

1 Run Title: Fine-scale nitrogen dynamics

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

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13 **Abstract:**

14 Monitoring nutrient concentrations at fine-scale temporal resolution contributes to a better
15 understanding of nutrient cycling in stream ecosystems. However, the mechanisms underlying
16 fine-scale nutrient dynamics and its implications for budget catchment fluxes are still poorly
17 understood. To gain understanding on patterns and controls of fine-scale stream nitrogen (N)
18 dynamics and to assess how they affect hydrological N fluxes, we explored diel variation in
19 stream nitrate (NO_3^-) concentration along a headwater stream with increasing riparian area and
20 channel width. At the down-stream site, the highest day-night variations occurred in early-spring
21 when stream NO_3^- concentrations were 13% higher at night than during day time. Such day-night
22 variations were strongly related to daily light inputs ($R^2=0.74$) and gross primary production
23 (GPP) ($R^2=0.74$), and they showed an excellent fit with day-night NO_3^- variations predicted from
24 GPP ($R^2=0.85$). These results suggest that diel fluctuations in stream NO_3^- concentration were
25 mainly driven by photoautotrophic N uptake. Terrestrial influences were discarded because no
26 simultaneous diel variations in stream discharge, riparian groundwater level, or riparian solute
27 concentration were observed. In contrast to the down-stream site, no diel variations in NO_3^-
28 concentration occurred at the up-stream site likely because water temperature was colder (10 vs.
29 12 °C) and light availability was lower (4 vs. 9 $\text{mol m}^{-2} \text{d}^{-1}$). Although daily GPP was between
30 10-100 folds lower than daily respiration, photoautotrophic N uptake contributed to a 10%
31 reduction in spring NO_3^- loads at the down-stream site. Our study clearly shows that the activity
32 of photoautotrophs can substantially change over time and along the stream continuum in

33 response to key environmental drivers such as light and temperature, and further that its capacity
34 to regulate diel and seasonal N fluxes can be important even in low productivity streams.

35 **Keywords**

36 Diel stream nitrate variation, photoautotrophic activity, in-stream nitrate uptake, stream
37 metabolism, headwater forested streams.

38

39 **Introduction**

40 Human activity has doubled the availability of bioreactive nitrogen (N) worldwide, which
41 compromises the function and biodiversity of terrestrial and freshwater ecosystems, as well as
42 soil and water quality (Schlesinger 2009, Sutton et al. 2011). Nonetheless, biological activity can
43 transform and retain a substantial amount of N inputs, and thus reduce the pervasive effects of
44 excessive N in ecosystems (Bernhardt et al. 2002, Goodale et al. 2004). Within catchments,
45 biogeochemical processes occurring at upland, riparian and aquatic ecosystems simultaneously
46 contribute to N cycling and retention, and ultimately determine N export downstream (Bernhardt
47 et al. 2005). In particular, there is a growing body of research demonstrating that streams and
48 rivers have a high capacity to transform and retain N (Peterson et al. 2001, Tank et al. 2008),
49 even though their ability to influence N export from catchments to downstream ecosystems is
50 still under debate (Brookshire et al. 2009). This is mostly because water chemistry of stream and
51 rivers integrates biogeochemical processes occurring at different spatial and temporal scales
52 throughout the catchment, which complicates assessing the relative influence of in-stream and
53 terrestrial processes on N exports (Sudduth et al. 2013). A better understanding of the
54 mechanisms and drivers of N dynamics within fluvial ecosystems is critical to evaluate their
55 capacity to modify N inputs from terrestrial sources.

56 Nitrate (NO_3^-) is the predominant form of dissolved inorganic N (DIN) in fluvial ecosystems,
57 and its uptake is mainly controlled by the metabolic activity of stream biota (Hall and Tank
58 2003, Mulholland et al. 2008). Recently, monitoring at fine-scale temporal resolution in streams
59 has provided examples of the close link between gross primary production and NO_3^- uptake (e.g.

60 Johnson et al. 2006, Roberts and Mulholland 2007, Heffernan and Cohen 2010). These studies
61 have found an inverse relationship between fine-scale stream NO_3^- and dissolved oxygen (DO)
62 concentrations, where lower NO_3^- and higher DO were observed during day- than night-time.
63 This diel pattern of stream NO_3^- concentration has been mainly associated with photoautotrophic
64 activity because the assimilation of NO_3^- by benthic algae needs light energy to reduce this form
65 of DIN to ammonium (Huppe and Turpin 1994). However, diel NO_3^- patterns can also be driven
66 by other processes such as diel fluctuations of riparian groundwater (Flewelling et al. 2013),
67 diurnal in-stream nitrification (Gammons et al. 2011) and nocturnal in-stream denitrification
68 (Baulch et al. 2012). Therefore, elucidating the potential mechanisms controlling diel variations
69 in stream nutrient concentration remains a great challenge in stream ecology (Scholefield et al.
70 2005, Pellerin et al. 2009). Moreover, the potential of fine-scale N dynamics to vary catchment N
71 fluxes is still poorly understood because studies so far have been mainly performed during short
72 time periods and within individual reaches.

73 The goal of this study was to investigate patterns and controls of diel variation in stream NO_3^-
74 concentration and to assess how these diel fluctuations influence N fluxes along a stream
75 continuum with increasing riparian area and channel width. We hypothesized that stream
76 metabolism will drive diel variations in stream NO_3^- concentration. We would expect a positive
77 relationship between daily GPP and diel variations in stream NO_3^- concentration if
78 photoautotrophic activity was the major control of fine-scale N dynamics. In this case, the largest
79 diel NO_3^- variations would be observed during spring and at the downstream-most site, which is
80 the widest and the most exposed to light. Conversely, if heterotrophic activity is the main control
81 of fine-scale N dynamics, diel NO_3^- variations would be positively related to ecosystem

82 respiration (ER). Since stream water chemistry integrates processes occurring within the entire
83 catchment, we also considered the alternative hypothesis that terrestrial or riparian processes will
84 control fine-scale N patterns. In this case, we expected a positive relationship between diel
85 variations in NO_3^- concentration in the stream and in riparian groundwater inputs, especially
86 during the vegetative period when water and nutrient uptake by trees is the highest.

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

93 **Materials and Methods**

94 *Study site*

95 The research was conducted at the Font del Regàs stream, which drains a 14.2 km² catchment in
96 the Montseny Natural Park, NE Spain (41°50'N, 2°30'E, 500-1500 m a.s.l.). The catchment is
97 dominated by biotitic granite (ICC 2010) and it is mainly covered by evergreen oak (*Quercus*
98 *ilex*) and beech (*Fagus sylvatica*) forests. The climate of the area is typical sub-humid
99 Mediterranean, with mild winters and warm summers. The meteorological station located at the
100 study catchment recorded a mean annual precipitation of 971.5 ± 140.7 mm (mean \pm SD) during
101 the study period (2010-2012), which falls within the long-term mean for this region ($924.7 \pm$

102 151.2 mm, period: 1940-2000). Similarly, mean annual temperature during the study period ($13 \pm$
103 $6 \text{ }^\circ\text{C}$) was close to the long-term mean ($12.1 \pm 2.5 \text{ }^\circ\text{C}$, period: 1940-2000).

