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 ºC) and light availability was lower (4 vs. 9 mol m^{-2} 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

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.

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 km^2 catchment in

96   the Montseny Natural Park, NE Spain (41º50'N, 2º30'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 ±

103   6 ºC) was close to the long-term mean (12.1 ± 2.5 ºC, 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 ± 18.7 L/s at the up-stream site, and increased to 78.3 ± 52.9 and 89.4 ± 58.1 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 ±

119   0.09, 0.17 ± 0.07, and 0.19 ± 0.08 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 ± 1.34 mg/L at the up-stream site

123   to 8.06 ± 1.02 mg/L at the down-stream site. The riparian groundwater level (~ 2 m from the

7

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).

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 mg O_2 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 ºC) until laboratory analysis

161 (< 24h 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 salicilate-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 ºC) and

172 accumulated daily PAR (ΣPAR, in mol m^{-2} 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 μmol m^{-2} s^{-1}) was optimal

175 for photosynthetic activity. We considered T = 10 ºC as the threshold upon which

176 photoautotrophs are not temperature limited (DeNicola 1996). A value of $\Sigma PAR = 4$ mol m^{-2} 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 $PAR_i > 200$ μmol m^{-2} 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 $$\Delta_{solute} = \frac{[solute]_{0h} - [solute]_{12h}}{[solute]_{0h}} \times 100 \, , \tag{1}$$

186   where $[solute]_{0h}$ and $[solute]_{12h}$ are the solute concentration (in mg/L) at midnight and noon,

187   respectively. Values of $\Delta_{solute} \sim 0$ 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 $\Delta_{solute} > 0$ indicate higher solute

190   concentrations at night than at day time, whereas values of $\Delta_{solute} < 0$ 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 mg O_2 L^{-1} min^{-1}). We multiplied GPP and ER by the mean reach

223   depth (in m) to obtain areal estimates (in g O_2 m^{-2} 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 Δ_{NO3} 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}, mg N L^{-1} min^{-1}) from instantaneous GPP (mg O_2 L^{-1} min^{-1}). We assumed that

232   (i) the molar ratio for CO_2: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 ($[NO_3]'_t$, in mg N/L) as follows:

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

242   where $[NO_3]'_{t-1}$ is the predicted stream NO_3^- concentration (in mg N L^{-1}) at sampling time t-1,

243     $\overline{U_{GPP}}$ 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_{NO3}

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_{NO3} were

251   examined for evaluating the ability of the model to predict changes in Δ_{NO3} 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 ºC 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 $\Sigma PAR > 4$ mol m^{-2} 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 ± 2.1 ºC from mid-March to mid-April, and then

265   it increased to 15 ºC until the end of the study period in July (Figure 3a). Diel variations in

266   temperature remained small during spring 2012, being 1.5 ± 0.8 ºC 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_{NO3}

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%).

15

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_{NO3} 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^-

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

16

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 g O_2 m^{-2} d^{-1}) to mid-May

313   (0.64 g O_2 m^{-2} d^{-1}), and then decreased until June (0.07 g O_2 m^{-2} 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 Δ_{NO3} and daily ER (l.r., $R^2 = 0.01$, $p > 0.1$, $n = 44$),

317   while daily Δ_{NO3} 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 Δ_{NO3} (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 Δ_{NO3} 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_{NO3} would then

342 have been 0.6% instead of 13% (Appendix A). Conversely, the strong relationship and

343 synchronicity between daily GPP and Δ_{NO3} 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 (GPP = 5-20 g O_2 m^{-2} d^{-1}, GPP:ER ~ 1) compared to the

349 values measured in this study (GPP < 0.7 g O_2 m^{-2} d^{-1}, 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.

18

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 µg 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 g O_2 m^{-2} d^{-1}), though diel NO_3^- variations were 4-6 fold lower at Walker

360 Branch (10-20 vs.75-100 µg 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_{NO3} 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,

19

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_{NO3} 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 ~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, ~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_{NO3} (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} (~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

Tables

586 *Table 1:* Mean daily stream water temperature (T), daily photosynthetically active radiation

587 (ΣPAR), hours per day with $PAR_i > 200$ µmol m^{-2} s^{-1} (PAR_{200}), days with T > 10 ºC (T_{10}), and

588 days with $\Sigma PAR > 4$ mol m^{-2} 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, ΣPAR

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 (ºC)	ΣPAR (mol m^{-2}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

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 (ΣPAR) 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 = 10ºC) or light (ΣPAR = 4 mol

607   m^{-2} 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 25$^{\text{th}}$ and 75$^{\text{th}}$ 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 25$^{\text{th}}$ and 75$^{\text{th}}$ 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_{NO3}), 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_{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.

