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4   Running Head: Litter species and nutrient uptake

5

6   Title: Leaf litter identity alters the timing of lotic nutrient dynamics

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8   Caleb J. Robbins[1,3,4], William J. Matthaeus[1], Stephen C. Cook[1], Lauren M. Housley[1], Sarah E.

9   Robison[1], Matt A. Garbarino[2], Erick S. LeBrun[1], Swastika Raut[1], Chi-Yen Tseng[1] and Ryan S.

10   King[1]

11

12   [1]Department of Biology, Baylor University, One Bear Place 97388, Waco, Texas 76798-7388,

13   USA

14   [2]Department of Environmental Science, Baylor University, One Bear Place 97266, Waco, Texas

15   76798-7388, USA

16   [3]Present address: Kansas Biological Survey, University of Kansas, 2101 Constant Ave,

17   Lawrence, Kansas 66047

18

19   Email addresses:

20   [4]Caleb_Robbins@ku.edu

21

22

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Summary

1. The effects of resource quality on ecosystems can shift through time based on preferential use and elemental needs of biotic consumers. For example, leaf litter decomposition rates are strongly controlled by initial litter quality, where labile litter is processed and depleted more quickly than recalcitrant litters.

2. We examined the effect of this "processing continuum" on stream nutrient dynamics. We added one of four different litter compositions differing in litter quality (Cottonwood (*Populus deltoides*; labile), Sycamore (*Platanus occidentalis*; recalcitrant), Bur Oak (*Quercus macrocarpa*, recalcitrant) and Mixed (equivalent mixture of previous three species)) to 12 large (~20 m long, with riffle, glide and pool sections) outdoor stream mesocosms to assess the effect of litter species composition on whole-stream nutrient uptake. Nutrients were dosed once weekly for eight weeks to measure uptake of NH_4-N, NO_3-N, and PO_4-P. We also measured changes in litter C, N, and P content on days 28 and 56 of the study.

3. Nutrient uptake rates were highly variable, but occasionally very different among litter treatments (~5x between highest and lowest uptake rates by species). Uptake rates were generally greatest in Cottonwood (labile) streams early in the study. However, during the last four weeks of the study, Bur Oak streams (recalcitrant) took up more nutrients than Cottonwood streams, resulting in more cumulative NO_3-N uptake in Bur Oak than in Cottonwood streams. Cumulative NO_3-N uptake was greater in Mixed streams than expected (non-additive) on two dates of measurement, but was generally additive.

4. Changes in litter nutrient content largely corroborated nutrient uptake patterns, suggesting strong N immobilization early in the study and some N mineralization later in the study. P was strongly retained by most litters, but especially Bur Oak. Nutrient content of litter also largely

52 changed additively, suggesting minimal evidence for non-additive diversity effects on nutrient

53 source/sink status.

54 5. Our results demonstrate that litter species identity can have whole-ecosystem effects on stream

55 nutrient dynamics, with important implications for the form and fate of nutrients exported

56 downstream. Further, diverse litter assemblages may serve as temporal stabilizers of ecosystem

57 processes, such as nutrient sequestration, due to microbial nutrient requirements and differential

58 decomposition rates, or the classic litter "processing continuum".

59

60

61 **Introduction**

62 Resource quality at the base of food webs controls biogeochemical rates and nutritional

63 demands of consumers, and is thus central to understanding ecosystem function (Cross *et al.*,

64 2005; Bukovinszky *et al.*, 2008; Hladyz *et al.*, 2009; Marcarelli *et al.*, 2011). In many streams,

65 leaf litter is a dominant basal resource, and decomposition of that litter is a key aspect of

66 ecosystem function where related processes and food webs can be centralized around litter

67 inputs. Shifts in the quality and compositional variety of stream detritus, such as those associated

68 with riparian species invasions, diversity loss or other anthropogenic activity (Sweeney *et al.*,

69 2004; Burton & Samuelson, 2008), are therefore highly likely to influence stream ecosystems

70 (Moore *et al.*, 2004; Lecerf *et al.*, 2005; Kominoski, Marczak & Richardson, 2011; Handa *et al.*,

71 2014).

72 Nutrient cycling is a central aspect of stream ecosystem function, but how detrital

73 resource quality influences this process is poorly understood. Leaf litter is a quantitatively

74 important nutrient sink in streams (Tank *et al.*, 2000; Webster *et al.*, 2000; Sebestyen *et al.*,

75 2014; Lin *et al.*, 2015), as litter decomposing microbes use water column nutrients to supplement

76 high C:nutrient ratios in leaf litter (Gulis & Suberkropp, 2003; Cheever *et al.*, 2013; Gulis *et al.*,

77 2017). This microbial dependence on water column nutrients is altered by litter quality, a

78 function of species identity, and often conceptualized as driven by C:N ratios (Stelzer, Heffernan

79 & Likens, 2003; Pastor *et al.*, 2014).

80   Nutrient cycling in leaf litter also changes through time with shifting nutrient demands of

81 the decomposing microbial community and reduction of litter quantity as decomposition

82 progresses. Terrestrial studies in particular suggest a predictable shift in litter microbial

83 decomposer communities from net immobilizers (uptake of inorganic nutrients) to net

84 mineralizers (release of inorganic nutrients from the litter substrate) as a function of time and

85 initial litter quality (Parton *et al.*, 2007; García-Palacios *et al.*, 2017; Yue *et al.*, 2018). Such

86 shifts have been less demonstrable in aquatic studies (Cheever, Kratzer & Webster, 2012;

87 Halvorson *et al.*, 2016). However, more recalcitrant litter species may serve as longer term

88 nutrient sinks due to a longer residence time than labile litters, pointing to the importance of litter

89 quantity-quality interactions (Mehring *et al.*, 2015).

90   In spite of a strong historical interest of stream ecologists in nutrient spiraling, there has

91 been little emphasis on the role of seasonally dominant inputs of leaf litter in driving patterns of

92 reach-scale uptake, and how that role might change throughout decomposition and in response to

93 the compositional variety of the detritus. More specifically, evidence for litter species effects on

94 nutrient cycling has been relegated to observations in microcosms or via measurements of litter

95 or microbial nutrient content (e.g., Quinn and others 2000; Pastor and others 2014; Mehring and

96 others 2015). Commonly used leaf disks in particular usually avoid any stem or large veins and

97 may poorly capture the nutrient demands of an entire leaf litter assemblage (i.e., multiple whole

98   leaf packs) due to differing surface areas, variable chemical composition, spatially heterogeneous

99   microbial colonization and growth, microsites of varying conditions (e.g., pH or O_2), and leaf

100   toughness that can control mechanical export of leaf litter from the system through breakdown.

101   Scaling results from leaf disks to whole-reach dynamics may therefore be problematic. Exclusion

102   techniques have been used to examine the functional role of leaf litter in streams (Wallace *et al.*,

103   1997), but experimentally altering the species identity of litters in natural streams is extremely

104   difficult, requiring exclusion of non-target litters and identifying streams similar enough for

105   comparability. Therefore, addressing questions regarding the influence of specific leaf litter

106   identity and diversity on whole ecosystem nutrient cycling is well-suited to large mesocosm

107   facilities where reach-scale nutrient uptake can be measured across whole leaf pack assemblages.

108   Here, we added one of four different litter compositions (Cottonwood (*Populus*

109   *deltoides*), Sycamore (*Platanus occidentalis*), Bur Oak (*Quercus macrocarpa*) and Mixed

110   (equivalent mixture of previous three species)) to 12 large (~20 m long, 2000 L), multiple habitat

111   (riffle, glide and pool sections) outdoor stream mesocosms to assess the effect of litter species

112   identity, and therefore quality, on litter nutrient dynamics over two months. We measured both

113   weekly uptake of nutrient slugs and changes in litter nutrient content to determine how litter

114   species identity influences inorganic nutrient demand. Litter nutrient content was used to help

115   explain nutrient uptake patterns and provide evidence for the fate of nutrient uptake, particularly

116   because the nutrient uptake patterns should be influenced by more than just litter-dominated

117   processes, even though litter was the dominant substrate in each mesocosm. Because quickly

118   decomposing litter should increase microbial metabolism and support faster microbial growth

119   and colonization, we hypothesized that streams with fast decomposing litter and high nutrient

120   content (Cottonwood) would initially take up more nutrients than streams with slowly

121 decomposing litter with low nutrient content (Bur Oak and Sycamore), but uptake rates in

122 streams with Cottonwood would slow down compared to those with Bur Oak and Sycamore,

123 leading to Bur Oak and Sycamore serving as longer-term nutrient sinks. In addition to the

124 relationship between breakdown rates and litter quality, mixing multiple litter species can result

125 in non-additive breakdown, where mixtures decompose at rates that would not be expected based

126 on the breakdown rates of its individual constituents (Schindler & Gessner, 2009; Lecerf *et al.*,

127 2011). Many of the mechanisms thought to underlie positive litter mixing effects on breakdown

128 rates involve the active transfer of nutrients between mixture constituents (Gessner *et al.*, 2010).

129 Therefore, we also hypothesized that litter mixtures would result in non-additive effects on

130 decomposition rates, which in turn could result in non-additive effects on nutrient demand.

131

132   **Methods**

133 *Experimental design*

134   The experiment was conducted in 12 large, outdoor stream mesocosms at the Baylor

135 Experimental Aquatic Research (BEAR) facility in McLennan County, Texas (Fig. S1-S4),

136 which receives low nutrient water (NO_3-N=12 µg/L, NH_4-N=7 µg/L, PO_4-P=6 µg/L) pumped

137 from an 80-ha constructed wetland. Each stream was comprised of an 8 meter long riffle

138 upstream of a 9 meter long glide, draining into a 1.7 m^2 pool. During the experiment, water was

139 either fully recirculated (pumped from the pools to the top of the riffles during nutrient uptake

140 assays) at a rate of 200 L/min or allowed to partially recirculate with approximately 6 L/min

141 fresh inflow from the wetland. Each stream drains excess water through a stand-pipe when fresh

142 inflow (or recirculating pump malfunction, see *Net nutrient uptake*) exceeds the capacity of the

143   pools, corresponding to ~2000 L maintained volume, resulting in a turnover rate of 4.2x/d during

144   partial recirculation. Velocity in each riffle was ~5.5 cm/s.

