002, Goodale et al. 2004). Within catchments, biogeochemical processes occurring at upland, riparian, and aquatic ecosystems simultaneously contribute to N cycling and retention, and ultimately determine N export downstream (Bernhardt et al. 2005). In

particular, there is a growing body of research demonstrating that streams and rivers have a high capacity to transform and retain N (Peterson et al. 2001, Tank et al. 2008), even though their ability to influence N export from catchments to downstream ecosystems is still under debate (Brookshire et al. 2009). This is mostly because water chemistry of stream and rivers integrates biogeochemical processes occurring at different spatial and temporal scales throughout the catchment, which complicates assessing the relative influence of in-stream and terrestrial processes on N exports (Sudduth et al. 2013). A better understanding of the mechanisms and drivers of N dynamics within fluvial ecosystems is critical to evaluate their capacity to modify N inputs from terrestrial sources.

Nitrate (NO_3^-) is the predominant form of dissolved inorganic N (DIN) in fluvial ecosystems, and its uptake is mainly controlled by the metabolic activity of stream biota (Hall and Tank 2003, Mulholland

et al. 2008). Recently, monitoring at fine-scale temporal resolution in streams has provided examples of the close link between gross primary production and NO_3^- uptake (e.g., Johnson et al. 2006, Roberts and Mulholland 2007, Heffernan and Cohen 2010). These studies have found an inverse relationship between fine-scale stream NO_3^- and dissolved oxygen (DO) concentrations, where lower NO_3^- and higher DO were observed during the day than at night. This diel pattern of stream NO_3^- concentration has been mainly associated with photoautotrophic activity because the assimilation of NO_3^- by benthic algae needs light energy to reduce this form of DIN to ammonium (Huppe and Turpin 1994). However, diel NO_3^- patterns can also be driven by other processes such as diel fluctuations of riparian groundwater (Flewelling et al. 2013), diurnal in-stream nitrification (Gammons et al. 2011) and nocturnal in-stream denitrification (Baulch et al. 2012). Therefore, elucidating the potential mechanisms controlling diel variations in stream nutrient concentration remains a great challenge in stream ecology (Scholefield et al. 2005, Pellerin et al. 2009). Moreover, the potential of fine-scale N dynamics to vary catchment N fluxes is still poorly understood because studies so far have been mainly performed during short time periods and within individual reaches.

The goal of this study was to investigate patterns and controls of diel variation in stream NO_3^- concentration and to assess how these diel fluctuations influence N fluxes along a stream continuum with increasing riparian area and channel width. We hypothesized that stream metabolism would drive diel variations in stream NO_3^- concentration. We would expect a positive relationship between daily gross primary production (GPP) and diel variations in stream NO_3^- concentration if photoautotrophic activity was the major control of fine-scale N dynamics. In this case, the largest diel NO_3^- variations would be observed during spring and at the most downstream site, which is the widest and the most exposed to light. Conversely, if heterotrophic activity is the main control of fine-scale N dynamics, diel NO_3^- variations would be positively related to ecosystem respiration (ER). Since stream water chemistry integrates processes occurring within the entire catchment, we also considered the alternative hypothesis that terrestrial or riparian processes would control fine-scale N patterns. In this case, we expected a positive relationship between diel variations in NO_3^- concentration in the stream and in riparian groundwater inputs, especially during the vegetative period when water and nutrient uptake by trees is the highest.

To evaluate these hypotheses, we measured diel variations in stream NO_3^- concentration together with stream metabolism, discharge, stream conservative tracer concentration (chloride), and riparian groundwater level and chemistry. Results from this study highlight the relevance of fine-scale temporal nutrient dynamics to understand the mechanisms underlying in-stream nutrient cycling, as well as to assess patterns of in-stream N removal and catchment nutrient fluxes at long-term scales.

MATERIALS AND METHODS

Study site

The research was conducted at the Font del Regàs stream, which drains a 14.2 km² catchment in the Montseny Natural Park, northeast Spain (41°50′ N, 2°30′ E; 500–1500 m above sea level [asl]). The catchment is dominated by biotitic granite (ICC 2010) and is mainly covered by evergreen oak (*Quercus ilex*) and beech (*Fagus sylvatica*) forests. The climate of the area is typical sub-humid Mediterranean, with mild winters and warm summers. The meteorological station located at the study catchment recorded a mean annual precipitation of 972 ± 141 mm (mean ± SD) during the study period (2010–2012), which falls within the long-term mean for this region (925 ± 151 mm, period: 1940–2000). Similarly, mean annual temperature during the study period (13° ± 6°C) was close to the long-term mean (12.1° ± 2.5°C, period: 1940–2000).

We selected three sampling sites along 3 km of the Font del Regàs stream (Fig. 1). The upstream site (800 m asl, 2.4 km from headwaters) was 1.7 m-wide stream with a poorly developed riparian forest composed of *Fagus sylvatica* and *Quercus ilex*. The midstream site (650 m asl, 4.1 km from headwaters) was a 2.5 m-wide stream flanked by a mixed forest of typically riparian tree species such as *Alnus glutinosa* and *Fraxinus excelsior*. The downstream site (500 m asl, 5.3 km from headwaters) was the widest (wetted width = 3.1 m), and it had a well-developed riparian forest (~30 m wide) consisting mainly of *Robinea pseudoacacia, Populus nigra*, and *A. glutinosa*.

