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Hydrologic control of nitrogen removal, storage, and export in a mountain

stream

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20   Running head: Hydrologic control of stream N cycling
21

35   Abstract

36   Nutrient cycling and export in streams and rivers should vary with flow regime, yet

37   most studies of stream nutrient transformation do not include hydrologic variability. We

38   used a stable isotope tracer of nitrogen (^{15}N) to measure nitrate (NO_3^-) uptake, storage, and

39   export in a mountain stream, Spring Creek, Idaho, USA. We conducted two tracer tests of 2-

40   week duration during snowmelt and baseflow. Dissolved and particulate forms of ^{15}N were

41   monitored over three seasons to test the hypothesis that stream N cycling would be

42   dominated by export during floods, and storage during low flow. Floods exported more N

43   than during baseflow conditions; however, snowmelt floods had higher than expected

44   demand for NO_3^- because of hyporheic exchange. Residence times of benthic N during both

45   tracer tests were longer than 100 days for ephemeral pools such as benthic algae and wood

46   biofilms. Residence times were much longer in fine detritus, insects, and the particulate N

47   from the hyporheic zone, showing that assimilation and hydrologic storage can be important

48   mechanisms for retaining particulate N. Of the tracer N stored in the stream, the primary

49   form of export was via seston during periods of high flows, produced by summer rainstorms

50   or spring snowmelt the following year. Spring Creek is not necessarily a conduit for

51   nutrients during high flow; hydrologic exchange between the stream and its valley represents

52   an important storage mechanism.

53

Introduction

Hydrologic transport by streams and rivers links processes in one region with those much farther away, a notable example being the eutrophication of estuaries and coastal oceans by nutrient sources high in the watershed (Rabalais et al. 2002). Rivers transport materials across the landscape while simultaneously transforming and storing them (Meyer and Likens 1979). Much recent research has examined uptake and storage of nutrients and has shown that streams can rapidly transform nutrients (Peterson et al. 2001; Hall et al. 2009), store them for the short term (<60 d) (Dodds et al. 2000; Tank et al. 2000; Hall et al. 2001) or in the case of nitrogen (N), be an outright sink through denitrification (Royer et al. 2004; Mulholland et al. 2008, 2009).

The balance between how much and how long streams retain elements vs. transport them downstream is central to our understanding of watershed nutrient export (Bernhardt et al. 2005; Roberts and Mulholland 2007, Brookshire et al. 2009). One obvious control on this balance between retention and transport is variation in stream discharge (Doyle 2005). Material transport should dominate during high flows, while material retention should dominate during low flows when streams have higher uptake rates and lower export. This pattern was first documented by budget studies (Meyer and Likens 1979), in which import and export of elements were measured across a range of stream flows. A central finding of budget studies in small streams is that most element export occurs during a small fraction of the time when stream flow is high (Meyer and Likens 1979; Webster et al. 1990). However, these budget approaches do not quantify the processes responsible for transforming or retaining nutrients within stream reaches; stream spiraling techniques are necessary (Newbold et al. 1981; Mulholland et al. 2001). Spiraling methods measure rates of uptake

76 and residence times of dissolved nutrient pools within a reach. Hundreds of stream spiraling

77 measurements show that nutrients are quickly removed by streams (Ensign and Doyle 2006;

78 Tank et al. 2008), yet few of these studies have linked uptake with turnover of particulate

79 nutrient pools because isotope tracers are required to estimate storage and fate of N.

80   Despite potentially large seasonal variation in stream flows, most N spiraling studies

81 in streams, whether isotope tracer studies (Peterson et al. 2001), or measures of uptake using

82 nutrient addition experiments (Hall and Tank 2003), are intentionally conducted at baseflow,

83 with few exceptions (Valett et al. 1996; Tank et al. 2000; Merriam et al. 2002). Spiraling

84 studies, by themselves, cannot consider the role of hydrologic variability, which is a

85 limitation of the spiraling approach (Fisher et al. 2004). Consequently, we do not know how

86 spiraling rates measured at baseflow change when the stream is flooding. Nor do we know

87 how subsequent spates control storage and export of N that is stored in the benthos. Isotope

88 labeling studies suggest that N pools in streams are resistant to scouring by spates when

89 flows increased 10-20-fold (Tank et al. 2000; Merriam et al. 2002), but the overall residence

90 times of tracer N were short, suggesting that small streams do not store N for long periods

91 (Ashkenas et al. 2004). It is not likely that rates of nutrient uptake are constant across large

92 variations of stream flow because high flows may alter nutrient inputs and uptake processes.

93 For example, nutrient concentration and export are often higher during high flow periods of

94 the year (Creed et al. 1996; Baron and Campbell 1997), which may saturate uptake

95 (Mulholland et al. 2008). High flows may scour algae and reduce benthic demand for

96 nutrients. On the other hand, high flows may exchange water and nutrients with floodplains

97 thereby increasing storage.

98   To consider the role of hydrologic processes in nutrient cycling, it not sufficient to

99   only examine variation in stream flow; it is also necessary to consider the spatial extent of

100   streams beyond the main channel (Triska et al. 1989; Fisher et al. 1998). Hydrologic

101   exchange between streams and groundwater increases the travel time of water down-gradient,

102   and puts stream water in contact with biofilms attached to alluvial sediments and buried

103   organic matter. This hyporheic exchange may contribute to N retention in many streams

104   (Triska et al. 1989; Holmes et al. 1994, 1996) by fostering denitrification through anoxic

105   flow paths with plentiful organic substrates (Holmes et al. 1996; Baker et al. 2000) or by

106   vegetation uptake (Peterjohn and Correll 1984; Ashkenas et al. 2004). The nature of these

107   surface-groundwater transformations may depend on hydrologic variability.

108   Here we show how a stream cycles N differently at hydrologic extremes, and then we

109   examine how variation in stream discharge controls the fate of stored N during one year. We

110   hypothesized that N uptake and storage would be highest during baseflow because of high

111   uptake rates and low transport conditions, and that N export would be highest during floods

112   because of high discharge (Doyle 2005). We used a ^{15}N tracer addition of nitrate to measure

113   N uptake, storage, and export for two seasons: during a snowmelt flood, and during baseflow.

114   The stable isotope tracer allowed us to combine budget and spiraling approaches to evaluate

115   the role of hydrologic variability as it controls nutrient cycling. Our approach was to

116   calculate a budget of ^{15}N by measuring standing stocks and export of ^{15}N tracer using a

117   mass-balance approach (Tank et al. 2000), and by measuring export as particles at the end of

118   the reach through one year following each isotope addition. We also related N cycling

119   parameters to changes in stream water and groundwater exchange during the two seasons.

120

121   **Methods**

122   *Study stream*—Spring Creek (44.29°N, 115.25°W) is a second-order mountain stream

123   in the Payette River drainage, at an elevation of 2116 m in the Sawtooth Mountains of Idaho

124   (Fig. 1). The 1.9-km study reach starts in a sparse forest dominated by lodgepole pine (*Pinus*

125   *contorta*) that gradually grades into a riparian meadow and delta plain dominated by willows

126   (*Salix* spp.) and sedges (*Carex* spp.) (Arp et al. 2006) (Fig. 1) and ends at Bull Trout Lake.

127   This reach occurs in a glacial outwash valley with the stream slope gradually declining from

128   0.007 to 0.003 m m^{-1}. The reach is normally covered with snow from December through

129   April. The stream has a typical snowmelt driven hydrograph (Fig. 2), with peak flows near

130   01 June of each study year. Mean wetted width was 3.8 m during snowmelt flows and 3.1 m

131   during baseflow. Substrate is composed of pebbles, gravels and sand, with a median size

132   (D_{50}) of 11 mm (Arp et al. 2007). The spring-fed creek was cold, with mean temperatures

133   during our June and August tracer additions of 4.6°C and 7.3°C, respectively. Nitrate

134   concentrations were low, 10.7 μg N L^{-1} during the snowmelt addition and 9.3 μg N L^{-1} during

135   baseflow. Phosphate phosphorus concentrations were near detection limit (1 μg P L^{-1}).

136   *Hydrology*—Stream depth was measured hourly using pressure transducers at gauging

137   stations located at the addition site (0 m) and 1559 m downstream in 2002 and 2003, with

138   additional stations at 900 and 1865 m downstream in 2004. At each station, we developed a

139   stage-discharge relationship (Arp et al. 2006).

140   To measure the degree of exchange between stream water and groundwater, we installed

141   sampling wells in the channel and riparian zone. These wells were augered to 1.5 m depth

142   and cased with 5-cm diameter machine-slotted (0.25 mm) polyvinyl chloride (PVC) well

143   screen below the water table and finished to the top with solid PVC. Wells were backfilled

144   with native material, capped with a bentonite clay plug, and purged before sampling. In-

145   channel wells were installed to 50 cm depth into gravel alluvium with a rod and cased with

146   2.5 cm diameter PVC pipe. Sampling transects, consisting of stream water, an in-channel

147   well, and 2 lateral wells (4-30 m from stream), were located at 150, 300, 800, and 1500 m

148   downstream of the addition site. Groundwater levels in the wells were measured using an

149   electronic beeper attached to a ruler. To estimate mixing of stream water in wells, we

150   sampled for Br^- in all wells before each experiment and on day 14. Stream water was labeled

151   with Br^- from a continuous injection (*see* below). Hyporheic mixing was calculated as the

152   ratio of groundwater to stream water Br^-, less background concentration (Triska et al. 1989).

