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Multiyear resource enrichment creates persistently higher species diversity in a landscape-scale field experiment

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1 Running head: Prolonged resource enrichment

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4 **Multi-year resource enrichment creates persistently higher species diversity in a landscape-scale**
5 **field experiment**

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18 *Abstract*

19 Short-term resource enrichment can increase species diversity in communities, but prolonged resource
20 enrichment may result in either a diversity collapse or persistent high species diversity if fluctuation-
21 dependent mechanisms of species coexistence are triggered. We tested the effects of resource
22 enrichment on stream invertebrates by boosting densities of benthic detritus. In a 22 km stream length,
23 we used wooden stakes to enhance retention of detritus at 40-m long sites, while other sites acted as
24 controls. Detritus and invertebrates were sampled prior to treatment and then 1, 2 and 5 years later.
25 Previously, we reported that detrital densities, species diversity and densities increased at enrichment
26 sites after 12 months. Here we report that similar increases occurred two and five years after
27 manipulation. Prolonged resource enrichment produced persistently higher species diversity without
28 loss of any taxa from the species pool, despite strong shifts in faunal composition in response to
29 environmental variation, including a 1-in-100 year flood. Detritus densities set upper limits to the
30 densities of common taxa. Positive relations between invertebrate and detritus densities (density-
31 resource relationships) took a variety of forms and showed that detritus was an essential resource for
32 some taxa and a substitutable resource for others. Species varied in the minimum amount of detritus
33 required for presence at a site, and population densities increased strongly from low densities when
34 detritus was increased. These outcomes suggest that fluctuation-dependent mechanisms of coexistence
35 enabled new taxa to coexist at manipulation sites, with relative non-linear averaging of competition and
36 the storage effect most likely to be in play. Two characteristics of the study stream underpin diversity
37 increases with resource enrichment: overall low background densities of detritus and species that are
38 able to disperse successfully from upstream areas where detritus is more abundant. Thus, the effects of
39 resource enrichment are context dependent.

40 *Key words:* aquatic invertebrates, community turnover, detritus, rivers, species coexistence

41 INTRODUCTION

42 The exchange of resources between ecosystems can influence community-level processes in the
43 recipient ecosystem (Polis et al. 1997, Polis et al. 2004). For resource-limited communities, resource
44 enrichment typically leads to increased consumer densities and species richness, at least in the short
45 term. Potential explanations for this diversity increase include: persistence of specialist species using
46 rare resources and of rare species that have extinction-prone populations, and increases in predators
47 consuming prey that exploit the resource directly (Srivastava and Lawton 1998). Such patterns could
48 arise via aggregative responses as individuals move from resource-poor to resource-rich locations and
49 numerical responses as populations grow via reproduction. The relative importance of aggregative vs
50 numerical responses depends on the duration of resource enrichment relative to generation times and
51 dispersal capabilities. Increased diversity in response to short-term resource enrichment is well known
52 (e.g. Yang et al. 2010); whether diversity increases persist over multiple generations is less clear.

53

54 Long-term resource enrichment is likely to encompass multiple generations and environmental
55 variation. Thus, effects on species diversity need to be considered within a theoretical framework of
56 species coexistence, because a persistent increase in diversity indicates a change in the mechanisms
57 that enhance coexistence (Chesson 2008). In theory, recurrent resource pulses may strengthen
58 coexistence mechanisms and increase diversity but they may also disrupt mechanisms and reduce
59 diversity (Holt 2008). Prolonged resource enrichment of sustained magnitude can lead to sustained
60 higher species richness (Schneider et al. 2011), but the quantity or quality of resources in natural
61 ecosystems is rarely constant. Typically, resources are patchy and fluctuate temporally via seasonal
62 cycles or interactions with the abiotic environment, which can influence coexistence mechanisms. The
63 suite of mechanisms that enable species coexistence may operate irrespective of environmental

64 variability (e.g. resource partitioning, frequency-dependent predation), but there are also fluctuation-
65 dependent mechanisms (e.g. relative non-linearity of competition, the storage effect), which are
66 enabled by variations in resource supply or environmental conditions (Chesson 2000b, 2008).

67

68 A key characteristic underpinning species coexistence is invasibility: the capacity of some species'
69 populations to grow strongly from low densities while other species are at typical (i.e. "normal")
70 densities (Chesson 2000b). Information on population growth rates over a range of resource density is
71 necessary to identify coexistence mechanisms but difficult to obtain empirically. Density–resource
72 relationships (DRR) are a practical alternative. Positive DRR are expected for taxa that benefit directly
73 or indirectly from the resource, but those relationships could take a variety of forms (Figure 1).
74 Negative or unimodal DRR are expected if competitive exclusion or species trade-offs occur, and
75 negative relationships may be diverse in form also. Non-linear relationships are of special interest
76 because they may indicate particular coexistence mechanisms (Chesson 2000b). Similarly, strong DRR
77 may manifest as deterministic relationships (data points cluster tightly around a regression line)
78 whereas, if local densities are influenced by multiple processes, then limiting relationships are likely
79 (data points are scattered below a regression line that denotes an upper limit to density). Clearly,
80 unravelling which mechanisms underpin community responses to enriched resources requires evidence
81 of the direction and shape of species-specific DRR.

82

83 A key resource for invertebrate communities of many freshwater systems is allochthonous detritus
84 (Moore et al. 2004, Gessner et al. 2010) and such donor-controlled systems have long been used for
85 experimental resource manipulations on scales ranging from small container habitats (Jenkins et al.
86 1992) to long river lengths (Wallace et al. 1999). Following classical ecological theory, resources are

87 any substance or factor that can be consumed or monopolized by organisms, potentially leading to
88 competition and density-dependent feedback loops (Tilman 1982). Accordingly, detritus can be
89 exploited in diverse ways by aquatic and terrestrial stages of many invertebrates (e.g. as food, hard
90 substrate to sit on, predator refuges, oviposition or emergence sites). In rivers, the focus of our
91 research, studies typically report increased density and richness of invertebrates with detritus, but most
92 have been short (≤ 2 years) and/or focus on a subset of the community (Smock et al. 1989, Richardson
93 1991, Dobson and Hildrew 1992, Wallace et al. 1997). Previous experiments often report taxon-
94 specific responses (e.g. Wallace et al. 1999), but most manipulations involve a categorical increase or
95 decrease in resources rather than continuous resource gradients, and DRR are rarely described
96 (Richardson and Wipfli 2016).

97

98 This study examines the community and species-level effects of experimental resource enrichment over
99 five years in a river with a pre-existing resource gradient. Of primary interest were changes in species
100 diversity, rather than modes of resource exploitation. Previously, we reported a one-year field
101 experiment conducted over large scales (22 km river length) in which boosting detrital resources
102 increased density and species richness of invertebrates and changed community composition (Lancaster
103 and Downes 2017). Dispersal within the metacommunity facilitated rapid species responses (< 1 year),
104 with no evidence that new taxa colonized from outside the catchment. Dispersal was not a constraint
105 for any species examined (Downes and Lancaster 2018). To enrich resources, we increased channel
106 retention and this boosted detrital densities (leaves and small woody debris) over five years, which
107 corresponds to at least five generations for invertebrates in that system.

108

109 We first tested whether increased densities, higher species richness and altered community composition
110 were maintained over five years. *A priori*, we erected three plausible community responses: (1) In the
111 *Gradual Increase* model, density and species richness continue to increase after the first year, with
112 increasing numbers of taxa that respond (i.e. higher colonization rates at manipulation sites) and
113 increasing divergence in community composition between manipulation and control sites. This pattern
114 is likely when species are slow to increase densities and/or to colonize resource-rich locations, perhaps
115 requiring periodic (e.g. seasonal) windows of opportunity for dispersal and reproduction. (2) In the
116 *Rapid Sustained Increase* model, the community does not change after the first year, i.e. species
117 richness and densities remain high, local colonization and extinction rates are equivalent after the first
118 year; treatments differ in community composition but not the magnitude of the difference. (3) In a
119 *Rapid Transient Increase*, densities remain high but richness decreases after the initial rise, e.g. high
120 local colonization followed by extinction as some species are displaced, potentially
121 by competitive dominants or increased predation (Polis and Strong 1996). Community composition
122 may return to the pre-manipulation configuration, depending on the effects of environmental variation
123 in space and time. Fluctuation-dependent mechanisms of species coexistence are strongly implied by
124 the first two models. In contrast, a collapse in species richness and return to a pre-manipulation
125 configuration suggests that fluctuation-independent mechanisms have returned the community to the
126 former status quo. The gradient of resource densities in space and time allowed us to examine species-
127 specific DRR and gain insight into the mechanisms underpinning community responses.

128

129 METHODS

130 *Study site and experimental design*

131 The study site and experimental design have been described in detail elsewhere (Downes et al. 2017,
132 Lancaster and Downes 2017, Downes and Lancaster 2018), so only a brief description is provided here.
133 The study was carried out in a 22 km length of Hughes Creek, a sand-bed stream in the Goulburn River
134 catchment of Victoria, Australia. Sample sites were in the upper reaches of Hughes Ck. Discharge is
135 monitored continuously at a gauge ~18 km downstream of our most downstream site. Along the study
136 length there were longitudinal gradients in the abiotic environment (physicochemistry, channel
137 morphology), resources (detrital standing stocks), benthic invertebrate density and diversity, which
138 have been fully documented in previous work (Downes et al. 2017, Lancaster and Downes 2017).