145   Streams were covered with 80% shade-cloth (i.e., 20% light transmittance) to simulate a

146   low-order stream with heavy riparian cover, except for the last downstream 3 meters of riffle,

147   which was left open. Streams were seeded with composite benthic invertebrate and algae

148   samples taken from Salado Creek, TX (30.94472 N, 97.533726 W) by collecting kick-screened

149   benthos into 12 buckets (2 m^2 benthic material in each bucket) and then distributing one bucket

150   evenly across the riffle and glide sections of each stream. This included minor contributions of

151   small gravels that were evenly distributed across mesocosms. The study ran for 56 days,

152   beginning on 19 February 2015 and ending on 16 April 2015. Fifty-six days is short for litter

153   decomposition studies, but litter export from reaches due to spates and the general acceleration

154   of litter decomposition due to high nutrient availability (see *Nutrient uptake*; Greenwood *et al.*,

155   2007; Tant, Rosemond & First, 2013; Rosemond *et al.*, 2015) makes this a reasonable timeframe

156   for evaluating the influence of litter species on nutrient dynamics in small streams. Further,

157   decomposition stage is relative to the rate at which a given litter decomposes, so 'late stages' of

158   decomposition occur sooner for some species than others. We expected our chosen litters (next

159   paragraph) to comprise a gradient from slow-to-fast decomposing species ("processing

160   continuum").

161

162   *Leaf litter*

163   Leaf litter was collected locally ~1 month after the time of initial abscission, and

164   comprised tree species described by Webster and Benfield (1986) as fast (Salicaceae -

165   Cottonwood, *Populus deltoides*), moderate-to-slow (Platanaceae - American Sycamore, *Platanus*

166   *occidentalis*), or slow (Fagaceae - Bur Oak, *Quercus macrocarpa*) with respect to breakdown

167   rates in freshwater systems. We chose tree species that are widely distributed in the United States

168   and at least partially riparian (i.e., no obligate upland species). Leaves with evidence of decay

169   were not used in the experiment. Leaves were dried at room temperature for at least one week.

170   Each stream was randomly assigned one of the four litter types (Cottonwood, Sycamore, Bur

171   Oak, or Mixed leaf; N = 3 per litter type). We distributed 1452 g of litter through the streams on

172   19 February 2015, with 472 g of litter in the riffle, 236 g in each of the caged glide sections, and

173   272 g in the pools. Litter in the riffle was submerged and secured in three large (60 cm wide x 50

174   cm long) sections of coarse plastic mesh (5 mm) to prevent drift. The final dry mass of leaf litter

175   added to each stream was approximately 100 g m^{-2}, within the range of observed litter standing

176   stocks in small, forested streams (Lugthart & Wallace, 1992; Webster *et al.*, 2003).

177   Leaf packs of 6 ± 0.05 g litter material were deployed in replicates of six in the riffle of

178   each stream. Mixed litter leaf packs were composed of 2 g of each litter species. Leaf packs were

179   also retained at the beginning of the study to determine species specific handling losses and

180   elemental content. Three deployed leaf packs were collected on days 28 and 56. Upon retrieval,

181   leaf packs were stored at 4 °C and rinsed with deionized water to remove collected sediments

182   and invertebrates. The remaining leaf tissue was dried at 40 °C for 72 hours and weighed to the

183   nearest 0.01 g. A subset of dried material was pulverized and combusted at 550 °C to obtain ash-

184   free dry mass (AFDM). We calculated decomposition rates (k, d^{-1}) for each litter type as the

185   slope of the regression of log-transformed AFDM against time. Another subset of dried material

186   was used to measure litter C, N, and P content (see below).

187

188   *Weekly net nutrient uptake rates*

189   Net inorganic nutrient uptake is an overall demand for external nutrients in different litter

190   types because it is the balance of immobilization (uptake) and mineralization (release). For

191   example, litters with either lower rates of immobilization due to low activity or high rates of

192   mineralization will exhibit lower net uptake. We measured net inorganic nutrient uptake by

193   measuring decline in daily nutrient concentrations from measured background concentrations

194   added to known nutrient addition concentrations. Nutrient additions were conducted by

195   distributing nutrient stock evenly within each stream at the beginning of weekly dosing periods.

196   Dosing periods lasted three days, except the first dosing period which lasted six days. Because

197   we knew the volume of each stream was 2000 L, spiked additions raised nutrient concentrations

198   in each stream by 5 mg/L NO_3-N, 1 mg/L NH_4-N and 0.5 mg/L PO_4-P. Full mesocosm

199   recirculation (i.e., no inputs of low-nutrient wetland water) was maintained during dosing

200   periods, and was followed by a four-day flushing period where wetland water (NO_3-N=12 µg/L,

201   NH_4-N=7 µg/L, PO_4-P=6 µg/L) replaced dosed mesocosm water before the next dosing period.

202   The purpose of this flushing period was to minimize accumulation of dosed nutrients that were

203   not taken up during a dosing period. Week 1 and 2 dosing periods were not separated by a

204   flushing period to facilitate microbial colonization, but net nutrient declines were calculated

205   separately for weeks 1 and 2 consistent with calculations for all other weeks.

206   Water samples were collected in 1 L dark bottles from the pool of each stream on days 0,

207   1, 2 and 3 of each recirculation period. Day 0 sampling occurred immediately prior to initiating

208   full recirculation for a dosing period. Samples were filtered through pre-rinsed 0.45 µm

209   polypropylene luer-lock filters. Water samples were then either frozen until analysis or

210   refrigerated and analyzed within 24 hours. Dissolved inorganic phosphorus (PO_4-P), nitrate-

211   nitrite, where we assume nitrite is negligible (NO_{2+3}-N = NO_3-N), and ammonium (NH_4-N) were

10

212 analyzed on a Lachat Quik Chem 8500 series 2 continuous flow injection analyzer (Hach

213 Company, Loveland, CO, USA). All analyses followed standard methods (APHA, 1998).

214 NH_4-N, NO_3-N and PO_4-P uptake rates (k) were calculated by regressing log-transformed

215 nutrient concentrations against time since nutrient addition (days). Because significant uptake

216 could occur between the time the nutrient slugs were added and completely mixed, day zero

217 nutrient concentrations for the regressions were calculated by summing the known added nutrient

218 concentration with the nutrient concentration measured during that day (immediately prior to

219 dosing). We set the y-intercept of each regression to the log initial nutrient concentration to make

220 sure that relationships where nutrients declined very strongly on the first day would still have

221 high slopes. Without this constraint, very fast nutrient declines could have lower k than slower

222 nutrient declines, but this was usually a problem only in NH_4-N uptake relationships. NH_4-N was

223 often depleted rapidly to near detection limits in all streams within two days, especially toward

224 the end of the experiment, and so such days (and any days after) were excluded from calculated

225 NH_4-N k rates.

226

227 *Cumulative nutrient uptake*

228 We used cumulative uptake as an indicator of relative demand of nutrients in each stream

229 through the decomposition process. Streams that take up more nutrients through time are more

230 retentive of that nutrient because mineralization and/or saturation of that nutrient is lower than in

231 less retentive streams. We calculated cumulative nutrient uptake for each stream as the sum of

232 nutrient removed (g) during the dosing period each week and previous weeks. This means that

233 significant unmeasured uptake can occur between dosing periods, but should be relatively small

234 compared to uptake during the dosing period because of water replacement with low nutrient

235   wetland water during the flushing period. Because one of the Cottonwood streams (S4) lost

236   water on week 7 due to a leak in the recirculation pipe for one day, we excluded S4 from k

237   calculations for that week. For cumulative uptake calculations, we used the relationship of S4

238   cumulative uptake with cumulative uptake in the other two streams to predict S4 uptake on week

239   7 only ($R^2 = 0.95$, P<0.001; that is, cumulative uptake in the other Cottonwood streams were

240   highly predictive of cumulative uptake in S4).

241

242   *Litter carbon and nutrient content*

243   Initial (non-incubated), day 28 and day 56 carbon (C) and nitrogen (N) content of each

244   litter type was determined by weighing out a known mass of pulverized leaf material and

245   measuring on a Thermo-Finnegan Flash 1200 elemental analyzer (ThermoQuest, Milan, Italy).

246   Phosphorus content (P) was analyzed using the molybdate method as in Taylor *et al.*, (2014). C,

247   N, and P content are calculated as % of AFDM, and analyzed in terms of percent change

248   between days 0 - 28 and days 28 – 56 because we were interested in the relative C and nutrient

249   changes among litter types to help interpret our nutrient uptake measures. Nutrient masses are

250   calculated by multiplying C, N, and P content by the total AFDM remaining in each stream

251   determined by breakdown rates.

252   To estimate the net immobilized mass of N (or P), N_{immob} (g), in the litter of each stream

253   during a time period (e.g., days 0 – 28), we calculated the difference between actual N mass

254   change during a time period (ΔN_{Mass}) and the nutrient mass change assuming there were only

255   breakdown losses of N ($\Delta N_{MassBrk}$, i.e., only fragmentation losses that excluded microbial

256   mineralization/immobilization changes as expressed by %N). Thus, we used

257   (Eqn. 1) $\Delta N_{Mass} - \Delta N_{MassBrk} = N_{immob}$ (g), where

12

258 $$\Delta N_{Mass} = (\%N_{28} \; x \; AFDM_{28}) - (\%N_0 \; x \; AFDM_0), \text{ and}$$

259 $$\Delta N_{MassBrk} = (AFDM_{28} - AFDM_0) \; x \; \%N_0,$$

260 so that if N_{immob} was positive, it indicated net immobilization, and if negative, net mineralization.

261 Positive net immobilization indicates that more nutrients have been immobilized than

262 mineralized *or* leached. This is important because, as our data corroborate, a substantial fraction

263 of litter nutrients can be leached within the first few days of submersion.