153   We constructed subsurface flow nets (Freeze and Cherry 1979) by measuring water surface

154   elevations of the stream surface, backwaters, and isolated ponds using a total station laser

155   theodolite.

156

157   *^{15}N additions*—We added ^{15}N as 28 atom-% $Na^{15}NO_3$ for two weeks during snowmelt

158   from 13-27 June 2002 and during baseflow from 05-19 August 2003 1895 m upstream from

159   the lake (Fig. 2). For the snowmelt addition we added 70 g ^{15}N, and 56 kg of Br^- (as NaBr) as

160   a conservative tracer of water. For the baseflow addition we added 75 g ^{15}N and 12.8 kg of

161   Br^-. Calculated enrichments of $\delta^{15}NO_3^-$ were 1704‰ during snowmelt and 11,670‰ during

162   baseflow. Solutes were mixed daily into a 20-L carboy and continuously pumped into the

163   stream at 10.4 mL min^{-1} using a Watson-Marlow peristaltic pump (Watson-Marlow). To

164   measure removal, storage, and residence times of N in the reach we collected samples

165   periodically at 62, 120, 225, 539, 1069, 1560, and 1895 m below the addition site.

166

167   *Solute sampling*—We measured the removal of ^{15}N-NO_3^- from the water column on

168   days 3, 7, and 14 following the start of each experiment. We collected 10-L of water for

169   analyses of ^{15}N-NO_3^-, ^{15}N-ammonium (^{15}N-NH_4^+) and total dissolved ^{15}N (^{15}N -TDN) at each

170   site. To estimate NO_3^- regeneration and/or transformation to NH_4^+ and dissolved organic N

171   (DON) we measured dissolved ^{15}N pools at each station on the day after the injection, then

172   weekly or biweekly thereafter at the top and bottom of the stream reach. For ^{15}N-NO_3^-, 3-L

173   samples were spiked with 150 μg NO_3-N and concentrated to 0.1 L by boiling. Devarda's

174   alloy catalyzed conversion of NO_3^- to NH_4^+ during a 48-h incubation (Mulholland et al.

175   2008). ^{15}N-NH_4^+ was collected on an acidified filter and $\delta^{15}N$ and N mass were measured at

176   the University of California Davis Stable Isotope Facility. We measured ^{15}N-NH_4^+ in 3-L

177   unamended samples using ammonia diffusion (Peterson et al. 2001). We measured ^{15}N-TDN

178   by persulfate oxidation of 0.75 L samples, followed by conversion of NO_3^- to NH_4^+ following

179   the same procedure for ^{15}N-NO_3^- above, and calculated ^{15}N- dissolved organic N (DON) as

180   the difference between ^{15}N-TDN and ^{15}N-NO_3^- + ^{15}N-NH_4^+.

181   We measured stream water NO_3^- and Br^- concentrations on a DIONEX-500 ion

182   chromatograph with concentrator and AS14 analytical and guard columns. Detection limits

183   were 0.5 μg L^{-1} NO_3-N and 2 μg L^{-1} Br^-. Water samples for TDN concentration were oxidized

184   using persulfate digestion (Valderrama 1981) followed by NO_3-N quantification on the

185   digested samples using second derivative spectroscopy (Crumpton et al. 1992), with a

186   detection limit of 15 μg L^{-1}. NH_4-N concentration was measured fluorometrically (Holmes et

187   al. 1999) with a detection limit of 2 μg L^{-1}.

188

189   *Particle sampling*—We collected samples for both ^{15}N and standing stock of N during

190   and after each addition to sequentially inventory ^{15}N in the benthos. At each of the 7 sites we

191   sampled the major benthic stocks of N. All particle samples were analyzed at the University

192   of Wyoming Light Stable Isotope facility using continuous-flow mass spectrometry.

193   Samples were combusted in an elemental analyzer (from which we could calculate mass of N

194   and C), linked to a VG IsoPrime or Finnegan Delta Plus mass spectrometer.

195   Fine benthic organic N (FBON) was sampled with a 20.3-cm diameter stovepipe

196   corer by setting the corer into sediment, swirling the sediment, and subsampling the

197   suspended water for ^{15}N and organic matter as ash-free dry mass (AFDM). We filtered

198   subsamples through Gelman A/E filters for both ^{15}N and organic matter quantification. In

199   2002 we separated surface from deep (to 10 cm) FBON. There was no difference in the δ^{15}N

200   of surface and deep FBON in 2002; therefore in 2003 and 2004 we collected only one sample

201   that combined surface and deep FBON. We measured organic matter standing stock (as

202   AFDM) and calculated N standing stock using the measured C:N ratio and assuming that

203   organic matter is 50% C. The ^{15}N in the samples was extrapolated to a ^{15}N standing stock (g

204   ^{15}N m^{-2}) by multiplying atomic ratio of ^{15}N excess (^{15}N$_{xs}$ which is background-corrected

205   atomic ratio of ^{15}N) of FBON by the standing stock of N at each site. We multiplied standing

206   stock by reach wetted area to extrapolate to the reach.

207   We sampled N from rock biofilms (epilithon) by collecting all stones in a 20.3-cm

208   core and scrubbing epilithon using a detail brush into a slurry. Processing and scaling of

209   AFDM and ^{15}N from this slurry was as for FBON samples as was scaling ^{15}N standing stock.

210   We also measured chlorophyll *a* by filtering 1-5 mL of slurry onto Gelman A/E filters,

211   extracting in 90% basic ethanol, and measuring on a calibrated fluorometer.

212   We sampled invertebrates at each site by collecting and freezing several individuals

213   of four dominant taxa for ^{15}N analysis. Taxa were *Yoraperla*, a shredder stonefly; *Sweltsa*, a

214 predatory stonefly; *Drunella* and *Cinygmula*, scraper mayflies. These four taxa constituted

215 44% to 67% of total assemblage biomass depending on year. For each isotope addition in

216 2002 and 2003, and in July 2004, we measured biomass of all taxa from 2 composite 20.3-cm

217 diameter stovepipe core samples at each of 7 sites, following Hall et al. (2006). From these

218 stovepipe core samples we also measured standing stock of coarse benthic organic matter

219 (CBOM) as AFDM which was mostly terrestrial leaf litter. We calculated $^{15}N_{xs}$ for CBOM as

220 for FBON and scaled the value to the stream reach.

221   After the 2002 snowmelt addition we discovered that hyporheic storage of N was

222 likely to be a large sink for N, so we sampled the shallow parafluvial zone to estimate

223 hyporheic storage of N in this region. We sampled 18 gravel bars adjacent to the stream 6

224 times following the 2003 baseflow addition (Fig. 1). We dug a hole to the water table in a

225 dry bar, and we inserted a plastic, 8.2 cm diameter corer 9-20 cm deep into the sediment

226 beneath the water surface. We plugged the top, excavated the core, and dropped the contents

227 into a bucket with a measured amount of stream water. We stirred the sample to suspend

228 particulate organic matter and sampled particulate organic matter as AFDM and ^{15}N from the

229 resultant slurry. We extrapolated ^{15}N standing stocks by multiplying area-specific mass of

230 $^{15}N_{xs}$ by the by bankfull area measured in 2002 as part of a wood survey.

231   We measured volume and surface area of wood in the stream by using the line-

232 intercept technique (Wallace and Benke 1984) once during each ^{15}N addition. We

233 established a transect every 25 m through the 1.9-km study reach and measured the diameter

234 of each piece of submerged wood. To sample for ^{15}N, we sampled a measured area of

235 loosely attached wood biofilm from each of three sticks. We dried and weighed the sample

236 and subsampled for ^{15}N and %N measurements. We calculated the mass of $^{15}N_{xs}$ per area of

237   wood at each site by multiplying $^{15}N_{xs}$ by mass of N per unit area of wood biofilm. We

238   scaled this value to the entire reach by multiplying by the total surface area of wood.

239   During the wood survey, we measured the percent cover of the limited macrophyte

240   vegetation and standing stocks of five areas where macrophytes constituted 100% cover to

241   estimate a reach-scale mass of macrophytes. On day 14 we sampled macrophytes at the 7

242   sample sites for ^{15}N and %N.

243   We measured enrichment of riparian willows at the end of the ^{15}N addition in August

244   2003. We collected leaves from three locations immediately adjacent to the stream upstream

245   from the addition site and 14 locations downstream and measured their ^{15}N.