139

140 Experimentally, we enriched detritus densities by increasing channel retentiveness at manipulation
141 sites, while control sites were unchanged. There were 12 sample sites (40 m channel lengths), four in
142 each of three zones (up-, middle, downstream) and, within each zone, two control and manipulation
143 sites were allocated at random. Sites within zones had similar abiotic environments. To increase
144 retentiveness, pairs of wooden stakes were hammered into the stream bed at densities of 0.13 – 0.21
145 pairs m⁻², in accordance with background, natural retention (Appendix S1: Table S1). Detritus
146 transported by currents accumulated on these stakes and in low flow areas on the bed, which the stakes
147 created (Lancaster and Downes 2017). This manipulation created very minor changes in local
148 hydraulics, which were too small to be ecologically significant or affect the results and interpretations.
149 Benthic invertebrates and detritus were sampled in the austral summer (Jan or Feb) before manipulation
150 (Year 0) and at 1, 2, and 5 years post-manipulation. Sample collection and processing used protocols
151 identical to Lancaster and Downes (2017). On each occasion we collected 10 (Year 0) or 15 (Years 1,
152 2, 5) Surber samples (0.09 m², 250 µm mesh) at each site according to a random stratified design that
153 ensured samples were collected along the full site length. Samples were composited to provide a single

154 estimate of invertebrate densities per site. Invertebrates were identified to the lowest taxonomic level
155 possible: species for most taxa; genus for most Chironomidae. Detritus >5 cm² was removed from
156 samples, dried and weighed to estimate detritus standing stocks.

157

158 *Numerical and statistical analyses*

159 Analysis was by three-way analysis of variance (ANOVA) with three fixed factors: Year, Treatment
160 (control vs manipulation), and Zone (up-, middle, downstream). The main tests of our hypotheses are
161 effects that include Treatment, which would indicate a response to increased detritus. Planned contrasts
162 within the Treatment × Year term determined in which years treatment effects were significant. Zone
163 effects were expected (Lancaster and Downes 2017) and were not central to the hypotheses posed, but
164 Zone was included in the model to account for spatial structure in the data. This model was used for all
165 univariate tests (detrital densities, taxon richness, total invertebrate densities, densities of particular
166 taxa). Data were log-transformed before analyses to satisfy assumptions of homogeneity of variance.

167

168 Local rates of species extinction and colonization between sample years were calculated for each site
169 following Lekve *et al.* (2002). Local extinction (E) was defined as the proportion of species lost from
170 the community between two sampling times, $E = S_{A \setminus B} / S_A$, where S_A is the number of species present
171 at time A, $S_{A \setminus B}$ is the number of species present at time A but not time B, and time A occurs before time
172 B. Local colonization, C , was defined as the proportion of species that were absent in the previous year,
173 $C = S_{B \setminus A} / S_A$, where $S_{B \setminus A}$ is the number of species present in time B but not time A. We used 3-way
174 ANOVA to test for the effects of Treatment, Year Pairs, and E vs C . Zone was not included because E
175 and C are independent of species richness and preliminary analyses indicated that Zone was not
176 significant. The main effects are uninteresting; instead we used *post hoc* pair-wise comparisons

177 (Tukey's test) to determine whether (i) *E* (and *C*) varied over time within treatments, (ii) *E* (and *C*)
178 differed between treatments within each year pair, and (iii) *E* differed from *C* within treatments and
179 each year pair.

180

181 For the multivariate data, we tested for treatment effects on community composition using three-way
182 ANOVA with three fixed factors: Year, Treatment and Zone. Tests were carried out using
183 PERMANOVA+ and PRIMER v.6 (Clarke and Gorley 2006, Anderson et al. 2008), based on a
184 resemblance matrix constructed from fourth-root transformed species abundance data and Bray-Curtis
185 similarity coefficients. The multi-species assemblage was also used to test for, and to assess, the
186 relative importance of the different environmental variables on community composition. Distance-
187 based linear models (DISTLM) were used to identify the combination of environmental variables that
188 explained the greatest variation in the resemblance matrix and to estimate the variance explained by
189 each. Models were fit using step-wise selection and the most parsimonious model chosen by AIC.
190 Detritus density was a continuous variable; Zone, Year and Treatment were categorical. Outcomes of
191 DISTLM were illustrated using distance-based redundancy analysis (dbRDA) in which ordination axes
192 were constrained to be combinations of the environmental variables that explained most variation.

193

194 For each of the common taxa, we tested for treatment effects using three-way ANOVA (Factors: Year,
195 Treatment, Zone) of log-transformed data. If treatment effects were significant, we tested for species-
196 specific DRR. Preliminary analyses indicated that most DRR were limiting relationships so we used
197 quantile regression for all tests. With $n = 48$ site/time combinations, we could estimate upper and lower
198 quantiles at $\tau = 0.85$ and $\tau = 0.15$ (i.e. 85th and 15th quantiles). Analyses were carried out using the
199 quantreg package in R Project Software (R Core Development Team 2017). Quantile regression

200 ANOVA (rqANOVA) was used to test whether a linear relationship described the 85th quantile and to
201 test for equality of slopes. A few taxa showed positive but not limiting DRR, and for these cases we fit
202 a central relationship, i.e. the 50th quantile. Finally, for positive relationships, upper and lower bounds
203 of the y -axis intercept were used to determine whether the intercept was positive, negative or did not
204 differ from zero, and determine which response model in Figure 1 best described the DRR.

205

206 RESULTS

207 Discharge varied over the five years with highest flows typically in winter (Appendix S1: Figure S1).
208 Discharge was abnormally low in Years 2 and 3, but high rainfall in Year 4 produced multiple high
209 discharge events. Extreme discharge 8 weeks before we collected samples in Year 5 was equivalent to
210 a 1-in-100 year flood.

211

212 Post-manipulation, densities of detritus varied between treatments, years and zones, and was higher in
213 manipulation sites in all years (Figure 2a, Appendix S1: Table S2). Pre-manipulation, detritus densities
214 did not differ between treatments (Lancaster and Downes 2017). In control sites, detritus fluctuated but
215 post-manipulation densities in manipulation sites were higher than controls in all years, although effect
216 sizes varied. Post-manipulation, the lowest detritus densities in manipulation sites and the smallest
217 difference between treatments occurred in Year 5 after a large flood (Appendix S1: Figure S1).

218

219 Over all years, we recorded >220 benthic invertebrate taxa, >95% of which were aquatic insects with
220 generation times of ≤ 1 year. Roughly half the taxa, 51%, occurred in at least three sample years,
221 whereas 32% occurred in only one year (typically in low densities). The number of taxa observed over
222 all sites in each year was 132, 182, 143 and 123, in Years 0, 1, 2 and 5, respectively. There was no

223 obvious colonization of new species from outside the catchment, nor loss of common species from the
224 community.

225

226 Taxon richness and total invertebrate densities were higher in manipulation than control sites in all
227 zones, in all post-manipulation years, although effect sizes varied (Figure 2, Appendix S1: Table S2).

228 There was no temporal gradient in the magnitude of differences, but the effect was smaller in the

229 upstream zone: on average, richness upstream was 1.34× higher in manipulation than control sites,

230 compared with 1.56× in the other zones. Similarly, there was no temporal trend in total invertebrate

231 densities or in the magnitude of the differences between treatments (Figure 2).

232

233 One year after increasing resources, the probabilities of local species extinction (*E*) was lower and

234 colonization (*C*) was higher, whereas controls did not differ in *E* and *C* (Figure 3, Appendix S1: Table

235 S3). *E* and *C* did not differ between treatments in Years 1–2, but *C* was lower than *E* in both treatments

236 in Years 2–5, after the flood. Within year pairs, in control sites, extinction rates did not differ from one

237 another but colonization rates were lower in Years 2–5 (Figure 3, Appendix S1: Tables S3 and S4). In

238 manipulation sites, *E* was lower and *C* was higher in Years 0–1 than other year pairs; neither *E* nor *C*

239 differed in Years 1–2 or 2–5 (i.e. with or without disturbance).

240

241 For the invertebrate assemblage, PERMANOVA revealed effects of Treatment and Treatment × Zone

242 (Table 1). Based on pair-wise tests, Treatment effects were strong in Years 1 and 5, but weak in Year 2.

243 Treatment effects varied between zones, with weaker effects upstream than in middle and downstream

244 zones (Appendix S1: Figure S2). After Year 1, there was no evidence that assemblages in control and

245 manipulation sites diverged or converged on the pre-manipulation composition. Relating the species

246 assemblage to environmental variables (DISTLM), all four variables were included in the best model
247 and together explained 49.3 % of the variation (Figure 4, Appendix S1: Table S5). The strongest
248 explanatory variables in marginal tests were Zone (22.8% variance explained) and Year (18.4%). Both
249 Detritus and Treatment each explained significant amounts of variation (5.6%; 2.5%), highlighting
250 temporal variations in detritus as well as treatment effects. The first dbRDA axis (Figure 4) was
251 associated with Zone, the second axis with Year and Detritus. Although Zone was a categorical
252 variable in the analysis, the spatial arrangement of sites in the dbRDA corresponds to the longitudinal
253 gradient. Similarly, Year was a categorical variable yet Figure 4 indicates a directional, temporal shift
254 in the community from Year 0 to 5, even though Years 1 and 2 cluster together in ordination space (see
255 also Appendix S1: Figure S2).