The three sampling sites showed well-preserved channel morphology with a riffle–run structure. The streambed was mainly composed of rock (~30%), cobbles (~25%), and gravel (~15%) at the up- and midstream sites, whereas rock (~25%), cobbles (~30%), and sand (~30%) were the dominant substrates at the downstream site. During the period of study, stream discharge (Q) averaged 22.6 ± 18.7 L/s at the upstream site, and increased to 78.3 ± 52.9 and 89.4 ± 58.1 L/s at the mid- and downstream sites, respectively, that were located downstream of the two main tributaries discharging to the mainstem (Fig. 1). Stream DIN concentration averaged 0.28 ± 0.09, 0.17 ± 0.07, and 0.19 ± 0.08 mg N/L at the up-, mid- and downstream sites, respectively, NO_3^- being the predominant form (>85%). In all cases, NH_4^+ concentration was low
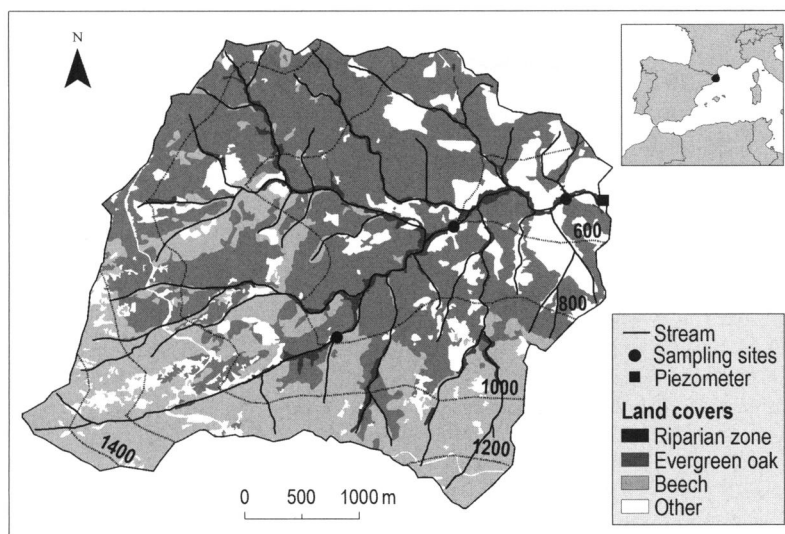
FIG. 1. Map of the Font del Regàs catchment (Montseny Natural Park, northeast Spain). The location of the three sampling sites along the stream continuum is shown with circles. The upstream site was located 0.6 km upstream of the first tributary discharging to the mainstem. The mid- and downstream sites were located 1.7 and 2.9 km downstream of the upstream site, respectively. The piezometer located in the riparian area of the downstream site is shown with a square. Numbers by dotted lines show meters above sea level.

(<0.02 mg N/L) and it represented a small fraction (<15%) of total DIN. Stream chloride (Cl^-) concentration increased along the stream continuum, from 6.21 ± 1.34 mg/L at the upstream site to 8.06 ± 1.02 mg/L at the downstream site. The riparian groundwater level (~2 m from the stream channel) was 0.5 ± 0.1 m below the soil surface (Bernal et al. 2015). At the downstream site, mean riparian groundwater concentration was 0.4 ± 0.2 mg N/L for NO_3^-, 11.4 ± 4 mg/L for Cl^-, and 4.2 ± 1.5 mg O_2/L for DO (averaged from seven piezometers; S. Poblador, *unpublished data*).

Field sampling and laboratory analysis

The field sampling was performed during two consecutive water years (2010–2011 and 2011–2012), each of which was devoted to accomplish different complementary objectives of our research. From September 2010 to August 2011 (water-year 2010–2011), we collected stream water samples twice a week at 12-h intervals at the three sampling sites (up-, mid-, and downstream) in order to explore the temporal pattern of diel variation in stream NO_3^- and Cl^- concentrations along the study elevation gradient. We considered Cl^- as a conservative solute, little affected by biogeochemical processes (Kirchner et al. 2001). Moreover, we collected water samples every day (at noon) to calculate stream solute loads. At each sampling site, water samples were collected with an auto-sampler (Model 1612, Teledyne Isco, Lincoln, Nebraska, USA), which was connected to a water pressure sensor (HOBO U20-001-04, Onset

Computer Corporation, Bourne, Massachusetts, USA) that monitored stream water level at 15-min intervals. Fortnightly, we measured Q at each sampling site by using the "slug" chloride addition method technique (Gordon et al. 1992). We inferred instantaneous Q from water level measurements by estimating the linear regression between stream water level and empirically measured Q ($n = 57$, 60, and 61 for up-, mid- and downstream sites, respectively; in all cases: $R^2 > 0.97$).

From March to July 2012 (spring 2012), we focused on investigating the relationship between the diel variation in stream NO_3^- concentration and daily stream metabolism. The sampling effort was concentrated at the downstream site, where both stream metabolism and diel variations in stream NO_3^- concentration were expected to be the highest. A Teledyne Isco autosampler was used to collect stream water samples at 6-h intervals: midnight (0 h), dawn (6 h), noon (12 h), and before sunset (18 h). Instantaneous Q was measured as in 2010–2011. Daily stream metabolism was calculated from stream DO (in mg O_2/L) recorded at 30-min intervals with an YSI ProODO oximeter (YSI Incorporated, Yellow Springs, Ohio, USA). We examined whether diel variations in stream solute concentration were related to riparian groundwater table fluctuations by monitoring riparian groundwater level (every 15 min), NO_3^- and Cl^- concentrations (every 12 h), and DO concentration (every 30 min) at a piezometer placed ~2 m from the stream channel. On average, riparian groundwater level and solute concentrations differed <9% between this piezometer and six others located nearby; and thus we considered this

piezometer representative of riparian groundwater at the downstream site (S. Poblador, *unpublished data*). In addition, we monitored the temporal pattern of temperature and light inputs to the stream along the study elevation gradient by installing HOBO sensors (HOBO U20-001-04) at the three sampling sites. The HOBOs recorded stream water temperature and photosynthetic active radiation (PAR) at 30-min intervals.

All water samples were filtered (Whatman GF/F) and kept cold (<4°C) until laboratory analysis (<24 h after collection). Water samples were analyzed for Cl^- and for DIN (NO_3^- and NH_4^+). Cl^- was analyzed by ionic chromatography (Compact IC-761, Methrom, Herisau, Switzerland). NO_3^- was analyzed by the cadmium reduction method (Keeney and Nelson 1982) using a Technicon Autoanalyzer (Technicon, Tarrytown, New York, USA). NH_4^+ was manually analyzed by the salicilate-nitropruside method (Baethgen and Alley 1989) using a spectrophotometer (PharmaSpec UV-1700, Shimadzu Corporation, Kyoto, Japan). Stream NH_4^+ concentration was low and show no diel variation for any of the three stream sites, and thus, NH_4^+ was not included in further data analysis.