246   We measured suspended particulate N (seston) flux at the bottom of the reach (1895

247   m) approximately weekly following the ^{15}N additions during June-Oct of each year. During

248   Spring 2004 we used an autosampler located at sta. 6, (1560 m) to capture samples during the

249   snowmelt flood. For each sample we filtered a known volume of water onto 25-mm Gelman

250   A/E filters, dried them, and analyzed them for both total N and ^{15}N. Instantaneous seston

251   export was calculated as the $^{15}N_{xs}$ of seston times discharge times concentration of seston N.

252   We fortuitously sampled seston export during one storm in each summer: one during

253   the snowmelt addition and the other 2 days following the baseflow addition (Fig. 2). In the

254   snowmelt addition we hand collected 8 samples during and following a storm that increased

255   discharge from 0.7 to 1.1 $m^3 s^{-1}$. Just following the baseflow addition we installed an ISCO

256   auto sampler at the bottom of the reach that sampled every 2 h, 19 times during a storm that

257   increased discharge from 0.11 to 0.55 $m^3 s^{-1}$.

258

259   *Denitrification*—In 2002 we measured potential denitrification rates *in situ* using a

260 modified acetylene block method (Baker and Vervier 2004) at three surface parafluvial sites

261 (gravel bars or abandoned side channels) and two subsurface parafluvial sites (wells).   For

262 surface sites, we excavated sediments and inserted a 20.3-cm diameter stovepipe 20 cm

263 below the water level. Water from the stovepipe and wells was pumped to a carboy where it

264 was bubbled with acetylene gas and mixed with Br^-, glucose, and NO_3^- to final concentrations

265 of 0.07 mg Br L^{-1}, 1.0 mg C L^{-1} and 0.25 mg N L^{-1}. We pumped this water back into the pipe

266 or well and collected samples for N_2O gas and Br^- via a syringe attached to the peristaltic

267 pump. Samples for N_2O gas were stored in evacuated glass vials for later analysis by gas

268 chromatography. Br^- concentrations were used to correct N_2O concentrations for dilution and

269 denitrification potential was calculated as the slope of the regression line of dilution-

270 corrected N_2O concentration vs. time (Baker and Vervier 2004).

271   During both snowmelt and baseflow additions we collected stream water for analysis

272 of $^{15}N_2$ and $^{15}N_2O$ as products of denitrification (Mulholland et al. 2008, 2009). In 2002 only

273 two samples were analyzed from samples collected at the top and bottom of the study reach

274 on day 12 of the tracer addition. In 2003, samples were collected in duplicate from each

275 station on days 2, 4, and 14. In all cases, samples were collected via a 60-mL syringe, and

276 were stored under water until headspace equilibration with ultra-pure helium following

277 Mulholland et al. (2008). Gas samples were analyzed for ^{15}N by mass spectrometry at the

278 U.C. Davis Stable Isotope Facility.

279   *Data analysis*—For display purposes we show $\delta^{15}N$, but for all calculations we used

280 the atomic ratio excess ($^{15}N_{xs}$) of ^{15}N, which corresponds to the ratio of masses, $^{15}N/(^{14}N+^{15}N)$,

281 from which the unlabeled background atomic ratio is subtracted. We measured background

282 ^{15}N prior the 2002 addition and from samples upstream of the addition site.

283   We calculated flux of ^{15}N as NO_3^- (mg N min^{-1}), $F_{NO3\ x}$ at each station (x) as:

284    $F_{15NO3x} =^{15} N_{xsNO3} \times [NO_3]_x \times Q_x$ (1)

285   where Q_x is the discharge at that station as calculated by mass balance of the added Br$^-$

286   conservative tracer. Per-meter removal (same as uptake) rate (k) is calculated by fitting ^{15}N

287   flux at each station to the following model using least squares regression:

288    $\ln F_{15NO3x} = \ln F_{15NO30} - kx$ (2)

289   The inverse of k is the uptake length of nitrate (S_{NO3}), that is the average distance a NO_3^- ion

290   travels before removal from the water column. Stream depth and velocity in part, control

291   uptake length (Hall et al. 2009), so we calculated uptake velocity (v_f, m min^{-1}) to compare

292   nutrient uptake at high and low discharge:

293        $v_f = Q/(w\ S_{NO3})$ (3)

294   where w is wetted channel width. We calculate uptake flux of NO_3^- (U_{NO3} mg N m^{-2} min^{-1}) as:

295    $U_{NO3} = v_f \times [NO_3]$ (4)

296   To estimate a mean and confidence interval for uptake parameters for the two

297   baseflow dates, we pooled data from both sampling dates because slopes and intercepts of the

298   regression (Eq. 2) did not differ (ANCOVA with interaction term) and we calculated a

299   confidence interval of the slope from the pooled regression. Slopes were significantly

300   different in the snowmelt addition so we simply calculated a 90%, two-tailed, t-based

301   confidence interval with $n=3$ measurement dates. To estimate uncertainty in mass of ^{15}N

302   stored in the reach, we calculated the 90% confidence interval of the mean of the $^{15}N_{xs}$ mass

303   ($n=7$ for most compartments, $n=14-17$ for hyporheic N). We calculated the propagated

304   uncertainty for the sum of all compartments assuming no covariance among the individual

305   compartments. If ^{15}N stocks declined with time we calculated residence time (d) as the

306   inverse slope of ln ^{15}N mass (g^{15}N$_{xs}$ reach^{-1}) vs. time.

307

308   **Results**

309   *Hydrology*—Spring Creek had a snowmelt-driven hydrograph during the three study

310   years (Fig. 2). During the snowmelt addition, discharge declined from 0.75 to 0.61 m^3 s^{-1}. A

311   rain-on-snow storm on 21 June 2002 increased discharge to 1.10 m^3 s^{-1} (Fig. 2). During the

312   baseflow addition, stream discharge declined evenly from 0.17 to 0.12 m^3 s^{-1} (Fig. 2).

313   Water table maps for the lower 500 m of the stream reach during snowmelt and

314   baseflow showed seasonal differences in surface water-ground water exchange. During

315   snowmelt the stream surface elevation exceeded that of the riparian zone; thus flow direction

316   was from the stream to the floodplain and hyporheic zone (Fig. 3A). During baseflow,

317   stream elevation was similar to the near stream water table, with much of the subsurface flow

318   parallel to the direction of stream flow, but with strong and isolated areas of water flow to the

319   hyporheic zone (Fig. 3B). These results were corroborated by observation of Br$^-$ in sampling

320   wells (Fig. 4). Overall, the fractions of hyporheic water that were derived from stream water

321   were highest during baseflow (10-70%) compared to snowmelt (1-40%) and were higher in

322   in-channel wells than lateral wells in all but one site (Fig. 4).

323   *Uptake and transformation*—Of the 70 g ^{15}N added to Spring Creek during snowmelt,

324   24.5 g, or 35%, was removed from stream water before the end of the 1.9-km reach. The

325   balance, 45.5 g ^{15}N, entered the lake at the end of the stream reach, and is no longer

326   considered in any calculations. During baseflow, 43.5 g ^{15}N or 58% of 75 g added was

327   removed from the stream water (Table 1). Uptake lengths were 2 times longer during

328   snowmelt than during baseflow (Table 1). Uptake velocity was about 70% higher during

329   snowmelt, although the 90% confidence intervals overlapped (Table 1).

330   We were able to collect enough water for ^{15}N analysis from only one well during each

331   addition. During snowmelt, the well contained 16% stream water, yet only 3% of the ^{15}N

332   concentration relative to the nearest stream sampling location, indicating 82% removal.

333   During baseflow, the percent stream water was much higher (97%) and 51% of the stream

334   water ^{15}N concentration was detected indicating 50% removal.

335   ^{15}N in epilithon was unrelated to sampling location during the snowmelt addition, in

336   part because long uptake lengths meant that decline in tracer concentration with distance was

337   small. However, variation in epilithon δ^{15}N was strongly positively correlated with

338   chlorophyll *a* standing stock suggesting that biofilms with higher photosynthetic biomass had

339   higher N uptake (Fig. 5). During the baseflow addition, a downstream decline in δ^{15}N of

340   epilithon was explained by distance from the addition site. The discharge-corrected rate of

341   decline was 0.00061 m^{-1}, close to the measured uptake rate of ^{15}N-NO$_3^-$ on day 14 (Table 1)

342   showing that enrichment of benthic pools mirrored the removal rate we measured by using

343   water column nitrate.

344   Little of the added ^{15}N-NO$_3^-$ was transformed to other dissolved pools. One day

345   following both additions there was detectable label in NO$_3^-$ which, when scaled for 15 d,

346   corresponded to a small amount of exported ^{15}N (Table 2). During snowmelt, NH$_4^+$ and

347   DON pools were not enriched by day 14 of the ^{15}N addition. In contrast, during baseflow,

348   the NH$_4^+$ pool was enriched by 147‰ on day 14, which represents 0.0026 μg ^{15}N L^{-1}. If NH$_4^+$

349   were this enriched for the two-week period, then ^{15}N-NH$_4^+$ export would have been 0.5 g.

