1   LRH: A. Argerich et al.

2   RRH: Hydrological exchange and hyporheic biogeochemistry

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4   **Temporal variation of hydrological exchange and hyporheic biogeochemistry in a headwater**

5   **stream during autumn**

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29   **Abstract.** The hyporheic zone is of great interest for stream ecologists because of its role in

30   stream biogeochemical processing. Our study addresses the effects of leaf-litter inputs and

31   varying discharge on surface–hyporheic water exchange and their possible consequences for the

32   hyporheic zone biogeochemistry. Our study was conducted during autumn in Riera de Santa Fe

33   (northeastern Iberian Peninsula), a stream with a well developed deciduous riparian canopy. We

34   placed 15 wells spaced at 5-m intervals longitudinally down the study reach and measured

35   surface and hyporheic nutrient and dissolved O_2 (DO) concentrations on 23 sampling dates (15

36   during the leaffall period and 8 after a flood that washed out 65% of the accumulated leaf

37   biomass). We assessed changes in surface-water exchange and in hyporheic NH_4-N and soluble

38   reactive P (SRP) uptake via coinjection of a conservative tracer and nutrients. Compared to

39   surface water, hyporheic water had lower DO, higher SRP and NO_3-N concentrations, and

40   similar NH_4-N concentration. Hyporheic water had higher DO saturation ($p = 0.00$) and higher

41   NH_4-N concentration ($p = 0.00$) in downwelling than in upwelling wells, whereas SRP and NO_3-

42   N concentrations did not differ significantly between well types ($p > 0.05$). Hydrologic

43   connectivity was higher in downwelling than in upwelling wells and decreased with leaf-litter

44   accumulation in the stream channel and increased with stream discharge. Increased connectivity

45   after a flood reduced the difference in DO between surface and hyporheic compartments in

46   upwelling and downwelling wells and in NO_3-N in upwelling wells. NH_4-N and SRP uptake

47   responded differently to these changes. Hyporheic SRP uptake rate was controlled by hyporheic

48   SRP concentration, which did not vary with changes in connectivity, whereas NH_4-N uptake rate

49   was indirectly affected by changes in connectivity through the influence of connectivity on DO

50   availability. Last, although no NO_3-N was added during the solute injections, we observed an

51   increase in hyporheic NO_3-N that probably was caused by nitrification. Together these results

52 illustrate how the combination of stream hydrology and organic matter accumulation can dictate

53 seasonal changes in hyporheic biogeochemistry.

54

55 **Key words:** stream, nutrients, surface–subsurface interactions, hyporheic zone, leaf litter, flood,

56 oxygen, upwelling, downwelling.

57   In temperate streams with well developed riparian forests, massive leaf-litter inputs

58   during autumn constitute a seasonal event of high ecological relevance even when assessed at an

59   annual scale. Leaf-litter inputs fuel stream heterotrophic metabolism by providing energy sources

60   to biological communities (Fisher and Likens 1973, Meyer et al. 1998, Acuña et al. 2004) and

61   have been associated with increases in stream phosphate uptake efficiency (Mulholland et al.

62   1985). In addition, accumulation of leaf litter on the stream bed can modify channel hydraulic

63   properties dictated by the geomorphological setting of the stream channel. For instance, previous

64   studies demonstrated that leaf inputs increase water transient storage, and thus, water residence

65   time (Haggard and Storm 2003, Argerich et al. 2008). This physical effect of leaf-litter inputs

66   can have indirect implications for nutrient retention because higher transient storage and

67   residence time is expected to extend the contact between dissolved nutrients and stream

68   microbial communities and may result in higher nutrient uptake (Argerich et al. 2008). Thus, in

69   temperate streams, leaffall can influence nutrient retention through both biological (i.e.,

70   increased biological demand) and physical (i.e., increased water transient storage) mechanisms.

71   Massive accumulations of leaves on the stream bed also might modify exchange between surface

72   water and the hyporheic zone, with additional implications for stream nutrient retention.

73   The hyporheic zone is a key stream compartment for biogeochemical nutrient processing

74   (Mulholland and DeAngelis 2000), and it is considered a major component of the transient

75   storage zone (Harvey and Wagner 2000). Physical, chemical, and biological conditions within

76   the hyporheic zone can differ greatly from those in stream surface waters. Hyporheic conditions

77   support a broad variety of biogeochemical processes, such as nitrification (Jones et al. 1995a)

78   and denitrification (Baker at al. 2000). In general, hyporheic waters are limited in O_2 availability

79   and are less subject to temporal variation in temperature relative to surface water (Anderson

80 2005). In addition, hyporheic sediments are metabolically active and have higher water residence

81 times than surface waters, which increase interactions between solutes and biota, thus enhancing

82 nutrient uptake and transformation in hyporheic zones (Triska et al. 1989). The size of the

83 hyporheic zone, exchange fluxes with surface water, and water residence time in it are

84 determined by hydrodynamic conditions, which are a function of the stream geomorphology

85 (reviewed by Tonina and Buffington 2009, Boulton et al. 2010) and discharge conditions

86 (Tonina and Buffington 2007, Zarnetske et al. 2007).

87   The stream surface and the hyporheic zone are hydrologically linked at discrete regions

88 of water exchange where hyporheic water discharges vertically to the surface (upwelling zones)

89 and where surface water reenters the hyporheic zone (downwelling zones; Dent et al. 2000). The

90 direction of this linkage (upwelling vs downwelling) affects nutrient concentrations, biofilm

91 characteristics, and microbial abundances and processes of surface and hyporheic habitats

92 (Grimm and Fisher 1984, Triska et al. 1989, 1990, Hendricks 1993, Dent et al. 2001, Franken et

93 al. 2001). The strength of these linkages determines the influence that the hyporheic zone exerts

94 on surface stream functioning and vice versa (Dent et al. 2000). Hyporheic water input can

95 modulate stream metabolic activity via control on water temperatures over diel and annual cycles

96 (Loheide and Gorelick 2006) and altered river-water alkalinity in a braided gravel-bed river

97 (Rodgers et al. 2004). Hyporheic influences on surface biology include effects on

98 microorganisms and microbial processes through changes in nutrient and O_2 availability (Jones

99 et al. 1995b), the distribution and abundance of primary producers and invertebrates (Pepin and

100 Hauer 2002), and the distribution and abundance of spawning fish (Baxter and Hauer 2000).

101   Hydrological exchange between the surface stream and the hyporheic zone also

102 influences key stream ecosystem services, such as nutrient cycling (reviewed by Boulton et al.

103 2010, Mulholland and Webster 2010). Results of several studies (Triska et al. 1989, 1990, 1993a,

104 Duff and Triska 1990, Valett et al. 1996) showed a relationship between surface–hyporheic

105 water exchange and stream nutrient retention. For instance, Valett et al. (1996) coinjected Br (a

106 conservative tracer) and NO_3-N into 3 headwater streams with contrasting geology and hydraulic

107 conductivities and found that NO_3-N uptake increased with increasing surface–hyporheic water

108 exchange. Results of other studies showed that the rate of hydrological exchange determined

109 whether the hyporheic zone would act as an NO_3-N source (via nitrification) or sink (via

110 denitrification) through the control of NO_3-N supply and redox conditions in the hyporheic zone

111 (Triska et al. 1989, 1990, 1993a, Duff and Triska 1990, Zarnetske et al. 2011). How these

112 responses vary over time and what factors influence them are less well understood.