264

265 *Data analysis*

266   We compared means of mass losses (as AFDM loss), nutrient uptake, and changes in

267 nutrient content and immobilization in the R package nlme (version 3.2.0, R Core Team, Vienna)

268 by producing fixed-effects, generalized least squares models (gls function). When appropriate,

269 we used the varIdent function to weight variance heterogeneity and the corAR1 function to

270 model covariance among repeated measures by stream (Zuur *et al.*, 2009; King *et al.*, 2016).

271 Inclusion of weighting terms was determined by model comparisons based on significant

272 reductions of AIC and better meeting the statistical assumptions of linear modelling (i.e., visual

273 analysis of residuals). We used the functions *emmeans* and *pairs* in the R package emmeans to

274 provide modeled estimates of means ("estimated marginal means") and standard errors, and to

275 perform post-hoc significance tests. Due to low replication of stream treatments, we attributed

276 statistical significance at $\alpha=0.10$ to increase the power of our statistical hypothesis tests and to

277 avoid "nearly significant" language (Gotelli & Ellison, 2004). Low replication in this case is a

278 trade-off to conduct an experiment at ecologically relevant scale (Carpenter, 1996, 1998;

279 Schindler, 1998). Where there could be disagreement about appropriate α between readers and

280 the authors, we report exact P values within 0.1>P>0.05 in the Results. The discrepancy

281 ultimately causes little or no differences in study conclusions.

282

283   **Results**

284 *Litter breakdown*

285   Cottonwood breakdown rates (k) were 5x faster than Sycamore and ~4x faster than Bur

286 Oak, and Bur Oak broke down 1.5x faster than Sycamore litter (Table 1). Mixed litter broke

287 down at a rate intermediate to the constituent three litter species, thus we observed no non-

288 additive effects of litter mixing on breakdown rates (P>0.10). Each individual litter type broke

289 down at a rate different from the other individual litter type (all P<0.001). At the end of the

290 study, Cottonwood had 28% initial AFDM remaining, Sycamore 78%, Bur Oak 71%, and Mixed

291 had 64% remaining.

292

293 *Weekly nutrient uptake*

294   Although highly variable within and between litter types, nutrient uptake rates (k) across

295 all inorganic nutrient analytes collectively showed that streams with fast decomposing litter

296 (Cottonwood) had reduced uptake rates through time compared to those with slower

297 decomposing litter (Bur Oak and Sycamore) and occasionally very different in magnitude. NH_4-

298 N uptake rates (Fig. 1A) particularly showed a shift in Bur Oak and Cottonwood stream uptake

299 rates, while NO_3-N and PO_4-P uptake suggested that Cottonwood uptake was reduced relative to

300 other litters primarily after week 4.

301   In week 1, NH_4-N uptake in Cottonwood streams was nearly 5x greater and 1.5x greater

302 than Bur Oak and Sycamore streams, respectively (both P<0.001), but similar to Mixed litter

303   streams (P>0.10). In weeks 2 and 3, differences in NH_4-N uptake rates were minimal, but uptake

304   in streams with Bur Oak was lower than those with Cottonwood in week 2 (P=0.07) and greater

305   in week 3 (P=0.06). In week 4, streams with Cottonwood took up NH_4-N at rates at least half of

306   any other treatment (all P<0.05).

307   In contrast to NH_4-N, NO_3-N (Fig. 1B) and PO_4-P (Fig. 1C) uptake rates were not

308   different by litter type through the first three weeks. However, Bur Oak streams in weeks 4 and 5

309   took up NO_3-N at least 2.5x faster than any other litter treatment (all P<0.05). Similarly, Bur Oak

310   stream PO_4-P uptake was ~2x greater than other litter treatments in weeks 4 and 5 (all P<0.05

311   except Week 4 PO_4-P: Bur Oak>Cottonwood, P=0.079; Week 5 PO_4-P:Bur Oak>Mixed,

312   P=0.070). In week 6, Sycamore streams took up NH_4-N, NO_3-N, and PO_4-P faster than

313   Cottonwood streams (P<0.05). In weeks 7 and 8, PO_4-P uptake was not different between litter

314   treatments, but NO_3-N uptake was typically lower in Cottonwood streams (Week 7:

315   Cottonwood<Mixed, P=0.08; Week 8: Bur Oak>Sycamore, P=0.09). By week 7 and 8, all added

316   NH_4-N in each stream was taken up through the first recirculation day, prohibiting uptake

317   calculations.

318   We did not observe non-additive (Mixed vs. Expected) differences in uptake of any

319   nutrient (Fig. S5).

320

321   *Cumulative nutrient uptake*

322   Cumulative NO_3-N uptake suggested that inorganic N sink status (total long term

323   demand) varied by litter type, but not for PO_4-P or NH_4-N. Cumulative NO_3-N uptake did not

324   significantly differentiate between litter treatments until weeks 7 and 8 of the study (Fig. 2A). By

325   week 7, cumulative NO_3-N uptake in Cottonwood streams was less than in Mixed (P=0.021) and

326 Bur Oak (P=0.034) streams, and these differences remained in week 8 (both P=0.02). Neither

327 cumulative NH_4-N uptake nor cumulative PO_4-P uptake were different between any litter

328 treatment in any week (Fig. S6). For NH_4-N, this was because almost all added NH_4-N was taken

329 up through most recirculation periods.

330   Cumulative NO_3-N uptake was predominantly additive. Cumulative NO_3-N uptake in

331 Mixed litter treatments trended higher than the mean of the three constituent individual species

332 (Expected) in all but week 1 (Fig. 2B), and the Litter*Week interaction term was significant

333 (P=0.037). Cumulative NO_3-N uptake was different only between Mixed and Expected uptake in

334 weeks 2 (P=0.099) and 8 (P=0.092), suggesting the possibility of non-additive effects on NO_3-N

335 uptake. Neither NH_4-N nor PO_4-P cumulative uptake was different between Mixed and Expected

336 comparisons (all P>0.10, Fig. S6), but cumulative PO_4-P uptake in Mixed streams did trend

337 higher than expected for most of the study (Fig. S6).

338

339 *Litter carbon and nutrient content*

340   We used changes in litter C, N, and P content to help explain patterns observed in uptake

341 rates and cumulative uptake (Fig. 3, top row). We observed few significant differences in C

342 content changes among species, as Cottonwood shifted to relative C loss in the latter half of the

343 study compared to the beginning of the study (P=0.053).

344   Changes in N content for all litters suggested a period of immobilization early in the

345 study (positive %N change), and either slower immobilization (Bur Oak) or net mineralization

346 (all other litters) later in the study (all P<0.022, except Bur Oak P=0.06). Bur Oak retained about

347 40% more (absolute percent differences) litter N than Cottonwood between days 28-56

348 (P=0.025), while Sycamore and Mixed N content changes fell intermediate to the other two litter

349 treatments.

350   Similar to N, P content for all litters increased between days 0-28, suggesting net

351 immobilization. Bur Oak was particularly retentive of P, nearly doubling in P content, and

352 immobilizing relatively more P than each other litter during days 0-28 (all P<0.005) and

353 relatively more than Cottonwood (P=0.035) and Mixed (P=0.013) litters during days 28-56.

354 Mixed litter also immobilized relatively more P than Cottonwood between days 0-28 (P=0.062).

355 Changes in P content through days 28-56 still suggested net P immobilization or a balance

356 between immobilization and mineralization (Mixed) in all litters, although P content generally

357 decreased compared to days 0-28, again suggesting a shift from immobilization to

358 mineralization.

359   Changes in C, N, and P mass (Fig. 3, bottom row) indicate both breakdown (export)

360 losses of litter-bound elements as well as shifts in microbial demand (i.e., immobilization and

361 mineralization). Changes in C mass largely reflected breakdown rates, particularly demonstrating

362 strong C losses in Cottonwood litter and weak C losses for Sycamore. Particularly fast

363 breakdown rates resulted in a net loss of litter N mass from Cottonwood streams early in the

364 study, whereas the overall mass of N increased in all other streams during days 0-28. However,

365 between days 28-56, N was strongly lost from most streams, partially because of lower N

366 demand (previous paragraph), with >70% of the litter N lost from Cottonwood streams. As such,

367 Cottonwood streams lost more litter N in each time period than all other litters (all P<0.007,

368 Mix-Cot days 28-56 P=0.06) except Mixed litter days 0-28.

369   Different litter identities had highly variant P mass losses. Between days 0-28,

370 Cottonwood streams lost far more litter P mass than all other streams (~20% loss, all P<0.05),

371 whereas Sycamore and Mixed streams similarly gained ~25% litter P mass, and Bur Oak streams

372 gained >75% litter P mass, more than any other stream (all P<0.05). Relative P losses increased

373 for Cottonwood and Mixed streams through the latter half of the study, and Bur Oak and

374 Sycamore streams only slightly increased in P mass in the latter half of the study. Cottonwood

375 streams lost more relative mass than any other litter type in that time period (all P<0.05).

376   Changes in litter C,N, and P stoichiometry largely corroborate changes in C,N, and P

377 content, particularly as a function of C,N, and P mass loss, and so are presented in Fig. S7 along

378 with the raw C,N, and P content and mass losses.

379   Estimates of total net N and P immobilized (Fig. 4) suggested each litter was a net sink of

380 nutrients through the study period, even if they were shifting toward net N mineralization. Bur

381 Oak was a particularly strong net sink of both N and P. Cottonwood litter net immobilized ~1.5-

382 2x more N than other litters through 28 days, in contrast to the low P immobilization in

383 Cottonwood compared to other litters. After 56 days, only Cottonwood litter had immobilized

384 significantly less N than on day 28 (P=0.085), although Sycamore and Mixed litters were

385 trending lower. The exception was that Bur Oak trended toward increasing cumulative net N

386 immobilized, and had significantly more N immobilized than Mixed litter (P=0.051). Cumulative

387 net P immobilized increased (Bur Oak, Sycamore, P<0.05) or stayed similar (Cottonwood,

388 Mixed) between days 28 and 56. Bur Oak and Mixed litters net immobilized ~1.5x more P than

389 Cottonwood and Sycamore litters through 28 days (all P<0.05). There was at least 2x more P

390 immobilized in Bur Oak litters than any other litter on day 56 (all P<0.05).