Data analysis

Temperature and light conditions

We explored whether environmental conditions favoring in-stream photoautotrophic activity (temperature and PAR) were similar along the study stream continuum. For each sampling site, we calculated mean daily temperature (T, in °C) and accumulated daily PAR (ΣPAR, in $mol \cdot m^{-2} \cdot d^{-1}$), and then we computed the number of days for which T and ΣPAR were optimal for photoautotrophic activity. Moreover, we computed the number of hours per day during which instantaneous PAR (PAR_i, in $\mu mol \cdot m^{-2} \cdot s^{-1}$) was optimal for photosynthetic activity. We considered $T = 10°C$ as the threshold upon which photoautotrophs are not temperature limited (DeNicola 1996). A value of $\Sigma PAR = 4$ $mol \cdot m^{-2} \cdot d^{-1}$ was considered the minimum daily input of light required to ensure the activity of photoautotrophs (Hill et al. 1995). Finally, we assumed that $PAR_i > 200$ $\mu mol \cdot m^{-2} \cdot s^{-1}$ was the optimal irradiance for photosynthetic activity (Hill et al. 1995). Differences in T, ΣPAR, and PAR_i between the three sampling sites were established with a Wilcoxon paired rank sum test (Zar 2010).

Temporal pattern of stream solute concentrations.—We examined the temporal pattern of day–night variations in Cl^- and NO_3^- concentrations by calculating the relative difference between midnight and noon solute concentrations (Δ_{solute}, in %) with the following equation:

$$\Delta_{solute} = \frac{[solute]_{0h} - [solute]_{12h}}{[solute]_{0h}} \times 100 \qquad (1)$$

where $[solute]_{0h}$ and $[solute]_{12h}$ are the solute concentration (in mg/L) at midnight and noon, respectively. Values of $\Delta_{solute} \sim 0$ indicate small or null variation in solute concentration between day and night, as expected for conservative solutes if the contribution of water sources to stream runoff does not vary between day and night time. Values of $\Delta_{solute} > 0$ indicate higher solute concentrations at night than during the day, whereas values of $\Delta_{solute} < 0$ indicate the opposite. Previous studies have shown that peaks of NO_3^- concentration often occur near predawn and minima later in the afternoon (Heffernan and Cohen 2010, Halliday et al. 2013). Therefore, values of Δ_{solute} may underestimate, to some extent, the amplitude of diel variation because we collected the nighttime sample at midnight.

To explore whether day–night variations in solute concentration were significant, we compared noon and midnight concentrations of either Cl^- or NO_3^- by applying a Wilcoxon paired rank sum test. For the water year 2010–2011, we compared midnight and noon solute concentrations for each month and for each sampling site. For spring 2012, we compared midnight and noon solute concentrations at the downstream site for each week and for both stream and riparian groundwater.

To examine the potential influence of day–night variations in NO_3^- concentration on the 2010–2011 stream NO_3^- flux, we calculated the stream NO_3^- flux from the downstream site with and without including day–night variations of NO_3^- concentration. The annual load of NO_3^- was calculated by multiplying instantaneous Q by stream NO_3^- concentration and integrating instantaneous NO_3^- loads over the water year (from 1 September to 31 August). To account for day–night variations, instantaneous stream NO_3^- concentration was estimated by linearly interpolating NO_3^- concentrations measured at noon and midnight, whereas only noon values of NO_3^- concentration were considered when excluding day–night variation. Because midnight samples were collected twice a week, instantaneous midnight stream NO_3^- concentration for each day was estimated by linearly interpolating midnight NO_3^- concentrations measured during consecutive sampling dates. Differences between the two approaches (with and without day–night NO_3^- concentration) were attributed to the effect of in-stream processes on stream NO_3^- concentrations. The same procedure was repeated to calculate stream NO_3^- loads in spring 2012.

Stream metabolism.—During spring 2012, we calculated daily rates of GPP and ER at the downstream site by using the single-station diel DO change method (Bott 2006). This method was appropriate because in-stream conditions were uniform throughout the reach and groundwater inputs were small compared to stream discharge (<10%; Bott 2006). DO curves were corrected for the reaeration flux by applying the nighttime regression method to estimate the reaeration coefficient (Young and Huryn 1998). Daily ER was estimated by averaging the change in nighttime reaeration-corrected

TABLE 1. Mean daily stream water temperature (T), daily photosynthetically active radiation (ΣPAR), hours per day with $PAR_i > 200$ μmol·m^{-2}·s^{-1} (PAR_{200}), days with $T > 10°C$ (T_{10}), and days with ΣPAR > 4 mol·m^{-2}·d^{-1} (ΣPAR_4) for the up-, mid-, and downstream sites of the Font del Regàs stream, Montseny Natural Park, Spain, during spring 2012.

Site	T (°C)	ΣPAR (mol m^{-2}d^{-1})	PAR_{200} (h/d)	T_{10} (d)	ΣPAR_4 (d)
Upstream	10.2[A] (8.6, 13.2)	4.1[A] (3.6, 4.8)	0.5[A] (0.0, 1.5)	57	66
Midstream	12.2[B] (10.4, 14.5)	5.2[B] (4.1, 6.1)	1.0[A] (0.5, 1.5)	99	83
Downstream	12.4[B] (10.4, 14.5)	8.9[C] (6.3, 11.9)	2.5[B] (1.5, 4.0)	103	104

Note: Values are medians and the 25th and 75th percentile are shown in parentheses. For T, ΣPAR, and PAR_{200}, different letters indicate statistical significant differences between sampling sites (Wilcoxon paired rank sum test, P value < 0.05, df $= 1$; for the three variables $n = 112$).

DO at the 30-min interval and multiplying it by 24 h, assuming that instantaneous ER was constant during the entire day (Bott 2006). Daily GPP was computed by integrating the difference between the change in reaeration-corrected DO and ER at 30-min intervals (both measures in mg O_2·L^{-1}·min^{-1}). We multiplied GPP and ER by the mean reach depth (in m) to obtain areal estimates (in g O_2·m^{-2}·d^{-1}). Mean reach depth was calculated weekly by averaging the water column depth measured at 20-cm intervals across five transects along a 40-m reach.