104 We selected three sampling sites along 3 km of the Font del Regàs stream (Figure 1). The up-
105 stream site (800 m a.s.l., 2.4 km from headwaters) was 1.7 m-wide stream with a poorly
106 developed riparian forest composed of *Fagus sylvatica* and *Quercus ilex*. The mid-stream site
107 (650 m a.s.l., 4.1 km from headwaters) was a 2.5 m-wide stream flanked by a mixed forest of
108 typically riparian tree species such as *Alnus glutinosa* and *Fraxinus excelsior*. The down-stream
109 site (500 m a.s.l., 5.3 km from headwaters) was the widest (wetted width = 3.1 m) and it had a
110 well-developed riparian forest (~30 m wide) consisting mainly of *Robinea pseudoacacia*,
111 *Populus nigra* and *A. glutinosa*.

112 The three sampling sites showed well-preserved channel morphology with a riffle-run structure.
113 The streambed was mainly composed of rock (~30%), cobbles (~25%) and gravel (~15%) at the
114 up- and mid-stream sites, whereas rock (~25%), cobbles (~30%) and sand (~30%) were the
115 dominant substrates at the down-stream site. During the period of study, stream discharge (Q)
116 averaged $22.6 \pm 18.7 \text{ L/s}$ at the up-stream site, and increased to 78.3 ± 52.9 and $89.4 \pm 58.1 \text{ L/s}$
117 at the mid- and down-stream sites, respectively, that were located downstream of the two main
118 tributaries discharging to the mainstem (Figure 1). Stream DIN concentration averaged $0.28 \pm$
119 0.09 , 0.17 ± 0.07 , and $0.19 \pm 0.08 \text{ mg N/L}$ at the up-, mid- and down-stream sites, respectively,
120 NO_3^- being the predominant form (> 85%). In all cases, NH_4^+ concentration was low (< 0.02 mg
121 N L^{-1}) and it represented a small fraction (< 15%) of total DIN. Stream chloride (Cl^-)
122 concentration increased along the stream continuum, from $6.21 \pm 1.34 \text{ mg/L}$ at the up-stream site
123 to $8.06 \pm 1.02 \text{ mg/L}$ at the down-stream site. The riparian groundwater level (~ 2 m from the

124 stream channel) was 0.5 ± 0.1 m below the soil surface (Bernal et al., 2015). At the down-stream
125 site, mean riparian groundwater concentration was 0.4 ± 0.2 mg N/L for NO_3^- , 11.4 ± 4 mg/L for
126 Cl^- , and 4.2 ± 1.5 mg O_2 /L for DO (averaged from 7 piezometers) (Poblador, unpublished data).

127 *Field sampling and laboratory analysis*

128 The field sampling was performed during two consecutive water years (2010-2011 and 2011-
129 2012), each of which was devoted to accomplish different complementary objectives of our
130 research. From September 2010 to August 2011 (water-year 2010-2011), we collected stream
131 water samples twice a week at 12-hour intervals at the three sampling sites (up-, mid-, and down-
132 stream) in order to explore the temporal pattern of diel variation in stream NO_3^- and Cl^-
133 concentrations along the study elevation gradient. We considered Cl^- as a conservative solute,
134 little affected by biogeochemical processes (Kirchner et al. 2001). Moreover, we collected water
135 samples every day (at noon) to calculate stream solute loads (see below). At each sampling site,
136 water samples were collected with an auto-sampler (Teledyne Isco Model 1612), which was
137 connected to a water pressure sensor (HOBO U20-001-04) that monitored stream water level at
138 15-min intervals. Fortnightly, we measured Q at each sampling site by using the “slug” chloride
139 addition method technique (Gordon et al. 1992). We inferred instantaneous Q from water level
140 measurements by estimating the linear regression between stream water level and empirically
141 measured Q (n = 57, 60 and 61 for up-, mid- and down-stream sites, respectively; in all cases: R^2
142 > 0.97).

143 From March to July 2012 (spring 2012), we focused on investigating the relationship between
144 the diel variation in stream NO_3^- concentration and daily stream metabolism. The sampling effort

145 was concentrated at the down-stream site, where both stream metabolism and diel variations in
146 stream NO_3^- concentration were expected to be the highest. A Teledyne Isco auto-sampler was
147 used to collect stream water samples at 6-hour intervals: mid-night (0h), dawn (6h), noon (12h)
148 and before sunset (18h). Instantaneous Q was measured as in 2010-2011. Daily stream
149 metabolism was calculated from stream DO (in $\text{mg O}_2 \text{ L}^{-1}$) recorded at 30-min intervals with an
150 YSI ProODO oxymeter. We examined whether diel variations in stream solute concentration
151 were related to riparian groundwater table fluctuations by monitoring riparian groundwater level
152 (every 15 min), NO_3^- and Cl^- concentrations (every 12 hours) and DO concentration (every 30-
153 min) at a piezometer placed ~ 2 m from the stream channel. On average, riparian groundwater
154 level and solute concentrations differed $< 9\%$ between this piezometer and 6 others located
155 nearby; and thus we considered this piezometer representative of riparian groundwater at the
156 down-stream site (Poblador, unpublished data). In addition, we monitored the temporal pattern of
157 temperature and light inputs to the stream along the study elevation gradient by installing HOBO
158 sensors (HOBO U20-001-04) at the three sampling sites. The HOBOs recorded stream water
159 temperature and photosynthetic active radiation (PAR) at 30-min intervals.

160 All water samples were filtered (Whatman GF/F) and kept cold (< 4 °C) until laboratory analysis
161 (< 24 h after collection). Water samples were analyzed for Cl^- and for DIN (NO_3^- and NH_4^+). Cl^-
162 was analyzed by ionic chromatography (Compact IC-761, Methrom). NO_3^- was analyzed by the
163 cadmium reduction method (Keeney and Nelson 1982) using a Technicon Autoanalyzer
164 (Technicon 1976). NH_4^+ was manually analyzed by the salicylate-nitropruside method (Baethgen
165 and Alley 1989) using a spectrophotometer (PharmaSpec UV-1700 SHIMADZU). Stream NH_4^+

166 concentration was low and show no diel variation for any of the three stream sites, and thus NH_4^+
167 was not included in further data analysis.

168 *Data analysis*

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

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

$$185 \quad \Delta_{\text{solute}} = \frac{[\text{solute}]_{0\text{h}} - [\text{solute}]_{12\text{h}}}{[\text{solute}]_{0\text{h}}} \times 100, \quad (1)$$

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

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

200 To examine the potential influence of day-night variations in NO_3^- concentration on the 2010-
201 2011 stream NO_3^- flux, we calculated the stream NO_3^- flux from the down-stream site with and
202 without including day-night variations of NO_3^- concentration. The annual load of NO_3^- was
203 calculated by multiplying instantaneous Q by stream NO_3^- concentration and integrating
204 instantaneous NO_3^- loads over the water year (from 1 September to 31 August). To account for
205 day-night variations, instantaneous stream NO_3^- concentration was estimated by linearly
206 interpolating NO_3^- concentrations measured at noon and midnight, whereas only noon values of

207 NO_3^- concentration were considered when excluding day-night variation. Because midnight
208 samples were collected twice a week, instantaneous midnight stream NO_3^- concentration for each
209 day was estimated by linearly interpolating midnight NO_3^- concentrations measured during
210 consecutive sampling dates. Differences between the two approaches (with and without day-
211 night NO_3^- concentration) were attributed to the effect of in-stream processes on stream NO_3^-
212 concentrations. The same procedure was repeated to calculate stream NO_3^- loads in spring 2012.