350   Similar to the snowmelt addition, the DON pool was not enriched during the baseflow

351   addition. We can constrain the amount or tracer N exported as DON in the baseflow

352   addition. If DON contained the minimal observable label of 4‰ above background, and

353   given DON concentration of 51 μg N L^{-1} during the two week addition, DO^{15}N could have

354   contributed 0.14 g ^{15}N of export, which is smaller than ^{15}N-NH$_4^+$ export, and 20-fold smaller

355   than seston export (Table 2).

356   Denitrification accounted for little N loss during both tracer additions. During

357   snowmelt, denitrification potential was not detectable in parafluvial sediments, and potential

358   rates were low in the two wells (mean and standard error = 0.052 $\pm$ 0.012 μg N$_2$O-N L^{-1} min^{-}

359   ^{1}). Dissolved gas pools were not labeled; mean δ^{15}N$_2$ and δ^{15}N$_2$O collected during tracer test

360   plateau were not different from background samples (t-test, N$_2$ t=0.75, df=37, p=0.46; N$_2$O

361   t=0.49, df=36, p=0.63). Given that δ^{15}N-NO$_3^-$ in the baseflow addition was about 60% of

362   enrichments in studies designed to measure denitrification (Mulholland et al. 2009), this level

363   of enrichment would have reduced our ability to detect enriched N$_2$ by less than one-half.

364   *Storage*—Both snowmelt and baseflow additions strongly enriched benthic pools.

365   During the snowmelt addition FBOM was enriched by 13‰, eplilithon 220‰, wood 240‰,

366   and invertebrates 250‰. Baseflow addition enrichments were 5-10 times higher.

367   Fine benthic organic N constituted a substantial stock of both total N and tracer ^{15}N

368   in the stream (Table 2). FBON standing stock and tracer ^{15}N were higher during baseflow

369   than snowmelt. The standing stock of FBON ^{15}N$_{xs}$ stayed roughly constant for the 60-80

370   days following the snowmelt addition (Fig. 6). For the baseflow addition, FBON ^{15}N$_{xs}$

371   remained nearly constant through the fall and winter (Fig. 6). However, in both additions the

372   ^{15}N standing stock of this pool declined greatly following the subsequent snowmelt flood

373   with zero ^{15}N$_{xs}$ remaining by August 2003, 1.2 y following the snowmelt addition and 0.6 g

374   ^{15}N$_{xs}$ in August 2004, 1y following the baseflow addition. Because storage was constant until

375   the following snowmelt, we did not calculate a residence time for FBON.

376   Epilithon had low standing stock of N, but high label (average $\delta^{15}N$ on day 14 in the

377   snowmelt and baseflow additions were 218‰ and 1610‰ respectively) such that it

378   represented a stock of $^{15}N_{xs}$ about equal to the much larger FBON pool. (Table 2). Mass of

379   epilithic tracer ^{15}N declined with time immediately following the baseflow addition, but not

380   the snowmelt addition (Fig. 6). Tracer ^{15}N stock in epilithon was zero in summer 2003, a

381   year after the snowmelt additio,n and only 0.2 g the summer following the baseflow addition,

382   showing that epilithon was not a substantial long-term store of N. Net residence time of

383   epilithon $^{15}N_{xs}$ was calculated for only the baseflow addition and was 117 d.

384   Wood volume was similar between 2002 and 2003, with 0.0032 and 0.0037 m^3 m^{-2} of

385   channel area, respectively. Surface area of wood was 0.095 in 2002 and 0.125 m^2 m^{-2} in

386   2003. Because the N content of wood biofilm was low, standing stock of N was low relative

387   to other pools (Table 2). Wood biofilm contained a small amount of tracer N mass at day 14

388   (Table 2), and this $^{15}N_{xs}$ declined exponentially with time following both additions, with an

389   estimated net residence time of 166 d following snowmelt addition and 117 d (coincidentally

390   the same as for epilithon) following the baseflow addition.

391   Hyporheic standing stocks of N measured in the parafluvial zone in 2003 were much

392   higher than all other pools combined (Table 2). Isotopic enrichment was considerably lower

393   than other pools averaging 31‰ during the 3 sampling dates following the baseflow addition

394   in 2003. However, because standing stock was so high, parafluvial organic matter contained

395   a large amount of tracer N following the addition. Standing stock of ^{15}N in summer 2004

396   was not different than that following the ^{15}N addition in 2003, showing that this store of ^{15}N

397   was not removed by the snowmelt flood (Fig. 7). We cannot calculate residence time using

398   an exponential model for this hyporheic N because the standing stock of $^{15}N_{xs}$ did not decline

399   with time, but residence time was much greater than 1 yr.

400   Willows collected from near the stream had a variable pattern of labeling from 42‰

401   to background (Fig. 8). Because of the spatial patchiness of labeling and not knowing the

402   mass of potentially labeled willows, we could not calculate the tracer ^{15}N mass stored in

403   willows; however, enriched willow leaves show that hyporheic flow transported stream water

404   $^{15}N\text{-}NO_3^-$ into riparian pools which was used by vegetation.

405   Coarse benthic organic matter and macrophytes constituted only a small fraction of

406   inventoried $^{15}N_{xs}$ immediately following both additions (Table 2).

407   Insects constituted a substantial stock of N, despite having relatively low biomass (0.7

408   to 1 g AFDM m^{-2}) (Table 2), but did not constitute a long-term store of N. By the following

409   summers, insects contained 0 (snowmelt addition) or 0.04 g $^{15}N_{xs}$ (baseflow addition).

410

411   *Seston export*—Seston ^{15}N export during the snowmelt addition was highest at the

412   end of the 14-d addition and declined rapidly afterward because of decreasing $\delta^{15}N$ of the

413   seston coupled with falling stream discharge (Figs. 2, 9). The storm during the snowmelt

414   addition exported 0.3 g $^{15}N_{xs}$. Although seston was still labeled during late summer and fall,

415   export was low. Total $^{15}N_{xs}$ export as seston was 2.7 g, (including the storm) which was 13%

416   of the 20.3 g $^{15}N_{xs}$ removed from the water column in the reach. We did not measure seston

417   export during the snowmelt flood the year following the addition. Label at day 80 was 12‰

418   above background and undetectable in one sample during the 2003 snowmelt flood. If seston

419   were enriched by 6‰ during snowmelt 2003, then based on discharge and estimated particle

420   N concentration, export could have been 2 g ^{15}N. Cumulative export of seston particles

421   through 2003 was about 2.5 times higher than the total amount measured in benthic pools on

422   day 14 of the snowmelt addition showing that these particles were coming from an

423   unmeasured pool.

424   As a proportion of ^{15}N removed, Spring Creek exported 6.5 times more ^{15}N as seston

425   in the baseflow addition relative to the snowmelt addition (Fig 9, Table 3). Like the

426   snowmelt addition, export was highest immediately following the baseflow addition and

427   declined through autumn reflecting declines in both seston δ^{15}N and discharge (Figs. 2, 9).

428   Seston δ^{15}N and discharge declined throughout autumn (Figs. 2, 9). During late summer and

429   fall the stream exported 12.1 g ^{15}N$_{xs}$ of seston, which included the storm on day 18. Export

430   presumably was low during the winter because of low discharge. Given δ^{15}N of seston in

431   autumn and a constant discharge of 0.07 m^3 s^{-1} throughout the winter, we estimate winter

432   seston flux as no higher than 2.4 g ^{15}N$_{xs}$. At the onset of snowmelt in spring 2004, discharge

433   increased, driving renewed export of particulate ^{15}N. Due to higher flow, seston export

434   during the following snowmelt was 16.1 g ^{15}N$_{xs}$, which was higher than export during the

435   previous summer and autumn. Total seston export following the baseflow addition was 31.0

436   g which represented 71% of the ^{15}N$_{xs}$ removed from the stream during the addition (Table 3).

437   The storm on day 18 during the baseflow addition had high instantaneous rates of

438   seston export, but it represented only 8% of total labeled seston export from the reach. (Figs.

439   9, 10). Particle N concentrations increased 1000-fold during this storm (Fig. 10B), and the

440   seston δ^{15}N declined to about 2‰ above background (Fig. 10C), suggesting that the high N

441   export was not of labeled, benthic N, but rather particle N from upstream or riparian areas.

442   As particle N concentration dropped, δ^{15}N rebounded, and even exceeded pre-storm values as

443   exported N reverted to N from the channel bottom in the study reach. Total export of tracer

20

444   $^{15}N_{xs}$ as seston during this storm (Fig. 10D) was 2.4 g $^{15}N_{xs}$ which represented 16% of the 15

445   g of $^{15}N_{xs}$ inventoried on the stream bed 3 d earlier (Table 2).

446

447   *Mass balance*—Of the 70-75 g $^{15}N-NO_3^-$ added each season 20.3 g was removed from

448   the water column during snowmelt season and 43.5 g was removed during baseflow season.