We examined the relationship between environmental variables (T and ΣPAR), metabolic rates (daily ER and daily GPP), and daily A_{NO3} using linear regression models. We further investigated the contribution of GPP to diel variations in stream NO_3^- concentration by comparing measured NO_3^- concentrations with those predicted based only on stoichiometric principles (Hall and Tank 2003). First, we inferred instantaneous NO_3^- uptake rates by the stream photoautotrophic community (U_{GPP}, mg N·L^{-1}·min^{-1}) from instantaneous GPP (mg O_2·L^{-1}·min^{-1}). We assumed that (1) the molar ratio for CO_2:O_2 was 1:1 during photosynthesis (Hall and Tank 2003), and (2) the C:N ratio of the epilithic photoautotrophic community was 14:1 (C:N = 13.7 ± 1.3 in light exposed epilithic biofilm at the study stream; Pastor et al. 2014). We acknowledge that these are rough estimates because not all GPP is translated into biomass accrual (Hall and Beaulieu 2013), and not all epilithic biofilm is composed of photoautotrophic organisms (Volkmar et al. 2011). However, this was a useful exercise for our purposes because we inferred N uptake by photoautotrophs from stoichiometric principles, independently of diel variations in stream NO_3^- concentration. Then, at each time step ($t = 0$, 6, 12, and 18 h), we calculated the predicted stream NO_3^- concentration ($[NO_3]_t'$, in mg N/L) as follows:

$$[NO_3]_t' = [NO_3]_{t-1}' - (\bar{U}_{GPP} \times \Delta t) \qquad (2)$$

where $[NO_3]_{t-1}'$ is the predicted stream NO_3^- concentration (in mg N/L) at sampling time $t-1$, $\bar{U}_{GPP}$ is the average U_{GPP} between sampling time intervals, and Δt is the time interval between sampling times (360 min; Heffernan and Cohen 2010). The initial condition to run the model was considered to be the observed stream NO_3^- concentration at the beginning of spring 2012. We evaluated the goodness of fit between predicted and observed NO_3^- concentration and A_{NO3} by ordinary least squares. Moreover, we tested whether the slope of the linear regression between predicted and observed values was similar to 1 with a slope test (Zar 2010). We expected a slope similar to 1 between predicted and observed values if GPP is the main driver of diel variations in stream NO_3^- concentration. Further, the residuals between predicted and observed A_{NO3} were examined for evaluating the ability of the model to predict changes in A_{NO3} over time.

All the statistical analyses were carried out with the R 2.15.1 statistical software (R Core Development Team 2012). We chose nonparametric tests for the statistical analysis because not all data sets had a normal distribution. In all cases, differences were considered statistically significant when $P < 0.05$.

RESULTS

Temperature and light inputs along the stream

During spring 2012, environmental conditions were more favorable for photosynthetic activity at the mid- and downstream sites than at the upstream site. Both T and ΣPAR were higher at the downstream than at the upstream site (Table 1). Moreover, $T > 10°C$ was reached during 50%, 85%, and 90% of the days at the up-, mid-, and downstream sites, respectively (Table 1, Fig. 2a). The percentage of days with ΣPAR > 4 mol·m^{-2}·d^{-1} increased along the stream continuum, being 59%, 74% and 93% at the up-, mid-, and downstream sites, respectively (Table 1, Fig. 2b).

At the downstream site, T remained around 9.6° ± 2.1°C from mid-March to mid-April, and then it increased to 15°C until the end of the study period in July (Fig. 3a). Diel variations in temperature remained small during spring 2012, being 1.5° ± 0.8°C higher at noon than at nighttime (Fig. 3a). Light inputs to the stream (PAR_i) increased from mid-March until two weeks after the riparian leaf-out in early April (Fig. 3b). As the riparian canopy developed (from
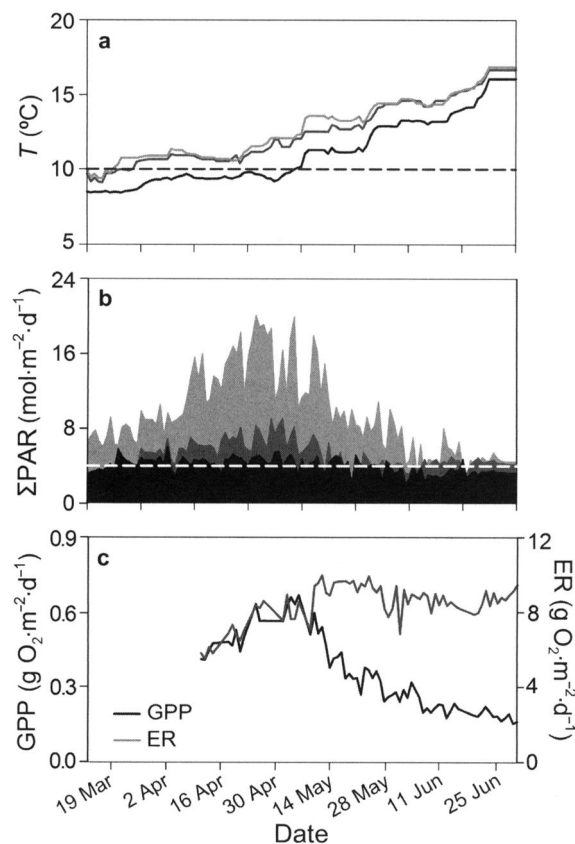
FIG. 2. Temporal pattern of (a) mean daily stream water temperature (T), (b) daily photosynthetically active radiation (ΣPAR), and (c) stream metabolism during spring 2012 at the downstream site. In panels (a) and (b), different shades showed data for the upstream (black), midstream (dark gray), and downstream (light gray) sampling site. Dashed lines indicate thresholds upon which photoautotrophs are not limited by temperature ($T = 10°C$) or light (ΣPAR = 4 mol·m^{-2}·d^{-1}). In panel (c), different shades showed data for gross primary production (GPP; black) and ecosystem respiration (ER; gray).

mid-April to late May), PAR_i and diel variation in PAR_i sharply decreased, and then remained low until the end of the experiment in July (Fig. 3b).