213 *Stream metabolism.* During spring 2012, we calculated daily rates of GPP and ER at the down-
214 stream site by using the single-station diel DO change method (Bott 2006). This method was
215 appropriate because in-stream conditions were uniform throughout the reach and groundwater
216 inputs were small compared to stream discharge (<10%) (Bott 2006). DO curves were corrected
217 for the reaeration flux by applying the night-time regression method to estimate the reaeration
218 coefficient (Young and Huryn 1998). Daily ER was estimated by averaging the change in night
219 time reaeration-corrected DO at 30 min interval and multiplying it by 24 hours, assuming that
220 instantaneous ER was constant during the entire day (Bott 2006). Daily GPP was computed by
221 integrating the difference between the change in reaeration-corrected DO and ER at 30-min
222 intervals (both measures in $\text{mg O}_2 \text{ L}^{-1} \text{ min}^{-1}$). We multiplied GPP and ER by the mean reach
223 depth (in m) to obtain areal estimates (in $\text{g O}_2 \text{ m}^{-2} \text{ d}^{-1}$). Mean reach depth was calculated weekly
224 by averaging the water column depth measured at 20-cm intervals across 5 transects along a 40-
225 m reach.

226 We examined the relationship between environmental variables (T and ΣPAR), metabolic rates
227 (daily ER and daily GPP) and daily ΔNO_3 using linear regression models. We further investigated
228 the contribution of GPP to diel variations in stream NO_3^- concentration by comparing measured

229 NO_3^- concentrations with those predicted based only on stoichiometric principles (Hall and Tank
230 2003). First, we inferred instantaneous NO_3^- uptake rates by the stream photoautotrophic
231 community (U_{GPP} , $\text{mg N L}^{-1} \text{ min}^{-1}$) from instantaneous GPP ($\text{mg O}_2 \text{ L}^{-1} \text{ min}^{-1}$). We assumed that
232 (i) the molar ratio for $\text{CO}_2:\text{O}_2$ was 1:1 during photosynthesis (Hall and Tank 2003), and (ii) the
233 C:N ratio of the epilithic photoautotrophic community was 14:1 (C:N = 13.7 ± 1.3 in light
234 exposed epilithic biofilm at the study stream, Pastor et al. 2014). We acknowledge that these are
235 rough estimates because not all GPP is translated into biomass accrual (Hall and Beaulieu 2013),
236 and not all epilithic biofilm is composed of photoautotrophic organisms (Volkmar et al. 2011).
237 However, this was a useful exercise for our purposes because we inferred N uptake by
238 photoautotrophs from stoichiometric principles, independently of diel variations in stream NO_3^-
239 concentration. Then, at each time step ($t = 0, 6, 12,$ and 18h), we calculated the predicted stream
240 NO_3^- concentration ($[\text{NO}_3^-]_t$, in mg N/L) as follows:

$$241 \quad [\text{NO}_3^-]_t = [\text{NO}_3^-]_{t-1} - (\overline{U_{\text{GPP}}} \times \Delta t) \quad (2)$$

242 where $[\text{NO}_3^-]_{t-1}$ is the predicted stream NO_3^- concentration (in mg N L^{-1}) at sampling time $t-1$,
243 $\overline{U_{\text{GPP}}}$ is the average U_{GPP} between sampling time intervals, and Δt is the time interval between
244 sampling times (360 min) (Heffernan and Cohen 2010). The initial condition to run the model
245 was considered to be the observed stream NO_3^- concentration at the beginning of spring 2012.
246 We evaluated the goodness of fit between predicted and observed NO_3^- concentration and Δ_{NO_3}
247 by ordinary least squares. Moreover, we tested whether the slope of the linear regression between
248 predicted and observed values was similar to 1 with a slope test (Zar 2010). We expected a slope
249 similar to 1 between predicted and observed values if GPP is the main driver of diel variations in

250 stream NO_3^- concentration. Further, the residuals between predicted and observed Δ_{NO_3} were
251 examined for evaluating the ability of the model to predict changes in Δ_{NO_3} over time.

252 All the statistical analyses were carried out with the R 2.15.1 statistical software (R-project
253 2008). We chose non-parametric tests for the statistical analysis because not all data sets had a
254 normal distribution. In all cases, differences were considered statistically significant when $p <$
255 0.05.

256 **Results**

257 *Temperature and light inputs along the stream*

258 During spring 2012, environmental conditions were more favorable for photosynthetic activity at
259 the mid- and down-stream sites than at the up-stream site. Both T and ΣPAR were higher at the
260 down- than at the up-stream site (Table 1). Moreover, $T > 10^\circ\text{C}$ was reached during 50%, 85%,
261 and 90% of the days at the up-, mid-, and down-stream sites, respectively (Table 1, Figure 2a).

262 The percentage of days with $\Sigma\text{PAR} > 4 \text{ mol m}^{-2} \text{ d}^{-1}$ increased along the stream continuum, being
263 59%, 74% and 93% at the up-, mid-, and down-stream sites, respectively (Table 1, Figure 2b).

264 At the down-stream site, T remained around $9.6 \pm 2.1^\circ\text{C}$ from mid-March to mid-April, and then
265 it increased to 15°C until the end of the study period in July (Figure 3a). Diel variations in
266 temperature remained small during spring 2012, being $1.5 \pm 0.8^\circ\text{C}$ higher at noon than at night-
267 time (Figure 3a). Light inputs to the stream (PAR_i) increased from mid-March until two weeks
268 after the riparian leaf-out in early-April (Figure 3b). As the riparian canopy developed (from

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

271 *Temporal patterns of day-night variation in stream and riparian groundwater solute*
272 *concentrations*

273 During the water year 2010-2011, Cl^- concentration did not differ between midnight and noon in
274 any month and at any of the three stream sites (for the 12 months and the 3 sites: Wilcoxon
275 paired rank sum test, $Z > Z_{0.05}$, $df = 11$, $p > 0.05$) (Figure 4, white circles). In contrast, the day-
276 night variation in NO_3^- concentration differed between stream sites. At the up-stream site, there
277 were no differences between midnight and noon stream NO_3^- concentrations in any month (for
278 the 12 months: $Z > Z_{0.05}$, $df = 11$, $p > 0.05$) (Figure 4a, black circles). At the mid- and down-
279 stream sites, stream NO_3^- concentrations at midnight were higher than at noon during spring
280 months (from April to June, and from April to May for the mid- and down-stream sites,
281 respectively; in all cases $Z < Z_{0.05}$, $df = 11$, $p < 0.05$). During this period, monthly median Δ_{NO_3}
282 ranged from 6.3 to 19.1% (Figure 4b and 4c, black circles). In November, stream NO_3^-
283 concentrations were 12.8% higher at noon than at midnight at the down-stream site ($Z = -1.825$,
284 $df = 11$, $p < 0.05$) (Figure 4c, black circles).