113   In summary, processes occurring in the hyporheic zone are highly relevant to stream

114 biogeochemical processing (Triska et al. 1989, McKnight et al. 2004) and metabolism (Grimm

115 and Fisher 1984, Mulholland et al. 1997, Fellows et al. 2001) at a whole-reach scale. Therefore,

116 the effects of in-channel accumulation of leaves on the stream bed may affect surface–hyporheic

117 water exchange, which will have implications for nutrient biogeochemistry in the hyporheic zone

118 and for whole-reach nutrient uptake. In part, changes in whole-reach nutrient uptake will be

119 caused by a shift in the relative dominance of different stream compartments contributing to

120 whole-reach water transient storage (e.g., in-channel vs hyporheic transient storage). However,

121 the effects of leaf-litter inputs on the surface–hyporheic water exchange have been seldom

122 studied. In general, published studies for changes in hydraulic variables in relation to leaf-litter

123 accumulation (Haggard and Storm 2003, Argerich et al. 2008) or coarse organic debris

124 (Nakamura and Swanson 1993, Lautz et al. 2006) were based on models that combined surface

125 and hyporheic transient storage zones. Consequently, water exchange coefficients reported in

126   those studies were not explicit measures of changes in surface–hyporheic water exchange.

127   We examined the effect of leaf-litter accumulation and variation in discharge on the

128   vertical hydrological exchange between the surface stream and the hyporheic zone and the

129   subsequent effects that variation in this exchange might have on hyporheic biogeochemistry. We

130   hypothesized that leaf litter accumulated on the stream bed during baseflow conditions would

131   decrease surface–hyporheic water exchange because of a clogging effect, and that clogging

132   would cause an increase in the chemical differences between the surface stream and the

133   hyporheic zone. On the other hand, sudden increases in discharge would reduce the amount of

134   leaf litter accumulated in the stream channel via scour and would increase surface–hyporheic

135   water exchange and subsequently induce a decrease in the chemical differences between the

136   surface stream and the hyporheic zone.

137

138   **Methods**

139   *Study site and sampling strategy*

140   Our study was conducted in Riera de Santa Fe, a 2nd-order tributary of La Tordera stream

141   in the natural preserved area of Parc Natural del Montseny (northeastern Iberian Peninsula; lat

142   41º46'34''N, long 2º27'40''E; Fig. 1). The study reach drains a 2.15-km^2 catchment that is

143   dominated by siliceous geology. The catchment is mostly forested by European beech (*Fagus*

144   *sylvatica*) and European Silver Fir (*Abies alba*) at higher elevations. Mean annual precipitation is

145   1200 mm, most of which occurs during autumn and spring. During the coldest months

146   (December and January), snow events occur regularly. Stream flow is permanent, with a median

147   discharge of 14.9 L/s (value obtained from biweekly data for 2004–2007). However, during storm

148   events (mainly in autumn) stream discharge can increase by >2 orders of magnitude.

149   We selected a morphologically homogeneous 70-m reach characterized by alternation of

150   pools and riffles and a slope of 0.072 m/m. The reach substrata are composed of cobbles (47.3%),

151   boulders (25.4%), and pebbles (21.1%) with patches of gravel (5.2%) and sand (1%). The tree

152   canopy along the reach is well developed and is dominated by European beech with some stems

153   of elder bush (*Sambucus nigra*). Previous studies in this reach have shown that metabolism is

154   dominated by heterotrophic activity (Haggerty et al. 2009, von Schiller et al. 2009) and that

155   demand for NH_4-N is higher than the demand for P (Argerich et al. 2008, von Schiller et al.

156   2008). Existing data on the molar ratio between dissolved inorganic N and P concentrations

157   indicate that this reach may be N-limited during summer and early autumn (von Schiller et al.

158   2007, Argerich et al. 2008).

159   We defined 5 surface sampling sites and 15 channel transects evenly distributed along the

160   reach (Fig. 1). In each transect, we placed a well at the thalweg of the stream channel. Wells

161   consisted of 2-cm-internal-diameter polyvinyl chloride (PVC) pipe screened across the last 15

162   cm. We set each well to a depth of 25 cm below bed surface. Wells remained in the reach during

163   the study period and were capped between sampling dates. We began the study during the

164   leaffall period of 2004 (1 October–30 November) and continued until the end of December to

165   examine the effects of a major flood that occurred during the 1st wk of December. This flood

166   washed out 65% of the leaf biomass that had accumulated in the reach. In total, 23 samplings

167   were completed during the study period (October–December 2004), 15 during the leaffall period

168   and 8 during the postflood period.

169

170   *Field sampling*

171   On each sampling date, we measured the channel wetted width (w, m) at each transect to

172   calculate the surface area of the reach. We visually estimated the percentage of the area between

173   transects covered by leaves and the percentage occupied by pools and riffles, which were

174   categorized according to flow type with River Habitat Survey guidelines (EA 2003). We used

175   these estimated percentages to calculate the total surface area of the reach associated with each

176   habitat type (i.e., pool or riffle) and leaf coverage. Leaf standing stock in the reach was

177   determined from twelve 20×20-cm^2 randomly selected samples. Half of the samples were

178   collected in pools and ½ in riffles to account for differences in leaf accumulation patterns

179   associated with each habitat type. Leaf samples were dried at 60ºC and weighed to determine dry

180   mass per unit area (g DM/m), which was then scaled to kg DM/reach.

181   We conducted 12 conservative-tracer (NaCl) injections to estimate surface–hyporheic

182   water exchange. During 8 of these 12 injections, we also added NH_4-N (as $ClNH_4$), and PO_4-P

183   (as $Na(H_2PO_4)\cdot2H_2O$) to estimate nutrient uptake in the hyporheic zone. On average, nutrient

184   injections increased hyporheic concentrations 15 ± 3 µg NH_4-N/L and 17 ± 4 µg PO_4-P/L above

185   background, and surface concentrations 104 ± 13 µg NH_4-N/L and 84 ± 15 µg PO_4-P/L above

186   background. Additions began at ~1100 h and lasted until conductivity reached plateau at the

187   bottom of the reach indicating complete solute mixing and steady-state conditions across the

188   reach (2–4 h). We used a conductivity meter (WTW model LF 340; WTW, Weilheim, Germany)

189   connected to a data logger (model CR510, Campbell Scientific, Logan, Utah) to record

190   conductivity at the bottom of the reach automatically every 5 s. We used conductivity to

191   calculate discharge (Q, L/s) by a tracer mass-balance approach (Shaw 1994).