256

257 Univariate analyses of the common taxa were consistent with the multivariate patterns. Of the >220
258 taxa, 54 were sufficiently abundant for analysis (a summary of responses is in Appendix S1: Table S6).
259 Some species were pooled into coarser taxonomic groups (genus or family) to enable meaningful
260 statistical analyses. A total of 52 taxa were also common in the seasonal survey of the first year
261 (Lancaster and Downes 2017); two taxa were added and four were lost (e.g. stoneflies that are scarce in
262 summer). Roughly half the common taxa (25 or 46%) were not affected by the manipulation overall or
263 in within-year, pair-wise tests. All treatment effects involved higher densities in manipulation than
264 control sites; differences were significant for 23 (43%) of the common taxa. For six taxa (11%),
265 treatment effects were marginally non-significant ($0.10 > P > 0.05$) largely owing to low densities in
266 Year 5 and associated loss of power. The identity of taxa that did or did not respond to the treatment
267 was not associated with particular ecological traits (Lancaster and Downes 2017), and was similar in
268 the one-year seasonal and five-year analyses. Two taxa responded in the one-year survey but not over

269 the five years; five taxa responded over five years but not one year. These small numbers are close to
270 the 5% error rate that could occur by chance when tests are carried out on many taxa.

271

272 In tests for DRR, there were clear relationships for 15 taxa that all showed significant Treatment effects
273 and were sufficiently abundant for meaningful quantile regression analysis (Appendix S1: Table S7).

274 No DRR occurred for taxa that did not show a Treatment effect. All significant DRR were positive
275 (density increased with detritus); we found no evidence of negative or unimodal relationships. Nine
276 taxa fit the tracking model (Figure 5a), two fit the threshold model (Figure 5b), four fit the expanding
277 model (Figure 5c), and none fit the saturation model. Among the responding taxa, there was a near
278 four-fold difference in the magnitude of the slopes in the log-log relationships at $\tau = 0.85$ (Appendix
279 S1: Table S7).

280

281 DISCUSSION

282 In response to prolonged enrichment of naturally-sourced detrital resources in a river, we found
283 persistently higher densities and species richness of invertebrates at manipulation sites, along with
284 continuous turnover in species composition, during a five-year, landscape-scale field experiment.

285 Treatment effects were maintained in all post-manipulation years, despite variation in resource
286 densities and community composition over time and space. For the 50% of taxa that responded, all
287 responses were positive with no evidence of population decline or extinction from the community.

288 Density–resource relationships varied among taxa: there were resource specialists and generalists,
289 differences in the minimum amount of detritus required to support populations, and detritus was often a
290 limiting resource. Although many studies have manipulated resources, this study is rare in that it tested
291 the effects of prolonged enrichment of natural resources over scales that are appropriate to assess long-

292 term community changes, and that encompassed natural environmental variation. Moreover, replication
293 in space and time allowed insights into the potential coexistence mechanisms that promoted increases
294 in species diversity.

295

296 In theory, prolonged resource enrichment with seasonal pulses can create sustained increases in species
297 diversity, but diversity can also crash after an initial increase (Holt 2008). We did not observe such a
298 fall in diversity, suggesting that resources were not depleted and predation did not increase, as
299 predicted by some food web models of donor-controlled communities (Polis and Strong 1996,
300 Subaluský and Post 2019). Thus, the *Rapid Transient Increase* model was not supported. The *Gradual*
301 *Increase* model also failed because taxon richness did not increase each year, local colonization rates
302 were not consistently higher than extinction rates at manipulation sites, and community composition
303 did not diverge between treatments. Community responses to resource manipulation may be slow in
304 some stream systems (e.g. Entrekín et al. 2009, Pilotto et al. 2018), but clearly not in all. Overall, our
305 results support the *Rapid Sustained Increase* model: prolonged resource enrichment produced
306 persistently high diversity and densities at manipulation sites, and was accompanied by low, and often
307 equivalent rates of local extinction and colonization after the first year. Community composition
308 continued to differ between treatments, but with no directional trend in the magnitude of difference.
309 Membership of the pool of common species remained fairly constant, as did the number and identity of
310 taxa that did or did not respond to the manipulation. Therefore, shifts in community composition likely
311 reflected changes in the relative abundance of common species or turnover in the identity of rare
312 species.

313

314 Spatial and temporal variations in resource densities created diverse effects beyond those of simple
315 resource enrichment, including context-dependent effects. Treatment effects occurred in all years and

316 all parts of the stream gradient (Zones), but the effect magnitude was often greatest downstream where
317 background densities of detritus were low owing to poor channel retentiveness. It has long been
318 recognized that resource subsidies are likely to have big effects in resource-poor systems (Polis et al.
319 1997) and theoretical models predict that effects will vary with the magnitude of enrichment relative to
320 background (Leroux and Loreau 2010), but empirical evidence is scarce. Background detrital densities
321 varied between stream zones because the supply of detritus depends on the density of riparian
322 vegetation, which was higher upstream than downstream. Additionally, the amount of benthic detritus
323 varied between years in concert with discharge fluctuations. Year 5 featured a 1-in-100 year flood that
324 scoured the channel and lowered background detrital densities across the entire river, but did not
325 obliterate the treatment effect. In contrast, below-average flows in Year 2 resulted in relatively high
326 densities of detritus at control sites, and differences in community composition between treatments
327 were marginally non-significant in that year. These results demonstrate that the status of a system as
328 resource-poor or resource-rich and the magnitude of resource enrichment can vary substantially with
329 environmental variation.

330

331 The experimental manipulation, in concert with environmental variation, produced a gradient of
332 resource density and species-specific responses. The density–resource relationships (DRR) do not
333 specifically describe changes over time (because resource densities fluctuated in both treatments), but
334 rather a species' potential to increase density with resources. For all responding taxa that were
335 sufficiently common for analysis, detritus was a limiting resource, i.e. it set upper limits or directly
336 controlled their densities. Moreover, these DRR demonstrated that detritus was an essential resource
337 for some taxa but not others, a condition that will affect the outcomes of species interactions and hence
338 coexistence (Letten et al. 2017). Densities of some species were relatively high at sites with virtually no

339 detritus (Figure 1: expanding) suggesting that detritus was a substitutable or complementary resource
340 rather than an essential resource (Tilman 1980), or that species benefitted indirectly (e.g. predatory
341 chironomids: Pentaneurini). In contrast, detritus was essential for other taxa because they were absent
342 when detritus density was near-zero. Some such taxa responded to even small increases in detritus
343 (Figure 1: tracking) whereas others required a higher, minimum amount of detritus to support any
344 individuals (Figure 1: threshold). There was no evidence of resource saturation (Figure 1), despite
345 detrital densities ranging over several order of magnitude. Species exploited detritus in diverse ways.
346 The two threshold species (larval elm mid beetles, *Notriolus* spp.; chironomids, *Harrissius* sp.) are
347 xylophages (specialist consumers of wood), but trackers included taxa that shred leaves (stoneflies in
348 the Gripopterygidae) or are collector-gatherers (chironomids, *Thienemanniella* and *Polypedilum*), or
349 that use detritus as attachment points for filter feeding (caddisflies, *Asmicridea*; chironomids,
350 *Rheotanytarsus*) or surfaces for grazing biofilm (limpets, *Ferrissia*) (Sephton and Hynes 1983, McKie
351 and Cranston 1998). Some other responding species require bark and wood for egg-laying (e.g.
352 caddisflies, *Cheumatopsyche* spp.) (Macqueen and Downes 2015), indicating that detritus can be an
353 essential resource for terrestrial adult stages rather than aquatic larvae. Overall, species responded to
354 detritus in qualitatively and quantitatively different ways and for purposes other than consumption for
355 food.

356

357 Persistently high species richness with resource enrichment, coupled with evidence that (i) detritus is a
358 limiting resource and (ii) some species show invasibility (positive population growth when detritus is
359 increased while other taxa are at typical densities), suggest that one or more fluctuation-dependent
360 mechanisms of species coexistence enabled new taxa to coexist (Holt 2008). Although coexistence
361 theory is well-developed, unambiguous tests of coexistence mechanisms are extremely challenging

362 (Chesson 2008). While we could not test coexistence mechanisms directly, the results implicate two
363 fluctuation-dependent mechanisms: relative non-linearity of competition and the storage effect.
364 Relative nonlinear averaging of competition can apply if the per capita growth rates of populations are
365 nonlinear as resource availability declines below optimal levels and becomes limiting (Chesson 2000b).
366 Species-specific relationships between growth rate and resource limitation must differ in the degree of
367 nonlinearity, as quantified by the extent of curvature (τ in coexistence models). Our DRR do not
368 illustrate population growth rates, but the line slopes imply that increases in detritus can trigger rapid,
369 species-specific increases in population densities. Moreover, because the DRR are nonlinear, variation
370 in the slopes of these log-log plots reflect different values for τ . Nevertheless, we could not address all
371 necessary conditions, such as the degree of resource variation each species creates through
372 consumption (Chesson 1994). Additionally, our data are for larvae and it is unknown whether a per
373 capita increase produces more adults in the next generation.

374

375 Coexistence via the storage effect (Chesson 2000b) requires several conditions and the first is that
376 species must differ in their responses to the environment. In Hughes Ck, many species are restricted to
377 or have high densities in particular zones, whereas others are widespread (Downes et al. 2017,
378 Lancaster and Downes 2017). These patterns may reflect spatial variation in the abiotic environment
379 that affect species' fitnesses. For example, the water temperature in upstream locations is cool (mean
380 18°C in summer), but can reach 36°C downstream (Downes et al. 2017). Water temperatures above
381 30°C can cause physiological stress for insects (Lancaster and Downes 2013), so it is likely that species
382 vary in their capacity to thrive in different parts of Hughes Ck. A second condition is that species vary
383 in their ability to disperse successfully between zones. As demonstrated independently (Downes and
384 Lancaster 2018), species that responded rapidly to resource enrichment either dispersed downstream

385 via the drift and colonized new sites, or increased densities at resource-rich sites via adult flight and
386 reproduction. In contrast, non-responders often failed to increase densities at sites with high detritus,
387 even when dispersal was successful. Limited dispersal that varies spatially and between species results
388 in environment–competition covariance, and this can be inferred even when species interactions (e.g.
389 competition, predation) have not been quantified (Chesson 2000a). Further support for the storage
390 effect lies in the finding that spatial patterns in faunal composition were consistent over time. If
391 temporal variation nullified spatial differences, then potential benefits of the storage effect would be
392 reduced (Chesson 2000b).

