Structural and functional responses of macroinvertebrate assemblages to long-term flow variability at perennial and non-perennial sites

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1 Structural and functional responses of macroinvertebrate assemblages to long-term

2 flow variability at perennial and non-perennial sites

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23 **Running headline:** Macroinvertebrate responses to flow variability

24 **Key-words:** functional traits, instream habitat, intermittent streams, stability,

25 heterogeneity, flow permanence, hydrology.

Abstract

27 Temporary streams constitute a significant proportion of rivers globally and are

28 common in wet, cool, temperate regions. These heterogeneous ecosystems harbour

29 high biodiversity associated with the dynamic turnover of taxa. Despite flow

30 permanence being widely recognised as an important environmental control, few

31 studies have characterised biotic responses to long–term hydrological variability in

32 temporary streams. We examined taxonomic and functional macroinvertebrate

33 communities of perennial and non-perennial river reaches over a 26-year period.

34 Flow permanence resulted in spatial variation in taxonomic and functional

35 macroinvertebrate communities. Non-perennial river reaches, which were

36 characterised by dynamic habitat provision (lotic, lentic and dry states) over the

37 study period, supported more heterogeneous communities than perennial river

38 reaches. Hydrological variables, in particular wetted width, water depth and zero-flow

39 states, were instrumental in structuring taxonomic and functional communities,

40 although the importance of substrate conditions increased in autumn. Hydrological

41 conditions resulted in separation of perennial and non-perennial taxonomic

42 communities regardless of season, whereas functional communities differed only in

43 spring. Our results emphasise that understanding of community responses to

44 hydrological variability is enhanced by analyses that concurrently explore taxonomic

45 and functional responses to long-term intra- and inter-annual hydrological variability.

46 Moreover functional responses represents a robust method to test ecological

47 responses to hydrological drivers. Further research that builds on our work is needed

48 to inform the protection of both perennial and non-perennial streams as they adapt to

49 ongoing environmental change.

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3   51   **1. Introduction**
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6   52   Hydrological variability and in particular flow permanence (Poff & Ward, 1989) are
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8   53   primary determinants of aquatic community composition in lotic ecosystems (Smith &
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10   54   Wood, 2002; Datry et al., 2014a). Non-perennial streams are defined by flow
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12   55   cessation, often experience partial or complete surface drying, and are characterised
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14   56   by high temporal and spatial variability (Leigh et al., 2015; Datry, Larned, & Tockner,
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16   57   2014b; Cid et al., 2017). The frequency, magnitude, duration and predictability of
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18   58   transitions between lotic, lentic and dry states influence the diversity and connectivity
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20   59   of habitat patches and, in turn, biotic responses (Bogan & Lytle, 2007; Leigh & Datry,
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22   60   2017). As such, non-perennial reaches often support high biodiversity as
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24   61   communities vary in space and time in response to changing habitat availability
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26   62   (Bogan, Boersma, & Lytle, 2013; Rolls, Heino, & Chessman, 2016; Tonkin et al.,
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29   63   2017).
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33   64   Historically, most research considering macroinvertebrate responses to flow
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35   65   permanence has focused on taxonomic changes. As discharge declines, flowing
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37   66   habitats are typically lost and rivers become dominated by connected pools inhabited
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39   67   by both lotic refugees and lentic colonists (Hill & Milner, 2018). Over time, pools
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41   68   become isolated and community composition continues to diverge from the lotic
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44   69   community as lentic colonists become increasingly dominant (Bonada, Rieradevall,
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46   70   Prat, & Resh, 2006). Flow resumption allows rapid recolonisation by resistant and
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48   71   resilient lotic species (Rolls et al., 2016; Vander Vorste, Corti, Sagouis, & Datry,
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50   72   2016). Communities within sites with contrasting flow permanence regimes may
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52   73   therefore converge as flowing phase durations increase (Wood, Gunn, Smith, &
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54   74   Abas-Kutty, 2005; Vander Vorste et al., 2016). However, studies of biotic responses
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56   75   to flow permanence are typically short-term (months to years), leaving long-term
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76 (decadal) patterns poorly characterised (but see Bêche, Connors, Resh, &

77 Merenlender, 2009; Stubbington, Wood, & Boulton, 2009; Chessman, 2015; Leigh &

78 Datry, 2017; Wilding et al., 2018).

79 In addition to taxonomic responses, biological traits based on life history, behaviour

80 morphology and physiology may be more informative when investigating community

81 responses to hydrological variability and flow permanence (Menezes, Baird, &

82 Soares, 2010; Schriever et al., 2015). Understanding life-history traits (such as body

83 size, morphology, lifecycle) associated with the habitat template via functional trait