192   On each sampling date, we collected water samples for analysis of nutrient

193   concentrations and Cl^- from each well and from each surface sampling site before 1100 h (3

194   replicates). When we conducted tracer injections, we repeated water sampling just before

195   stopping the injection (5 replicates). All water samples were collected with 100-mL syringes,

196   immediately filtered through Whatman[®] GF/F glass-fiber filters (0.7-µm pore diameter), and

197   refrigerated until analysis. We analyzed NH_4-N, NO_3-N, and soluble reactive P (SRP)

198   concentrations by standard colorimetric methods (APHA 1998) with a Bran+Luebbe TRAACS

199   2000 (Norderstedt, Germany) autoanalyzer for NO_3-N and SRP and a Skalar San[+] (Breda, The

200   Netherlands) autoanalyzer for NH_4-N. Cl^- concentration was analyzed using an ion

201   chromatograph (Metrohm Compact 761, column 6.1006.520 Metrosep A Supp 5; Herisau

202   Switzerland). On 18 sampling dates, dissolved O_2 (DO) concentration and temperature also were

203   measured in each well and at the adjacent surface-water location with a WTW 340i portable O_2

204   meter. Percent DO saturation was estimated using DO and temperature data and a standard

205   altitude–air-pressure algorithm to correct for site altitude.

206

207   *Characterization of surface–hyporheic water exchange*

208   We assessed the direction of water exchange between the stream surface and the

209   hyporheic zone in each well by measuring the vertical hydraulic gradient (VHG). VHG was

210   calculated following Dahm and Valett (1996).

$$VHG = \Delta h / \Delta l$$

211

212   where Δh is the difference measured between the hydraulic head inside and outside the well (the

213   hydraulic head differential, cm) and Δl is the depth from the substratum surface to the first

214   opening in the well sidewall (the elevation head differential, cm). Thus, VHG is a unitless

215   measure that is positive under upwelling conditions (i.e., hyporheic discharge) and negative

216   under downwelling conditions (i.e., hyporheic recharge). We measured VHG twice, once during

217   the leaffall period (11 November) and again 1 mo after finishing the study (3 February). In

218   November, VHG values were positive in 7 wells, negative in 7 wells, and ~0 in 1 well. In

219   February, VHG values were positive in 9 wells, negative in 5 wells, and ~0 in 1 well. For data

220   analysis, we considered only wells that had consistent upwelling or downwelling on both

221   sampling dates: 5 in upwelling and 4 in downwelling areas (Fig. 1).

222   We measured the magnitude of water exchange between stream surface and the

223   hyporheic zone (*connectivity*) for each well and sampling date by calculating the percentage of

224   surface water that exchanged with water in the well. We measured connectivity with data from

225   the Cl⁻-tracer injections by comparing the increase in Cl⁻ concentration between background and

226   plateau conditions in wells with increases in Cl⁻ concentration at the adjacent surface-sampling

227   point with the equation proposed by Wondzell (2006):

228   $$connectivity = \left(\frac{Cl^h_{plat} - Cl^h_{bckg}}{Cl^s_{plat} - Cl^s_{bckg}} \right) \times 100$$

   [1]

229   where Cl is Cl⁻ concentration, subscripts *plat* and *bckg* indicate concentrations at plateau and at

230   background, respectively, and superscripts *h* and *s* indicate hyporheic water (in the well) and

231   water in the stream surface, respectively.

232

233   *Characterization of hyporheic biogeochemistry*

234   We assessed the effects of leaf-litter accumulation and variation in Q on the hyporheic

235   biogeochemistry with 2 approaches. First, we examined temporal variation in the ratio between

236   hyporheic and surface nutrient concentrations and DO saturation. Values of the ratio <1 indicated

237   that surface water was nutrient- or DO-enriched compared to hyporheic water. Values >1

238   indicated that hyporheic water was nutrient- or DO-enriched compared to surface water. Second,

239   we examined the temporal variation in apparent nutrient gain or loss in the wells (hereafter

240   referred to as hyporheic nutrient regeneration and nutrient uptake, respectively) based on data

241   from nutrient and Cl⁻ injections. We calculated nutrient uptake or regeneration for each well on 9

242   sampling dates (4 dates during the leaffall period and 5 dates during the postflood period). We

243   made calculations by comparing observed nutrient concentrations in wells when surface plateau

244   was achieved to those predicted assuming hydrological exchange only (i.e., assuming that the

245   nutrients were behaving like the conservative tracer).

246   We calculated predicted nutrient concentrations with the equation:

247
$$C^h_{pred,plat} = \left(C^s_{obs,plat} - C^s_{obs,bckg} \right) \times \left(\frac{connectivity}{100} \right) + C^h_{obs,bckg} \quad [2]$$

248   where C is nutrient concentration; superscripts h and s refer to water in the well and in the

249   surface stream, respectively; subscripts $pred$ and obs refer to predicted and observed values,

250   respectively; subscript $plat$ refers to surface plateau conditions; subscript $bckg$ refers to

251   background conditions; and connectivity is based on Cl⁻ concentration using Eq. 1. We

252   calculated hyporheic nutrient uptake or regeneration in each well as the ratio between observed

253   plateau nutrient concentrations and predicted plateau nutrient concentrations. Values <1 indicate

254   nutrient uptake, whereas values >1 indicate nutrient regeneration.

255

256   *Statistical analysis*

257   We examined the effect of leaf accumulation and Q on surface–hyporheic water

258   exchange and the implications for hyporheic biogeochemistry separately for upwelling and

259   downwelling wells. We used a linear-regression approach to assess the relationship between

260   connectivity and leaf-litter biomass and Q. We used the average value of connectivity on each

261   sampling date because we had only 1 value of Q and leaf-litter biomass per sampling date.

262   We used a mixed-model analysis of variance to test if nutrient and DO concentrations in

263   surface water during the leaffall and the postflood periods were significantly different from those

264   in hyporheic water. We treated sampling date and sampling site as random-effect factors and

265   water compartment (surface or hyporheic water) and period (leaffall or postflood) as fixed-effect

266   factors. We used a mixed-model analysis of variance to test if nutrient and DO concentrations in

267   upwelling wells during the leaffall and postflood periods differed significantly from those in

268   downwelling wells. In this case, we treated sampling date and sampled well as random-effect

269   factors and type of well (upwelling or downwelling) and period (leaffall or postflood) as fixed-

270   effect factors. We assessed the influence of connectivity on surface–hyporheic differences in DO

271   and nutrient concentrations and the influence of connectivity and hyporheic nutrient

272   concentrations on hyporheic nutrient uptake or regeneration with a linear-regression analysis of

273   the data from each well on each sampling date on which connectivity and the dependent

274   variables were measured simultaneously (12 sampling dates for nutrient concentrations, 8

275   sampling dates for DO measurements).