393

394 Competition-colonization trade-offs, another fluctuation-dependent mechanism that can underpin
395 increased diversity after physical disturbance, did not play a role in this study. In this mechanism,
396 species that are good at colonizing newly disturbed sites establish briefly but are subsequently ousted
397 by species that are superior competitors and poorer colonists; this mechanism underpins the
398 intermediate disturbance hypothesis (Connell 1978, Chesson 2000b). The flood preceding sampling in
399 Year 5 was a major disturbance that reduced invertebrate densities at all sites. Consequently, local
400 extinction exceeded colonization in both treatments, but there was no shift in species composition
401 brought about by new colonists. Studies on flood disturbance similarly often report short-term declines
402 in diversity and density, but not species replacement related to colonization ability (e.g. Lake et al.
403 1989, Lake 2000).

404

405 In multiple ecosystems, invertebrates depend directly or indirectly on detrital resources, but how
406 resource pulses affect species diversity or food webs is unclear (e.g. Moore et al. 2004, Stoler and
407 Relyea 2013). Unlike our study, most studies of increased detritus in rivers have not recorded increased

408 diversity (e.g. Entekin et al. 2009), which suggests that outcomes are context-dependent. The
409 experiment described in this paper was repeated over one year in another stretch of Hughes Ck and
410 delivered an equally strong effect on species diversity, which was also greater than responses in five
411 different rivers (Bovill et al. submitted ms). Hughes Ck had the lowest background levels of detritus
412 and was also the only stream in which dispersal from intact, upstream sections was implicated in
413 increases in diversity. This combination of characteristics may explain why Hughes Ck showed the
414 strong, consistent responses over time reported here. Context-dependency suggests that tests of the
415 effects of resource enrichment on species diversity need to be field-based, conducted over large spatial
416 and temporal scales, and subject to natural rates and identities of dispersers, because results can be
417 qualitatively different to laboratory or mesocosm studies (Brooks et al. 2020). Our simple, low-
418 maintenance method of indirectly manipulating resources via direct manipulation of retention
419 effectively captured natural rates and seasonal pulses of detritus over multiple years. The technique can
420 be applied to any wade-able stream. As such, rivers may be particularly useful ecosystems to facilitate
421 further insights into the consequences of prolonged resource enrichment.

422

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429

430 Author contributions: Both authors designed and executed the experiment, collected samples, analysed
431 data and wrote the paper.

432

433 LITERATURE CITED

434 Anderson, M. J., R. N. Gorley, and K. R. Clarke. 2008. PERMANOVA+ for PRIMER: Guide to
435 Software and Statistical Methods. PRIMER-E Ltd, Plymouth, UK.

436 Brooks, A. J., J. Lancaster, B. J. Downes, and B. Wolfenden. 2020. Just add water. Rapid assembly of
437 new communities in previously dry riverbeds, and limited long-distance effects on existing
438 communities. *Oecologia* **194**:709-722.

439 Chesson, P. 2000a. General theory of competitive coexistence in spatially-varying environments.
440 *Theoretical Population Biology* **58**:211-237.

441 Chesson, P. 2000b. Mechanisms of maintenance of species diversity. *Annual Review of Ecology and*
442 *Systematics* **31**:343-366.

443 Chesson, P. 2008. Quantifying and testing species coexistence mechanisms. Pages 119-164 *in* F.
444 Valladares, A. Camacho, A. Elosegui, C. Gracia, M. Estrada, J. C. Senar, and J. M. Gili, editors.
445 *Unity in Diversity: Reflections on Ecology after the Legacy of Ramon Margalef*. Fundacion
446 BBVA, Bilbao.

447 Chesson, P. L. 1994. Multispecies competition in varying environments. *Theoretical Population*
448 *Biology* **45**:227-276.

449 Clarke, K. R., and R. N. Gorley. 2006. PRIMER v6: User Manual/Tutorial. PRIMER-E Ltd.,
450 Plymouth.

451 Connell, J. H. 1978. Diversity in tropical rain forests and coral reefs. *Science* **199**:1302-1310.

- 452 Dobson, M. K., and A. G. Hildrew. 1992. A test of resource limitation among shredding detritivores in
453 low order streams in southern England. *Journal of Animal Ecology* **61**:69-78.
- 454 Downes, B. J., and J. Lancaster. 2018. Itinerant, nomad or invader? A field experiment sheds light on
455 the characteristics of successful dispersers and colonists. *Freshwater Biology* **63**:1394-1406.
- 456 Downes, B. J., J. Lancaster, A. Glaister, and W. Bovill. 2017. A fresh approach reveals how dispersal
457 shapes metacommunity structure in a human-altered landscape. *Journal of Applied Ecology*
458 **54**:588-598.
- 459 Entekin, S. A., J. L. Tank, E. J. Rosi-Marshall, T. J. Hoellein, and G. A. Lamberti. 2009. Response of
460 secondary production by macroinvertebrates to large wood addition in three Michigan streams.
461 *Freshwater Biology* **54**:1741-1758.
- 462 Gessner, M. O., C. M. Swan, C. K. Dang, B. G. McKie, R. D. Bardgett, D. H. Wall, and S.
463 Hättenschwiler. 2010. Diversity meets decomposition. *Trends in Ecology & Evolution* **25**:372-
464 380.
- 465 Holt, R. D. 2008. Theoretical perspectives on resource pulses. *Ecology* **89**:671-681.
- 466 Jenkins, B., R. L. Kitching, and S. L. Pimm. 1992. Productivity, disturbance and food web structure at
467 a local spatial scale in experimental container habitats. *Oikos* **65**:249-255.
- 468 Lake, P., T. Doeg, and R. Marchant. 1989. Effects of multiple disturbance on macroinvertebrate
469 communities in the Acheron River, Victoria. *Australian Journal of Ecology* **14**:507-514.
- 470 Lake, P. S. 2000. Disturbance, patchiness, and diversity in streams. *Journal of the North American*
471 *Benthological Society* **19**:573-592.
- 472 Lancaster, J., and B. J. Downes. 2013. *Aquatic Entomology*. Oxford University Press, UK.
- 473 Lancaster, J., and B. J. Downes. 2017. A landscape-scale field experiment reveals the importance of
474 dispersal in a resource-limited metacommunity. *Ecology* **98**:565-575.

- 475 Lekve, K., T. Boulinier, N. C. Stenseth, J. Gjørseter, J.-M. Fromentin, J. E. Hines, and J. D. Nichols.
476 2002. Spatio-temporal dynamics of species richness in coastal fish communities. Proceedings of
477 the Royal Society of London. Series B: Biological Sciences **269**:1781-1789.
- 478 Leroux, S. J., and M. Loreau. 2010. Consumer-mediated recycling and cascading trophic interactions.
479 Ecology **91**:2162-2171.
- 480 Letten, A. D., P. J. Ke, and T. Fukami. 2017. Linking modern coexistence theory and contemporary
481 niche theory. Ecological Monographs **87**:161-177.
- 482 Macqueen, A., and B. J. Downes. 2015. Large-scale manipulations of oviposition substrata affects egg
483 supply to populations of some stream-dwelling caddisflies. Freshwater Biology **60**:802-812.
- 484 McKie, B. G., and P. S. Cranston. 1998. Keystone coleopterans? Colonization by wood-feeding elmids
485 of experimentally immersed woods in south-eastern Australia. Marine and Freshwater Research
486 **49**:79-88.
- 487 Moore, J. C., E. L. Berlow, D. C. Coleman, P. C. de Ruiter, Q. Dong, A. Hastings, N. C. Johnson, K. S.
488 McCann, K. Melville, and P. J. Morin. 2004. Detritus, trophic dynamics and biodiversity.
489 Ecology Letters **7**:584-600.
- 490 Pilotto, F., C. Nilsson, L. E. Polvi, and B. G. McKie. 2018. First signs of macroinvertebrate recovery
491 following enhanced restoration of boreal streams used for timber floating. Ecological
492 Applications **28**:587-597.
- 493 Polis, G. A., W. B. Anderson, and R. D. Holt. 1997. Toward an integration of landscape and foodweb
494 ecology: The dynamics of spatially subsidized food webs. Annual Review of Ecology and
495 Systematics **28**:289-316.
- 496 Polis, G. A., M. E. Power, and G. R. Huxel, editors. 2004. Food webs at the landscape level. The
497 University of Chicago Press, Chicago, IL, USA.