449   The remainder was exported as NO_3^- to Bull Trout Lake. Of the $^{15}N-NO_3^-$ that was removed

450   from the water column, the fraction found in benthic pools was much lower in the snowmelt

451   compared to the baseflow addition (Table 3). By day 14 of the snowmelt addition we

452   recovered only 15% of the $^{15}N_{xs}$ that was removed from the water column, but much more

453   (41%) in the baseflow addition (Table 2). We did not sample the parafluvial zone in the

454   snowmelt addition, but assuming that the fraction of N transported into this zone was similar

455   to that in the baseflow addition, we would not have increased the inventory by much.

456   Total seston export following each addition accounted for 13% of the N removed

457   during snowmelt and 71% of N during baseflow (Table 3). Much (39%) of ^{15}N removed

458   during the baseflow addition exited the stream during the next snowmelt flood. For both

459   additions, the total amount of N exported as seston exceeded the amount inventoried at any

460   one time in the benthic pools, showing that an unaccounted pool must have supplied seston

461   for export.

462

463   **Discussion**

464   Spring Creek was highly retentive of N, both during snowmelt and baseflow

465   hydrologic regimes, with long residence times of benthic pools and unmeasurable (i.e., >1 yr)

466   turnover of parafluvial sediment pools of N. This high retention was despite a large annual

467   snowmelt flood and streambed composed of gravels with high mobility during such flows

468   (Arp et al. 2007; Myers et al. 2007). Thus, N residence time in the stream is on the order of

469   at least one year, possibly longer, and strongly points to storage as an important fate for N

470   that has been assimilated from the water column.

471

472   *Benthic uptake, hydrologic storage, and denitrification*—Nitrate removal, measured

473   as uptake velocity was similar between the snowmelt addition and the baseflow addition.

474   Demand for this N was high; v_f value during snowmelt was in the top 20% of 72 tracer

475   experiments in streams (Hall et al. 2009). High assimilatory N removal in Spring Creek is

476   perhaps not surprising given its low dissolved inorganic N concentrations (5-20μg N L^{-1}) and

477   high demand for this nutrient. However, high demand for N during snowmelt is somewhat

478   surprising because streams have higher biofilm biomass during baseflow than during or after

479   floods (Fisher et al. 1982), and this higher biomass should take up NO_3^- more rapidly.

480   During the baseflow experiment epilithon and FBON standing stocks were higher and

481   temperatures were warmer than during snowmelt. In fact, benthic uptake of N was higher in

482   the baseflow addition (after correcting for higher ^{15}N in the water), and thus we might have

483   expected uptake velocity to be higher because of this higher biological demand. However,

484   the similar uptake velocity in snowmelt was more likely due to groundwater-surface water

485   exchange, and not because of biological demand on the stream bed. This hyporheic loss of N

486   during snowmelt probably explains why we were able to account for little of the tracer as

487   exported particles. Because of high hydrologic connectivity between the stream and its

488   riparian zone during snowmelt, most of the ^{15}N likely ended up far from the stream.

489   Conversely, during the baseflow addition, it is more likely that the ^{15}N tracer was located in

490   zones closer to the channel where particulate tracer could be more easily mobilized during

491   high flows (Fig 9). Our Br$^-$ data (Fig. 4) support this notion in that near-channel exchange

492   was stronger during baseflow compared to snowmelt.

493   We have five lines of evidence that suggest that the hyporheic zone was where much

494   of the ^{15}N was stored. 1) Based on water table elevation, there was a spatially consistent net

495   flow of water from the stream to the hyporheic zone. This flow would have carried N from

496   the stream to the riparian zone, where it could be immobilized by soil microbes or vegetation.

497   2) We measured high standing stocks of particle ^{15}N in the near-stream parafluvial zone.

498   This region contained about 25% of the measurable benthic ^{15}N$_{xs}$ standing stock, but it

499   probably only constituted a small fraction of the hyporheic zone. Given that the hyporheic

500   zone was much larger than what we sampled, it likely contained much more immobilized

501   ^{15}N. 3) We measured ^{15}N tracer in streamside willows showing unequivocally that some

502   tracer was transported away from the stream channel into riparian vegetation. 4)

503   Groundwater wells lateral to the stream channel contained 5-40% stream water as measured

504   by Br$^-$ concentration, and the magnitude of stream water in the subsurface increased with

505   distance from the release point during both tracer tests. 5) Hydrometric analysis from four

506   discharge gauging stations maintained during 2004 suggested that the lower portion of Spring

507   Creek study reach was losing during both snowmelt peakflow and summer storms.

508   Comparison of mean annual flows however, suggested that the stream was either slightly

509   gaining or neutral (Arp et al. 2006). Thus the loss and exchange of water with the riparian

510   floodplain in our study reach varied with flow conditions. Additionally, transient storage was

511   high in the reach with transient storage area ÷ channel cross section area $(A_s/A) = 0.64$

512   suggesting high exchange of water with backwater or hyporheic zones (Arp and Baker 2007).

513   Low denitrification rates were also not surprising given low ambient NO_3^- and

514   dissolved organic carbon concentrations (Arp and Baker 2007). Further, the denitrification

515   potential rates we measured in Spring Creek were an order of magnitude lower than ambient

516   rates in parafluvial groundwater of a eutrophic river (Baker and Vervier 2004). While

517   denitrification certainly accounts for some amount of N loss in our study (Mulholland et al.

518   2008) ambient rates were likely too low to detect significant change in the isotopic

519   composition of the dissolved gas pool.

520

521   *Long residence time in benthic and hyporheic pools*—Residence time of N stored on

522   the stream bed and in the near-hyporheic zone was much longer than those reported from

523   other N isotope tracer studies in streams. We compared residence time of FBON, epilithon

524   N, and wood N with 6 studies from the Lotic Intersite Nitrogen eXperiment (LINX 1) and an

525   isotope addition to an estuary (Dodds et al. 2000; Tank et al. 2000; Hamilton et al. 2001;

526   Mulholland et al. 2001; Merriam et al. 2002; Tobias et al. 2003; Ashkenas et al. 2004).

527   Residence times for those studies averaged 26 d (range 7-53 d) for FBON, 20 d (range 1.5-47

528   d) for epilithon, and 33 d (range 19-47 d) for wood. Residence times of any pool in Spring

529   Creek were much longer than the longest residence times from the LINX 1 study. The

530   shortest residence time we were able to measure was 117 d for both epilithon and wood

531   biofilm during the baseflow addition. FBON residence times were >200 d, and hyporheic N

532   exceeded 1 yr because hyporheic standing stocks of ^{15}N were not depleted 1 year after the

533   baseflow addition. These residence times were longer than those for an estuary where 60%

534   of stored N was in sediment samples and observed at least 2 months following the isotope

535   addition (Tobias et al. 2003). The cold climate in Spring Creek may have contributed to

536   longer storage of N, but even if this stream were as biologically active all year as during the

537   6-month growing season, it would still have much longer residence times for N as some of

538   the LINX streams because residence times exceeded twice the values from other streams.

539   Year-scale retention of isotope has been noted in only a few studies. Peterson et al.

540   (1997) found labeled insects and epilithon in the Kuparuk River one summer later. The

541   Kuparuk freezes solid from October to May, thus shutting down mineralization and fluvial

542   export. Insects have 2-3 year life cycles which will promote interannual storage of ^{15}N in

543   their tissue (Peterson et al. 1997). In Mack Creek, Oregon, mosses and epilithon each

544   contained 5% of their peak label one year following ^{15}N addition (Ashkenas et al. 2004).

545   Our data suggest that uptake in the hyporheic zone is an important mechanism by

546   which N can be stored for long periods as shown for the hyporheic zone in Mack Creek

547   where riparian plants were enriched following ^{15}N addition (Ashkenas et al. 2004). Streams

548   similar to Spring Creek, at mountain fronts with valley and slope transitions, gain and lose

549   water over long (100-1000's of m) spatial scales (Covino and McGlynn 2007). A similar

550   process likely operates here to affect N retention in the subsurface, because of the

551   hydrogeomorphic transition of a stream approaching a lake and its delta (Arp et al. 2006; Arp

552   et al. 2007).

553   If in fact the hyporheic zone was a primary storage zone for particulate N as the

554   hydrologic data and labeled willows suggest, then it posits a mechanism by which residence

555   time can be so long in some of the shallow benthic pools. Either mineralization followed by

556   transport and reuptake of N by the benthos, or movement of particle N from deeper to

557   shallow pools may have allowed benthic pools to remain enriched for a long time. We could

558   find no direct evidence in the literature to support either mechanism, but Holmes et al. (1994)

559   suggested that stream-derived particles were mineralized in the hyporheic zone and

560   contributed to high nitrification rates and supported algal growth in areas where hyporheic

561   water re-entered the stream (Valett et al. 1994; Henry and Fisher 2003).

562

563   *Floods and seston export*—Although most measurements of nutrient cycling occur

564   during baseflow conditions, it is important to recognize that in snowmelt-dominated

565   watersheds, little nutrient transport occurs during baseflow, but rather during high flows

566   (Meyer and Likens 1979; Creed et al. 1996; Baron and Campbell 1997). The form of this N

567   can be dissolved (as NO_3^-) (Baron and Campbell 1997) or particulate (Wurtsbaugh et al.