276   We log(x)-transformed nutrient and DO concentrations and arcsin($\sqrt{x}$)-transformed

277   connectivity and hyporheic nutrient uptake and regeneration to meet assumptions of normality

278   prior to statistical analysis. We ran statistical analyses in SPSS for Windows (version 17.0;

279   SPSS, Chicago, Illinois), and we set levels of significance at $\alpha = 0.05$ for all tests.

280

281   **Results**

282   *Temporal variation of physical and chemical parameters in surface and hyporheic water*

283   Surface water temperature declined steadily from 12ºC to 3ºC during the study period.

284   During the leaffall period (7 October–2 December), leaves gradually accumulated in the stream

285   channel (from 4.0 to 163.4 kg DM/reach), the wetted area of the reach gradually increased (from

286   156 m^2 to 260 m^2; Table 1), and Q remained relatively constant at ~7 L/s, with the exception of a

287   slight increase (18.5 L/s) between 25 October and 1 November after a small episode of

288   precipitation (Fig. 2). On 10 December, a large storm increased Q 19.7× (from ~6 to 119.5 L/s;

289   Fig. 2, Table 1). After the storm, Q receded rapidly, but at the end of the study, Q was still higher

290   than before the storm. The wetted area of the reach increased up to 323 m^2 during the flood and

291   gradually decreased after the flood to 239 m^2 by the end of the study period (Table 1). As a

292   consequence of the flood, 62.6% of the leaf benthic standing stock was removed from the reach,

293   and standing stock decreased to 61.2 kg DM/reach. Two weeks after the flood, leaf benthic

294   standing stock in the channel had recovered to preflood levels.

295   Average surface DO saturation per sampling date ranged from 70.4 to 85.9% during the

296   leaffall period, decreased to 51.8% during the flood, and gradually increased after the flood to

297   reach the highest values by the end of the study period (Fig. 3A). Average surface DO saturation

298   was negatively correlated with Q ($r = -0.53$, $n = 18$, $p = 0.02$) and showed no relationship to

299   leaf-litter biomass. Average surface SRP concentration per sampling date ranged from 9.1 to

300   34.9 µg P/L during the leaffall period and tended to decrease except for an increase in between

301   21 October and 2 November. SRP concentration decreased after the flood and then gradually

302   increased to recover preflood values 5 d after the flood (Fig. 3B). Average surface NH_4-N

303   concentration per sampling date ranged from 2.4 to 25.4 µg N/L and did not show any clear

304   temporal pattern (mean ± SE = 8.1 ± 0.9 µg N/L; Fig. 3C). Among the analyzed nutrients,

305   average surface NO_3-N concentration per sampling date had the largest temporal variation. NO_3-

306   N concentration decreased from 124.6 to 38.8 µg N/L during leaffall, increased sharply after the

307   flood (to 249.8 µg N/L), and decreased again to preleaffall values by the end of the study (Fig.

308   3D). Surface SRP concentration was negatively correlated with leaf-litter biomass ($r = -0.56$, $p <$

309   0.01, $n = 23$), and surface NO_3-N concentration was positively correlated with leaf-litter biomass

310   ($r = 0.47$, $p = 0.02$, $n = 23$) and Q ($r = 0.83$, $p = 0.00$, $n = 23$). No correlations were observed

311   between NH_4-N concentration and leaf litter or Q or among nutrient concentrations.

312   Temporal variation in DO and nutrient concentrations in surface water was similar to that

313   in hyporheic water (Fig. 3A–D). Average hyporheic DO and NO_3-N concentrations per sampling

314   date were highly correlated with surface concentrations ($r = 0.98$, $p = 0.00$, $n = 23$ for DO; $r =$

315   0.97, $p = 0.00$, $n = 23$ for NO_3-N), whereas hyporheic NH_4-N and SRP concentrations were

316   moderately correlated with surface values ($r = 0.52$, $p < 0.01$, $n = 23$ for NH_4-N; $r = 0.85$, $p = 0.00$, n

317    $= 23$ for SRP). Nevertheless, compared to surface water, hyporheic water presented lower DO

318   saturation ($67.5 \pm 1.1\%$ vs $82.1 \pm 1.0\%$ in surface water), higher SRP concentration (19.7 ± 0.9 µg

319   P/L vs 14.3 ± 0.6 µg P/L in surface water), higher NO_3-N concentration (107.5 ± 3.3 µg N/L vs

320   93.6 ± 3.2 µg N/L in surface water), and similar NH_4-N concentration (8.4 ± 0.4 vs 8.1 ± 0.3 in

321   surface water; Table 2). Hyporheic SRP concentration was negatively correlated with leaf-litter

322   biomass ($r = -0.58$, $p = 0.00$, $n = 23$), and hyporheic NO_3-N concentration was positively

323   correlated with Q ($r = 0.80$, $p = 0.00$, $n = 23$). No correlations were observed between NH_4-N and

324   DO concentrations and leaf litter or Q or among hyporheic nutrients and DO concentrations.

325   Hyporheic water had higher DO saturation and NH_4-N concentration ($70.6 \pm 1.5\%$, $8.9 \pm$

326   0.5 µg N/L, respectively) at downwelling wells than at upwelling wells ($65.0 \pm 1.6\%$, 7.9 ± 0.5 µg

327   N/L, respectively), whereas no significant differences between well types were observed for SRP

328   and NO_3-N concentrations (Table 3).

329

330   *Effects of leaf-litter accumulation and discharge on surface–hyporheic water exchange*

331   Cl^- from tracer injections reached all wells on all sampling dates during the duration of the

16

332   injection (2–4 h), and connectivity ranged between 2 and 100%. During the leaffall period,

333   connectivity tended to decrease in upwelling wells, whereas in downwelling wells, it decreased

334   during the first 2 wk of the experiment, increased after the 1st episode of precipitation, and

335   tended to decrease again until the end of the leaffall period (Fig. 4). After the flood, connectivity

336   increased by an average of 2.6× in upwelling wells and of 1.8× in downwelling wells. As the

337   flood receded and leaf litter re-accumulated in the channel, connectivity decreased in both

338   upwelling and downwelling wells, but by the end of the study period, it remained 1.5× higher

339   than before the flood (Fig. 4).

340   On average, connectivity was lower during the leaffall period (51.9 ± 3.1%) than after the

341   flood (66.7 ± 3.7%; Table 3). In general, downwelling wells showed higher connectivity than

342   upwelling wells (Fig. 4), but those differences were significant only during the leaffall period

343   (57.7 ± 3.2% in downwelling wells vs 46.1 ± 4.3% in upwelling wells; Table 3).

344   Temporal variability in hydrologic connectivity was related to variability in both Q and

345   leaf-litter accumulation. Connectivity was negatively related to the accumulation of leaf litter

346   and positively related to increasing Q in both upwelling wells ($R^2 = 0.65$, df = 11, $p = 0.01$;

347   Connectivity = 40.9log[Q] – 9.0log[leaf-litter biomass] + 55.3) and downwelling wells ($R^2 =$

348   0.76, df = 11, $p = 0.00$; Connectivity = 24.7log[Q] – 7.9log[leaf-litter biomass] + 71.7). The

349   standardized coefficients for log(Q) and log(leaf-litter biomass) were 0.92 and –0.29 in

350   upwelling wells, and 1.03 and –0.47 in downwelling wells, indicating that Q was the main factor

351   explaining variation in connectivity in both types of wells and that the effects of leaf litter were

352   more relevant for downwelling wells than for upwelling wells.

353

354   *Influence of connectivity on hyporheic chemistry*

355   Hyporheic and surface DO became more similar with higher connectivity. The ratio

356   between hyporheic and surface DO saturation increased with increasing connectivity in both

357   upwelling ($R^2 = 0.63$, $n = 39$, $p = 0.00$) and downwelling wells ($R^2 = 0.46$, $n = 32$, $p = 0.00$; Fig.