285 Such day-night variations in stream NO_3^- concentration influenced stream N fluxes mainly
286 during spring, reducing the NO_3^- load at the down-stream site by 11%. The reduction in stream
287 NO_3^- load was similar during spring 2012 (9%). During autumn, winter and summer, diel
288 variations in NO_3^- concentration had a small effect on stream NO_3^- loads (< 5%).

289 During spring 2012, the diel pattern of stream solute concentrations at the down-stream site was
290 similar to spring 2011. Stream Cl^- concentration averaged 8.3 ± 0.3 mg/L and it slightly
291 increased from March to July, showing the opposite pattern than stream Q (Figure 3c and Figure
292 3d). Diel variations for both Q and Cl^- concentration remained low ($< 5\%$) and did not differ
293 between midnight and noon throughout the sampling period (from March to June: $Z > Z_{0.05}$, $df =$
294 6 , $p > 0.1$) (Figure 5a, white circles). Stream NO_3^- concentration ranged from 0.12 to 0.23 mg
295 N/L, and showed higher values at midnight than at noon from mid-March to late-May (for each
296 of the 12 weeks: $Z < Z_{0.05}$, $df = 6$, $p < 0.05$) (Figure 3e). The Δ_{NO_3} increased from mid-March to
297 the beginning of May (three weeks after the riparian leaf-out), and then declined until the
298 riparian canopy was fully closed in June (Figure 5a, black circles). No day-night variations in
299 stream NO_3^- concentration were found later on (for all June weeks: $Z > Z_{0.05,6}$, $df = 6$, $p > 0.1$).

300 During spring 2012, riparian groundwater DO concentration averaged 4.72 ± 1.47 mg O_2 /L and
301 it slightly decreased from March to June, showing the same pattern than riparian groundwater
302 level. Riparian groundwater concentration averaged 11.3 ± 0.5 mg/L for Cl^- and 0.46 ± 0.08 mg
303 N/L for NO_3^- . Diel variations in riparian groundwater level, DO, Cl^- and NO_3^- concentration did
304 not differ between midnight and noon throughout the sampling period (for the four variables and
305 for each of the 15 weeks: $Z > Z_{0.05}$, $df = 6$, $p > 0.1$) (Figure 5b).

306 *Relationship between diel variation in nitrate concentration and stream metabolism*

307 During spring 2012, daily rates of ER at the down-stream site ranged from 5.5 to 10.0 g O_2 m^{-2} d^{-1}
308 1 , increasing from April to mid-May and then remaining relatively constant at 8.4 ± 1.0 g O_2 m^{-2}
309 d^{-1} (Figure 2c). This temporal pattern was positively related to the temporal pattern of T (linear

310 regression [l.r.], $R^2 = 0.38$, $p < 0.05$, $n = 44$). Daily rates of GPP were between 10-100 fold lower
311 than daily rates of ER, indicating that stream metabolism was dominated by heterotrophic
312 activity during spring. Daily rates of GPP increased from April ($0.35 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$) to mid-May
313 ($0.64 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$), and then decreased until June ($0.07 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$) (Figure 2c). This temporal
314 pattern was positively related to the temporal pattern of ΣPAR (Figure 6a). No relationship was
315 found between daily rates of GPP and ER (l.r., $R^2 = 0.02$, $p > 0.1$, $n = 44$).

316 There was no relationship between daily ΔNO_3 and daily ER (l.r., $R^2 = 0.01$, $p > 0.1$, $n = 44$),
317 while daily ΔNO_3 was positively related to daily GPP (Figure 6b). There was a good fit between
318 observed stream NO_3^- concentrations and those predicted from stoichiometric principles as
319 indicated by both the strong relationship between observed and predicted values (l.r., $R^2 = 0.73$, p
320 < 0.001 , $n = 201$), and non-significant divergences from the 1:1 line (slope test, $F = 1.01$, $df =$
321 200 , $p > 0.1$). Similarly, there was a good fit between observed and predicted ΔNO_3 (l.r., $R^2 =$
322 0.85 , $p < 0.001$, $n = 44$; slope test, $F = 0.55$, $df = 43$, $p > 0.1$) (Figure 6c). Divergences between
323 observed and predicted ΔNO_3 were $< 4\%$ during March, April and May, while on average
324 predicted values were overestimated by 14% in June.

325 **Discussion**

326 This study aimed to investigate the importance of terrestrial and in-stream biogeochemical
327 processes on controlling fine-scale temporal N dynamics along a stream continuum, and to assess
328 the influence of such diel NO_3^- fluctuations on stream N fluxes at seasonal scale. Our results
329 indicated that the temporal pattern of diel variation in stream NO_3^- concentration varied
330 substantially along the stream. No diel NO_3^- variations were observed at the up-stream site, while

331 day-night variations in NO_3^- concentration peaked during the onset of riparian leaf emergence at
332 the mid- and down-stream sites as reported in previous studies (Roberts and Mulholland 2007,
333 Rusjan and Mikoš 2009). These contrasting patterns in fine-scale N dynamics were accompanied
334 by longitudinal increases in temperature and light availability, suggesting that these two
335 environmental factors were controlling the extent to which in-stream processes modified fine-
336 scale NO_3^- dynamics along the stream continuum.

337 The results obtained during spring 2012 convincingly showed that terrestrial processes did not
338 control diel variations in NO_3^- concentration because no simultaneous diel variations in stream
339 discharge, riparian groundwater level or N concentration were observed. Moreover, simple mass
340 balance calculations indicate that hydrological mixing with riparian groundwater inputs could
341 not explain midnight increases in stream NO_3^- concentration because median Δ_{NO_3} would then
342 have been 0.6% instead of 13% (Appendix A). Conversely, the strong relationship and
343 synchronicity between daily GPP and Δ_{NO_3} supports the hypothesis that in-stream
344 photoautotrophic activity was a major driver of the observed diel variations in stream NO_3^-
345 concentration. These results are in agreement with findings from lowland rivers (Heffernan and
346 Cohen 2010), headwater forested streams (Roberts and Mulholland 2007), and even coastal
347 ecosystems (Johnson et al. 2006). Yet, these previous studies were performed during periods of
348 relatively high photoautotrophic activity ($\text{GPP} = 5\text{-}20 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$, $\text{GPP:ER} \sim 1$) compared to the
349 values measured in this study ($\text{GPP} < 0.7 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$, $\text{GPP:ER} < 0.01$). Therefore, our study is
350 novel in showing the potential of photoautotrophic activity to regulate in-stream NO_3^- dynamics
351 even in extremely low productivity streams dominated by heterotrophic metabolism.