84 analysis has been cited as strong basis for the theoretical development of ecological

85 indicators (Statzner & Bêche, 2010). Macroinvertebrates within non-perennial

86 streams may exhibit *r*-selected traits such as high dispersal, short life histories, rapid

87 growth and opportunistic feeding modalities that promote resilience to drying and

88 enable persistence in dynamic environments (Williams, 1996; García-Roger et al.,

89 2013; Giam et al., 2017). Taxa may also possess traits promoting resistance to

90 drying, such as desiccation-tolerant forms or respiration systems which permit air

91 breathing (Leigh et al., 2016; Datry et al., 2017a). The prevalence of taxa possessing

92 resistance traits may result in functional redundancy increasing with intermittence

93 (García-Roger et al., 2013; Boersma, Bogan, Henrichs, & Lytle, 2014). In turn,

94 functional redundancy can stabilise community trait composition over time (Bêche,

95 Mcelravy, & Resh, 2006), potentially facilitating clearer discrimination between

96 assemblages from sites with contrasting flow permanence compared to taxonomic

97 approaches (Cid et al., 2016; García-Roger, 2017). Communities in reaches which

98 dry infrequently or unpredictably may be decoupled from such adaptations, and may

99 therefore exhibit stronger taxonomic and functional responses (Bêche et al., 2009;

100 Chessman, 2015).

4

101   Recognition of their extensive and increasing occurrence, biodiversity, and

102   ecosystem service provision has resulted in increased research examining

103   temporary streams (Leigh et al., 2015; Datry et al., 2018). Despite this increase,

104   most ecological studies are from Mediterranean and dryland climates (e.g. Bonada,

105   Rieradevall, & Prat, 2007; Bogan et al., 2015; Cid et al., 2016; Leigh et al., 2016),

106   whereas research in cooler, wetter temperate climates remains restricted to a few

107   well-studied rivers including groundwater-dominated streams (Wood & Petts, 1999;

108   Hill & Milner, 2017; White et al., 2018). Such systems support high biodiversity

109   including rare species but are particularly sensitive to hydrological alterations, which

110   may manifest instream as reduced discharge in perennial reaches, as flow cessation

111   or drying in near-perennial reaches, and as prolonged ponded or dry states in non-

112   perennial reaches. This spatial variability makes groundwater-dominated streams

113   useful systems in which to examine ecological responses to hydrological variability

114   (Wright, 1992).

115   This study examined taxonomic and functional macroinvertebrate assemblage

116   responses to antecedent hydrological variability (including flow cessation and drying

117   events) at sites with contrasting flow permanence regimes in a UK groundwater-

118   dominated stream. We analysed a long-term dataset consisting of macroinvertebrate

119   samples and continuous, site-specific hydrological measurements. Hydrological data

120   were supplemented by observations of flow states aiding discrimination between

121   lentic conditions and streambed drying events. The following research questions

122   were addressed:

123   (i)   Do perennial and non-perennial river reaches support distinct taxonomic

124   and functional macroinvertebrate assemblages?

125   (ii)   Does heterogeneity of taxonomic and functional macroinvertebrate

126   assemblages differ between perennial and non-perennial river reaches?

127   (iii)   Which environmental parameters are influential in structuring the

128   composition of taxonomic and functional macroinvertebrate assemblages

129   in perennial and non-perennial river reaches?

2. Methods

2.1 Study sites

132   The River Misbourne is a small chalk stream in southern England which flows for

133   approximately 27 km to its confluence with the River Colne (Figure 1). Land use

134   within the 94.8 km^2 catchment is predominately arable (34.07%) with some grassland

135   (28.33%), mixed woodland (21.50%) and urban land cover (16.67%); NRFA, 2017).

136   The Misbourne experiences predictable annual discharge variation and loses water

137   from its upper reaches and the middle section upstream of Chalfont St. Peter (Figure

138   1). Natural variation in water loss is exacerbated by anthropogenic influences (e.g.,

139   channel realignment to provide water to historic mills and instream channel

140   modifications).

141   Six sampling sites (1-6 from upstream to downstream) with contrasting flow

142   permanence were examined during the 26-year study period (1990–2016; Table 1;

143   Figure 1). For the purposes of this study, sites were categorised as 'non-perennial'

144   being those that dry regularly (sites 1, 3 and 4), or 'perennial' being those that never

145   cease to flow or maintain flow except during extreme drought events (sites 2, 5 and

146   6.) Table 1 summarises the physical characteristics of each site and the flow

147   permanence regime according to two hydrological metrics: the six-month

148   predictability of dry periods (Sd6; which ranges from 0 - no predictability to 1 - total

149   predictability) and the mean number of days with zero flow (Gallart et al., 2012).