358   5A). Differences between hyporheic and surface SRP and NH_4-N were not related to

359   connectivity (Fig. 5B, C), but differences between hyporheic and surface NO_3-N decreased with

360   increasing connectivity in upwelling wells. The ratio between hyporheic and surface NO_3-N

361   concentration in upwelling wells was >1 at lower connectivity and tended to be ~1 at higher

362   connectivity ($R^2 = 0.20$, $n = 59$, $p = 0.00$; Fig. 5D).

363   Data from nutrient injections showed hyporheic SRP and NH_4-N uptake (i.e., values of

364   the ratio between observed and predicted concentrations <1) on all sampling dates except for 4

365   wells on 23 December that showed values for SRP between 1.2 and 1.4. On average the

366   observed/predicted ratio for NH_4-N was 2× lower than that for SRP (i.e., the hyporheic zone was

367   2× more effective in taking up NH_4-N than SRP). In addition, no significant differences in

368   nutrient uptake were found between upwelling and downwelling wells (Table 3).

369   SRP uptake in the hyporheic zone was greater during the leaffall period ($SRP_{obs}/SRP_{pred} =$

370   0.46 ± 0.05) than during the postflood period ($SRP_{obs}/SRP_{pred} = 0.65 \pm 0.03$; Table 3). Temporal

371   variation in hyporheic SRP uptake was not significantly related to variation in connectivity or DO,

372   but it increased with increasing hyporheic SRP concentration in both upwelling ($R^2 = 0.19$,

373    $SRP_{obs}/SRP_{pred} = 0.72\exp[-0.01 \times SRP_h]$, $p = 0.00$, $n = 44$) and downwelling wells ($R^2 = 0.36$,

374    $SRP_{obs}/SRP_{pred} = 0.73\exp[-0.02 \times SRP_h]$, $p = 0.00$, $n = 36$; Fig. 6A).

375   NH_4-N uptake in the hyporheic zone was similar between the leaffall and postflood

376   periods (Table 3). Temporal variation in hyporheic NH_4-N uptake was not significantly related to

377   variation in connectivity, but it increased with higher hyporheic DO saturation in upwelling ($R^2 =$

378   0.21, NH_4-N_{obs}/NH_4-N_{pred} = 2.12 – 0.41ln(DO_h), p = 0.01, n = 29) and downwelling wells (R^2 =

379   0.42, NH_4-N_{obs}/NH_4-N_{pred} = 3.13 – 0.65ln(DO_h), p = 0.00, n = 24; Fig. 6B) and with higher

380   background NO_3-N concentrations in downwelling wells (R^2 = 0.26, NH_4-N_{obs}/NH_4-N_{pred} = 0.51

381   + 0.002(NO_3-N_h), p = 0.00, n = 36).

382   Last, no NO_3-N was added during the solute injections, but we observed an increase in

383   background concentration of NO_3-N in some of the wells during the injections. This increase was

384   higher in upwelling (25.03 ± 4.37 μg N/L) than in downwelling wells (17.09 ± 4.21 μg N/L;

385   Table 3) and was significant during the postflood period (35.02 ± 4.61 μg N/L) but not during the

386   leaffall period (3.66 ± 91 μg N/L; Table 3). The increase in NO_3-N concentration was not related

387   to any of the independent variables considered, but increases >25 μg N/L were positively related to

388   hyporheic DO saturation in upwelling wells (R^2 = 0.35, ΔNO_3-N_h = 0.71$(DO_h)^{1.1}$, p < 0.01, n = 19;

389   Fig. 7A) and to the amount of NH_4-N predicted at wells if no reaction occurred in both upwelling

390   (R^2 = 0.36, ΔNO_3-N_h = 40.45 + 0.60(NH_4-N_{pred}), p = 0.01, n = 16) and downwelling wells (R^2 =

391   0.63, ΔNO_3-N_h = 25.58 + 0.73(NH_4-N_{pred}), p = 0.00, n = 13; Fig. 7B).

392

393   **Discussion**

394   *Effects of leaf-litter accumulation and discharge on surface–hyporheic water exchange*

395   Leaf-litter inputs during autumn in Riera de Santa Fe modify channel morphology and

396   habitat availability of surface stream, increasing the cross-sectional area of the channel and the

397   number and extension of pools because of generation of small leaf-pack debris dams (Table 1;

398   Argerich et al. 2008). Results from our study show that leaf-litter inputs also can affect the

399   hyporheic zone of the stream by reducing the vertical hydrological exchange between surface

400   stream and the hyporheic zone.

401 During our study, leaffall inputs occurred over a period without intense precipitation

402 events and relatively constant baseflow Q, which facilitated gradual accumulation of leaves in

403 the channel of the study reach. During this period, the percentage of surface water present in the

404 wells tended to decrease as leaf litter accumulated in the stream channel, a result indicating a

405 decrease in the hydrological connectivity between the surface stream and the hyporheic zone,

406 especially at downwelling locations. We are not sure if the hyporheic zone had reached plateau

407 conditions when we sampled the wells, so the observed decrease could indicate either that less

408 surface water reached the well (if we sampled at hyporheic plateau conditions) or that water

409 traveled to the well more slowly (if we sampled at the rising limb of the hyporheic breakthrough

410 curve). In any case, our results reflect a weaker hydrologic connection between the surface

411 stream and the hyporheic zone at short time scales (i.e., a few h) when large amounts of leaves

412 accumulate in the stream channel. However, the leaf-litter effect was counterbalanced by the

413 sudden increase in Q caused by a flood. Under these flood conditions, surface–hyporheic water

414 exchange increased considerably (hydrologic connectivity values increased up to 85%) in both

415 upwelling and downwelling locations. The flood removed a high proportion of the leaves

416 accumulated in the reach, so these results reaffirm the relevance of leaf-litter inputs for surface–

417 hyporheic hydrological connections during autumn. However, our results also indicate that

418 variation in Q is an important factor influencing hydrological connections either directly or

419 through leaf removal. Butturini and Sabater (1999) also found that the percentage of surface

420 water present in wells at a similar depth to those used in our study varied as a function of Q

421 (from 100% at high Q to 33–46% at baseflow Q) in Riera Major (northeastern Barcelona, Spain).

422 Other investigators have found similar effects on Q when measuring exchange between free-

423 flowing water and transient storage at whole-reach scale (Hart et al. 1999, Zarnetske et al. 2007),

424   but others reported the opposite effect (Martí et al. 1997). Lack of common effects among

425   studies could be explained in part by the influence of other factors besides Q, such as channel

426   morphology, sediment type, or leaf accumulation as we showed in our study. However,

427   assessment of the relevance of the 2 factors separately is difficult because accumulation of leaves

428   over the study period also depends on Q. Our results indicate the relevance of leaf-litter inputs as

429   an additional factor to be considered to understand solute transport and hydrologic exchange

430   among stream compartments in streams with well-forested riparian zones.