352 Our results add to the growing body of research demonstrating that GPP is a strong driver of in-
353 stream NO_3^- uptake (Hall and Tank 2003, Mulholland et al. 2008), though the relationship
354 between stream metabolism and fine-scale N dynamics can vary among streams. For instance,
355 diel NO_3^- variations in April were similar (10-20 $\mu\text{g N/L}$) between Walker Branch (TN, USA;
356 Roberts and Mulholland 2007) and Font del Regàs (this study), despite daily rates of GPP that
357 were 10 fold larger at Walker Branch. On the other hand, GPP at Walker Branch was similar to
358 Sycamore Creek (AZ, USA; Grimm 1987) and Ichetucknee river (FL, USA; Heffernan and
359 Cohen 2010) (7-14 $\text{g O}_2 \text{ m}^{-2} \text{ d}^{-1}$), though diel NO_3^- variations were 4-6 fold lower at Walker
360 Branch (10-20 vs. 75-100 $\mu\text{g N/L}$). Midday decline in stream NO_3^- concentrations is likely driven
361 by photoautotrophic N demand relative to N supply (Sterner and Elser 2002, Appling and
362 Heffernan 2014). Thus, divergences between GPP and diel NO_3^- variations among streams could
363 be explained by differences in both N availability (from 0.12 to 0.42 mg N/L at Font del Regàs
364 and Ichetucknee river, respectively) and the C:N ratio of primary uptake compartments (from
365 14:1 in Font del Regàs epilithic biofilms to 25:1 in Ichetucknee macrophytes). A good
366 assessment of the stream biota stoichiometry is thus crucial to constrain the uncertainty
367 associated with mechanistic models linking stream metabolism and fine-scale nutrient dynamics.

368 Despite the strong match between day-night variations measured at the down-stream site and
369 those predicted from GPP instantaneous rates during early spring, divergences between measured
370 and predicted Δ_{NO_3} were evident in late spring. These biases in model prediction could be
371 explained by changes in the stoichiometry of the algal community (Sterner and Elser 2002,
372 Heffernan and Cohen 2010) or in the respiration rate of photoautotrophs (Hall and Beaulieu
373 2013), which could be induced by decreased light inputs after riparian leaf-out. Additionally,

374 these mismatches could be explained by shifts in the main processes regulating diel NO_3^-
375 variations after leaf-out such as in-stream nitrification or denitrification (Gammons et al. 2011,
376 Baulch et al. 2012). Diel cycles of these two processes could probably be suited for day-night
377 NO_3^- variations during the peak of leaf litter accumulation in November, which resulted in
378 midnight decline in stream NO_3^- concentrations (Laursen and Seitzinger 2004). However, it
379 seems unlikely that nitrification could account for the observed diel NO_3^- patterns in spring
380 because no diel variations in NH_4^+ concentration occurred to support nitrification, while
381 relatively high DO concentrations in the stream (10.7 ± 0.5 mg O_2/L) and hyporheic zone ($7.8 \pm$
382 1.6 mg O_2/L ; Poblador, unpublished data) suggest low denitrification in stream sediments (Kemp
383 and Dodds 2002, Johnson and Tank 2009). The lack of correlation between Δ_{NO_3} and ER, further
384 support that GPP was a major player regulating fine-scale NO_3^- dynamics. The current
385 understanding of the influence of metabolism on stream N dynamics has been mostly based on
386 correlational analysis (e.g. Hall and Tank 2003). Nonetheless, our study shows that
387 stoichiometric models based on diel nutrient variation are complementary and powerful tools that
388 can contribute to disentangle the mechanisms driving stream nutrient cycling over time and
389 space.

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

396 photoautotrophic N uptake led to a decrease in catchment NO_3^- export of ~ 20 g N/ha, which was
397 equivalent to a $\sim 10\%$ reduction in the stream NO_3^- export. Since maxima NO_3^- and minima DO
398 concentrations usually coincide over a daily cycle (Heffernan and Cohen 2010, Halliday et al.
399 2013), our estimates may be slightly underestimated because we measured NO_3^- at 0h, while
400 minima DO occurred between 0-3h. Nevertheless, we estimated a similar decrease in spring
401 NO_3^- loads (15 g N/ha, $\sim 12\%$) for Walker Branch (38.4 ha, 6-14 L/s) based on mean NO_3^-
402 concentration (0.2-0.5 mg N/L) and Δ_{NO_3} (2-15 μg N/L) reported by Roberts and Mulholland
403 (2007). These estimations for Font del Regàs and Walker Branch suggest that benthic algae are
404 an important transitory sink of DIN in these headwater forested streams, similarly to the vernal
405 dam described for spring ephemeral plants by Muller and Bormann (1976). Nonetheless, the
406 relevance of photoautotrophic N retention at longer time scales will ultimately depend on the
407 turnover rates of the primary uptake compartments, which can vary widely between epilithic
408 biofilms (few days) to macrophytes (months) (Riis et al. 2012).

409 The influence of fine-scale N patterns on N fluxes could be even higher in open-canopy and
410 lowland streams for which reported diel NO_3^- variations are larger than for headwater forested
411 streams (Grimm 1987, Heffernan et al. 2010, Halliday et al. 2013). For instance, we estimated
412 that spring diel NO_3^- variation may reduce catchment NO_3^- exports by ~ 70 g N ha^{-1} ($\sim 16\%$) at the
413 Ichetucknee river (770 km^2 , 8900 L/s), based on mean daily minima and maxima NO_3^-
414 concentrations (0.38 and 0.46 mg N/L) reported by Hefferman and Cohen (2010). The
415 contribution of fine-scale N dynamics to reduce catchment N export was even larger at the Upper
416 Hafren river in UK (122 ha, 60 L/s), an open stream where spring diel NO_3^- variations (from
417 0.014 to 0.018 mg N/L) reduced stream NO_3^- loads by 154 g N/ha (22 %) (Halliday et al. 2013).

418 These back-of-the-envelope calculations highlight that fine-scale N dynamics can not only
419 indicate the preferential mechanisms of in-stream N uptake, but also provide a relevant
420 evaluation of their contribution on regulating NO_3^- downstream fluxes at the catchment scale.

421 **Conclusions**

422 This study adds to the growing evidence demonstrating that in-stream processes can substantially
423 modify stream N concentration and fluxes (Peterson et al. 2001, Bernhardt et al. 2005, Arango et
424 al. 2008, Bernal et al. 2012). In-stream GPP was the major driver of diel variations in stream
425 NO_3^- concentration in this highly heterotrophic headwater stream, while the contribution of other
426 in-stream, riparian, and upland processes was minimal. From a network perspective, the temporal
427 pattern of such diel NO_3^- variations, and thus their influence on stream N fluxes, varied along the
428 stream continuum depending on light and temperature regimes. Finally, and in line with previous
429 work, our study indicates that discrete measurements performed at midday can limit our
430 understanding of in-stream nutrient cycling as well as the assessment of reliable nutrient budgets
431 at long time scales even in low productivity streams (Mulholland et al. 2006). These biases could
432 be even larger (up to 15-20%) for highly productive streams given that the capacity of stream
433 biota to regulate diel and seasonal stream N dynamics could increase along the river continuum,
434 as observed in this study. Overall, monitoring of nutrient data at fine-scale temporal resolution
435 can provide mechanistic explanations about the relevance of in-stream and terrestrial processes
436 on regulating stream nutrient concentrations and their implications on long-term fluxes at the
437 catchment scale.