Temporal patterns of day–night variation in stream and riparian groundwater solute concentrations

During the water year 2010–2011, Cl$^-$ concentration did not differ between midnight and noon in any month and at any of the three stream sites (for the 12 months and the 3 sites: Wilcoxon paired rank sum test, $Z > Z_{0.05}$, df = 11, $P > 0.05$; Fig. 4, open circles). In contrast, the day–night variation in NO$_3^-$ concentration differed between stream sites. At the upstream site, there were no differences between midnight and noon stream NO$_3^-$ concentrations in any month (for the 12 months: $Z > Z_{0.05}$, df = 11, $P > 0.05$; Fig. 4a, solid circles). At the mid- and downstream

sites, stream NO$_3^-$ concentrations at midnight were higher than at noon during spring months (from April to June, and from April to May for the mid- and downstream sites, respectively; in all cases $Z < Z_{0.05}$, df = 11, $P < 0.05$). During this period, monthly median Δ_{NO3} ranged from 6.3% to 19.1% (Fig. 4b,c; solid circles). In November, stream NO$_3^-$ concentrations were 12.8% higher at noon than at midnight at the downstream site ($Z = -1.825$, df = 11, $P < 0.05$; Fig. 4c, solid circles).

Such day–night variations in stream NO$_3^-$ concentration influenced stream N fluxes mainly during spring, reducing the NO$_3^-$ load at the downstream site by 11%. The reduction in stream NO$_3^-$ load was similar during spring 2012 (9%). During autumn, winter, and summer, diel variations in NO$_3^-$ concentration had a small effect on stream NO$_3^-$ loads (<5%).

During spring 2012, the diel pattern of stream solute concentrations at the downstream site was similar to spring 2011. Stream Cl$^-$ concentration averaged 8.3 ± 0.3 mg/L (mean ± SD), and it slightly increased from March to July, showing the opposite pattern than stream Q (Fig. 3c,d). Diel variations for both Q and Cl$^-$ concentration remained low (<5%) and did not differ between midnight and noon throughout the sampling period (from March to June: $Z > Z_{0.05}$, df = 6, $P > 0.1$; Fig. 5a, open circles). Stream NO$_3^-$ concentration ranged from 0.12 to 0.23 mg N/L, and showed higher values at midnight than at noon from mid-March to late-May (for each of the 12 weeks: $Z < Z_{0.05}$, df = 6, $P < 0.05$; Fig. 3e). The Δ_{NO3} increased from mid-March to the beginning of May (3 weeks after the riparian leaf-out), and then declined until the riparian canopy was fully closed in June (Fig. 5a, solid circles). No day–night variations in stream NO$_3^-$ concentration were found later on (for all June weeks: $Z > Z_{0.05,6}$, df = 6, $P > 0.1$).

During spring 2012, riparian groundwater DO concentration averaged 4.72 ± 1.47 mg O$_2$/L, and it slightly decreased from March to June, showing the same pattern than riparian groundwater level. Riparian groundwater concentration averaged 11.3 ± 0.5 mg/L for Cl$^-$ and 0.46 ± 0.08 mg N/L for NO$_3^-$. Diel variations in riparian groundwater level, DO, Cl$^-$, and NO$_3^-$ concentration did not differ between midnight and noon throughout the sampling period (for the four variables and for each of the 15 weeks: $Z > Z_{0.05}$, df = 6, $P > 0.1$; Fig. 5b).

Relationship between diel variation in nitrate concentration and stream metabolism

During spring 2012, daily rates of ER at the downstream site ranged from 5.5 to 10.0 g O$_2$·m^{-2}·d^{-1}, increasing from April to mid-May and then remaining relatively constant at 8.4 ± 1.0 g O$_2$·m^{-2}·d^{-1} (Fig. 2c). This temporal pattern was positively related to the
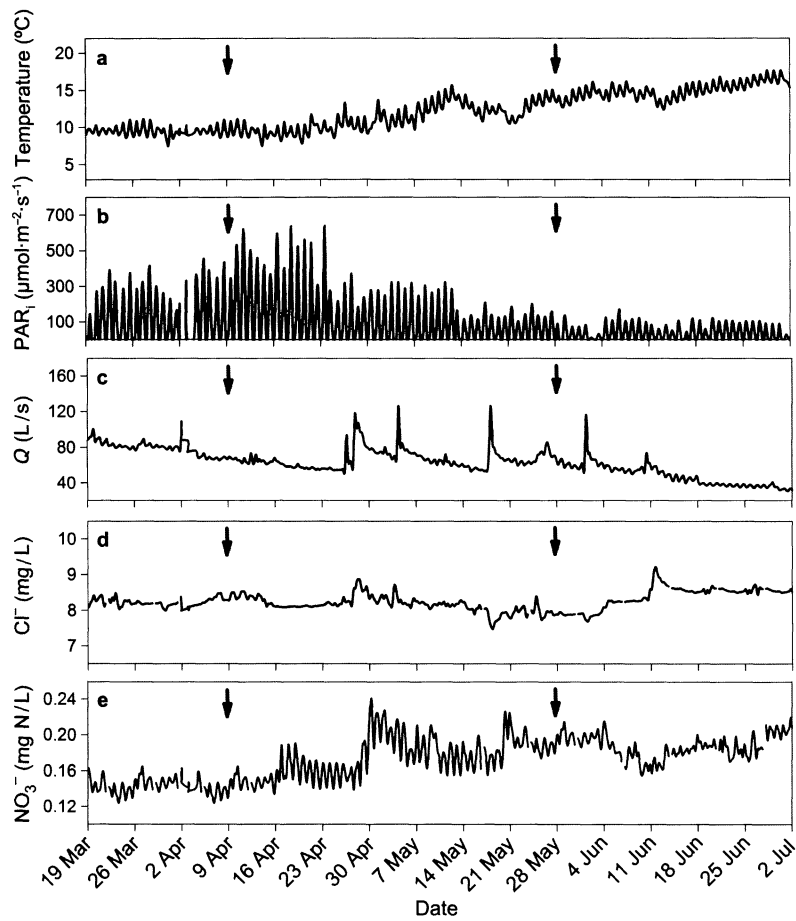
Fig. 3. Diel variation of (a) stream water temperature, (b) instantaneous photosynthetically active radiation (PAR$_i$), (c) stream discharge (Q), (d) stream Cl$^-$ concentration, and (e) stream NO$_3^-$ concentration during spring 2012 at the downstream site. Black arrows indicate the beginning and the end of the leaf-emergence period (S. Poblador, *unpublished data*).