431   In a previous paper, Argerich et al. (2008) examined the variation in water transient

432   storage size and exchange coefficient in this stream reach during the same study period by

433   applying an advection dispersion–transient storage zone model (OTIS; Runkel 1998) to the

434   surface data. They observed that the coefficients of water exchange between the channel and the

435   transient storage zones decreased during the leaffall period and increased after the flood (Table

436   1). Results from our study further support these findings and provide a possible mechanistic

437   explanation of the variation of the exchange coefficient with transient storage observed at whole-

438   reach scale. In addition, our results suggest that the observed increases in relative transient

439   storage size during leaffall (Argerich et al. 2008; Table 1) were mostly associated with the

440   generation of pools in the surface channel. Taken together, the results of these 2 studies indicate

441   that the hyporheic zone may contribute to some extent to the overall transient storage of the

442   study reach, but under periods of leaf-litter accumulation, its influence is reduced.

443

444   *Surface and hyporheic biogeochemistry during the study period*

445   Surface SRP and NO_3-N concentrations were within the range reported by von Schiller et

446   al. (2008) in the same reach in a 2-y biweekly data set (2004–2006). NH_4-N concentrations

21

447   remained very low throughout the study period. Our values were in the lower end of the range

448   reported by von Schiller et al. (2008), who also indicated that surface SRP and NO_3-N

449   concentrations were positively correlated and that both were negatively related with Q. Our

450   results do not agree with the results of von Schiller et al. (2008). The range of nutrient

451   concentrations and Q in our study were similar to those observed by von Schiller et al. (2008)

452   over 2 y, but temporal variation of NO_3-N and SRP concentrations captured within a single

453   season (i.e., autumn) were not correlated, and NO_3-N concentration was positively correlated

454   with Q. The contrasting results of the 2 studies suggest that the relevance of controlling factors

455   on nutrient concentrations varies at different temporal scales and emphasizes the importance of

456   performing studies at different temporal scales for a full understanding of the biogeochemistry

457   dynamics in stream ecosystems.

458   Temporal variation in DO and nutrient concentrations over the study period was similar

459   between surface and hyporheic water. However, the 2 stream compartments were chemically

460   distinct. In particular, hyporheic water was DO depleted and more enriched in nutrient

461   concentrations than surface water, especially for SRP and NO_3-N. Several authors (Triska et al.

462   1989, Valett et al. 1990, Hendricks and White 1991, Jones et al. 1995a, b) have found similar

463   patterns in different streams and have attributed the differences to distinct biogeochemical

464   processes occurring within each stream compartment. Among these studies, some have included

465   a temporal variation component in the comparison of surface and hyporheic water. For instance,

466   Jones et al. (1995a, b) measured temporal variation in hyporheic chemistry on a monthly basis

467   over 15 mo, and Hendricks and White (1991) examined seasonal variation over 1 y. Our study

468   complements this previous work by providing information on temporal variability in hyporheic

469   chemistry and how it compares with the surface stream in a headwater stream with deciduous

470   vegetation during a season of high ecological relevance (autumn). The data for our study were

471   obtained at a relatively high frequency (≤ 1 wk) within this season and from wells spaced 5 m

472   apart along the entire reach. Therefore, results provide a very detailed picture of both temporal

473   and spatial variability of hyporheic chemistry. We also were able to capture differences between

474   hyporheic locations of upwelling and downwelling. Upwelling wells presented lower DO and

475   NH_4-N concentrations and similar SRP and NO_3-N concentrations to downwelling wells. This

476   result indicates that the direction and strength of water exchange can contribute to the spatial

477   chemical variability in the hyporheic zone. Our results support previous findings observed in

478   other streams (e.g., Sycamore Creek, Arizona, Valett et al. 1990; Maple River, Michigan,

479   Hendricks and White 1995; Speed River, Southern Ontario, Franken et al. 2001; Hunter River,

480   New South Wales, Hancock and Boulton 2005) in which DO differences among well types were

481   attributed to the downward flow of oxygenated surface water into the hyporheic zone at

482   downwelling sites. In the case of the difference in NH_4-N concentration, high rates of

483   decomposition and mineralization of the large amount of accumulated organic matter during

484   autumn might increase the NH_4-N concentration within the leaf packs compared to in free-

485   flowing water. This NH_4-N-enriched surface water exchanges with the hyporheic zone at

486   downwelling locations leading to the observed difference in NH_4-N concentration between

487   upwelling and downwelling wells.

488

489   *Effects of varying connectivity on hyporheic biogeochemistry*

490   As found in previous studies (Triska et al. 1993b, Findlay 1995), we found that the

491   supply of DO to the hyporheic compartment was influenced by hydrologic exchange. Assuming

492   that all DO found in hyporheic water had a surface origin, differences between hyporheic and

493 surface DO could be attributable to community respiration and water residence time in the

494 hyporheic zone (Valett et al. 1996, Zarnetske et al. 2011). Downwelling wells showed smaller

495 DO differences than upwelling wells, and the DO difference increased with decreasing

496 connectivity, which was associated with accumulation of leaf litter in the channel. This temporal

497 variation in the surface–hyporheic DO difference can be explained by slower downward

498 movement of oxygenated water when leaf litter is present causing a longer exposure of water to

499 biota, and by the occurrence of higher respiration rates in the hyporheic zone that probably were

500 enhanced by higher availability of dissolved organic C (DOC) from the leaf-litter accumulation.

501 However, we did not measure DOC, so we cannot provide evidence to support this hypothesis. In

502 fact, we may have observed the results of the combination of both phenomena because leaf-litter

503 inputs are a source of organic C that fuels stream metabolism and increases ecosystem

504 respiration rates (Crenshaw et al. 2002, Roberts et al. 2007).

505 Changes in connectivity did not appear to affect SRP biogeochemistry in the hyporheic

506 zone. Hyporheic SRP uptake was controlled by hyporheic SRP concentration, which was higher

507 than in surface water, a result that is in agreement with findings in other studies (Grimm and

508 Fisher 1984, Ford and Naiman 1989, Valett et al. 1990, Hendricks and White 1991, 1995, Jones

509 et al. 1995a, b). Hyporheic SRP uptake increased with increasing SRP concentration in the

510 hyporheic zone. Higher SRP concentration was observed at the beginning of the leaffall period

511 when fresh leaves started to accumulate in the reach. Higher availability of SRP together with the

512 input of fresh organic matter may have enhanced SRP uptake by microbial communities.

513 Mulholland et al. (1985) also found that higher whole-reach SRP uptake efficiency at the annual

514 scale coincided with higher benthic organic matter in autumn, although the sampling frequency

515 in that study did not allow assessment of variability in autumn. A similar relationship was found

516   with measurements of whole-reach SRP uptake and surface-water SRP concentration in Riera de

517   Santa Fe during the leaffall period in our study (Argerich et al. 2008). This result suggests that

518   mechanisms controlling SRP uptake were similar at both whole-reach and hyporheic scales.

519   Nevertheless, apparent SRP uptake in the hyporheic zone was $2\times$ lower than that for NH_4-N.

520   This difference in uptake between nutrients is consistent with previous findings in Riera de Santa

521   Fe at the whole-reach scale (Argerich et al. 2008, von Schiller et al. 2008) and in another nearby

522   Mediterranean stream (Martí and Sabater 1996, Butturini and Sabater 1999). This difference may

523   be attributed to the fact that NH_4-N uptake can be influenced by assimilation, nitrification, and

524   sorption onto sediments or organic substrates, but SRP uptake is affected only by assimilation in

525   this stream dominated by siliceous geology.