391   We observed mixing effects on litter nutrient content changes only for N days 0-28,

392 where Mixed litter gained less N than expected based on the other three litter species (Fig. 5,

393 P=0.086). This stands in contrast to the observed differences in cumulative NO_3-N, which

394 showed greater uptake of added N in Mixed litter.

395

396 **Discussion**

397   Litter quality and quantity could have a critical temporal interaction on nutrient dynamics

398 in stream ecosystems (Gibson & O'Reilly, 2012). We showed that litter species quality, as a

399 function of species identity, can mediate stream ecosystem nutrient dynamics, possibly through

400 reduction of litter quantity. Moreover, relative effects on nutrient uptake of one litter type versus

401 another (fast versus slow decomposing) are a function of decomposition stage of the leaf litter.

402 Here, Cottonwood (labile litter) streams initially had relatively high N demands compared to

403 those with Bur Oak and Sycamore (recalcitrant). In contrast, we observed that nutrient uptake

404 rates in streams containing Bur Oak eventually surpassed those in streams containing

405 Cottonwood, corroborated by decreasing N content in Cottonwood litters. Further, lower

406 inorganic N demand in Cottonwood streams relative to other litter compositions in the later

407 weeks of the study led to lower long-term nutrient demand (i.e., cumulative uptake and net

408 immobilization) in Cottonwood streams in comparison to those with Bur Oak and Sycamore. In

409 contrast, all litters were generally quite retentive of P.

410   Resource quality (e.g., C:N and breakdown rate) underlies both the 1) shifts in exogenous

411 nutrient needs of microbial decomposers and 2) the depletion of resource quantity (labile litter C)

412 available to drive microbial metabolism coupled to nutrient uptake (e.g., denitrification or

413 assimilative uptake; Pastor and others 2014; García-Palacios and others 2017). The decrease in

414 Cottonwood litter uptake rates relative to other litters was at least partially due to shifts in

415 microbial immobilization rates, leading to net mineralization earlier than other litter types. Vastly

416 faster C losses compared to other litters suggested Cottonwood probably contained relatively

417 more labile C available to drive decomposition (Danger, Gessner & Bärlocher, 2016), manifested

418 in higher NH_4-N uptake rates very early in the study and strong increase in litter N content and

419 net immobilized N. Although also true for Sycamore and Mixed litters, decreases in litter N

420 content suggest rapid N mineralization was particularly prevalent in Cottonwood litters through

421 the latter half of the study. As microbes further the decomposition process, they become more C

422 limited and shift to net mineralization of nutrients (Pastor *et al.*, 2014). Microbial communities

423 using substrate that decomposes quickly (high labile C and nutrient content) typically reach this

424 shift (at a critical C:Nutrient value) from net immobilization to net mineralization sooner than on

425 slower decomposing litters (Melillo and others 1984; García-Palacios and others 2016).

426   Fast decomposition also reduces the quantity of substrate available to drive metabolism

427 and associated nutrient demand. A reduction in labile C quantity should be a strong constraint on

428 microbial metabolism for both assimilative and dissimilative N uptake pathways (Quinn *et al.*,

429 2000; Stelzer *et al.*, 2014b). If quantity of substrate was the only factor driving nutrient uptake,

430 we would expect Sycamore streams to take up a greater mass of nutrients than Bur Oak and

431 Mixed streams if we continued the experiment. However, litter N content suggested that

432 Sycamore litter at the end of the study was shifting to net N mineralization rather than

433 immobilization. Sycamore uptake may be limited by labile C to support significant microbial

434 biomass to continue immobilizing N (Melillo *et al.*, 1984). Further, while litters were beginning

435 to mineralize N, they were still generally immobilizing P and taking up added PO_4, suggesting

436 that there was still substrate driving net P acquisition rather than release. This underscores the

437 idea that both litter C quality and nutrient content are probably important in determining the

438   magnitude and timing of nutrient demand from litters, as they are intimately tied in driving

439   decomposition.

440   While qualitatively similar to NO_3-N uptake, differences in water column PO_4-P uptake

441   rates were more difficult to detect and cumulative uptake never significantly diverged by species

442   – in stark contrast to the differences observed in litter P content and immobilization. All litters

443   were highly retentive of P, exhibiting net P immobilization throughout the study. This strong

444   retention, even with substantial N mineralization in Cottonwood and other litters, is not

445   unexpected. Critical thresholds for a shift from net immobilization to net mineralization for one

446   nutrient (e.g., N) will likely not occur at the same time as others (e.g., P), as timing for each

447   threshold is dependent on microbial nutrient requirements relative to availability (Manzoni *et al.*,

448   2010). In our study, N:P ratios of both organic and inorganic resources suggested P limitation

449   (dosing inorganic N:P=27). In fact, litter N:P ratios generally declined through the study,

450   indicating microbes retained P more strongly than N. Mehring et al. (2015) found that litters with

451   similar N:P ratios (~54-93) as litters here (except Cottonwood) were also longer term sinks for P

452   than N. The retention of P over N is probably dependent on N:P supply ratios and quantities (i.e.,

453   nutrient slugs), though, and we caution against extending our N:P retention results outside of

454   those inorganic parameters (Güsewell & Gessner, 2009; Gulis *et al.*, 2017; Jabiol *et al.*, 2018).

455   Differences in N and P dynamics do, however, suggest that the coupling of N and P demand in

456   detritus-dominated streams could depend on riparian detritus composition and inputs (e.g.,

457   Gibson & O'Reilly, 2012).

458   Metabolic rates driving nutrient uptake and mass loss through fragmentation could be

459   important controls on the form which immobilized nutrients are exported from forested reaches.

460   Comparisons of litter nutrient mass loss versus net litter nutrients mineralized (comparison not

461   shown) suggest that even in Cottonwood, which was strongly mineralizing N in the latter half of

462   the study, the vast majority of nutrient loss across litter types was particulate, not mineralized

463   inorganic forms. This suggests that litter can transform labile, inorganic nutrients into (eventually

464   exported) organic forms that are more recalcitrant downstream. Further, consumers such as

465   invertebrate shredders will contribute to this process by increasing fragmentary losses,

466   potentially increasing organic:inorganic nutrient export, as well as assimilating organic forms

467   and excreting some nutrients in inorganic forms. Here, invertebrate densities were not quantified

468   but were generally low. Litter identity may also matter in determining nutrient form, because Bur

469   Oak and Sycamore litters were relatively retentive of nutrients later in the study, tending to lose

470   much less proportional nutrient mass than Cottonwood. Particularly for temperate deciduous

471   forested catchments during winter months, functional species diversity, spanning a processing

472   continuum, could be important to the long-term organic:inorganic nutrient export out of stream

473   reaches.

474   Although our results line up with predictions based on both terrestrial and aquatic

475   literature surrounding shifts in microbial decomposer nutrient demands (Melillo *et al.*, 1984;

476   Manzoni *et al.*, 2010; Cheever *et al.*, 2012; García-Palacios *et al.*, 2017), not all of the uptake in

477   our mesocosms can be attributed to immobilization and mineralization in added litter, as is clear

478   from the cumulative net immobilization estimates in comparison to cumulative uptake of added

479   nutrients. For example, litter quality and quantity can drive denitrification (Stelzer *et al.*, 2014b;

480   O'Brien *et al.*, 2017). Non-litter associated processes should also influence our observed uptake

481   rates. Microbes attaching to non-litter mesocosms such as walls or pipes could be a large sink for

482   added nutrients, although algal uptake could be limited because light was heavily attenuated by

483   80% shadecloth over most of the mesocosms. Further, abiotic PO_4 adsorption was probably an

484   important sink for P, and could have contributed to the large gap between net litter P

485   immobilized and cumulative PO_4-P uptake (Froelich, 1988). Here, we are unable to estimate

486   those processes, but studies in natural systems should contextualize litter specific uptake within

487   the overall stream nutrient cycling which will also have large contributions from non-litter

488   uptake and mineralization processes.

489   We observed some evidence for positive non-additive effects of litter mixing on

490   cumulative NO_3-N uptake and percent N content changes. However, these results were

491   conflicting, with greater cumulative NO_3-N than expected in Mixed litter streams and %N

492   content increasing slower than expected in Mixed litter. We also failed to observe any mixing

493   effects on litter N immobilization, and observed only additive effects on breakdown rates. Non-

494   additive breakdown effects are by no means ubiquitous, often weak and are more likely to occur

495   under specific environmental and temporal scales of measurement (Lecerf *et al.*, 2011; Frainer *et*

496   *al.*, 2014). In particular, the nutrient additions used here probably dampened or precluded any

497   non-additive effects (Rosemond *et al.*, 2010). The concept of non-additive litter effects on

498   nutrient dynamics deserves further study even if any effects are likely to be small, but we

499   conclude riparian species composition and diversity probably serve as more important controls

500   on stream nutrient dynamics additively, at least under high nutrient scenarios.

501   Like any other study focusing on the interaction of detrital resource quality and nutrient

502   cycling, our results must be interpreted in the context of nutrient availability. Our chosen dosing

503   concentrations are, for example, found more commonly in agricultural ditches (although the total

504   flux of nutrients is smaller than some press additions at lower concentrations, e.g., Greenwood *et*

505   *al.*, 2007). High inorganic nutrient supply strongly accelerates litter decomposition, increasing

506   microbial carbon use efficiency and decreasing nutrient use efficiency, allowing microbes to

507   shunt more resources into C acquisition while mineralizing nutrients (Manzoni *et al.*, 2012;

508   Mooshammer *et al.*, 2012, 2014; Tant *et al.*, 2013; Gulis *et al.*, 2017). In contrast, nutrient-

509   limited microbes will mine more nutrients from litter substrate and be more nutrient retentive;

510   however, the low resource supply causes a longer time to reach the critical C:Nutrient ratio for

511   that litter (García-Palacios *et al.*, 2017; Gulis *et al.*, 2017). Nutrient availability also interacts

512   with resource quality. Inorganic nutrient additions increase decomposition of low quality (low N

513   or P content) litter more than high quality litter (Greenwood *et al.*, 2007; Tant *et al.*, 2013;

514   Manning *et al.*, 2015, 2016). Similarly, nutrient enrichment homogenizes litter C:N and C:P

515   ratios through decomposition, suggesting that higher C:N or C:P litters acquire nutrients more

516   rapidly (Manning *et al.*, 2015, 2016). Thus, the critical C:Nutrient ratio for each litter under

517   nutrient enrichment should not only be reached sooner but should also be more similar for each

518   litter type. In this sense, we suggest that the differences in nutrient uptake observed here were

519   somewhat dampened by resource homogenization (Biasi *et al.*, 2017), and possibly amplified by

520   denitrification. In lower nutrient scenarios, differences in litter nutrient uptake may be more

521   obvious, and shifts to mineralization will likely occur later in the decay sequence.