- 498 Polis, G. A., and D. R. Strong. 1996. Food web complexity and community dynamics. *American*
499 *Naturalist* **147**:813-846.
- 500 R Core Development Team. 2017. R: A language and environment for statistical computing. R
501 Foundation for Statistical Computing, Vienna, Austria.
- 502 Richardson, J. S. 1991. Seasonal food limitation of detritivores in a montane stream: an experimental
503 test. *Ecology* **72**: 873-887.
- 504 Richardson, J. S., and M. S. Wipfli. 2016. Getting quantitative about consequences of cross-ecosystem
505 resource subsidies on recipient consumers. *Canadian Journal of Fisheries and Aquatic Sciences*
506 **73**:1609-1615.
- 507 Schneider, K., M. C. Christman, and W. F. Fagan. 2011. The influence of resource subsidies on cave
508 invertebrates: results from an ecosystem-level manipulation experiment. *Ecology* **92**:765-776.
- 509 Sephton, D. H., and H. B. N. Hynes. 1983. Food and mouthpart morphology of the nymphs of several
510 Australian Plecoptera. *Australian Journal of Marine and Freshwater Research* **34**:893-908.
- 511 Smock, L. A., G. M. Metzler, and J. E. Gladden. 1989. Role of debris dams in the structure and
512 functioning of low-gradient headwater streams. *Ecology* **70**:764-775.
- 513 Srivastava, D. S., and J. H. Lawton. 1998. Why more productive sites have more species: an
514 experimental test of theory using tree-hole communities. *American Naturalist* **152**:510-529.
- 515 Stoler, A. B., and R. A. Relyea. 2013. Bottom-up meets top-down: leaf litter inputs influence predator-
516 prey interactions in wetlands. *Oecologia* **173**:249-257.
- 517 Subalusky, A. L., and D. M. Post. 2019. Context dependency of animal resource subsidies. *Biological*
518 *Reviews* **94**:517-538.
- 519 Tilman, D. 1980. Resources: a graphical-mechanistic approach to competition and predation. *The*
520 *American Naturalist* **116**:362-393.

- 521 Tilman, D. 1982. Resource competition and community structure. Princeton University Press.
- 522 Wallace, J. B., S. L. Eggert, J. L. Meyer, and J. R. Webster. 1997. Multiple trophic levels of a forest
523 stream linked to terrestrial litter inputs. *Nature* **277**:102-104.
- 524 Wallace, J. B., S. L. Eggert, J. L. Meyer, and J. R. Webster. 1999. Effects of resource limitation on a
525 detrital-based ecosystem. *Ecological Monographs* **69**:409-442.
- 526 Yang, L. H., K. F. Edwards, J. E. Byrnes, J. L. Bastow, A. N. Wright, and K. O. Spence. 2010. A
527 meta-analysis of resource pulse–consumer interactions. *Ecological Monographs* **80**:125-151.

529 Table 1 Summary of PERMANOVA comparing invertebrate assemblages between treatments in
 530 different zones on the stream gradient and over time. Pair-wise, *post hoc* comparisons and
 531 associated *t*-statistics shown below the Treat \times Year term, indicate whether Treatment effects
 532 were significant for each year See Appendix S1: Figure S2 for NMDS plot.

533

Source	df	MS	Pseudo- <i>F</i>	<i>t</i> _{1,6}	<i>P</i>
Year	3	2971	5.13		<0.001
Treatment	1	2209	3.81		<0.001
Zone	2	5529	9.54		<0.001
Treat \times Year	3	794	1.37		0.087
¹ Year 0				1.08	0.329
Year 1				1.68	0.007
Year 2				1.40	0.081
Year 5				1.46	0.049
Treat \times Zone	2	1154	1.99		0.007
Zone \times Year	6	901	1.55		0.006
Treat \times Zone \times Year	6	385	0.664		0.992
Error	24	580			
Total	47				

534 ¹ The non-significant treatment effect in Year 0 has been reported previously (Lancaster and Downes
 535 2017), a pair-wise test is included here for completeness.

536

537 **Figure legends**

538 Figure 1 – Hypothetical relationships between population density and resource availability. A,
539 threshold: a minimum amount of resource is required before a species can occur, densities increase
540 with resource abundance above that threshold, y -intercept $a < 0$ (e.g. a response that may be seen in
541 predators). B, expanding: species can occur when the resource is absent, $a > 0$, but densities increase
542 with resource abundance (e.g. generalist species). C, tracking: maximum density is closely related to
543 resource abundance, $a = 0$ (e.g. fugitive species: Richardson 1991). D, saturation: density increases but
544 reaches an asymptote, such that further resource increases do not affect density. Line D shows $a = 0$,
545 but a could be positive or negative. Relationships need not be linear but are illustrated that way for
546 simplicity.

547 Figure 2 – Summary of (a) detrital densities, (b) taxon richness and (c) total invertebrate densities per
548 site (mean \pm SE), in control and treatment sites (stippled and solid bars respectively) in relation to time
549 since the manipulations started (time 0 = pre-manipulation). For simplicity, sites from the three zones
550 were pooled to calculate means. Pairs of bars indicated by an asterisk were significantly different
551 (planned contrasts). White numbers on black bars indicate the magnitude of the treatment effect. See
552 Appendix S1: Table S2 for statistical tests.

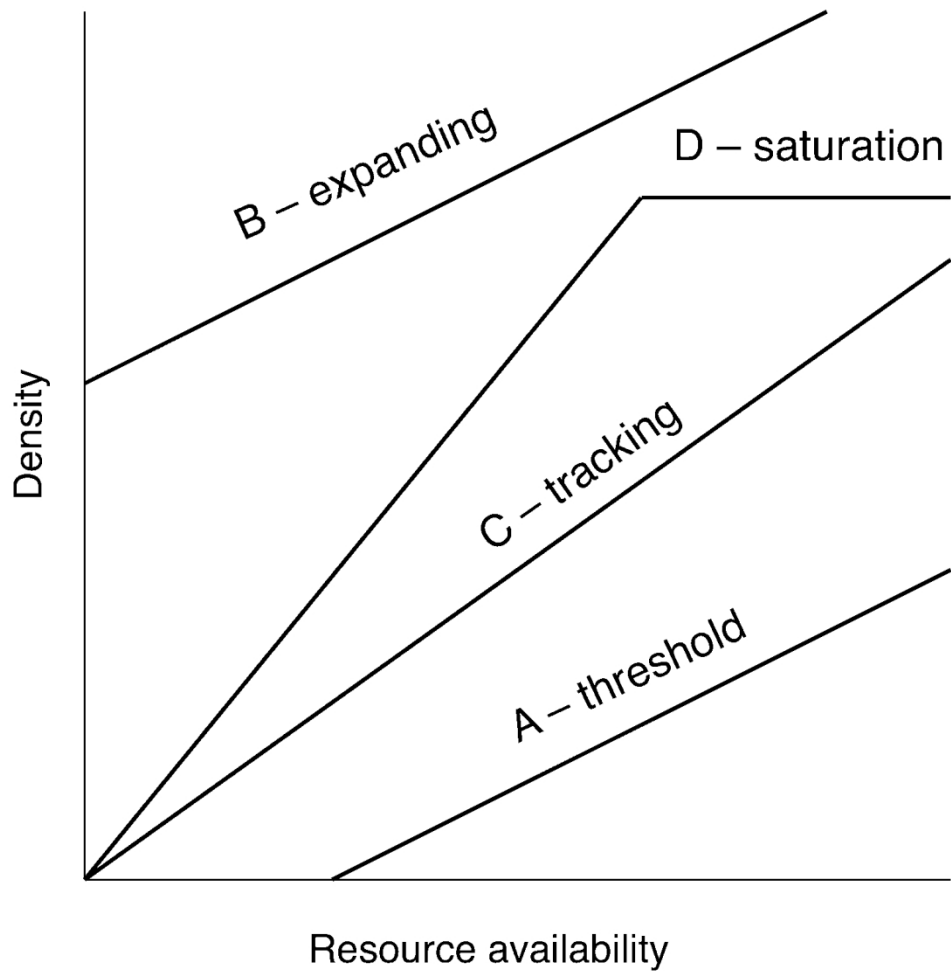
553 Figure 3 – Probability of local extinction, E , and colonization, C , of species in control and manipulation
554 sites for each pair of years (a) – (c). Within each year-pair, bars designated by different letters are
555 different. There were no significant differences in (b). See Appendix S1: Table S3 for statistical tests.

556 Figure 4 – Ordination by dbRDA relating environmental variables to the invertebrate assemblage. Open
557 and closed symbols represent control and manipulation sites, respectively. Years 0, 1, 2 and 5 are
558 represented by blue circles, black diamonds, red squares and green triangles, respectively. Sites are
559 labelled by zone (U, M, D). Environmental variables are shown as vectors whose direction and length

560 indicate the strength and sign of their correlation with the dbRDA axes. The relative size and position
561 of the unit circle is arbitrary. Each vector begins at the circle origin and terminates at the coordinates
562 describing its correlation with the two axes. See Appendix S1: Table S5 for summary of DISTLM
563 analysis.

564 Figure 5 – Examples of three DRR. Solid lines indicate the 85th quantiles ($\tau = 0.85$). In these log-log
565 plots, the origin is represented by the coordinates 1, 1. Where the line intersects the y -axis differs
566 between panels: (a) at the origin for a tracking species, (b) below the origin for a threshold species, (c)
567 above the origin for an expanding species. The lower bounds of the quantile relationships ($\tau = 0.15$) are
568 represented by the x -axes, i.e. slopes did not differ from 0. F -values are the results of rqANOVA
569 testing for a significant relationship at $\tau = 0.85$; b -values are line slopes at $\tau = 0.85$. Tests for equality
570 of slope were significant in each case (Appendix S1: Table S7). Each point is a site/time sample ($n =$
571 48). Open and filled circles indicate control and manipulation sites, respectively.

572



Hypothetical relationships between population density and resource availability. A, threshold: a minimum amount of resource is required before a species can occur, densities increase with resource abundance above that threshold, y -intercept $a < 0$ (e.g. a response that may be seen in predators). B, expanding: species can occur when the resource is absent, $a > 0$, but densities increase with resource abundance (e.g. generalist species). C, tracking: maximum density is closely related to resource abundance, $a = 0$ (e.g. fugitive species: Richardson 1991). D, saturation: density increases but reaches an asymptote, such that further resource increases do not affect density. Line D shows $a = 0$, but a could be positive or negative. Relationships need not be linear but are illustrated that way for simplicity.