526   Connectivity did not appear to affect NH_4-N uptake in the hyporheic zone directly, but

527   the positive relationship between hyporheic NH_4-N uptake and hyporheic DO indicate an indirect

528   effect through changes in DO availability. DO strongly influences N transformations because of

529   the wide range of available redox conditions for various reactions (Duff and Triska 2000). NH_4-

530   N uptake in the hyporheic zone could be attributed either to the use of amended NH_4-N as a

531   source of N through assimilation processes or to the aerobic oxidation of NH_4-N as a source of

532   energy through nitrification processes. NO_3-N was not used in the solute injections, but we

533   observed an increase in hyporheic NO_3-N concentrations during plateau conditions, a result that

534   suggested that nitrification of injected NH_4-N was taking place in the relatively well-oxygenated

535   hyporheic zone of this stream. This finding is in agreement with previous results in hyporheic

536   zones of different streams. Jones et al. (1995a) presented a conceptual model of hyporheic N

537   cycling in Sycamore Creek (Arizona) and demonstrated that reduced N in organic matter could

538   be oxidized to NO_3-N in subsurface flow paths. Hyporheic tracer studies at Little Lost Man

539 Creek (Triska et al. 1990) confirmed that NH_4-N dissolved in stream water added directly to an

540 oxygenated hyporheic flow path could be nitrified based on the increase in NO_3-N concentration

541 (i.e., up to 55 μg N/L) observed after a 9-d NH_4-N injection. The NH_4-N injections in our study

542 were much shorter (a few h), but they resulted in increases in NO_3-N concentration up to 130

543 μg/L. Differences in the magnitude of the NO_3-N increases between the 2 studies could be the

544 result of differences in the level of NH_4-N enrichment. Nevertheless, our results indicate a rapid

545 response and high potential for nitrification in the hyporheic zone of the study stream.

546   NH_4-N enrichment levels from the injections and apparent NH_4-N uptake were similar on

547 all sampling dates, but increases in NO_3-N were higher after the flood than during the leaffall

548 period. This temporal difference could be explained by either lower assimilative demand for

549 NH_4-N by heterotrophic bacteria after the flood, from which nitrifiers would benefit (Bernhardt

550 et al. 2002), or a decrease in denitrification rates caused by an increase in DO availability in the

551 hyporheic zone after the flood. We think that the 1st option is more likely because rates of

552 nitrification at whole-reach scale in this stream are measurable, whereas rates of denitrification

553 are almost negligible (von Schiller et al. 2009). Other results from our study provide additional

554 evidence to support the relevance of nitrification as a control of NH_4-N uptake in the hyporheic

555 zone of Riera de Santa Fe in autumn. For instance, the relationship between apparent NH_4-N

556 uptake and background NO_3-N concentrations is consistent with previous findings on the

557 controls on nitrification rates (Bernhardt et al. 2002). In addition, the observed increase in NO_3-

558 N concentration was closely related to the amount of NH_4-N predicted in the wells in absence of

559 transformation (i.e., in the amount of NH_4-N supplied by the injection). Kemp and Dodds (2002)

560 observed a similar relationship in a suite of experiments, in which they found very strong

561 responses of rates of nitrification in most stream biotic compartments to NH_4-N amendment.

562   In summary, leaf-litter inputs play an important role in the dynamics of nutrient cycling

563   and surface–hyporheic water exchange in this forested stream. Leaf litter introduces organic

564   matter that fuels stream metabolism and alters morphology of the channel, and it modifies

565   surface–hyporheic water exchange by altering vertical head gradients and decreasing the velocity

566   at which surface water arrives in the hyporheic zone. These effects are counterbalanced by the

567   effects of variability in Q, especially under flood conditions, which can be common in temperate

568   climates during autumn. Increases in Q during our study led to an increase in surface–hyporheic

569   water exchange and to homogenization of upwelling and downwelling wells in terms of

570   connectivity. These Q-related changes in surface–hyporheic exchange dynamics can be the result

571   of increasing Q in itself (by changing the local head gradients), but changes in Q also provided

572   flow conditions leading to the removal of leaves from the stream bed. Our results show that,

573   together, these hydrologic changes have implications for the temporal variation of hyporheic

574   water chemistry and nutrient uptake. Nevertheless, N and P responded differently to these

575   changes. P uptake was controlled by hyporheic SRP concentration, which did not respond to

576   changes in connectivity, whereas N biogeochemistry was indirectly affected by connectivity

577   through changes in DO availability. This differential response may induce shifts in the

578   stoichiometry of available bioreactive elements in the hyporheic zone, which could influence the

579   activity of the microbial communities in the hyporheic zone.

580

581

582 **Acknowledgements**

592

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767

768 **Figure Captions**

769 Fig. 1. Contour map (0.5-m intervals) for Riera de Santa Fe and a diagram of the reach showing

770 positions of wells (squares) and vertical hydraulic gradient (VHG) values measured on 11

771 November 2004. Black squares represent wells that presented consistent upwelling or

772 downwelling on the 2 dates when VHG was measured. Gray squares represent wells with

773 inconsistent upwelling or downwelling. Inset shows the location of the stream in Spain.

774 Fig. 2. Temporal variation of stream discharge (dashed line), standing stocks of leaf litter

775 accumulated in the study reach (black line), and precipitation (grey bars) registered

776 during the study period (October–December 2004).

777 Fig. 3. Mean (±1 SE) dissolved O_2 (DO) saturation (A), soluble reactive P (SRP) concentration (B),

778 NH_4-N concentration (C), and NO_3-N concentration (D) in stream surface water and in

779 hyporheic water during the study period (October–December 2004). Surface values are

780 the average of data from 5 sampling stations along the reach. Hyporheic values are the

781 average of data from samples collected at 15 wells along the reach. The break between

782 continuous lines indicates the time when the flood occurred.

783 Fig. 4. Mean (±1 SE) hydraulic connectivity between surface and hyporheic water, expressed as

784 percentage of surface water found in the wells, during the sampling period (October–

785 December 2004). Values are the average of data from 5 wells for upwelling and 4 wells

786 for downwelling. The break between continuous lines indicates the time when the flood

787 occurred.

788 Fig. 5. Relationship between hydrologic connectivity and the ratio of hyporheic (h)/surface (s)

789 dissolved O_2 (DO) saturation (sat) (A), the ratio of hyporheic/surface soluble reactive P

790 (SRP) concentration (B), the ratio of hyporheic/surface NH_4-N (C), and the ratio of

791   hyporheic/surface NO_3-N concentration (D) for upwelling and downwelling wells.

792   Significant relationships are expressed as solid lines for upwelling wells and as dashed

793   lines for downwelling wells. Dots represent data for all sampling dates on which paired

794   measurements were available (A: $n = 39$ upwelling wells, $n = 32$ downwelling wells; B–D:

795    $n = 59$ upwelling wells, $n = 48$ downwelling wells).