522   The goal of our study was not to determine the potential quantitative contribution of leaf

523   litter to stream nutrient uptake, so we caution readers against using the rates of nutrient uptake

524   here to estimate the exact quantity of microbial uptake in other systems with lower nutrient

525   concentrations. For example, high dosing concentrations underestimate ambient uptake by

526   decreasing the efficiency of the system uptake kinetics (Mulholland *et al.*, 2002). Further,

527   multiple sources of uptake likely existed, not just litter. Instead, our results primarily demonstrate

528   temporal shifts in nutrient demand, based on differences in litter quality stemming from species

529   identity, expressed at the reach scale. The terrestrial literature predicts that these shifts will vary

530   in timing based on environmental factors such as temperature and nutrient availability (e.g.,

531   Parton *et al.*, 2007; Manzoni *et al.*, 2012; Yue *et al.*, 2018), but much more testing is needed to

532   see whether terrestrial theory and our results generalize to natural lotic systems. Especially, more

533   studies are needed at the reach scale that can more effectively determine how temporal shifts in

534   detrital nutrient cycling affect reach or network scale nutrient budgets.

535   Our study suggests that the effects of inherently microbial level processes (e.g., critical

536   thresholds, Manzoni *et al.*, 2010) may interact with leaf scale processes (breakdown reducing

537   litter quantity) to determine reach scale processes (nutrient uptake). In forested watersheds where

538   leaf litter seasonally inundates stream networks, the expression of these microbial level processes

539   may be disproportionately important to predicting the biogeochemical cycling of whole regions

540   at intraannual timescales. The following may inform hypotheses underlying future studies

541   examining the role of litter in nutrient cycling at the reach or network scale. First, functional

542   diversity of riparian tree species could maintain long-term nutrient retention after litterfall

543   (Schellhorn, Gagic & Bommarco, 2015). Diverse or recalcitrant litters may be a longer-term

544   nutrient storage compartment, contributing to temporal stability of nutrient retention by

545   containing both fast-decomposing litter that will quickly exhaust nutrient demand and slow-

546   decomposing species that are initially slow nutrient removers. Second, litter may shift from

547   nutrient sink to source, and this shift could happen sooner for litters containing high N and P, but

548   also depending on the ambient streamwater nutrient availability. Little data exist to suggest

549   critical nutrient thresholds for freshwater detritus, as opposed to terrestrial systems. At the same

550   time, the fast decomposition of such species may limit the quantity of litter potentially

551   contributing to reach scale immobilization/mineralization. Third, the interplay between litter

552   breakdown and mineralization timing could control the downstream transport of bioavailable

553   inorganic nutrients versus relatively recalcitrant particulate nutrients (i.e., fragmented litter that

554   contains immobilized, biologically-bound N, of unknown mineralization state). Taken together,

555   the timing of spates with the phenology of leaf abscission may play a significant role in addition

556   to litter chemical composition on the form and quantity of nutrient export from detrital-based

557   watersheds. Although inputs of leaf litter are seasonally important nutrient sinks, more work

558   needs to be done to determine the importance of riparian species composition in watershed

559   nutrient retention and exports relative to other ecosystem factors.

560   Alterations of riparian communities stemming from biodiversity losses or invasions that

561   change the composition or biochemical character of stream litter could influence whole-stream

562   nutrient uptake over timescales that span much of the litter decay sequence (Kominoski *et al.*,

563   2011), but more testing in natural systems is sorely needed. Our study shows that detrital

564   resource quality can underlie temporal effects in microbial to reach scale detrital nutrient cycling,

565   shedding light on litter quality as a determinant not just of breakdown rates, but also for how

566   litter might interact with other ecosystem facets. The interaction of litter quality and quantity has

567   long been understood in terms of a 'processing continuum' of slow and fast decomposing litter

568   recognized by Petersen and Cummins (1974). Whereas the importance of this processing

569   continuum has notably been applied to consumer resource availability, e.g., for shredding

570   macroinvertebrates (Cummins *et al.*, 1989), the processing continuum could also extend to

571   whole-stream nutrient dynamics and potentially other ecosystem functions and services

572   (Schellhorn *et al.*, 2015).

573

574

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Data Availability Statement

582 Upon acceptance of this manuscript for publication, data will be archived in Dryad or a

583 similar repository.

Conflict of Interest

585 The authors claim no conflicts of interest.

586

587

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752

753 Table 1. Characteristics of litter deployed in 12 streams: Cottonwood (*Populus deltoides*), Sycamore (*Platanus occidentalis*), Bur Oak

754 (*Quercus macrocarpa*), and an equivalent mixture of the three (Mixed). Total ash free dry mass loss (AFDM; g) at days 28 and 56,

755 and breakdown rates (-k is the negative slope of the relationship of log-transformed AFDM remaining against day of experiment).

756 Litter bags (N=3) were initially 6 ±0.05 g dry mass. Masses of initial C, N, and P are the masses of litter nutrients extrapolated to

757 whole mesocosms based on AFDM. C:N, C:P, and N:P are molar ratios. Leachate dissolved inorganic nitrogen (DIN) and PO_4-P are

758 based on leachate concentrations per 6 g dry litter and scaled to total initial dry mass in each stream. Where noted, standard errors are

759 in parentheses.

760
761 Tables

762

Litter	AFDM Loss (g) Day 28	AFDM Loss (g) Day 56	Breakdown rate, k (d^{-1})	Initial C (%)	Initial N (%)	Initial P (%)	Initial C (g)	Initial N (g)	Initial P (g)	Initial C:N	Initial C:P	Initial N:P	Leachate DIN (g)	Leachate PO_4-P (g)
Cottonwood	2.06 (0.07)	3.38 (0.06)	0.022 ($1.22*10^{-4}$)	50.7	2.63	0.131	608	31.6	1.57	22.5	1007	45.0	0.36	0.76
Mixed	1.16 (0.04)	1.88 (0.04)	0.008 ($5.03*10^{-5}$)	51.4	2.03	0.071	674	26.6	0.93	29.9	1880	63.0	0.0092	0.33

Bur Oak	0.913	1.58	0.006 (6.97*10⁻⁵)	51.4	1.45	0.043	693	19.5	0.59	41.9	3098	73.9	0.012	0.13
	(0.09)	(0.04)												
Sycamore	0.833	1.04	0.004 (3.02*10⁻⁵)	50.0	1.74	0.053	681	23.7	0.73	34.2	2449	72.0	0.0045	0.16
	(0.01)	(0.04)												

763

764

766   Figure Legends

767   Fig. 1. Weekly A) NH_4-N, B) NO_3-N, C) PO_4-P uptake rates (k, d^{-1}) in 12 mesocosm streams

768   supplied with different leaf litters. Cottonwood (Populus deltoides) = yellow square, Sycamore

769   (Platanus occidentalis) = green triangle, Bur Oak (Quercus macrocarpa) = blue diamond, Mixed

770   = black circle. Points are means (± 1 SE from Estimated Marginal Means models; N=3).

771   Different letters indicate statistically significant (P≤0.10) difference within weeks. Asterisks

772   indicate inability to calculate uptake rates for those weeks due to all NH_4-N being taken up on

773   the first day of dosing.

774

775   Fig. 2. A) Cumulative NO_3-N uptake in 12 mesocosm streams supplied with different leaf litters.

776   Cottonwood (Populus deltoides) = yellow square, Sycamore (Platanus occidentalis) = green

777   triangle, Bur Oak (Quercus macrocarpa) = blue diamond, Mixed = black circle. Different letters

778   indicate statistically significant (P≤0.10) difference within weeks. B) Cumulative NO_3-N uptake

779   for Mixed litter (N=3, black circle) and the modelled mean of the three constituent litter species

780   (N=9, red square). Non-additive effects are evidenced by deviations from expected uptake. Error

781   bars indicate ± 1 SE from Estimated Marginal Means models. Significant (P≤0.10) differences

782   are indicated by asterisks. Cumulative uptake is based only on measurements during the

783   recirculation periods.

784

785   Fig. 3. Percent changes in litter C, N, and P content (top row) and changes in masses of litter C,

786   N, and P (bottom row) between days 0-28 and 28-56. Positive percent change indicates an

787   increase in litter C, N, P content or mass (% C, N, or P applied to remaining AFDM in whole

788 mesocosm) during that time period, negative indicates a decrease. Points (Cottonwood (*Populus*

789 *deltoides*) = yellow square, Sycamore (*Platanus occidentalis*) = green triangle, Bur Oak

790 (*Quercus macrocarpa*) = blue diamond, Mixed = black circle) are means and error bars are ± 1

791 SE from Estimated Marginal Means models. Significant (P≤0.10) differences among litter types

792 are indicated by different letters, whereas differences within litter types but between time periods

793 are indicated by an asterisk. N=3 per litter type.

794

795 Fig. 4. Estimated cumulative N and P immobilized (g) in total mesocosm litter at 28 and 56 days,

796 calculated with Eqn. 1. Points (Cottonwood (Populus deltoides) = yellow square, Sycamore

797 (Platanus occidentalis) = green triangle, Bur Oak (Quercus macrocarpa) = blue diamond, Mixed

798 = black circle) are means and error bars are ± 1 SE from Estimated Marginal Means models.

799 Significant (P≤0.10) differences within days among litter types are indicated by different letters.

800

801 Fig. 5. Percent changes in litter N content between days 0-28 and 28-56. Points (Mixed = black

802 circle, Expected based on three constituent species = red square) are means and error bars are ± 1

803 SE from Estimated Marginal Means models. Significant (P≤0.10) differences among litter types

804 are indicated by asterisk. Mixed N=3, Expected N=9.