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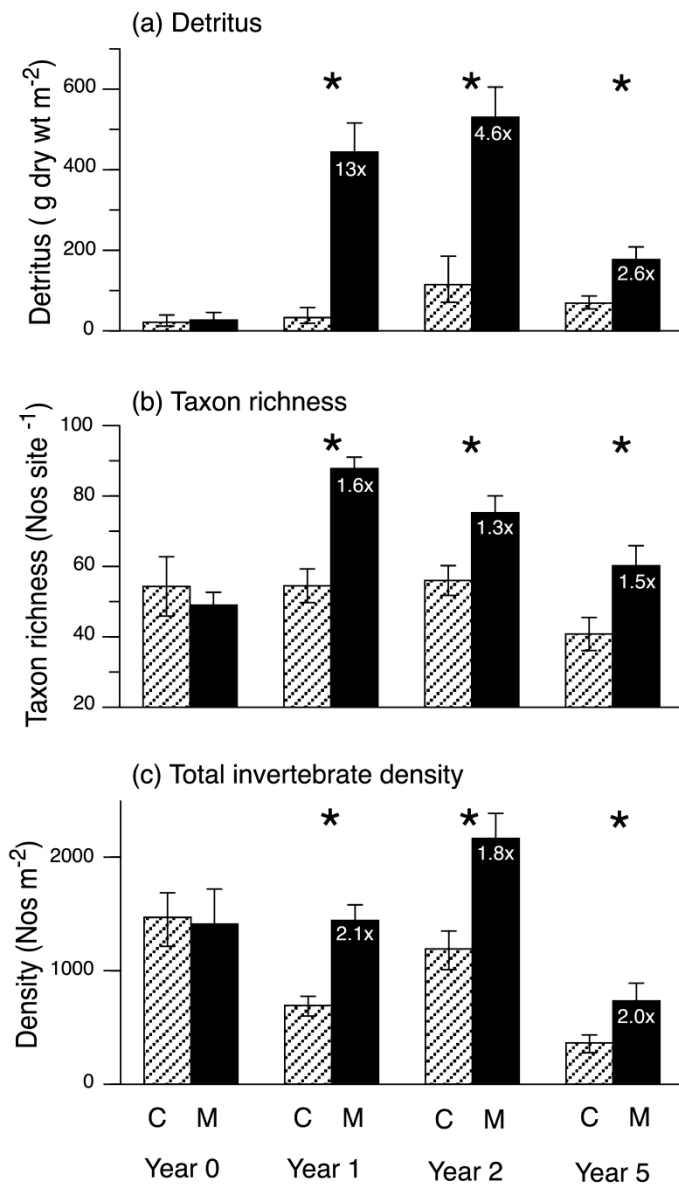


Figure 2. Summary of (a) detrital densities, (b) taxon richness and (c) total invertebrate densities per site (mean \pm SE), in control and treatment sites (stippled and solid bars respectively) in relation to time since the manipulations started (time 0 = pre-manipulation). For simplicity, sites from the three zones were pooled to calculate means. Pairs of bars indicated by an asterisk were significantly different (planned contrasts). White numbers on black bars indicate the magnitude of the treatment effect. See Appendix S1: Table S2 for statistical tests.

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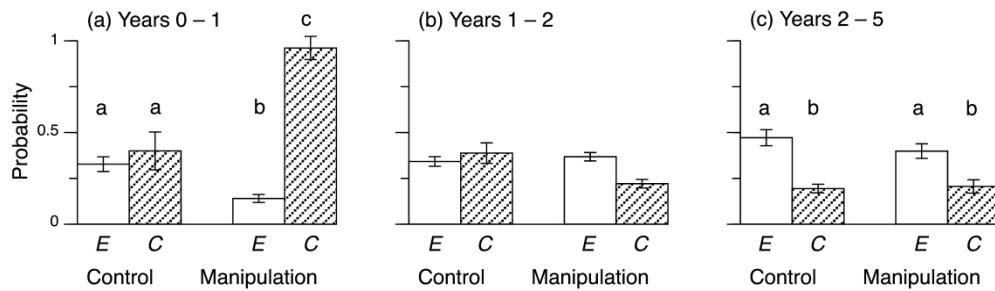


Figure 3. Probability of local extinction, *E*, and colonization, *C*, of species in control and manipulation sites for each pair of years (a) – (c). Within each year-pair, bars designated by different letters are different. There were no significant differences in (b). See Appendix S1: Table S3 for statistical tests.

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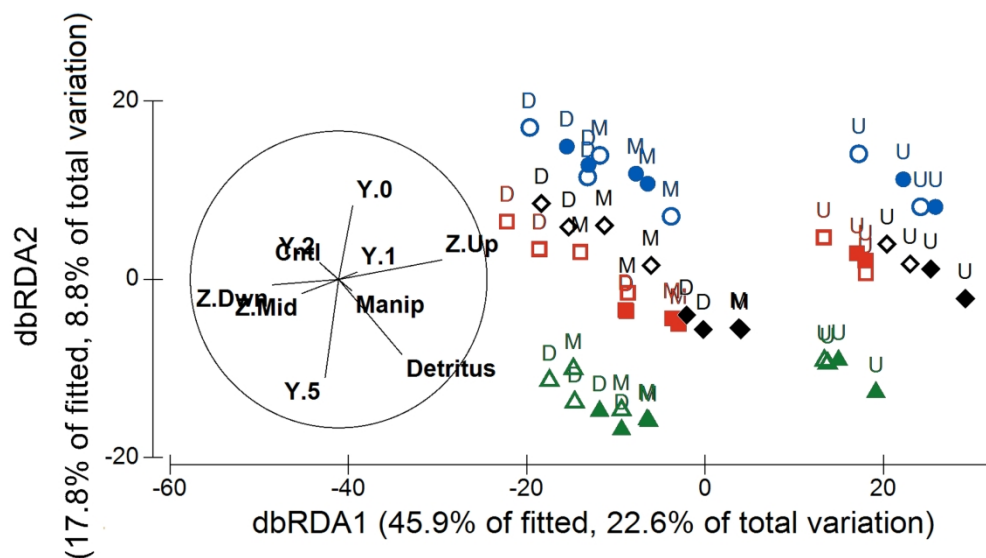


Figure 4. Ordination by dbRDA relating environmental variables to the invertebrate assemblage. Open and closed symbols represent control and manipulation sites, respectively. Years 0, 1, 2 and 5 are represented by blue circles, black diamonds, red squares and green triangles, respectively. Sites are labelled by zone (U, M, D). Environmental variables are shown as vectors whose direction and length indicate the strength and sign of their correlation with the dbRDA axes. The relative size and position of the unit circle is arbitrary. Each vector begins at the circle origin and terminates at the coordinates describing its correlation with the two axes. See Appendix S1: Table S5 for summary of DISTLM analysis.

237x135mm (300 x 300 DPI)

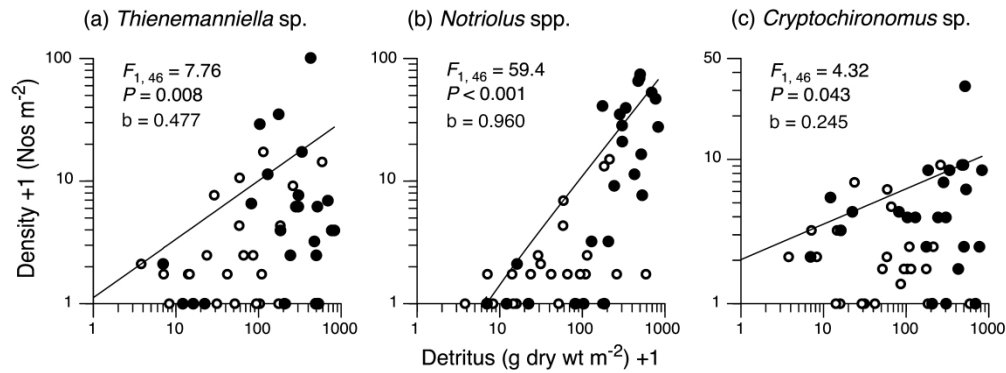


Figure 5. Examples of three DRR. Solid lines indicate the 85th quantiles ($\tau = 0.85$). In these log-log plots, the origin is represented by the coordinates 1, 1. Where the line intersects the y -axis differs between panels: (a) at the origin for a tracking species, (b) below the origin for a threshold species, (c) above the origin for an expanding species. The lower bounds of the quantile relationships ($\tau = 0.15$) are represented by the x -axes, i.e. slopes did not differ from 0. F -values are the results of rqANOVA testing for a significant relationship at $\tau = 0.85$; b -values are line slopes at $\tau = 0.85$. Tests for equality of slope were significant in each case (Appendix 1: Table S7). Each point is a site/time sample ($n = 48$). Open and filled circles indicate control and manipulation sites, respectively.

131x50mm (1200 x 1200 DPI)

Appendix S1

Multi-year resource enrichment creates persistently higher species diversity in a landscape-scale field experiment

Jill Lancaster & Barbara J. Downes

Ecology

Table S1 Summary of treatment site area, number and density of stake pairs on three occasions during the first year (2013). Stake pairs were arranged haphazardly within each site, we avoided placing pairs immediately up-downstream of one another, and we attempted to create an even spread across each site. Stake densities were lowest in the upstream zone where natural channel retention was highest. Stake densities were maintained (e.g., lost or buried stakes were replaced) and were roughly constant over the first three years, despite a dynamic channel morphology.