628   White circles in panel (c) indicated day-night variations in stream nitrate concentration in June.
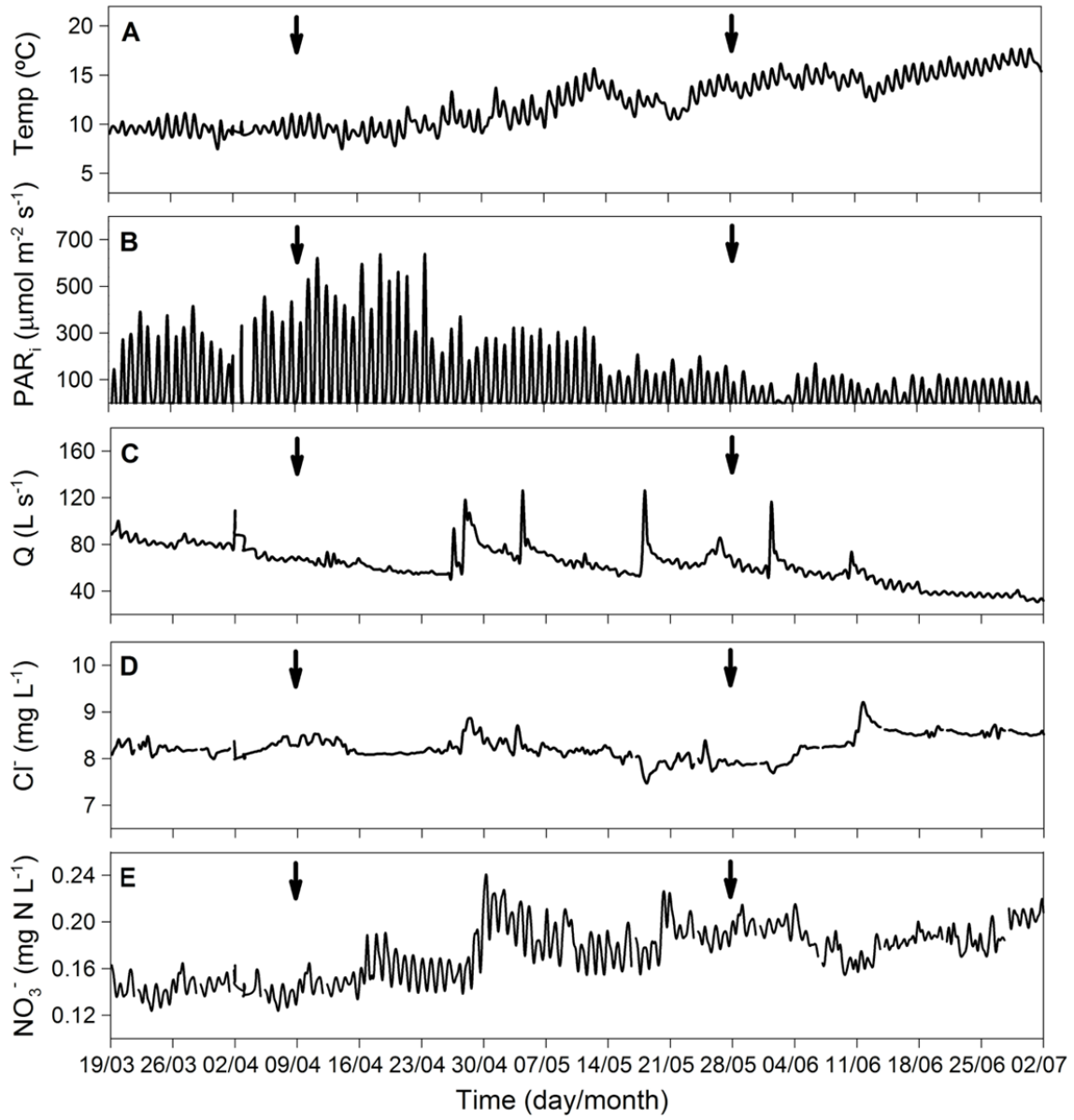
Figures