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582 **Appendix A: Contribution of riparian groundwater inputs to day-night variations in**

583 **stream nitrate concentration**

584

585 **Tables**

586 *Table 1:* Mean daily stream water temperature (T), daily photosynthetically active radiation
 587 (Σ PAR), hours per day with $\text{PAR}_i > 200 \mu\text{mol m}^{-2} \text{s}^{-1}$ (PAR_{200}), days with $T > 10 \text{ }^\circ\text{C}$ (T_{10}), and
 588 days with $\Sigma\text{PAR} > 4 \text{ mol m}^{-2} \text{d}^{-1}$ (ΣPAR_4) for the up-, mid-, and down-stream sites during spring
 589 2012. Values are medians and the 25th and 75th percentile are shown in brackets. For T, Σ PAR
 590 and PAR_{200} , different letters indicate statistical significant differences between sampling sites
 591 (Wilcoxon paired rank sum test, p-value < 0.05, df = 1; for the three variables n = 112).

592

Site	T ($^\circ\text{C}$)	ΣPAR ($\text{mol m}^{-2}\text{d}^{-1}$)	PAR_{200} (hours/day)	T_{10} (days)	ΣPAR_4 (days)
Up-stream	10.2 [8.6, 13.2] ^A	4.1 [3.6, 4.8] ^A	0.5 [0.0, 1.5] ^A	57	66
Mid-stream	12.2 [10.4, 14.5] ^B	5.2 [4.1, 6.1] ^B	1.0 [0.5, 1.5] ^A	99	83
Down-stream	12.4 [10.4, 14.5] ^B	8.9[6.3, 11.9] ^C	2.5 [1.5, 4.0] ^B	103	104

593

594

595 **Figure captions**

596 *Figure 1.* Map of the Font del Regàs catchment (Montseny Natural Park, NE Spain). The
597 location of the three sampling sites along the stream continuum is shown with circles. The up-
598 stream site was located 0.6 km upstream of the first tributary discharging to the mainstem. The
599 mid- and down-stream sites were located 1.7 and 2.9 km downstream of the up-stream site,
600 respectively. The piezometer located in the riparian area of the down-stream site is shown with a
601 square.

602 *Figure 2.* Temporal pattern of (a) mean daily stream water temperature (T), (b) daily
603 photosynthetically active radiation (Σ PAR) and (c) stream metabolism during spring 2012 at the
604 down-stream site. In panel (a) and (b), different colors showed data for the up-stream (black),
605 mid-stream (dark grey) and down-stream (grey) sampling site. Dashed lines indicate thresholds
606 upon which photoautotrophs are not limited by temperature ($T = 10^{\circ}\text{C}$) or light ($\Sigma\text{PAR} = 4 \text{ mol}$
607 $\text{m}^{-2} \text{d}^{-1}$). In panel (c), different colors showed data for GPP (black) and ER (grey).

608 *Figure 3.* Diel variation of (a) stream water temperature (Temp), (b) photosynthetically active
609 radiation (PAR_i), (c) stream discharge (Q), (d) stream Cl^- concentration, and (e) stream NO_3^-
610 concentration during spring 2012 at the down-stream site. Black arrows indicate the beginning
611 and the end of the leaf emergence period (Poblador, unpublished data).

612 *Figure 4.* Temporal pattern of the relative difference between midnight and noon stream water
613 concentrations (Δ_{solute}) for both chloride (white) and nitrate (black) at the (a) up-stream, (b) mid-
614 stream, and (c) down-stream sites during the water-year 2010-2011. Circles are the median of

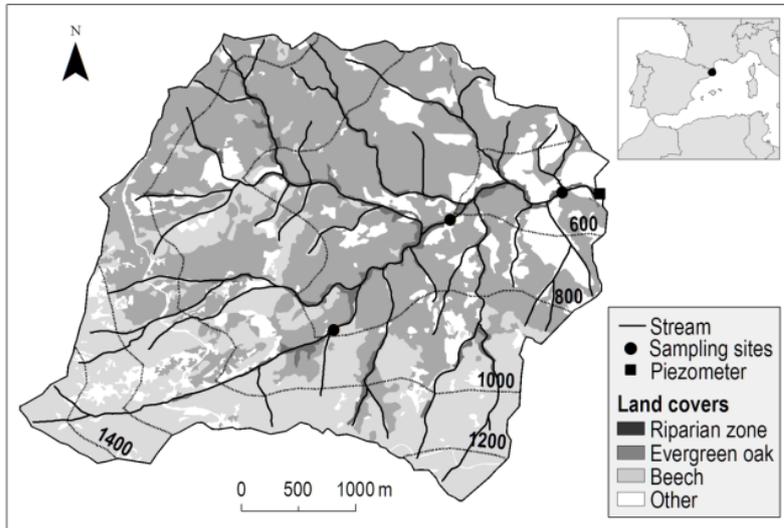
615 Δ_{solute} for each month and whiskers denote the 25th and 75th percentile. The black line indicates
616 no differences between midnight and noon solute concentrations.

617 *Figure 5.* Temporal pattern of the relative difference between midnight and noon concentrations
618 (Δ_{solute}) for both chloride (white) and nitrate (black) in (a) stream water, and (b) riparian
619 groundwater during spring 2012 at the down-stream site. Circles are the median of Δ_{solute} for
620 each week and whiskers denote the 25th and 75th percentile. The black line indicates no
621 differences between midnight and noon solute concentrations.

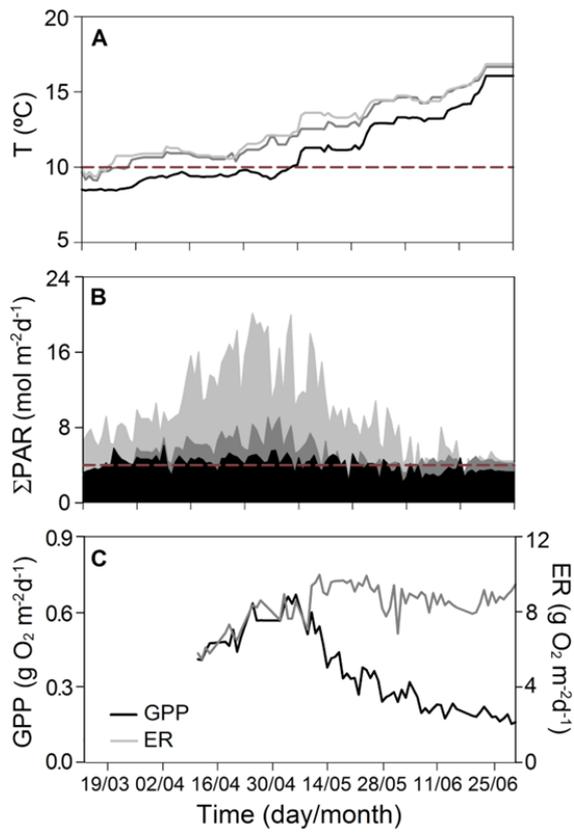
622 *Figure 6.* Relationship between (a) daily photosynthetically active radiation (ΣPAR) and daily
623 gross primary production (GPP), (b) daily GPP and day-night variations in stream nitrate
624 concentration (Δ_{NO_3}), and (c) observed and stoichiometrically predicted day-night variations in
625 stream nitrate concentration during spring 2012 at the down-stream site. The black line in panels
626 (a) and (b) is the linear regression between variables (GPP vs. ΣPAR : l.r., $R^2 = 0.74$, $p < 0.001$;
627 Δ_{NO_3} vs. GPP: l.r., $R^2 = 0.74$, $p < 0.001$). The 1:1 line is indicated in panel (c) with a dashed line.
628 White circles in panel (c) indicated day-night variations in stream nitrate concentration in June.