796   Fig. 6. Relationship between soluble reactive P (SRP) uptake (expressed as the ratio between

797   observed [*obs*] and predicted [*pred*] SRP concentration) and hyporheic (*h*) SRP

798   concentration (A), and between NH_4-N uptake and hyporheic dissolved O_2 (DO)

799   saturation (B) at upwelling and at downwelling wells. Significant relationships are

800   expressed as solid lines for upwelling wells and as dashed lines for downwelling wells.

801   Dots represent data for all sampling dates on which paired measurements were available

802   (A: $n = 44$ upwelling wells, $n = 36$ downwelling wells; B: $n = 29$ upwelling wells, $n = 24$

803   downwelling wells).

804   Fig. 7. Relationship between increase in hyporheic NO_3-N (measured as the difference between

805   plateau sampling and background sampling [Δ]) and hyporheic (*h*) O_2 saturation (A) and

806   predicted (*pred*) hyporheic NH_4-N concentration at plateau in absence of reaction (B).

807   Dots represent data for all sampling dates on which paired measurements were available

808   (A: $n = 19$ upwelling wells, $n = 14$ downwelling wells; B: $n = 16$ upwelling wells, $n = 13$

809   downwelling wells). Only increases >25 µg N/L were considered. Significant relationships

810   are expressed as solid lines for upwelling wells and as dashed lines for downwelling

811   wells.

812

813   Table 1. Discharge (Q), wetted area of the reach (A_w), cross-sectional area of the stream (A),

814   ratio of the cross-sectional area of the transient storage zone to the cross-sectional area of the

815   stream (A_s/A), and water exchange rates between the main channel and the transient storage zone

816   (α) during the study period (October–December 2004). A_s/A and α were estimated using OTIS

817   (Runkel 1998) considering a reach of 140 m (Argerich et al. 2008). nm = not measured.

Date	Q (L/s)	A_w (m^2)	A (m^2)	A_s/A	α (s^{-1})
7 October	5.9	156	0.164	nm	nm
14 October	5.6	203	0.164	0.219	0.00025
18 October	4.6	218	0.218	0.206	0.00014
21 October	5.3	189	0.182	0.214	0.00014
28 October	18.5	230	0.579	0.100	0.00008
2 November	8.8	239	0.239	0.150	0.00014
4 November	7.3	231	0.220	0.186	0.00012
8 November	7.2	237	0.244	nm	nm
11 November	6.5	233	0.237	nm	nm
16 November	7.3	253	0.241	0.208	0.00010
19 November	7.4	237	0.278	0.227	0.00012
23 November	6.8	236	0.250	0.216	0.00010
26 November	5.7	243	0.220	nm	nm

30 November	6.1	253	0.211	0.251	0.00012
2 December	12.2	260	0.236	0.161	0.00025
10 December	119.5	323	0.332	nm	nm
13 December	28.5	276	0.19	0.114	0.00050
15 December	22.6	275	0.199	0.146	0.00022
17 December	18.0	257	0.184	0.174	0.00020
19 December	14.8	248	0.174	0.190	0.00021
21 December	13.2	248	0.178	0.169	0.00021
23 December	14.5	248	0.209	0.196	0.00020
29 December	10.6	239	0.180	0.239	0.00019

818

819

820 Table 2. Statistical results of the mixed-model analysis of variance to test for differences in the

821 dependent variables (dissolved O_2 [DO] saturation, concentrations of soluble reactive P [SRP],

822 NH_4-N, and NO_3-N) between surface and hyporheic water (stream compartment factor), between

823 the leaffall and the postflood periods (period factor), and the interaction between the 2 factors. **

824 $= p < 0.01$.

Dependent variable	Factor	df Numerator	df Denominator	F	p
DO saturation	Stream compartment	1	37.7	61.7	0.00**
	Period	1	151.1	2.7	0.10
	Stream compartment × period	1	151.1	0.0	0.88
SRP concentration	Stream compartment	1	71.1	45.7	0.00**
	Period	1	79.3	90.7	0.00**
	Stream compartment × period	1	79.3	0.7	0.39
NH_4-N concentration	Stream compartment	1	82.6	2.3	0.13
	Period	1	91.5	0.2	0.68
	Stream compartment × period	1	91.5	0.1	0.71
NO_3-N concentration	Stream compartment	1	65.4	93.8	0.00**
	Period	1	66.6	394.8	0.00**
	Stream compartment × period	1	66.6	2.9	0.94

825

826   Table 3. Statistical results of the mixed-model analysis of variance to test for differences in the

827   dependent variables (dissolved O_2 [DO] saturation, nutrient concentrations, connectivity, nutrient

828   uptake, and increase in hyporheic NO_3-N as a result of the injections) between upwelling and

829   downwelling wells (well type factor), between the leaffall and the postflood periods (period

830   factor), and the interaction between the 2 factors. $* = p < 0.05$, $** = p < 0.01$.

Dependent variable	Factor	df Numerator	df Denominator	F	p
Hyporheic DO	Well type	1	18.9	10.12	0.00**
	Period	1	99.2	1.83	0.18
	Well type × period	1	99.2	0.13	0.72
Hyporheic SRP	Well type	1	30.5	0.56	0.46
	Period	1	35.1	17.87	0.00**
	Well type × period	1	35.1	0.01	0.93
Hyporheic NH_4-N	Well type	1	36.0	10.12	0.00**
	Period	1	39.6	0.01	0.93
	Well type × period	1	39.6	0.98	0.33
Hyporheic NO_3-N	Well type	1	40.4	0.51	0.48
	Period	1	41.6	120.18	0.00**
	Well type × period	1	41.6	1.97	0.17
Connectivity	Well type	1	17.4	0.19	0.67
	Period	1	86.4	19.59	0.00**

	Well type × period	1	86.4	6.38	0.01*
SRP uptake	Well type	1	10.9	0.14	0.71
	Period	1	14.2	5.32	0.04*
	Well type × period	1	14.2	0.18	0.67
NH_4-N uptake	Well type	1	12.4	0.85	0.37
	Period	1	17.3	1.79	0.20
	Well type × period	1	17.3	0.21	0.62
Increase in hyporheic NO_3-N	Well type	1	16.0	5.49	0.03*
	Period	1	16.3	4.62	0.04*
	Well type × period	1	16.3	2.30	0.15

831

1186 1184 1182 1180 1182 1180 1182 1184

E

injection site

Mixing region

VHG (cm/cm)

Distance downstream (m)

Connectivity (%)

O Downwellings
● Upwellings

1 Oct   15 Oct   29 Oct   12 Nov   26 Nov   10 Dec   24 Dec

A

$(DO\ sat)_h/(DO\ sat)_s$

B

○ Downwellings
● Upwellings

SRP_h/SRP_s

C

$NH_4\text{-}N_h/NH_4\text{-}N_s$

D

$NO_3\text{-}N_h/NO_3\text{-}N_s$

Connectivity (%)

A

Lower SRP uptake

Higher SRP uptake

SRP_{obs}/SRP_{exp}

SRP_h (μg P/L)

B

Lower NH$_4$-N uptake

Higher NH$_4$-N uptake

$NH_4\text{-}N_{obs}/NH_4\text{-}N_{exp}$

DO_h (% saturation)

○ Downwellings
● Upwellings