Figure 1

632   *Figure 2*

633
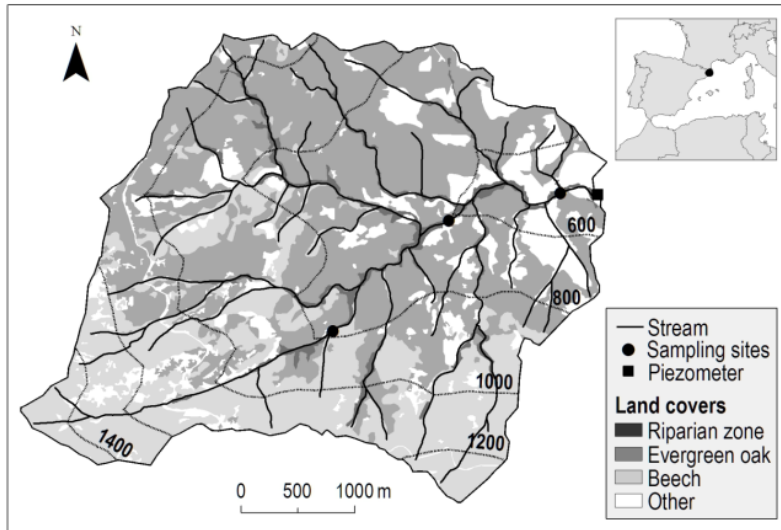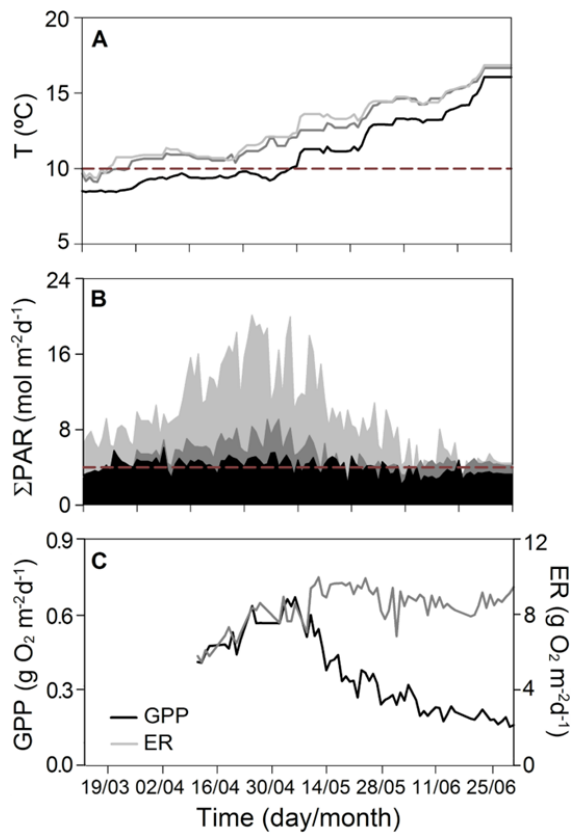
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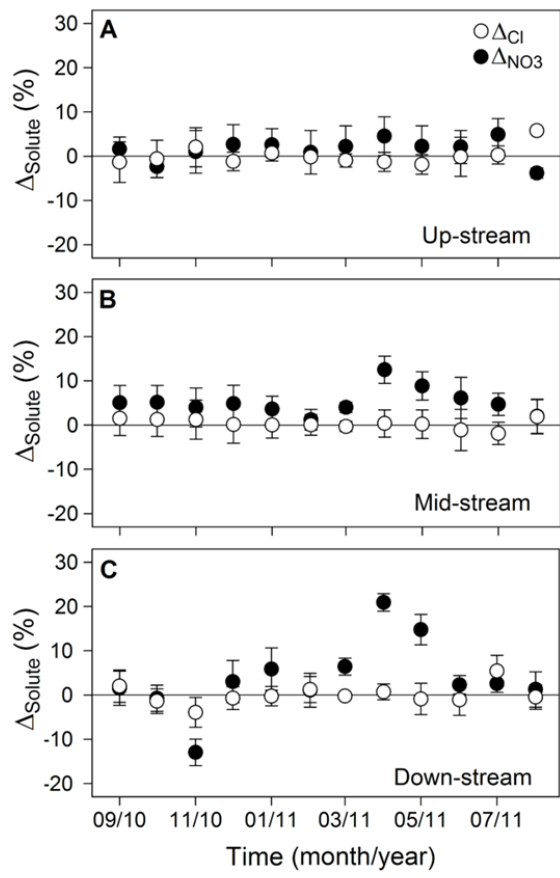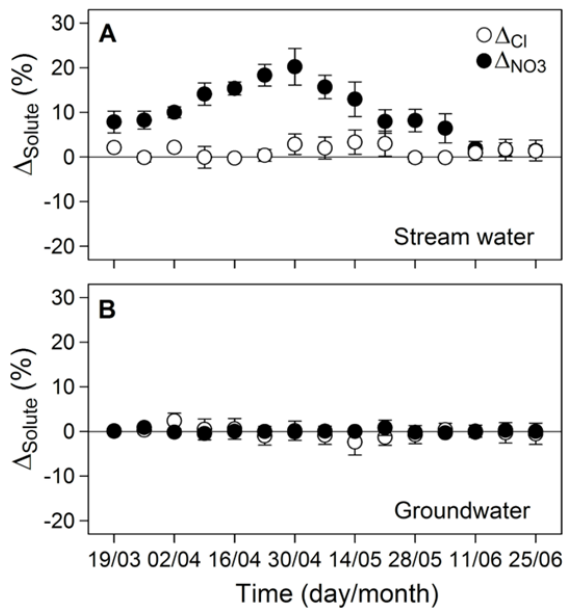
635   *Figure 3*