568   2005).

569   In Spring Creek, seston dominated ^{15}N export. Other studies have shown that

570   mineralized N (as DON or NH_4^+) can be important losses (Hamilton et al. 2001; Merriam et

571   al. 2002; Tobias et al. 2003). The amount of tracer exported in dissolved pools was low in

572   Spring Creek. In addition, the mass balance for ^{15}N in the baseflow addition shows that most

573   of the ^{15}N-NO_3^- removed by the stream was subsequently accounted for as seston (Table 3).

574   Despite that nearly all ^{15}N export was as seston, summer floods scoured little of the

575   labeled N from the stream. The summer spate that occurred three days following the

576   baseflow addition scoured 16% of the ^{15}N$_{xs}$ found on the stream bed and constituted 8% of

577   the total seston ^{15}N$_{xs}$ export. This resistance to particle export occurred despite gravelly bed

578   sediments that are highly mobile during floods (Myers et al. 2007), which we assume would

579   have facilitated organic matter loss during storms. Other stream tracer studies that have had

580   serendipitous floods showed similar resistance to benthic N export. A spate during a ^{15}N

581   addition to a Puerto Rican rainforest stream increased discharge 20-fold, but only removed

582   37% of the N tracer in stream detrital pools (Merriam et al. 2002). A storm increased

583   discharge 10-fold in Ball Creek, North Carolina but did not reduce $\delta^{15}N$ in benthic pools

584   (Tank et al. 2000). At first glance, these results suggest a paradox. Tracer studies show that

585   benthic N is resistant to flooding (Tank et al. 2000; Merriam et al. 2002; our data), yet

586   budget studies show that most export occurs during storms (Webster et al. 1990). Using our

587   mass balance approach, we suggest these two findings are congruent. Because of the strong

588   decline in $\delta^{15}N$ during the storm, much of what was exported during the flood was not from

589   the labeled benthos in the study reach, but from upstream, the unwetted channel, or from

590   upland sources. With moderate storms, a fraction of stream benthic $^{15}N_{xs}$ is exported and this

591   amount is larger than what would have been exported over the same time interval during

592   baseflow. However, the bulk of $^{15}N_{xs}$ stored was resistant to export by any one storm. Over

593   long timescales (and several floods) much of the benthic N in Spring Creek will eventually

594   be winnowed out as fine particles.

595

596   *A longer-term view of stream element cycling*—In order to understand and predict

597   how streams transport and transform elements such as N, we show that it is necessary to

598   account for these processes over time and over a range of environmental variability, i.e.,

599   snowmelt floods, rain storms, and baseflow. Ecologists have a body of theory and data

600   showing mechanisms of N uptake across a range of streams (Tank et al. 2008; Hall et al.

601   2009), but we have not incorporated hydrologic variability into this theory, despite knowing

602   for 30 yr that hydrologic variation regulates element export (Meyer and Likens 1979).

603   Additionally, it is necessary to consider the stream as part of a larger parafluvial and riparian

604   ecosystem because elements in transport do not necessary remain in the channel (Fisher et al.

605   1998). Hyporheic exchange represented an important nitrate sink, especially during

606   snowmelt when benthic biomass and uptake was low. The fate of this missing N is unknown,

607   but we hypothesize that this N is in long-term soil and vegetation pools where residence

608   times of N will be long simply because the pools are so large (Likens and Bormann 1995).

609   In this regard, we consider streams with a large connected floodplain or hyporheic zone to be

610   analogous to a small watershed (Bormann and Likens 1967; Likens and Bormann 1995).

611   Over long timescales (millennia) streams with floodplains are probably in steady state with

612   respect to N cycling (Brookshire et al. 2009), but over shorter timescales (month-years), they

613   may not be in steady state because the storage pool is so large, that slight changes to the size

614   of this pool could dramatically alter export. Incorporating hyporheic storage and hydrologic

615   variation in our model of N cycling, we suggest that removal of N from the water column is

616   decoupled from hydrologic export; i.e., exported N is not from the same time or place as N

617   removed from streamwater. Therefore, Spring Creek is not in steady state with respect to its

618   nutrient budget at months-years time scales, similar to phosphorus in Bear Brook (Meyer and

619   Likens 1979).

620   There is much current interest in the role of streams and rivers in removing N

621   (Alexander et al. 2000; Seitzinger et al. 2002). Denitrification is thought to be the primary

622   process in which streams and rivers remove N from transport (Seitzinger et al. 2006;

623   Mulholland et al. 2008), though, on average, 84% of NO_3^- removal is via assimilation (Hall et

624   al. 2009, Mulholland et al. 2009). We show that storage in the stream bed can retard N

625   export, and may be an important fate especially if streams have intact hyporheic zones and

626   floodplains. Additionally, assimilatory uptake may precede denitrification in riparian zones.

627   Contrary to our hypothesis, we show that Spring Creek was not solely a conduit for nutrients

628   at high flows, but had as high an uptake velocity for N during the snowmelt flood as during

629   summer baseflow. Given that most dissolved nutrient transport occurs at high flows (Baron

630   and Campbell 1997), streams with connected hyporheic zones or floodplains may be able to

631   absorb some of this N at a time when biotic uptake is low. Variable flows may promote

632   dissolved nutrient transformation and storage in streams with connected hyporheic zones and

633   floodplains.

634

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789

Table 1. Nitrate uptake during the snowmelt and baseflow isotope additions. Fraction removed describes the amount of ^{15}N removed by the 1895-m stream reach relative to what we added at the measurement time; the balance was exported to the lake. No data are shown for baseflow at the 3-day sampling period because regression of dilution-corrected ^{15}N flux vs. distance downstream was not statistically significant, thus uptake rate could not be calculated. Ninety percent confidence intervals are shown below each value in parentheses.

	Day	Discharge	Uptake rate	Uptake length	Uptake velocity	Fraction removed
		$(m^3 \ s^{-1})$	$(1000 \ m^{-1})$	(m)	$(mm \ min^{-1})$	
Snowmelt	3	0.754	0.134 (0.069-0.198)	7460 (5040-14300)	1.60	0.22
	8	0.576	0.312 (0.164-0.461)	3210 (2170-6100)	2.84	0.45
	14	0.488	0.248 (0.207-0.289)	4030 (3460-4810)	1.91	0.37
	Average		0.232 (0.045-0.28)	4320 (740-5640)	2.12 (1.24-3.36)	0.35 (0.19-0.53)
Baseflow	8	0.160	0.369 (0.081-0.658)	2710 (1520-12410)	1.15	0.50
	14	0.127	0.528 (0.408-0.648)	1894 (1540-2450)	1.30	0.63
	Average		0.456 (0.338-0.573)	2190 (1740-2950)	1.27 (0.95-1.60)	0.58 (0.47-0.66)

Table 2. Mass of ^{15}N excess ($^{15}N_{xs}$) in benthic pools and seston for the two additions at the end of each tracer injection (day 14). Hyporheic fine benthic organic N (FBON) is the average of three dates collected in summer and autumn 2003. 'Percent of removed accounted for' is the total $^{15}N_{xs}$ inventoried in the reach relative to the amount of $^{15}N\text{-}NO_3^-$ removed by the stream during the 14 d addition (i.e., not including what was exported to the lake as unremoved $^{15}N\text{-}NO_3^-$).

	Snowmelt		Baseflow	
	N stock (mg N m^{-2})	tracer stock (g ^{15}N reach^{-1})	N stock (mg N m^{-2})	tracer stock (g ^{15}N reach^{-1})
Fine benthic organic N	200	0.1	1200	4.3
Epilithon	40	0.3	110	4.0
Wood biofilm	17	0.1	14	0.3
Coarse benthic organic N	110	0.3	120	0.4
Macrophytes	NM	NM	20	0.5
Insects	72	0.3	82	1.3
Hyporheic FBON	NM	NM	6100	4.1
Seston export to day 14		1.5		2.6
NH_4^+ export to day 15		0*		0.5
NO_3^- export to day 15 [†]		0.5		0.1
DON export to day 15		0*		0*
Denitrification		0*		0*
Total		3.1		18.1
90% confidence interval		(2.3-3.9)		(11.8-23.7)
Percent of removed accounted for		15%		41%

790 NM means not measured
791 * ^{15}N enrichment not detectable above background
792 † Export only includes NO_3^- that was mineralized and not tracer that was directly exported
793 from the reach during the 14-d addition without being taken up.

Table 3. Mass balances of nitrogen for the snowmelt and baseflow additions on the final day of the ^{15}N addition (day 14) and the summers following each addition. We added 70 g ^{15}N for the snowmelt addition and 75 g ^{15}N for the baseflow addition. Percent removed is the % of the ^{15}N$_{xs}$ that was removed from the water column to the benthos and hyporheic zone during the 2-week addition. Because we found little export as dissolved N after the addition was shut off, we assumed that the cumulative amount of dissolved loss was that which was exported by day 15.