temporal pattern of T (linear regression [l.r.], $R^2 = 0.38$, $P < 0.05$, $n = 44$). Daily rates of GPP were between 10- and 100-fold lower than daily rates of ER, indicating that stream metabolism was dominated by heterotrophic activity during spring. Daily rates of GPP increased from April (0.35 g O$_2$·m^{-2}·d^{-1}) to mid-May (0.64 g O$_2$·m^{-2}·d^{-1}), and then decreased until June (0.07 g O$_2$·m^{-2}·d^{-1}; Fig. 2c). This temporal pattern was positively related to the temporal pattern of ΣPAR (Fig. 6a). No relationship was found between daily rates of GPP and ER (l.r., $R^2 = 0.02$, $P > 0.1$, $n = 44$).

There was no relationship between daily Δ_{NO3} and daily ER (l.r., $R^2 = 0.01$, $P > 0.1$, $n = 44$), while daily Δ_{NO3} was positively related to daily GPP (Fig. 6b). There was a good fit between observed stream NO$_3^-$ concentrations and those predicted from stoichiometric principles as indicated by both the strong relationship between observed and predicted values (l.r., $R^2 = 0.73$, $P < 0.001$, $n = 201$), and nonsignificant divergences from the 1:1 line (slope test, $F = 1.01$, df = 200,

$P > 0.1$). Similarly, there was a good fit between observed and predicted Δ_{NO3} (l.r., $R^2 = 0.85$, $P < 0.001$, $n = 44$; slope test, $F = 0.55$, df = 43, $P > 0.1$; Fig. 6c). Divergences between observed and predicted Δ_{NO3} were < 4% during March, April, and May, while, on average, predicted values were overestimated by 14% in June.

DISCUSSION

This study aimed to investigate the importance of terrestrial and in-stream biogeochemical processes on controlling fine-scale temporal N dynamics along a stream continuum, and to assess the influence of such diel NO$_3^-$ fluctuations on stream N fluxes at seasonal scale. Our results indicated that the temporal pattern of diel variation in stream NO$_3^-$ concentration varied substantially along the stream. No diel NO$_3^-$ variations were observed at the upstream site, while day–night variations in NO$_3^-$ concentration peaked during the onset of riparian leaf emergence
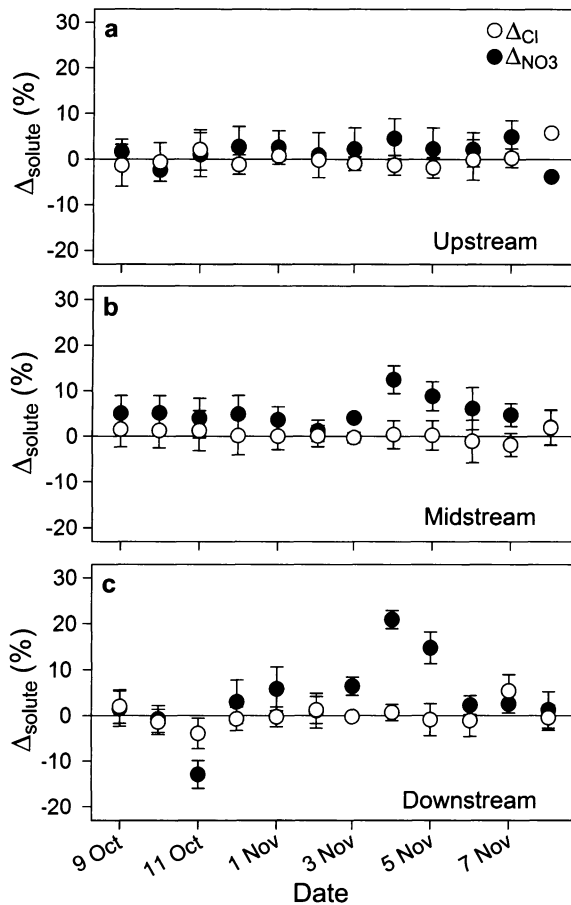
FIG. 4. Temporal pattern of the relative difference between midnight and noon stream water concentrations (Δ_{solute}) for both chloride (open circles) and nitrate (solid circles) at the (a) upstream, (b) midstream, and (c) downstream sites during the water-year 2010–2011. Circles are the median of Δ_{solute} for each month, and whiskers denote the 25th and 75th percentile. The black line indicates no differences between midnight and noon solute concentrations.

Δ_{NO3} would then have been 0.6% instead of 13% (Appendix S1 and Figure S1). Conversely, the strong relationship and synchronicity between daily GPP and Δ_{NO3} supports the hypothesis that in-stream photoautotrophic activity was a major driver of the observed diel variations in stream NO_3^- concentration. These results are in agreement with findings from lowland rivers (Heffernan and Cohen 2010), head-water forested streams (Roberts and Mulholland 2007), and even coastal ecosystems (Johnson et al. 2006). Yet, these previous studies were performed during periods of relatively high photoautotrophic activity (GPP = 5–20 g $O_2 \cdot m^{-2} \cdot d^{-1}$, GPP:ER ~ 1) compared to the values measured in this study (GPP < 0.7 g $O_2 \cdot m^{-2} \cdot d^{-1}$, GPP:ER < 0.01). Therefore, our study is novel in showing the potential of pho-toautotrophic activity to regulate in-stream NO_3^- dynamics even in extremely low-productivity streams dominated by heterotrophic metabolism.