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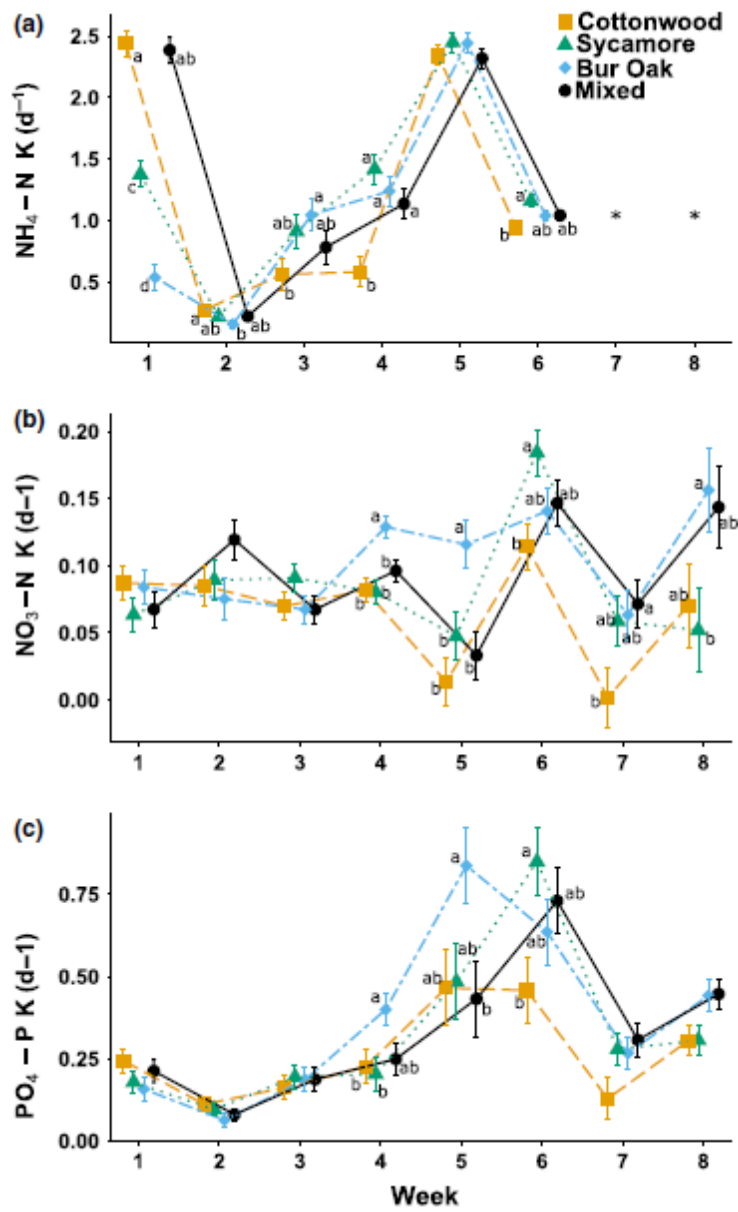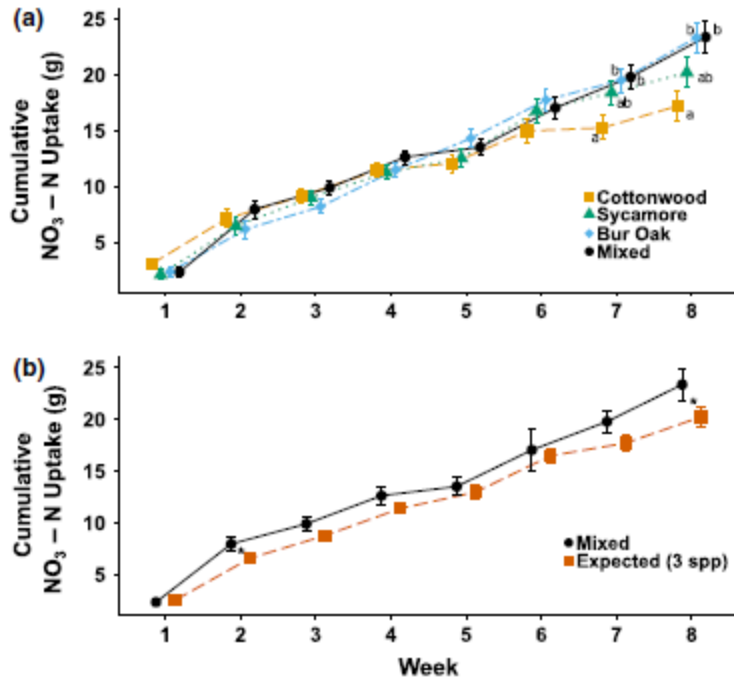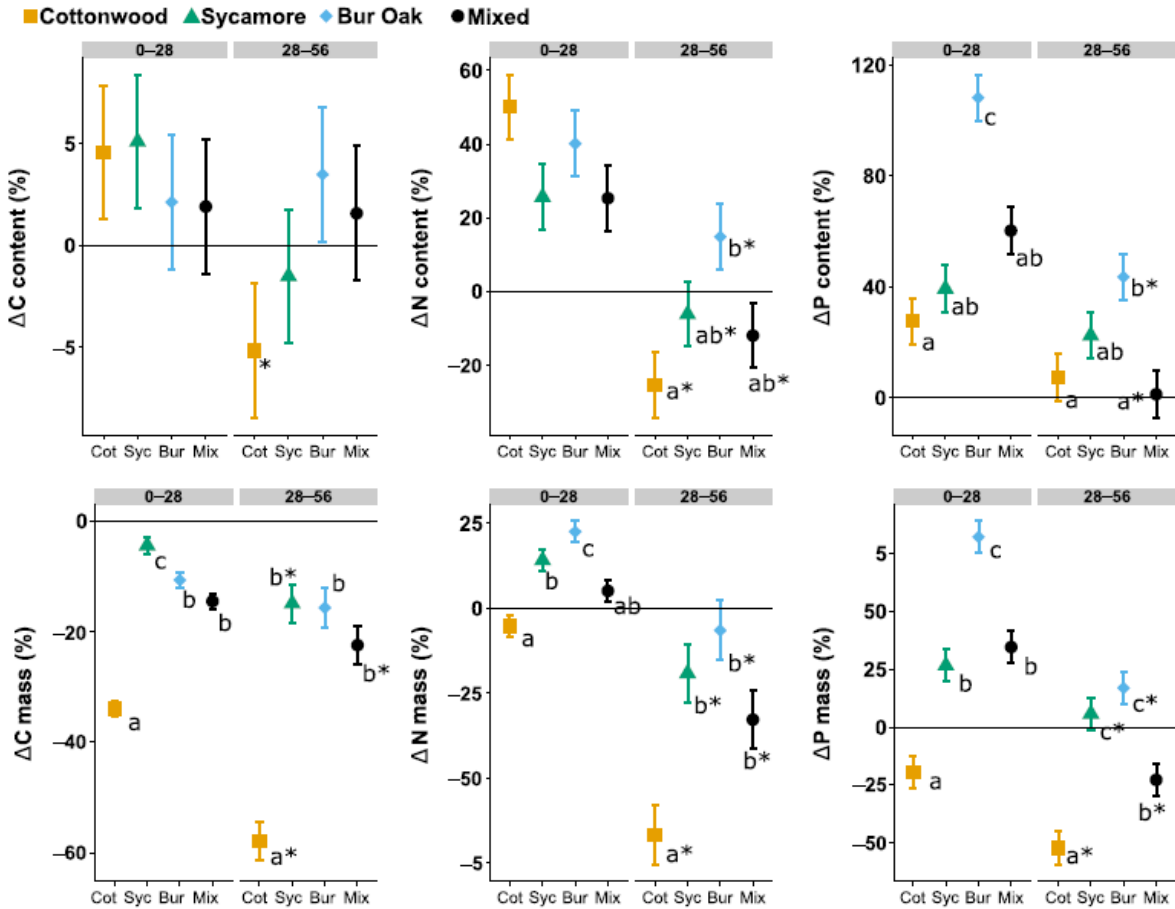
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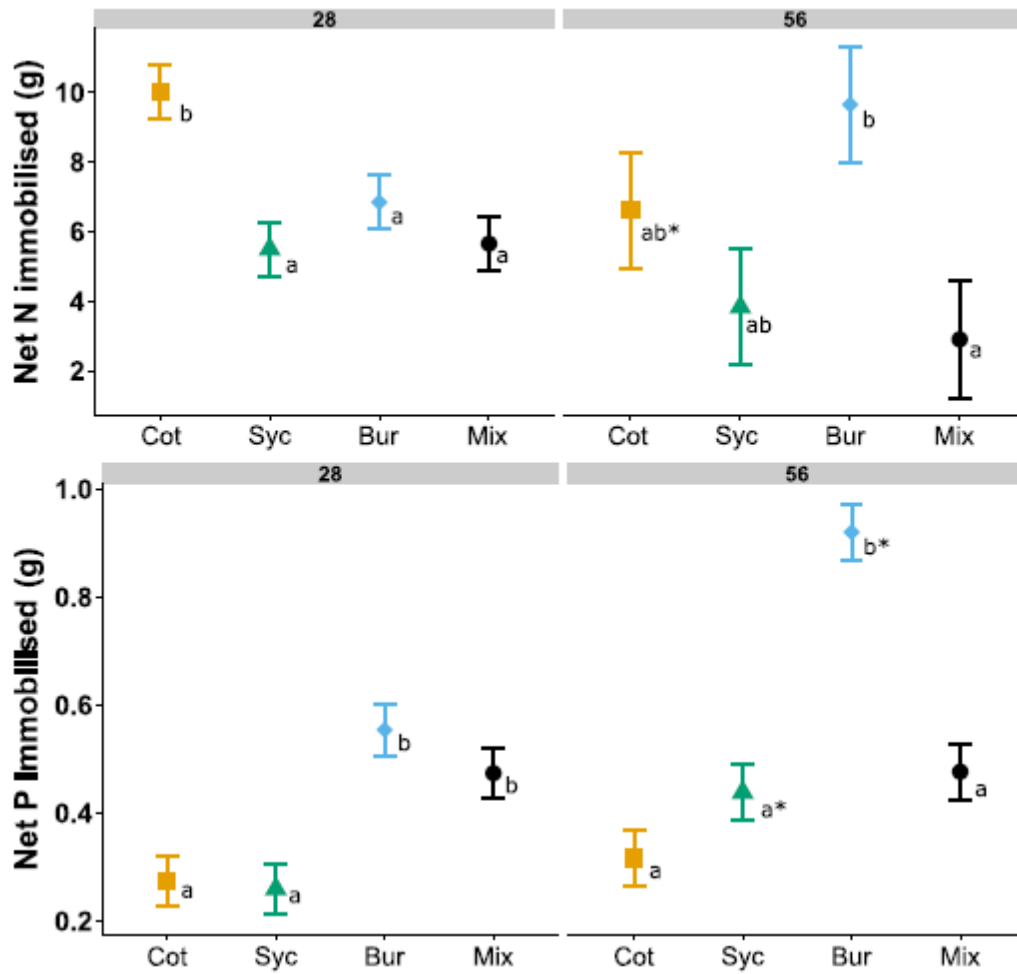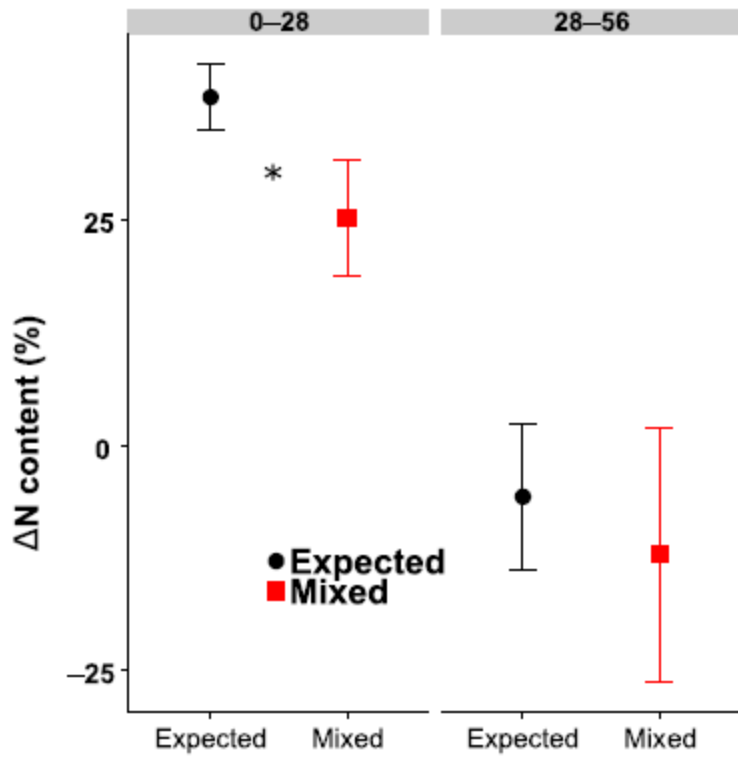
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816   Figure S1. Glide sections and pools. During recirculation, water is pumped from the pools, back up to the