Month	Zone	Site	Wetted area (m ²)	Stake pairs (<i>n</i>)	Stake density (m ⁻²)	Mean density for Zone (m ⁻²)
Feb	Up	2	189	24	0.13	0.13
	Up	3	216	30	0.14	
	Mid	6	257	43	0.17	0.17
	Mid	8	303	50	0.17	
	Dwn	9	237	52	0.22	0.21
	Dwn	12	279	54	0.19	
May	Up	2	209	24	0.11	0.12
	Up	3	228	30	0.13	
	Mid	6	290	43	0.15	0.17
	Mid	8	340	62	0.18	
	Dwn	9	271	52	0.19	0.17
	Dwn	12	430	60	0.14	
Oct	Up	2	215	24	0.11	0.12
	Up	3	238	30	0.13	
	Mid	6	376	48	0.13	0.17
	Mid	8	297	62	0.21	
	Dwn	9	297	52	0.18	0.15
	Dwn	12	492	60	0.12	

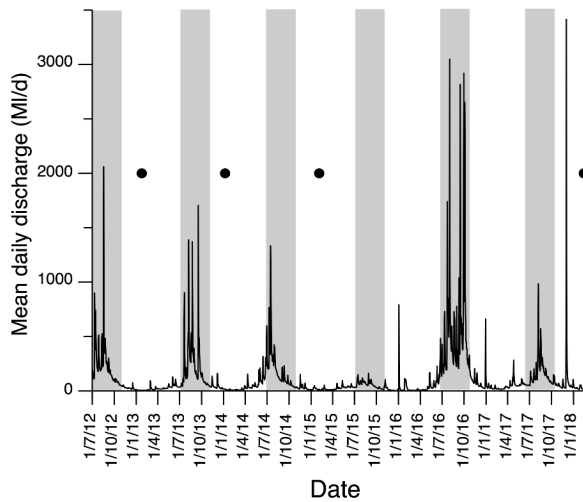


Figure S1 Mean daily discharge in Hughes Creek over six years, including the sampling period (State Government of Victoria Monitoring Site 405228: Hughes Creek @ Tarcombe Road). Circles indicate when invertebrate samples were collected; grey bars indicate the season when rainfall and high discharge are most likely (1 July to 1 November). The extreme discharge event in Dec 2017 is equivalent to a daily Q_1 i.e. daily flow exceeded this level on only 1% of the days over 45 years (1975-2020). Data accessed from the State Government of Victoria: <http://data.water.vic.gov.au/static.htm>.

Table S2 Summary of 3-way ANOVA comparing standing stocks of detritus, taxon richness and total invertebrate densities between treatments in different zones on the stream gradient over time. Planned contrasts within the Treat \times Year term, indicate whether Treatment effects were significant for each year. Limits on degrees of freedom meant that planned contrasts for Year 0 were not possible, but previous work has demonstrated no difference between treatments pre-manipulation, for these response variables (Lancaster & Downes, 2017). See Figure 2 for illustration

Source	df	Detritus standing stock			Taxon richness			Total density		
		MS	<i>F</i>	<i>P</i>	MS	<i>F</i>	<i>P</i>	MS	<i>F</i>	<i>P</i>
Year	3	2.19	22.9	<0.001	1261	10.4	<0.001	0.592	32.9	<0.001
Treatment	1	3.97	41.6	<0.001	3317	27.3	<0.001	0.560	31.1	<0.001
Zone	2	1.32	13.8	<0.001	1472	12.1	<0.001	0.156	8.67	0.001
Treat \times Year	3	0.573	6.00	0.003	775	6.39	0.002	0.075	4.16	0.017
Year 1	1	3.83	40.1	0.000	3333	27.5	<0.001	0.304	16.9	<0.001
Year 2	1	1.33	13.9	0.001	1102	9.09	0.006	0.201	11.2	0.003
Year 5	1	0.507	5.32	0.030	1121	9.25	0.006	0.278	15.5	0.001
Treat \times Zone	2	0.339	3.55	0.045	84.4	0.696	0.508	0.011	0.618	0.547
Zone \times Year	6	0.209	2.19	0.080	35.2	0.290	0.936	0.090	5.02	0.002
Treat \times Zone \times Year	6	0.114	1.20	0.341	26.2	0.216	0.968	0.015	0.836	0.554
Error	24	0.095			121			0.018		
Total	47									

Table S3 Summary of 3-way ANOVA testing for differences between Treatments, Year-Pairs and the probability of Extinction vs Colonization. See Table S4 and Figure 3 for summary of *post hoc*, pair-wise comparisons.

Source	df	MS	<i>F</i> -ratio	<i>P</i> -value
Treatment	1	0.0214	1.01	0.319
Year-Pairs	2	0.0421	1.99	0.145
Extinction vs Colonization	1	0.00004	0.0019	0.965
Treatment × Year Pairs	2	0.0254	1.20	0.309
Treatment × <i>E</i> vs <i>C</i>	1	0.192	9.09	0.004
Year Pairs × <i>E</i> vs <i>C</i>	2	1.01	48.2	<0.001
Treatment × Year Pairs × <i>E</i> vs <i>C</i>	2	0.451	21.3	<0.001
Error	60	0.0211		

Table S4 Summary of *P*-values for *post hoc*, pair-wise comparisons (Tukey's tests) between year pairs to determine whether extinction and colonization probabilities (*E* and *C*) varied over time within control and manipulation sites. Tests significant at $\alpha < 0.05$ are shown in bold.

Variable	Treatment	Years 0–1	Years 0–1	Years 1–2
		vs Year 1–2	vs Year 2–5	vs Year 2–5
<i>C</i>	Control	1.00	0.074	0.039
	Manipulation	<0.001	<0.001	1.00
<i>E</i>	Control	1.00	0.679	0.989
	Manipulation	<0.001	<0.001	1.00

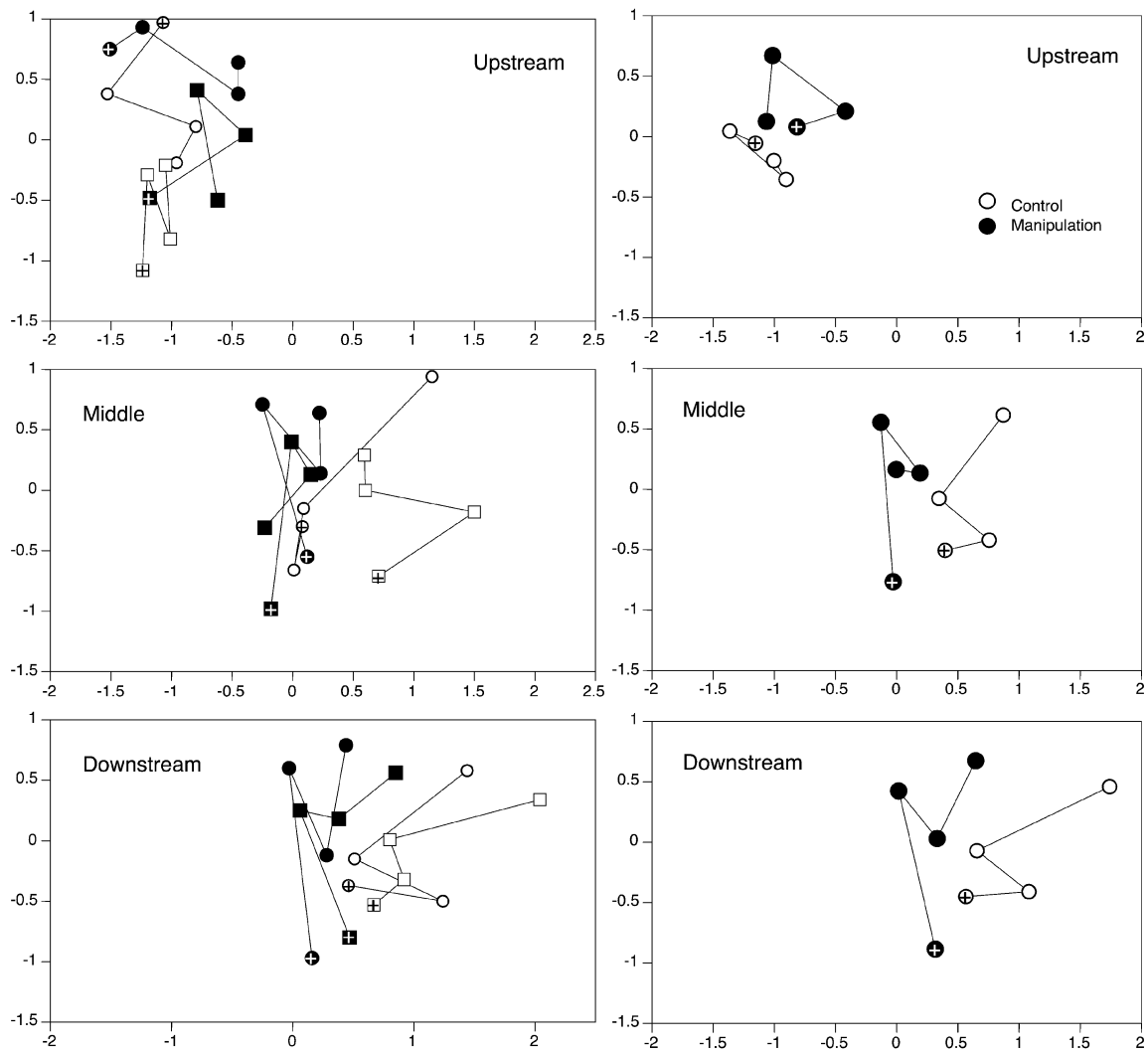


Figure S2 Non-metric multi-dimensional scaling (NMDS) ordination of (left panels) sites at different time and (right panels) centroids of replicate sites at different times. For convenience, the three zones are shown in separate panels with the same scales, although all sites and times were included in the ordination (stress for 2D configuration = 0.16). Different sites are indicated by different symbols (circles, squares); open symbols indicate control sites, closed symbols manipulation sites; symbols with crosses indicate pre-manipulation samples (Year 0). Lines connect treatments over the temporal trajectory.