	Snowmelt				Baseflow			
	Day 14 (2002)		Following summer (2003)		Day 14 (2003)		Following summer (2004)	
	g^{15}N$_{xs}$	% of removed	g^{15}N$_{xs}$	% of removed	g^{15}N$_{xs}$	% of removed	g^{15}N$_{xs}$	% of removed
Amount of tracer exported	49.7	-	-	-	31.5	-	-	-
Amount removed by stream	20.3	100	-	-	43.5	100	-	-
Inventoried in stream channel	1.1	6	0.03	0.1	14.9	34	5.0	12
Exported as particles	1.5	7	2.7	13	2.6	6	31.0	71
Exported as dissolved	0.5	2	0.5	2	0.6	1	0.6	1
Missing	17.2	85	17.1	84	25.4	58	7.5	16

Figure legends

Figure 1. Spring Creek near where it exits forest onto a glacial outwash floodplain. Note the dry gravel bar where we sampled parafluvial sediment.

Figure 2. Hydrographs of Spring Creek during the three years of this study. Grey bars indicate the period of the two-week snowmelt and baseflow ^{15}N additions.

Figure 3. Water table maps showing higher flow of streamwater to the subsurface zone during (A) snowmelt compared to (B) baseflow. Predominance of black arrows during snowmelt show the stream loses water to the hyporheic zone, while during baseflow, white arrows show that most of the subsurface flow is parallel to the stream. Stars indicate water table monitoring points. Dotted lines represent 0.1 m contours of water table height above an arbitrary datum descending to Bull Trout Lake.

Figure 4. Percent stream water measured from Br$^-$ in wells located in the channel and lateral to the stream show substantial hyporheic exchange during both experiments.

Figure 5. During the 2002 snowmelt addition, epilithon δ^{15}N increased as a function of epilithon chlorophyll standing stock.   Data are from day 14, which was the end of the snowmelt ^{15}N addition.

Figure 6. Mass of ^{15}N$_{xs}$ in FBON, epilithon, and wood biofilms during the snowmelt and baseflow additions. Note differences in Y-axis scaling, indicating the much higher tracer

mass during the baseflow addition than during the snowmelt experiment. The tracer also

persisted in the baseflow addition relative to the snowmelt addition. Grey bars on each

figure indicate the snowmelt flood following the additions the previous year. Y-axis is g

$^{15}N_{xs}$ in a particular pool scaled to the entire reach. Error bars are 90% t-based confidence

intervals of the mean.

Figure 7. Hyporheic storage of $^{15}N_{xs}$ did not decline with time for one year following the

baseflow ^{15}N addition in 2003. Y-axis is g $^{15}N_{xs}$ in the entire stream reach of hyporheic

zone immediately beneath and adjacent to the wetted area of the stream, but within the

active channel. Error bars are 90% t-based confidence intervals of the mean.

Figure 8. Some willows downstream of the addition site contained ^{15}N label. X-axis is

the distance downstream of the addition site, and Y-axis is the $\delta^{15}N$ of willow leaves.

Open symbols are from three unlabeled willows upstream of the addition site. Solid

symbols are willows that could potentially be labeled.

Figure 9.   Much of the stored ^{15}N was exported from the stream as seston. The top two

panels show the $\delta^{15}N$ of seston through time for the snowmelt and baseflow additions.

The bottom panels show both instantaneous export (solid circles, left-axes) and

cumulative export (open circles, right-axes). Units for instantaneous export are g $^{15}N\,d^{-1}$,

and cumulative export is the fraction of ^{15}N relative to the total amount of ^{15}N removed by

the stream.

Figure 10. A storm 3 days after the baseflow addition ended removed a small fraction of the tracer ^{15}N standing stock. (A) shows the storm hydrograph. (B) shows that particulate N concentration (g N m^{-3}) increased during the storm. (C) shows that the δ^{15}N of seston decreased during the storm, and (D) shows export of ^{15}N$_{xs}$ as seston (g^{15}N h^{-1}).

Fig 2

A) Snowmelt

B) Baseflow

100 m

Fig 3

A) Snowmelt

streambank (4-6 m lateral)
streambed (50 cm depth)

B) Baseflow

Percent stream water (mean ± 1 SE)