Our results add to the growing body of research demonstrating that GPP is a strong driver of in-stream NO_3^- uptake (Hall and Tank 2003, Mulholland et al. 2008), though the relationship between stream metab-olism and fine-scale N dynamics can vary among streams. For instance, diel NO_3^- variations in April were similar (10–20 μg N/L) between Walker Branch

at the mid- and downstream sites as reported in previous studies (Roberts and Mulholland 2007, Rusjan and Mikoš 2009). These contrasting patterns in fine-scale N dynamics were accompanied by lon-gitudinal increases in temperature and light availa-bility, suggesting that these two environmental factors were controlling the extent to which in-stream pro-cesses modified fine-scale NO_3^- dynamics along the stream continuum.

The results obtained during spring 2012 convinc-ingly showed that terrestrial processes did not control diel variations in NO_3^- concentration because no simultaneous diel variations in stream discharge, riparian groundwater level, or N concentration were observed. Moreover, simple mass balance calculations indicate that hydrological mixing with riparian groundwater inputs could not explain midnight in-creases in stream NO_3^- concentration because median
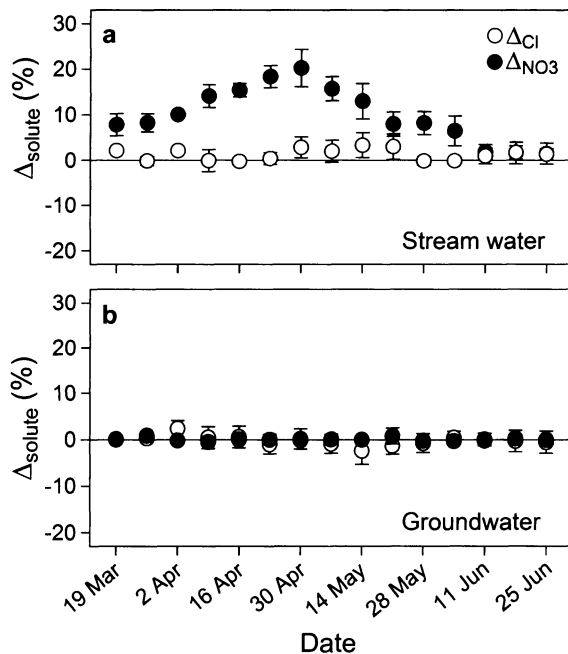

FIG. 5. Temporal pattern of the relative difference between midnight and noon concentrations (Δ_{solute}) for both chloride (open circles) and nitrate (solid circles) in (a) stream water, and (b) riparian groundwater during spring 2012 at the downstream site. Circles are the median of Δ_{solute} for each week, and whiskers denote the 25th and 75th percentile. The black line indicates no differences between midnight and noon solute concentrations.
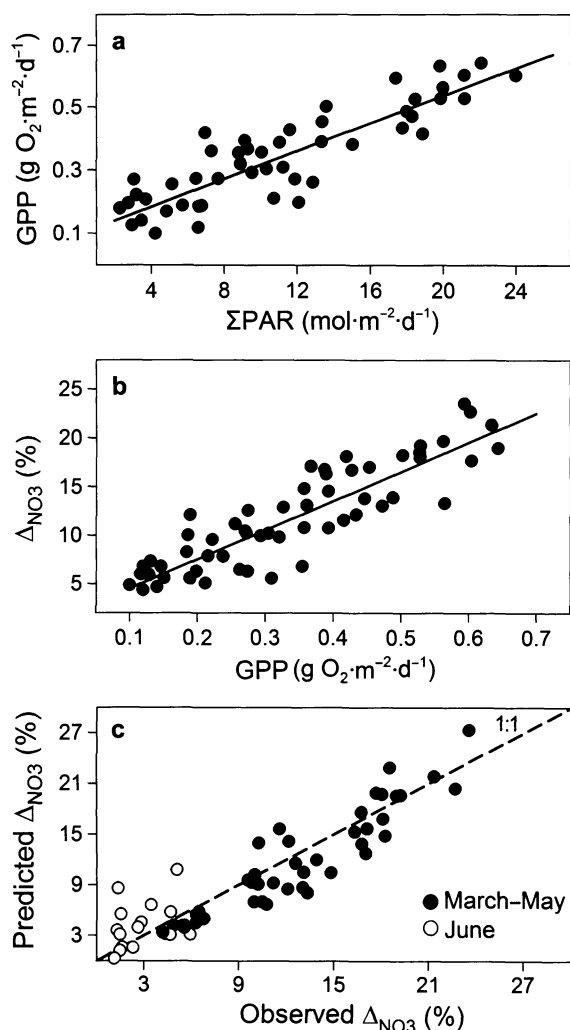
Fig. 6. Relationship between (a) daily photosynthetically active radiation (ΣPAR) and daily gross primary production (GPP), (b) daily GPP and day–night variations in stream nitrate concentration (Δ_{NO3}), and (c) observed and stoichiometrically predicted day–night variations in stream nitrate concentration during spring 2012 at the downstream site. The black line in panels (a) and (b) is the linear regression between variables (GPP vs. ΣPAR: linear regression [l.r.], $R^2 = 0.74$, $P < 0.001$; Δ_{NO3} vs. GPP: l.r., $R^2 = 0.74$, $P < 0.001$). The 1:1 line is indicated in panel (c) with a dashed line. Open circles in panel (c) indicated day–night variations in stream nitrate concentration in June.

(Tennessee, USA; Roberts and Mulholland 2007) and Font del Regàs (this study), despite daily rates of GPP that were 10-fold larger at Walker Branch. On the other hand, GPP at Walker Branch was similar to Sycamore Creek (Arizona, USA; Grimm 1987) and Ichetucknee River (Florida, USA; Heffernan and Cohen 2010) (7–14 g $O_2 \cdot m^{-2} \cdot d^{-1}$), though diel NO_3^- variations were four- to six-fold lower at Walker Branch (10–20 vs.75–100 µg N/L). Midday decline in stream NO_3^- concentrations is likely driven by photoautotrophic N demand relative to N supply (Sterner and Elser 2002,

Appling and Heffernan 2014). Thus, divergences between GPP and diel NO_3^- variations among streams could be explained by differences in both N availability (from 0.12 to 0.42 mg N/L at Font del Regàs and Ichetucknee River, respectively) and the C:N ratio of primary uptake compartments (from 14:1 in Font del Regàs epilithic biofilms to 25:1 in Ichetucknee macrophytes). A good assessment of the stream biota stoichiometry is thus crucial to constrain the uncertainty associated with mechanistic models linking stream metabolism and fine-scale nutrient dynamics.