629 **Figures**

630 *Figure 1*

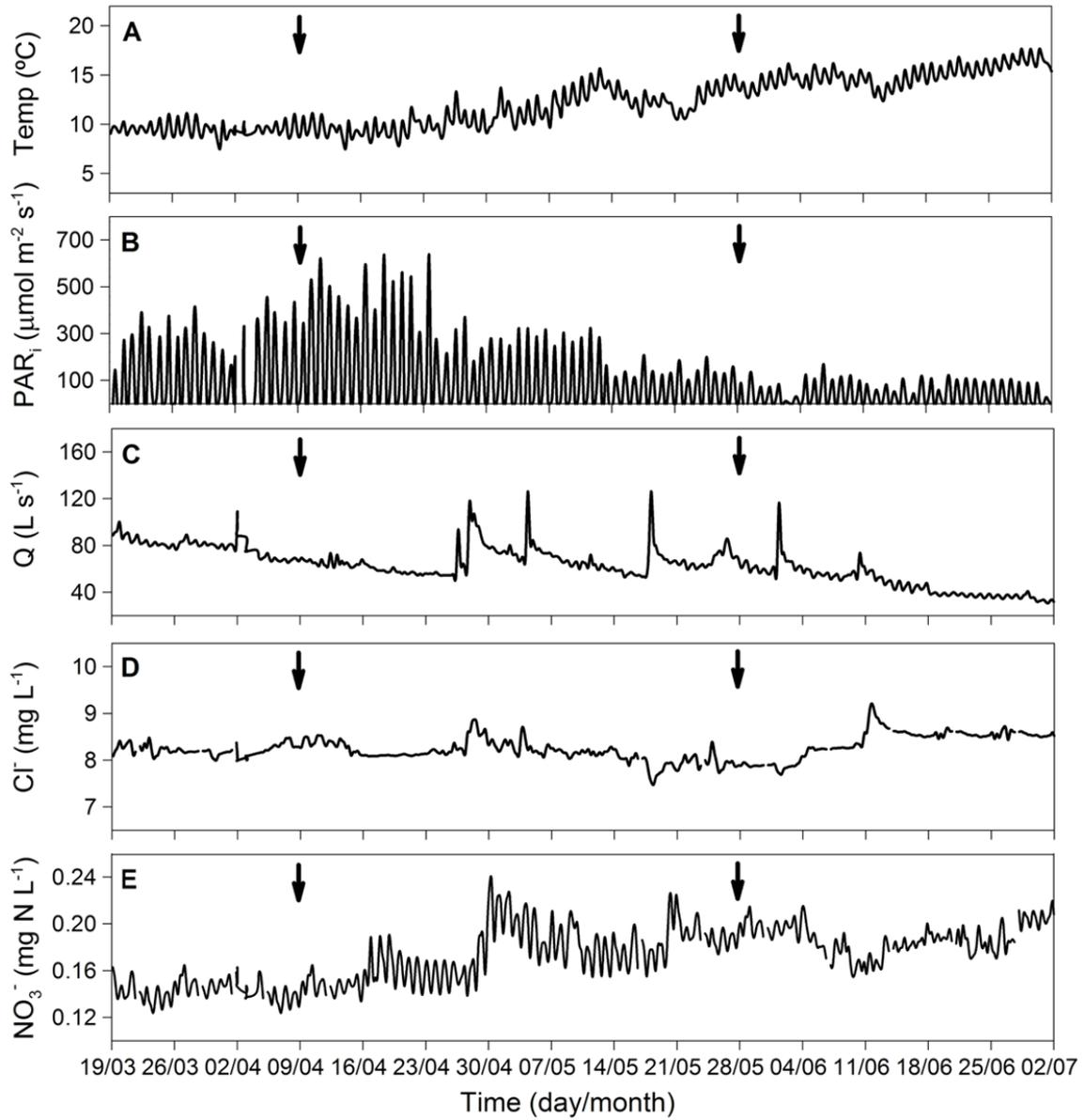


631



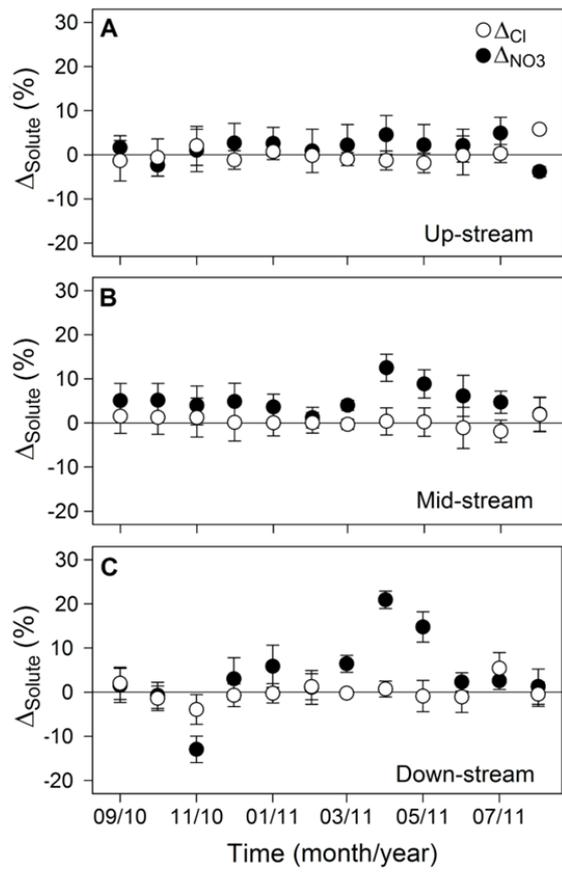
634

635 *Figure 3*



636

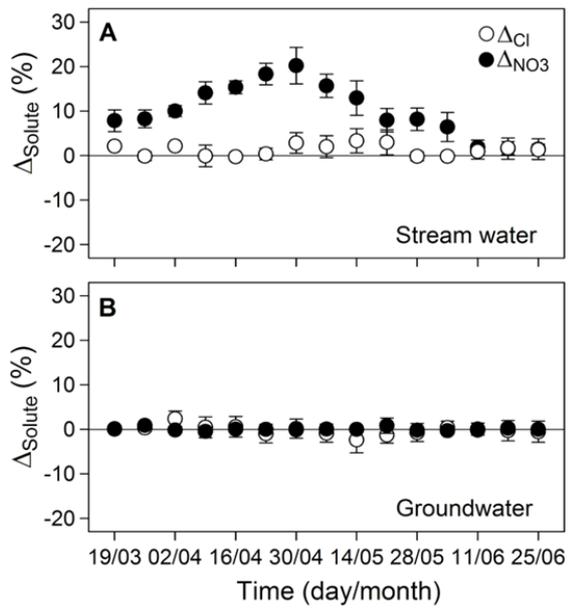
637 *Figure 4*



638

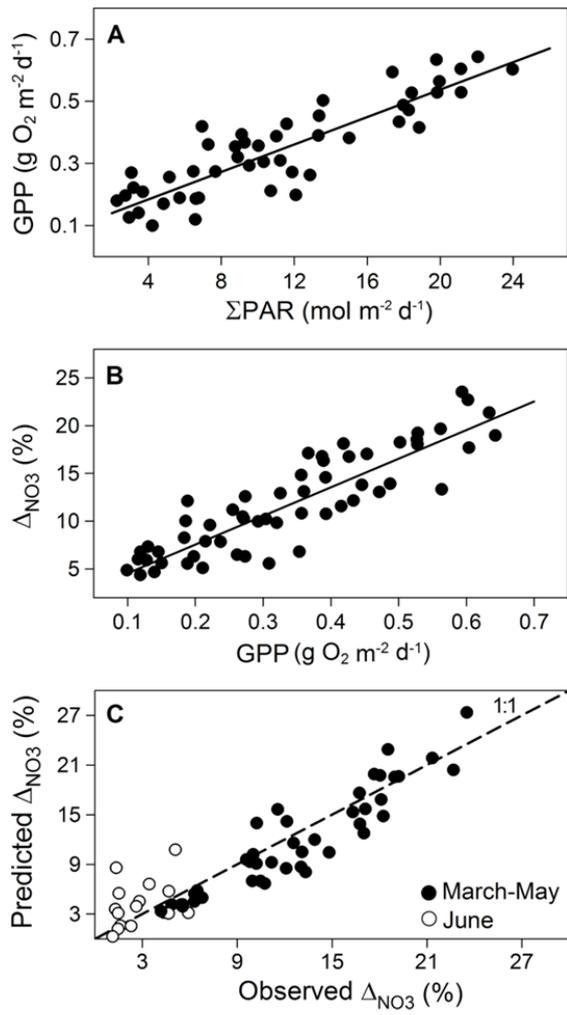
639

640 *Figure 5*



641

642 *Figure 6*



643

644

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

3 **Authors**

4 *Anna Lupon¹, Eugènia Martí², Francesc Sabater¹ Susana Bernal^{1,2}*

5 **Appendix A**

6 **Title: Contribution of riparian groundwater inputs to day-night variations in stream**
7 **nitrate concentration**

8 We considered the possibility that day-night fluctuations in riparian groundwater inputs suffice
9 to explain the observed diel variations in stream nitrate (NO_3^-) concentration during spring 2012
10 at the down-stream site. We used a mass balance approach to calculate midnight NO_3^-
11 concentrations based solely on hydrological mixing. For each day:

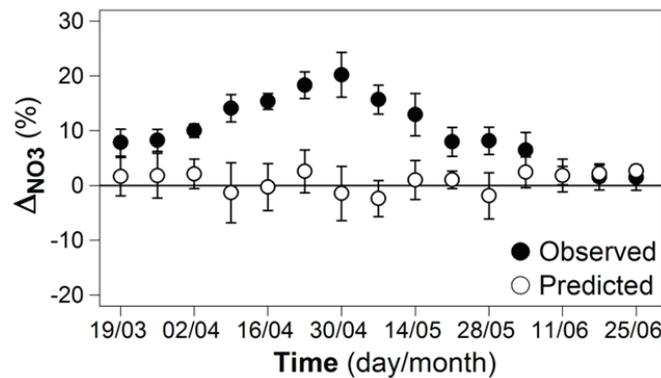
$$12 \quad [\text{NO}_3^-]_{\text{sw}(0\text{h})} = \frac{[\text{NO}_3^-]_{\text{sw}(12\text{h})} * Q_{\text{sw}(12\text{h})} + [\text{NO}_3^-]_{\text{gw}} * Q_{\text{sw}(0\text{h}-12\text{h})}}{Q_{\text{sw}(0\text{h})}}, \quad (\text{A.1})$$

13 where $[\text{NO}_3^-]_{\text{sw}}$ is stream NO_3^- concentration and $[\text{NO}_3^-]_{\text{gw}}$ is the average of riparian groundwater
14 NO_3^- concentration between midnight and noon (all in mg N L^{-1}). Q_{sw} is stream discharge and
15 $Q_{\text{sw}(0\text{h}-12\text{h})}$ is riparian groundwater input estimated as the difference in Q_{sw} between midnight and
16 noon (all in L s^{-1}). The subscripts (0h) and (12h) denote time of the day, midnight and noon
17 respectively. We calculated the relative difference between midnight NO_3^- concentrations