Table S5 Summary of distance-based linear model (DISTLM) relating species data with environmental variables in a step-wise selection procedure. Table shows the best model according to AIC selection criterion. See Figure 4 for dbRDA plot.

Variable	Pseudo- F	P	Variance explained (%)	Cumulative variance (%)
Zone	6.65	0.001	22.8	22.8
Year	4.38	0.001	18.4	41.1
Detritus	4.32	0.001	5.6	46.8
Treatment	1.94	0.022	2.5	49.3

Table S6 List of taxa that were sufficiently abundant for univariate ANOVA, organized according to whether their response to the experimental treatment was nonsignificant, significant or marginally non-significant ($0.10 > P > 0.05$). Also shown is whether each taxon showed a Treatment effect over the seasonal cycle of the first year, as reported in (Lancaster & Downes, 2017); "rare" indicates that densities were too low for statistical analyses in the 1-year survey. Beetles were fully aquatic and separate analyses were carried out on larvae (L) and adults (A) for many taxa.

Response to manipulation	Order	Taxon	1-year seasonal response	
No treatment effect	Ephemeroptera	<i>Offadens</i> spp.	signif.	
	Ephemeroptera	<i>Ulmerophlebia</i> spp.	ns	
	Hemiptera	<i>Micronecta annae</i>	ns	
	Hemiptera	<i>Micronecta australiensis</i>	ns	
	Megaloptera	<i>Archichauliodes</i> sp.	rare	
	Coleoptera	<i>Berosus involutus</i> (A)	rare	
	Coleoptera	<i>Berosus</i> sp. (L)	ns	
	Coleoptera	<i>Austrolimnius waterhousei</i> (A)	ns	
	Coleoptera	<i>Austrolimnius waterhousei</i> (L)	ns	
	Coleoptera	<i>Austrolimnius resa</i> (A)	ns	
	Coleoptera	<i>Austrolimnius resa</i> (L)	ns	
	Coleoptera	<i>Laccobius</i> sp. (L)	ns	
	Diptera	Orthocladiinae early instars	ns	
	Diptera	<i>Riethia</i> sp.	ns	
	Diptera	Tanypodinae (excl. Pentaneurini)	ns	
	Diptera	<i>Chironomus</i> sp.	ns	
	Diptera	<i>Dicrotendipes</i> sp.	ns	
	Diptera	<i>Microchironomus</i> sp.	ns	
	Diptera	<i>Cladotanytarsus</i> sp.	ns	
	Diptera	<i>Corynoneura</i> sp.	ns	
	Diptera	Ceratopogonidae sp.	ns	
	Diptera	Empididae sp.	ns	
	Diptera	Tipulidae spp.	ns	
	Trichoptera	<i>Ecnomus cygnitus</i>	ns	
	Trichoptera	Hydrobiosidae	signif.	
	Treatment effect	Hygrophila, Neotaenioglossa & Basommatophera	Gastropoda spp.	ns
		Hygrophila	<i>Ferrissia petterdi</i>	signif.
Ephemeroptera		<i>Tasmanocoenis tillyardi</i>	ns	
Ephemeroptera		<i>Atalophlebia</i> spp.	signif.	
Ephemeroptera		<i>Nousia</i> spp.	signif.	
Plecoptera		Gripopterygidae (excl. <i>Dinotoperla</i>)	signif.	
Coleoptera		<i>Notriolus</i> spp.	signif.	
Diptera		<i>Botryocladus</i> sp.	signif.	
Diptera		<i>Rheotanytarsus</i> & <i>Paratanytarsus</i> spp.	signif.	
Diptera		<i>Thienemanniella</i> sp.	signif.	

	Diptera	<i>Parakiefferiella</i> sp.	signif.
	Diptera	<i>Cricotopus</i> spp.	signif.
	Diptera	<i>Polypedilum</i> sp.	signif.
	Diptera	Pentaneurini	signif.
	Diptera	<i>Tanytarsus</i> sp. 1	ns
	Diptera	<i>Tanytarsus</i> sp. 2	signif.
	Diptera	<i>Harrissius</i> sp.	signif.
	Diptera	<i>Cryptochironomus</i> sp.	ns
	Trichoptera	Hydroptilidae spp	ns
	Trichoptera	<i>Cheumatopsyche</i> sp. AV1	signif.
	Trichoptera	<i>Asmicridea edwardsi</i>	signif.
	Trichoptera	<i>Cheumatopsyche</i> sp. AV2	signif.
Weak treatment effect			
	Trichoptera	<i>Cheumatopsyche deani</i>	signif.
	Trichoptera	<i>Ecnomus continentalis</i>	signif.
	Trichoptera	<i>Triplectides ciuskus ciuskus</i>	signif.
	Trichoptera	<i>Oecetis</i> spp.	signif.
	Trichoptera	<i>Anisocentropus</i> sp.	rare
	Trichoptera	<i>Coenoria</i> AV1	signif.

LITERATURE CITED

Lancaster J. & Downes B. J. (2017). A landscape-scale field experiment reveals the importance of dispersal in a resource-limited metacommunity. *Ecology*, 98, 565-575.

Table S7 Population response models: List of common taxa that responded to the treatment and for which we could establish a relationship between population density and detrital standing stocks, organized according to the response model. All relationships were positive (slope $b > 0$). All but two taxa fit limiting relationships at $\tau = 0.85$ (85th quantile); we report F -tests for the 85th quantile, coefficients for the y -intercept (a) and slope (b), and F -tests for equality of slope (comparing slopes of lines at $\tau = 0.85$ and $\tau = 0.15$). The two exceptions were not limiting relationships so details are for a central relationship at $\tau = 0.50$ (50th quantile); tests for equality of slopes are not germane in these cases. See Figure 5 for illustrations.

Model	Order	Taxon	85th quantile	a (upper, lower bounds)	b (upper, lower bounds)	Equality of slopes
Tracking	Hygrophila	<i>Ferrissia petterdi</i>	$F_{46} = 26.1, P < 0.001$	-0.446 (-0.702, 0.020)	0.773 (0.564, 0.911)	$F_{95} = 9.51 P = 0.003$
	Ephemeroptera	<i>Atalophlebia</i> spp.	$F_{46} = 10.0 P = 0.003$	-0.308 (-0.474, 0.373)	0.534 (0.133, 0.627)	$F_{95} = 8.91 P = 0.005$
	Plecoptera	Gripopterygidae (excl. <i>Dinotoperla</i>)	$F_{46} = 5.49 P = 0.023$	-0.254 (-0.370, 0.435)	0.444 (0.203, 0.488)	$F_{95} = 5.72 P = 0.019$
	Trichoptera	<i>Asmicridea edwardsi</i>	$F_{46} = 7.01 P = 0.011$	-0.031 (-0.530, 0.132)	0.469 (0.353, 0.767)	$F_{95} = 7.01 P = 0.011$
	Diptera	<i>Botryocladus</i> sp.	$F_{46} = 7.08 P = 0.011$	-0.014 (-0.107, 0.181)	0.298 (0.223, 0.330)	$F_{95} = 7.08 P = 0.009$
	Diptera	<i>Thienemanniella</i> sp.	$F_{46} = 7.76 P = 0.008$	0.049 (-0.194, 1.82)	0.477 (0.226, 0.826)	$F_{95} = 7.42 P = 0.008$
	Diptera	<i>Tanytarsus</i> sp. 2 (spur)	$F_{46} = 4.20 P = 0.046$	-0.283 (-0.409, 0.271)	0.359 (0.134, 0.495)	$F_{95} = 4.20 P = 0.043$

	Diptera	¹ <i>Rheotanytarsus</i> & <i>Paratanytarsus</i> spp.	$F_{46} = 4.61 P = 0.037$	0.282 (-0.244, 0.800)	0.377 (0.243, 0.606)	
	Diptera	¹ <i>Polypedilum</i> sp.	$F_{46} = 10.3 P = 0.002$	-0.166 (-0.858, 0.536)	0.581 (0.279, 0.706)	
Threshold	Coleoptera	<i>Notriolus</i> spp. (L)	$F_{46} = 59.4 P < 0.001$	-0.808 (-0.909, -0.462)	0.960 (0.751, 1.01)	$F_{95} = 20.7 P < 0.00$
	Diptera	<i>Harrissius</i> sp.	$F_{46} = 30.1, P < 0.001$	-0.438 (-0.523, -0.337)	0.514 (0.476, 0.574)	$F_{95} = 11.6 P < 0.001$
Expanding	Ephemeroptera	<i>Nousia</i> spp.	$F_{46} = 5.31 P = 0.026$	0.678 (0.167, 0.977)	0.442 (0.351, 0.680)	$F_{95} = 3.05 P = 0.084$
	Diptera	<i>Cryptochironomus</i> sp.	$F_{46} = 4.32 P = 0.043$	0.306 (0.151, 0.629)	0.245 (0.130, 0.324)	$F_{95} = 4.72 P = 0.032$
	Diptera	<i>Tanytarsus</i> sp. 1 (no spur)	$F_{46} = 5.18 P = 0.027$	2.58 (1.90, 3.16)	0.549 (0.204, 0.762)	$F_{95} = 11.5 P = 0.001$
	Diptera	Pentaneurini	$F_{46} = 8.71 P = 0.005$	0.978 (0.662, 1.34)	0.365 (0.264, 0.517)	$F_{95} = 2.87 P = 0.093$

¹ Line fit for 50th quantile ($\tau = 0.50$). No test for equality of slopes.