817   tops of the riffles (next photo). During partial recirculation, excess water volume exits through an

818   overflow pipe and drains into the Lake Waco Wetland.

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821   Figure S2. Riffle sections at the tops of the mesocosms (left). The downstream section of each riffle was
822   left unshaded.
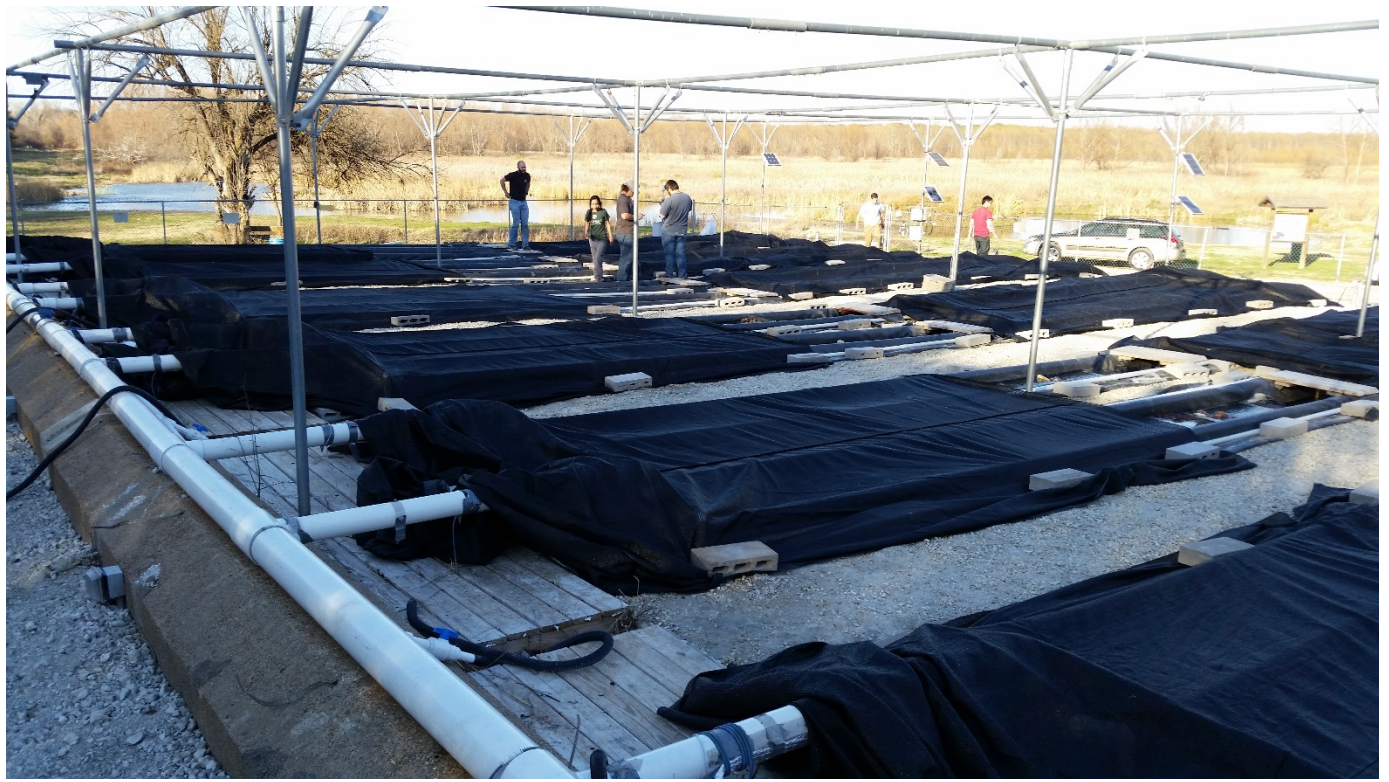
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825 Figure S3. Riffle sections, uncovered (flow from top left to lower right). Litter packs (bags collected for
826 mass and nutrient contents are white, larger packs were secured using black mesh) are shown here
827 immediately after litter installation. Within hours, they were inundated and submersed.
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833
834 Figure S4. Looking downstream at the glide section immediately after placement of litter in the three
835 glide cages.
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Figure S5. Weekly A) NH_4-N, B) NO_{3+2}-N, C) PO_4-P uptake in 12 mesocosm streams for Mixed litter (N=3) and the mean of the three constituent litter species (N=9). Points are means ± 1 SE from LS Means models. Significant (P≤0.10) differences were not observed.