636

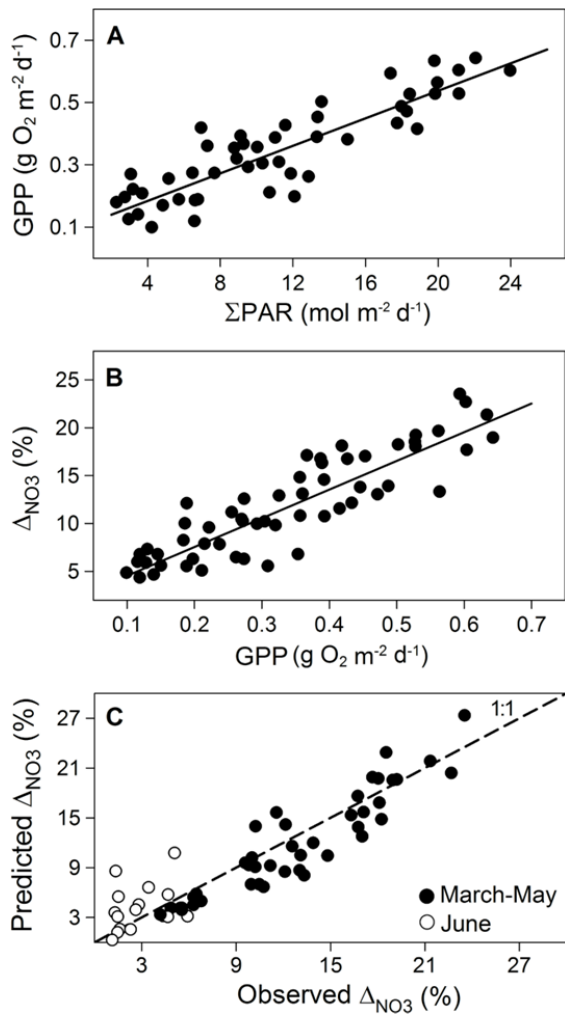637   *Figure 4*

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640   *Figure 5*

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642   *Figure 6*

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645

1   **Title: Green light: gross primary production influences seasonal stream N export by**

2   **controlling fine-scale N dynamics**

3   **Authors**

4   *Anna Lupon [1], Eugènia Martí [2], Francesc Sabater [1] 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   $$[NO_3]_{sw\,(0h)} = \frac{[NO_3]_{sw\,(12h)} * Q_{sw\,(12h)} + [NO_3]_{gw} * Q_{sw\,(0h-12h)}}{Q_{sw\,(0h)}},$$ (A.1)

13   where $[NO_3]_{sw}$ is stream NO_3^- concentration and $[NO_3]_{gw}$ 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   $Q_{sw\,(0h-12h)}$ 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_{NO3}, 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_{NO3} calculated from predicted midnight NO_3^- concentrations was 0.6%

25   (Figure A1, white circles). This value was 20 fold lower than the Δ_{NO3} 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**
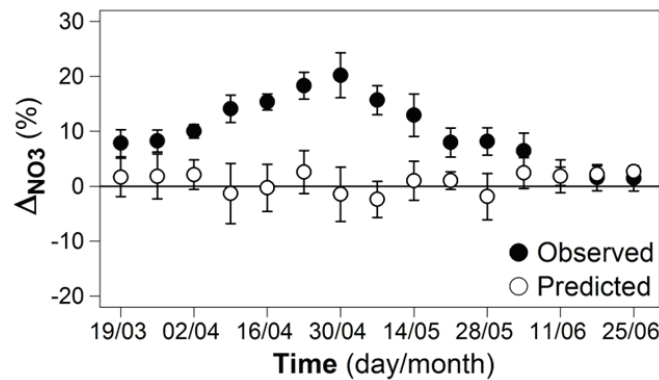
32

33   *Figure A1*. Temporal pattern of the relative difference between midnight and noon stream nitrate

34   concentrations (Δ_{NO3}) during spring 2012 at the down-stream site. The Δ_{NO3} 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_{NO3} 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.