Distance from solute addition site (m)
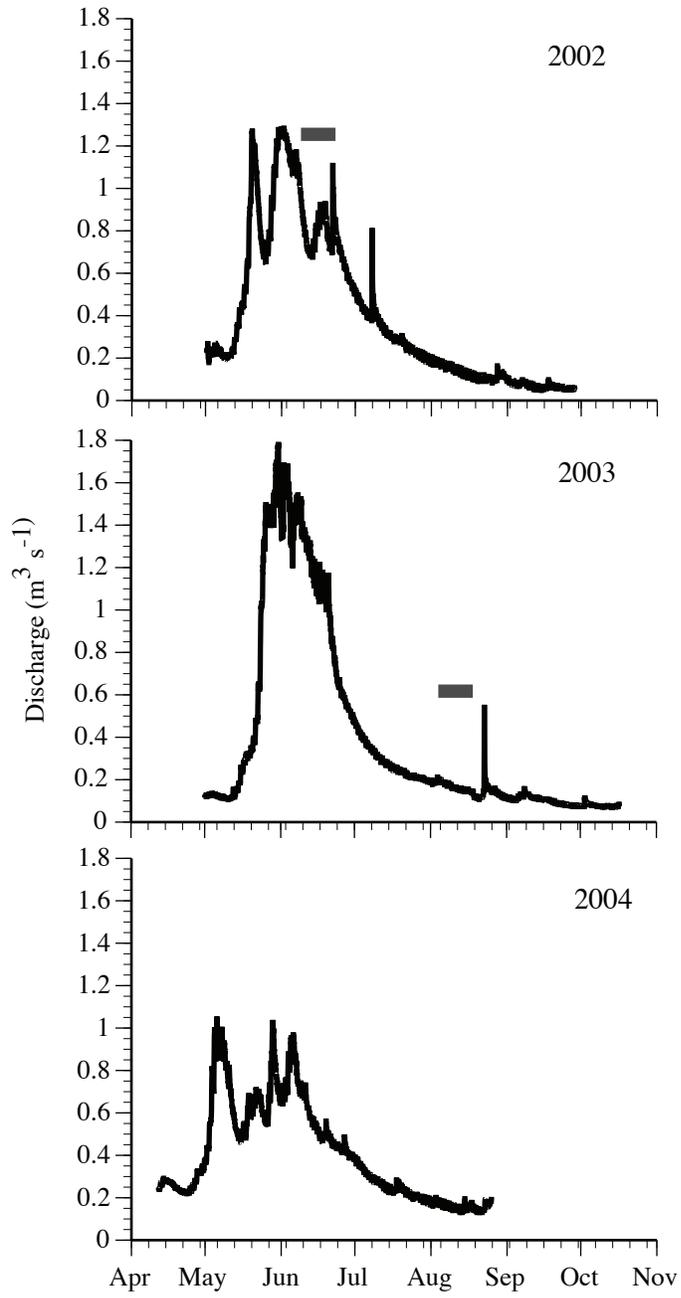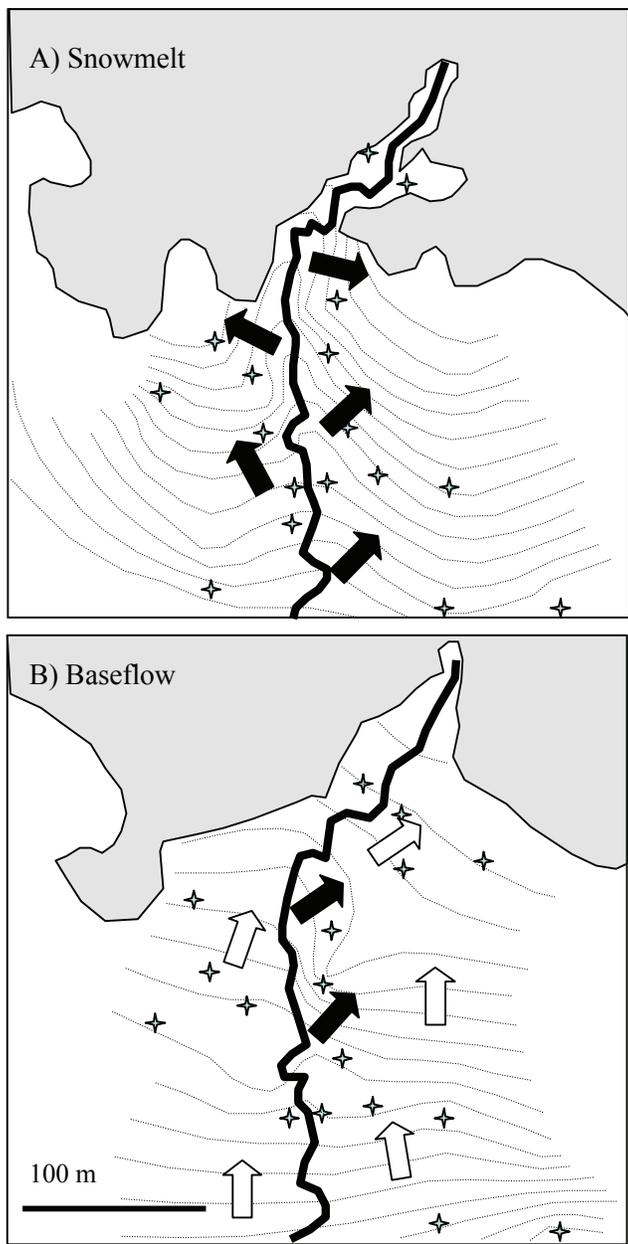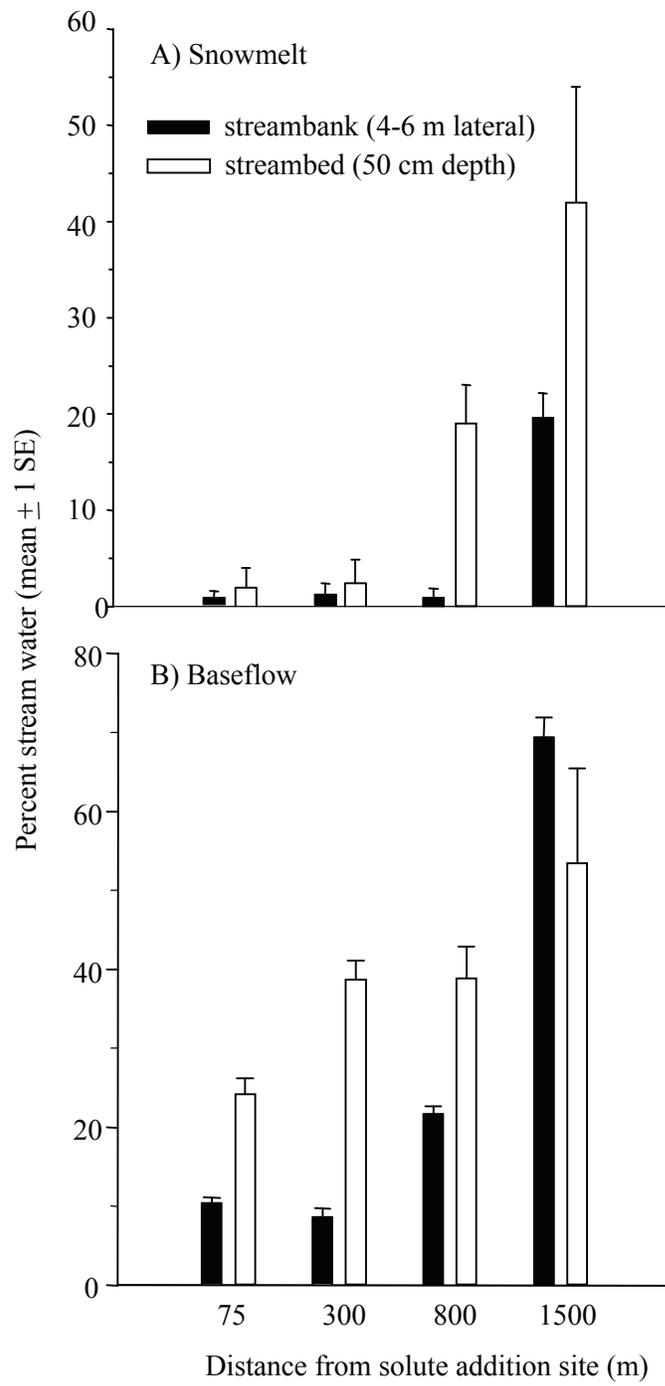
Fig 4
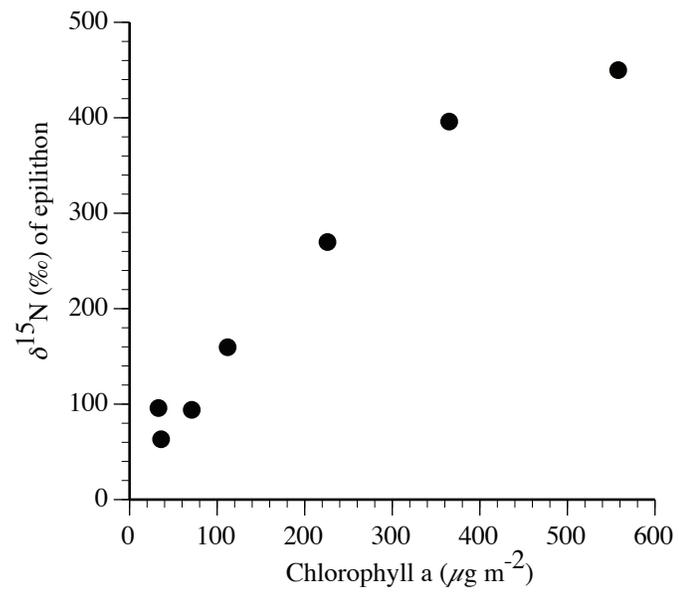
Fig. 5
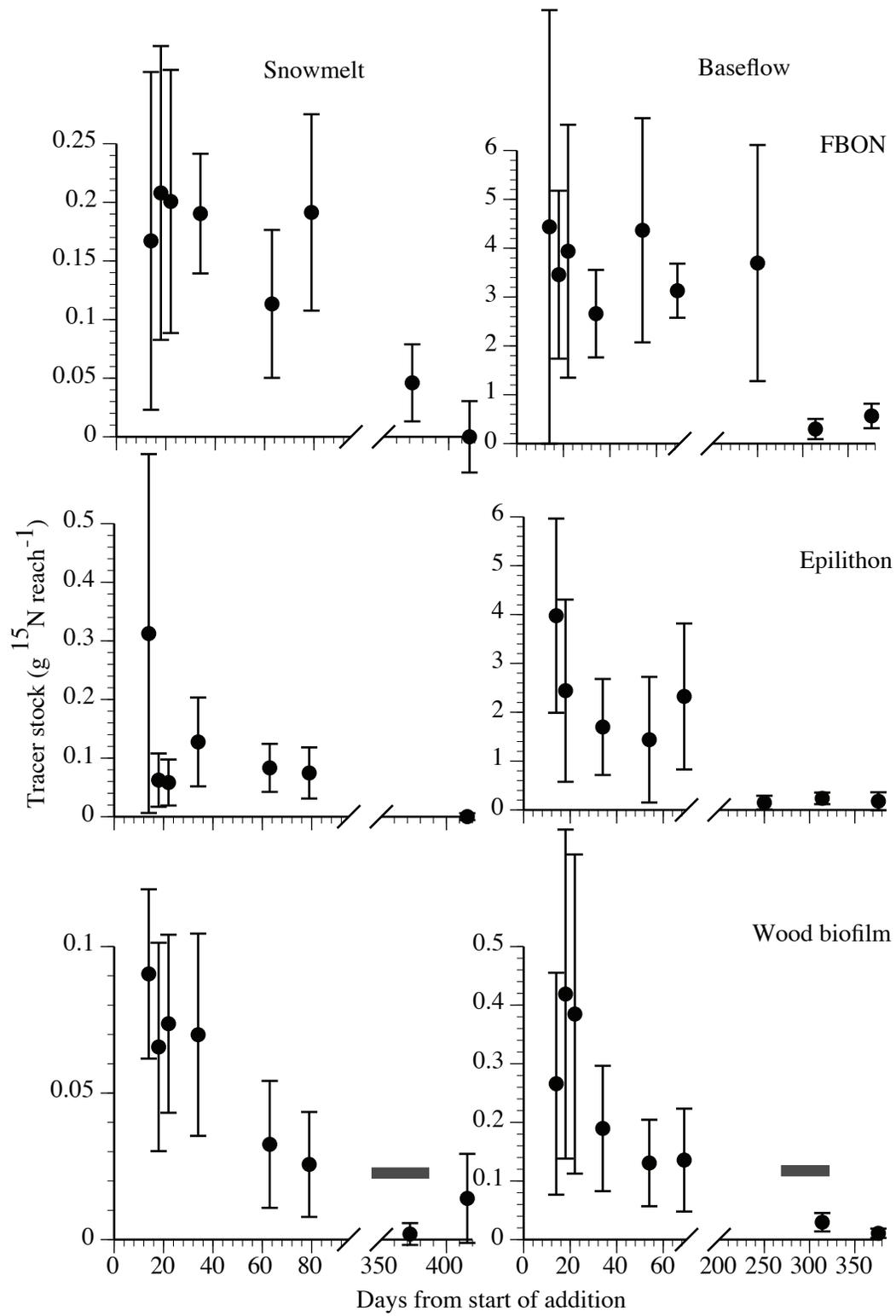
Fig. 6

Fig 7

Fig 8

Fig 9

Fig 10