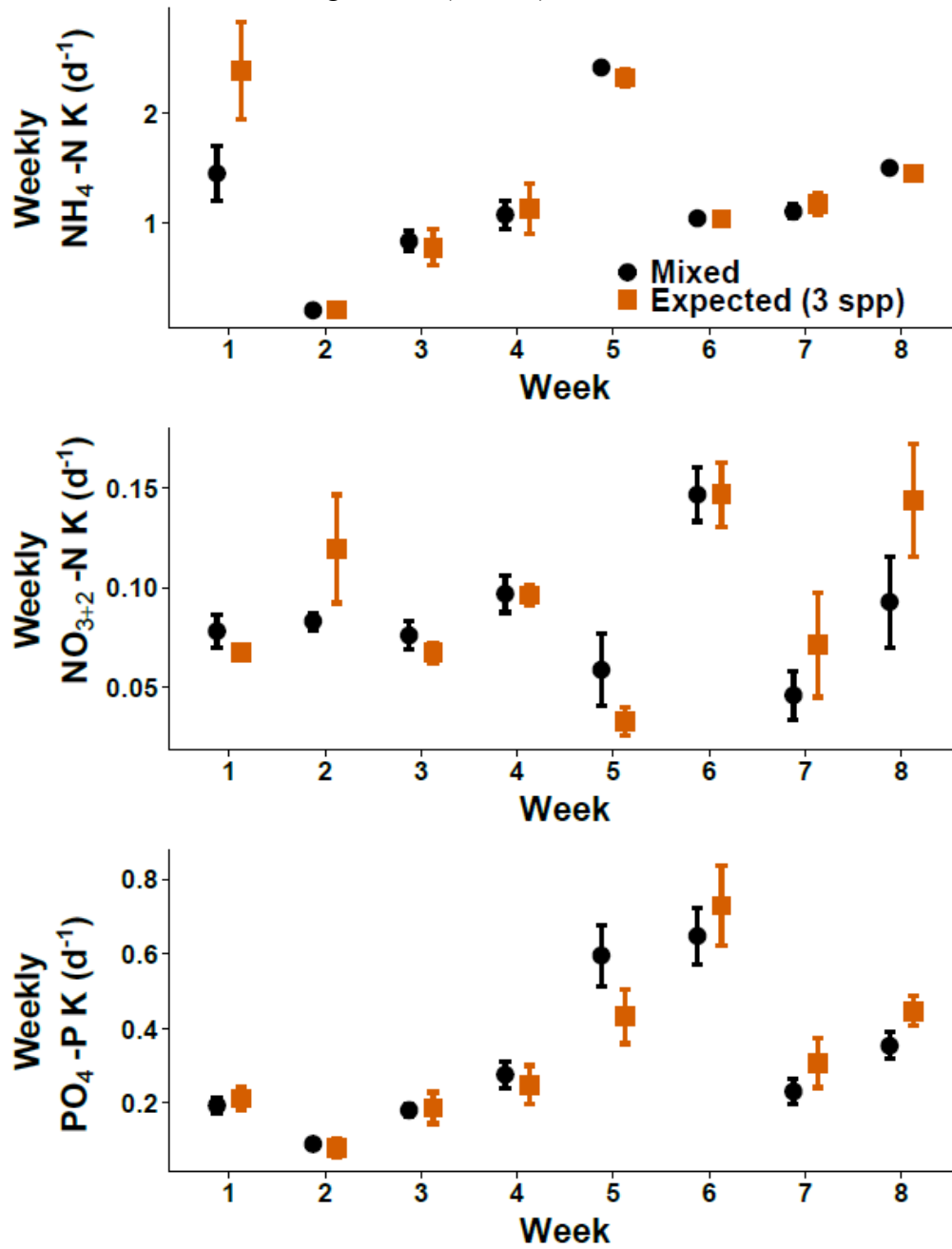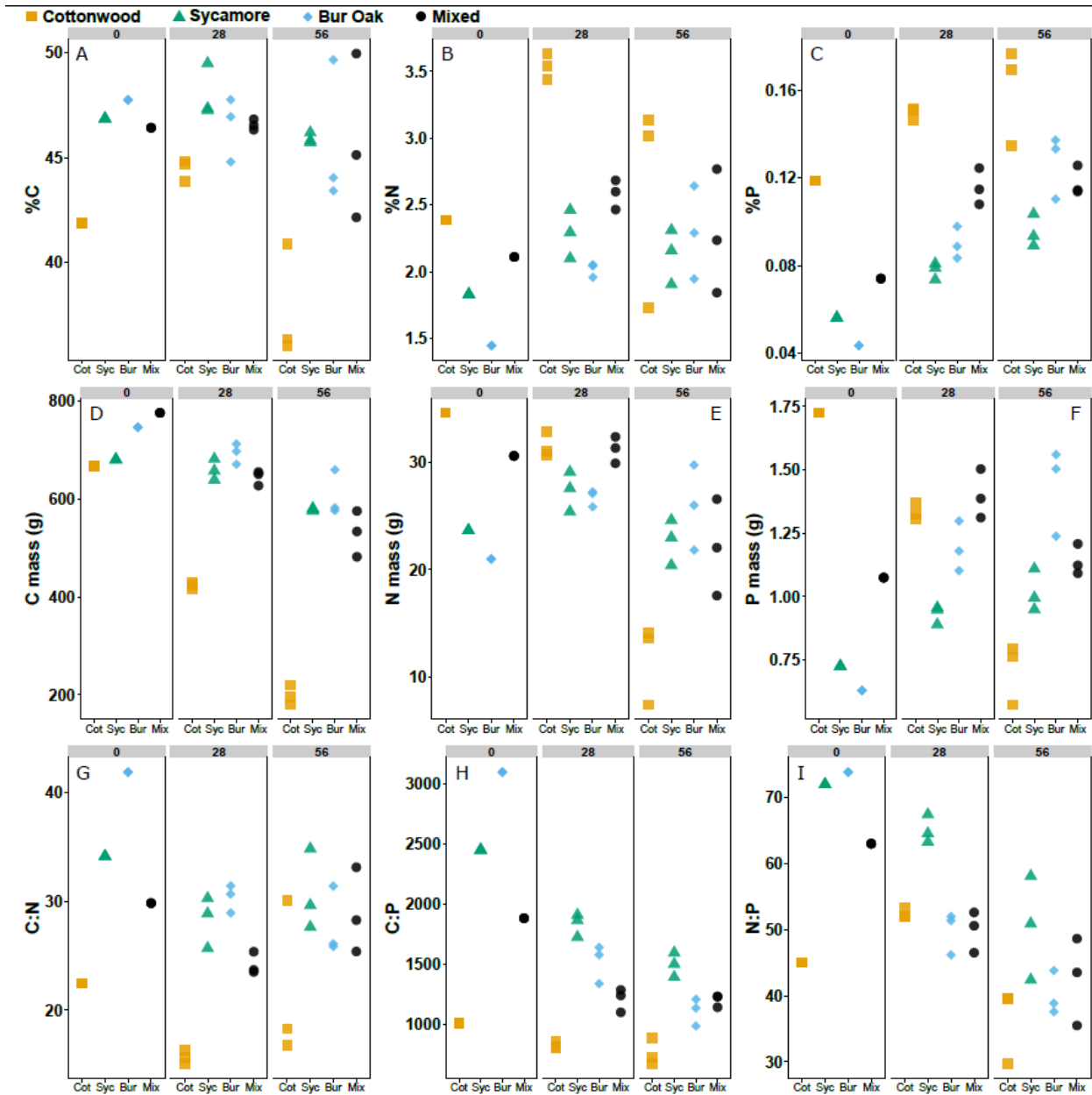
Figure S6. Cumulative NH_4-N (panels A & C) and PO_4-P (panels B & D) uptake in 12 mesocosm streams supplied with different leaf litters (Bur Oak (*Quercus macrocarpa*) = blue diamond, Cottonwood (*Populus deltoides*) = yellow square, Mixed = black circle, Sycamore (*Platanus occidentalis*) = green triangle). Panels C & D show Mixed litter (N=3, black circle) versus the mean of the three constituent litter species (N=9, red square). Points are means ± 1 SE from LS Means models. Significant (P≤0.10) differences were not observed.
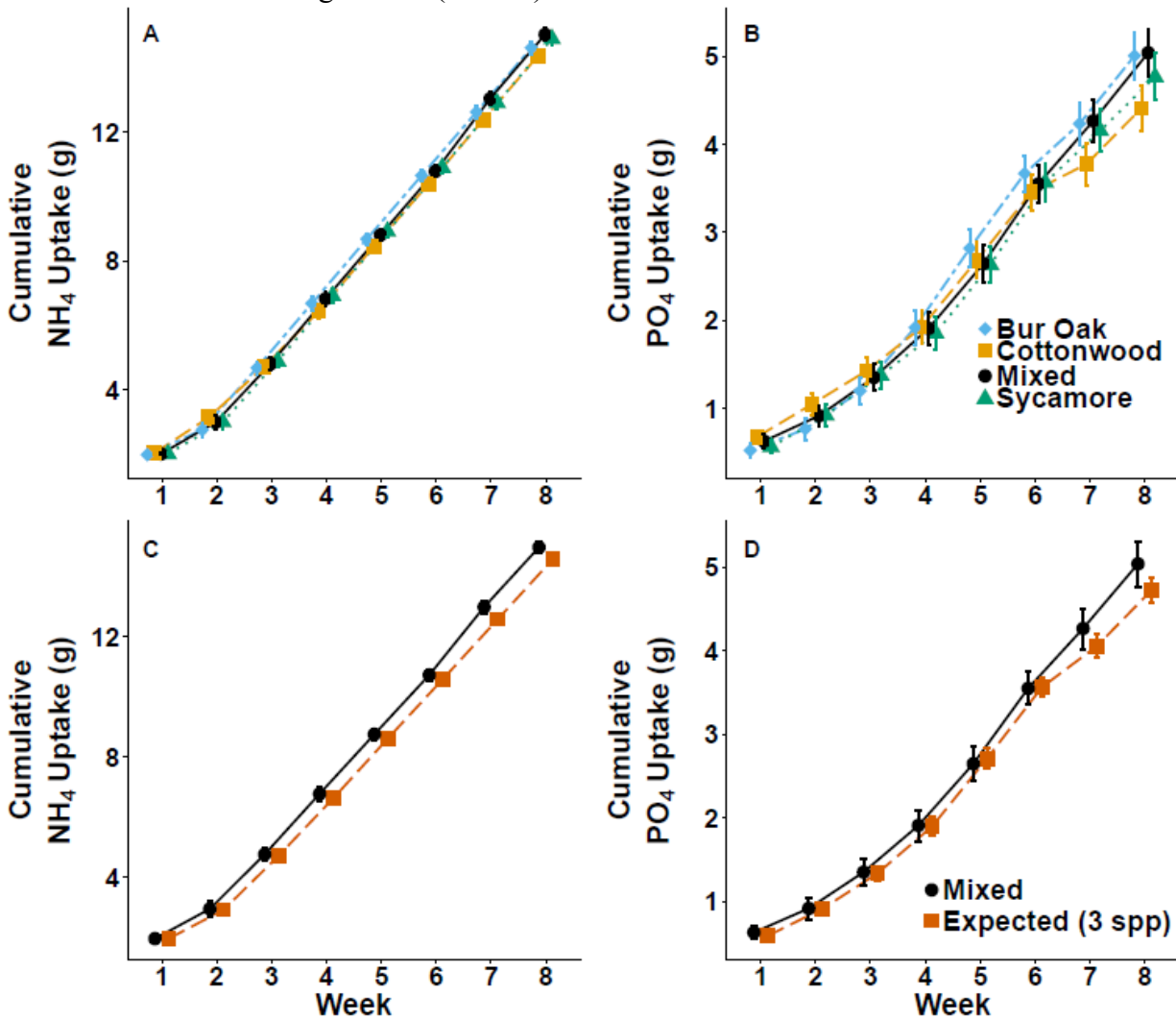
Figure S7. Litter C, N, and P content (%AFDM, Panels A-C), where points on day 0 represent the mean from three replicates based on the same litter type initially, and on days 28 and 56 are the means of three litter packs (one C,N and P sample analyzed per leaf pack) from each stream of a litter type. C, N, and P Mass (g, Panels D-F) are the %C,N, and P content extrapolated with ash-free dry mass remaining to total mass of each element in each stream. Molar ratios of C, N, and P (Panels G-I) based on %C,N, and P content.