Despite the strong match between day–night variations measured at the downstream site and those predicted from GPP instantaneous rates during early spring, divergences between measured and predicted Δ_{NO3} were evident in late spring. These biases in model prediction could be explained by changes in the stoichiometry of the algal community (Sterner and Elser 2002, Heffernan and Cohen 2010) or in the respiration rate of photoautotrophs (Hall and Beaulieu 2013), which could be induced by decreased light inputs after riparian leaf-out. Additionally, these mismatches could be explained by shifts in the main processes regulating diel NO_3^- variations after leaf-out such as in-stream nitrification or denitrification (Gammons et al. 2011, Baulch et al. 2012). Diel cycles of these two processes could probably be suited for day–night NO_3^- variations during the peak of leaf litter accumulation in November, which resulted in midnight decline in stream NO_3^- concentrations (Laursen and Seitzinger 2004). However, it seems unlikely that nitrification could account for the observed diel NO_3^- patterns in spring because no diel variations in NH_4^+ concentration occurred to support nitrification, while relatively high DO concentrations in the stream (10.7 ± 0.5 mg O_2/L) and hyporheic zone (7.8 ± 1.6 mg O_2/L; S. Poblador, *unpublished data*) suggest low denitrification in stream sediments (Kemp and Dodds 2002, Johnson and Tank 2009). The lack of correlation between Δ_{NO3} and ER further support that GPP was a major player regulating fine-scale NO_3^- dynamics. The current understanding of the influence of metabolism on stream N dynamics has been mostly based on correlational analysis (e.g., Hall and Tank 2003). Nonetheless, our study shows that stoichiometric models based on diel nutrient variation are complementary and powerful tools that can contribute to disentangle the mechanisms driving stream nutrient cycling over time and space.

There is still little research available on whether diel variations in nutrient concentration can have any implication at larger spatial and temporal scales, and how the mechanisms underlying such fine-scale patterns can ultimately modify catchment nutrient fluxes. Our study indicated that the contribution of photoautotrophic N uptake to regulate NO_3^- fluxes at the downstream site was small in annual terms (4%), as expected for a low-productivity stream such as Font del Regàs (Battin et al. 2008, Valett et al. 2008).

However, during spring, increased photoautotrophic N uptake led to a decrease in catchment NO_3^- export of ~20 g N/ha, which was equivalent to a ~10% reduction in the stream NO_3^- export. Since maxima NO_3^- and minima DO concentrations usually coincide over a daily cycle (Heffernan and Cohen 2010, Halliday et al. 2013), our estimates may be slightly underestimated because we measured NO_3^- at 0 h, while minima DO occurred between 0–3 h. Nevertheless, we estimated a similar decrease in spring NO_3^- loads (15 g N/ha, ~12%) for Walker Branch (38.4 ha, 6–14 L/s) based on mean NO_3^- concentration (0.2–0.5 mg N/L) and Δ_{NO3} (2–15 µg N/L) reported by Roberts and Mulholland (2007). These estimations for Font del Regàs and Walker Branch suggest that benthic algae are an important transitory sink of DIN in these headwater forested streams, similarly to the vernal dam described for spring ephemeral plants by Muller and Bormann (1976). Nonetheless, the relevance of photoautotrophic N retention at longer time scales will ultimately depend on the turnover rates of the primary uptake compartments, which can vary widely between epilithic biofilms (few days) to macrophytes (months) (Riis et al. 2012).

The influence of fine-scale N patterns on N fluxes could be even higher in open-canopy and lowland streams for which reported diel NO_3^- variations are larger than for headwater forested streams (Grimm 1987, Heffernan et al. 2010, Halliday et al. 2013). For instance, we estimated that spring diel NO_3^- variation may reduce catchment NO_3^- exports by ~70 g N/ha (~16%) at the Ichetucknee River (770 km², 8900 L/s), based on mean daily minima and maxima NO_3^- concentrations (0.38 and 0.46 mg N/L) reported by Heffernan and Cohen (2010). The contribution of fine-scale N dynamics to reduce catchment N export was even larger at the Upper Hafren River in UK (122 ha, 60 L/s), an open stream where spring diel NO_3^- variations (from 0.14 to 0.18 mg N/L) reduced stream NO_3^- loads by 154 g N/ha (22%) (Halliday et al. 2013). These back-of-the-envelope calculations highlight that fine-scale N dynamics can not only indicate the preferential mechanisms of in-stream N uptake, but also provide a relevant evaluation of their contribution on regulating NO_3^- downstream fluxes at the catchment scale.

CONCLUSIONS

This study adds to the growing evidence demonstrating that in-stream processes can substantially modify stream N concentration and fluxes (Peterson et al. 2001, Bernhardt et al. 2005, Arango et al. 2008, Bernal et al. 2012). In-stream GPP was the major driver of diel variations in stream NO_3^- concentration in this highly heterotrophic headwater stream, while the contribution of other in-stream, riparian, and upland processes was minimal. From

a network perspective, the temporal pattern of such diel NO_3^- variations, and thus, their influence on stream N fluxes, varied along the stream continuum depending on light and temperature regimes. Finally, and in line with previous work, our study indicates that discrete measurements performed at midday can limit our understanding of in-stream nutrient cycling as well as the assessment of reliable nutrient budgets at long time scales even in low-productivity streams (Mulholland et al. 2006). These biases could be even larger (up to 15–20%) for highly productive streams given that the capacity of stream biota to regulate diel and seasonal stream N dynamics could increase along the river continuum, as observed in this study. Overall, monitoring of nutrient data at fine-scale temporal resolution can provide mechanistic explanations about the relevance of in-stream and terrestrial processes on regulating stream nutrient concentrations and their implications on long-term fluxes at the catchment scale.

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