6

150   Hydrological variability was evident within and among the six sampling sites during

151   the study period. Droughts occurred in England in 1997-1998, 2006-2007 and 2011-

152   2012, and wet phases in 2001 and 2014 (Marsh, Cole, & Wilby, 2007; Marsh et al.,

153   2013).

154   *2.2 Macroinvertebrate data*

155   Benthic macroinvertebrate samples were collected by the Environment Agency of

156   England using their standard sampling protocol for statutory monitoring purposes: a

157   3-minute kick sample (1-mm mesh size) encompassing all habitats in proportion to

158   their occurrence, followed by a 1-minute hand search (Murray-Bligh, 1999).

159   Sampling was conducted in spring (March – May) and autumn (September –

160   November; UKTAG, 2014) and began between 1990 and 1998 (dependent on site)

161   and continued until 2016. For sites surveyed more than once in a single sampling

162   season, the most recent replicate was retained to ensure independence of samples,

163   leaving a total of 180 samples. Taxa were mainly identified to family level, except

164   Hydracarina and Collembola, which were recorded as such. Due to the semi-

165   quantitative technique, abundance data were standardised into ordinal classes (1 =

166   1–9, 2 = 10–32, 3 = 33–99, 4 = 100–332, 5 = 333–999, 6 = ≥1000 individuals per

167   sample; Durance & Ormerod, 2009; Mathers et al., 2016).

168

169   The functional composition of macroinvertebrate communities was characterised by

170   assigning taxa into 11 categories comprising 61 biological traits from the Tachet,

171   Bournaud, Richoux, & Usseglio-Polatera (2010) database (Table S1). Traits were

172   assigned to genera based on a fuzzy-coding approach with scores ranging from zero

173   (indicating no affinity) to five (the strongest affinity based on available literature;

174   Chevene, Doléadec, & Chessel, 1994). Because we worked at the family level, we

7

175 averaged the affinities of all genera recorded within a family and these affinity scores

176 were rescaled as proportions for each category (sum=1) for each taxon (*sensu*

177 Gayraud et al., 2003). To produce a trait abundance matrix, taxon-trait categories

178 were multiplied by the ordinal abundances and subsequently scaled such that each

179 trait equalled one (Larsen & Ormerod, 2010a; Descloux, Datry, & Usseglio-Polatera,

180 2014; White et al., 2017a). Trait abundance and diversity can be achieved at the

181 family level (i.e.) regardless of spatial scales (Gayraud et al., 2003; García-Roger et

182 al., 2013). Chironomidae and taxa recorded at a coarser resolution than family level

183 were excluded, because these taxa are likely to include multiple representatives with

184 highly variable responses (Monk et al., 2012; Cañedo-Argüelles, Bogan, Lytle, &

185 Prat, 2016) Of the 90 taxa recorded, 78 were assigned functional traits. Alpha

186 diversity of each sample was determined via taxa richness and the inverse

187 Simpson's diversity value (Oksanen et al., 2015), which accounts for the fixed

188 number of traits and the lack of independence between traits (Larsen & Ormerod,

189 2010b; White et al., 2017b).

2.3 Hydrological data

191 Discharge has been gauged continuously in the lower Misbourne (51°40' N, 00°39'

192 W) since 1985 and in the upper river (51°34' N, 00°29' W) since 1993 (Figure 1) with

193 additional spot discharge measurements taken along the river under varying flow

194 conditions (Figure 1). Table S2 shows the time periods and number of spot gauge

195 measurements used to establish flow relationships at each site. Daily mean

196 discharge data provided by the Environment Agency of England (available from

197 NRFA, 2017) and spot-gauging measurements closest to the biological monitoring

198 sites were used to generate site-specific discharge time series using linear

199 regressions (see Gordon, McMahon, Finlayson, Gippel, & Nathan 2004). This

200 approach provides sufficient representation of hydrological conditions at a site, gives

201 a good description of relative patterns but may lack precision for low flows (Malcolm

202 et al., 2012a), and has been used successfully in hydroecological assessments

203 (Gordon et al., 2004; Westwood et al., 2017). For sites 2-5, between one and four

204 linear regressions were constructed reflecting different hydrological periods

205 associated with discharge levels (Table S2). Figure 2 provides hydrographs for

206 typical perennial and non-perennial river reaches.