18 predicted from hydrological mixing and those observed at noon (Δ_{NO_3} , in %) (Eq. 1, main
19 manuscript). Moreover, we used a Wilcoxon paired rank sum test to examine whether

20 differences between NO_3^- concentrations observed at noon and those predicted for midnight were
21 statistically significant (Zar 2010).

22 During spring 2012, midnight stream NO_3^- concentration predicted from hydrological mixing
23 were similar to those observed at noon (for each week from March to June: $Z > Z_{0.05}$, $df = 6$, $p >$
24 0.1). The average Δ_{NO_3} calculated from predicted midnight NO_3^- concentrations was 0.6%
25 (Figure A1, white circles). This value was 20 fold lower than the Δ_{NO_3} obtained from observed
26 midnight and noon NO_3^- concentrations (13%) (Figure A1, black circles). Similar results were
27 obtained when using midnight rather than average riparian groundwater NO_3^- concentration.
28 These findings, together with the fact that no simultaneous diel variations in discharge, riparian
29 groundwater level and N concentrations were observed, support the idea that terrestrial processes
30 did not control diel variations in NO_3^- concentrations at the study site.

31 Figures



32
33 *Figure A1.* Temporal pattern of the relative difference between midnight and noon stream nitrate
34 concentrations (Δ_{NO_3}) during spring 2012 at the down-stream site. The Δ_{NO_3} is shown for
35 observed values and for values predicted from hydrological mixing (black and white circles,

36 respectively). Symbols are the median of Δ_{NO_3} for each week and whiskers denote the 25th and
37 75th percentiles. The black line indicates no differences between midnight and noon nitrate
38 concentrations.

39 **Refernces**

40 Zar, J. H. 2010. *Biostatistical analysis in* Prentice-Hall/Pearson, *editors*. Upper Saddle River, NJ.