207

208 Using the derived daily mean discharge data, we calculated antecedent hydrological

209 metrics previously shown to be relevant when examining macroinvertebrate

210 response to flows (Dunbar et al. 2010a, 2010b). We divided the year into two periods

211 which we termed summer flows (April–September) and winter flows (October–

212 March). Each flow period for each site thus provides a series of blocks of discharge

213 data, each of 183 days for summer and 182/183 days for winter. For each block, two

214 statistics were calculated: the % of zero-flow days for the previous year (% of winter

215 zero-flow days – % W1 zero-flow days; % of summer zero-flow days – % S1 zero-

216 flow days) and two characterising mean discharge (mean winter discharge – Mean

217 W1; mean summer discharge – Mean S1). In addition, mean discharge (Mean S2)

218 and % of zero-flow days (% S2 zero-flow days) were calculated for the summer

219 before S1, resulting in six hydrological statistics.

220 **2.4 Data analysis**

221 *2.4.1 Macroinvertebrate responses in perennial and non-perennial river reaches*

222 All analyses were conducted in R version 3.4.3 (R Development Core Team, 2017).

223 To assess whether alpha diversity varied in association with flow permanence

224 regime (perennial or non-perennial), season, site and the interaction of these factors,

225   differences in alpha diversity were tested via a linear mixed-effects model using the

226   '*lme*' function in the *'nlme'* package (Pinheiro et al., 2016). Season, site and flow

227   permanence regime were fitted as fixed interacting effects and year as a random

228   effect to account for community compositions from individual sites being potentially

229   correlated over time. Significance values were obtained using Type II analysis of

230   variance (ANOVA) in the *´car´* package (Fox & Weisberg, 2011). Marginal r^2

231   (proportion of variance explained by the fixed effects alone) and conditional r^2

232   (proportion of variance explained by the fixed and random effects) values were

233   extracted using the '*MuMIn*' package (Bartoń, 2016).

234   Differences in community composition were explored as a function of the interactive

235   explanatory factors of site, flow permanence regime and season via a permutational

236   multivariate ANOVA (PERMANOVA) using the '*adonis*' function in the '*vegan'*

237   package (Oksanen et al., 2015). Indicator taxa and traits were identified for perennial

238   and non-perennial sites using the '*multipatt*' function within the '*indicspecies*'

239   package (De Cáceres & Jansen, 2016). An indicator value of >0.25 was accepted as

240   ecologically relevant (Dufrêne & Legendre, 1997), and all significant indicators with a

241   fidelity value of <0.25 removed to exclude rare taxa and traits (De Cáceres,

242   Legendre, Wiser, & Brotons, 2012; Trivellone et al., 2014). Indicator values were

243   assigned based on global, spring and autumn datasets to assess variation between

244   seasons.

245   *2.4.2 Heterogeneity in macroinvertebrate community composition in perennial and*

246   *non-perennial river reaches*

247   To examine heterogeneity in macroinvertebrate community composition,

248   homogeneity of multivariate dispersions were calculated for functional and taxonomic

249   communities based on Bray-Curtis distances using the '*betadisper'* function in

10

250   '*vegan*'. Tests were run on flow permanence regime (perennial and non-perennial)

251   on global, spring and autumn datasets. Statistical differences in multivariate

252   dispersion between the flow permanence groups and within flow permanence regime

253   by season were tested using one-way ANOVA. Heterogeneity of all taxonomic and

254   functional samples (regardless of factors) were determined to establish overall

255   temporal variability of the two approaches and tested using ANOVA.

256   *2.4.3 Perennial and non-perennial macroinvertebrate community associations with*

257   *environmental variables*

258   Redundancy analyses (RDA) were performed using the '*ordistep*' function in '*vegan*'

259   to examine relationships between taxonomic / functional composition and 13

260   environmental parameters, specifically six hydrological metrics (described above),

261   five substrate characteristics assessed via visual estimates (% boulders, cobbles,

262   pebbles, gravel, silt), and two mean wetted channel dimensions (width, depth;

263   Murray-Bligh, 1999). Prior to analysis, a Hellinger transformation was applied to the

264   taxonomic data. A stepwise (forward and backward) selection procedure using

265   permutational-based significance tests (999 permutations) was used to identify

266   factors that influenced assemblages, with only significant variables included in the

267   final model. Final variables were checked for collinearity using the '*vif*' function in the

268   '*car*' package to ensure that all 'variance inflation factors' were <3 (Zuur et al., 2010).

269   Analyses were conducted on spring and autumn datasets for taxonomic and

270   functional communities.

271   **3. Results**

272   ***3.1 Macroinvertebrate responses in perennial and non-perennial river reaches***

273   Taxa richness for the 26-year study period was higher at perennial sites (mean ± SE

274   34 ± 0.56, range 9-39) compared to non-perennial sites (18 ± 0.81, range 4-30) with

11

275 15 families being unique to perennial river reaches (most notably Odontoceridae and

276 Psychomyiidae). Functional diversity was higher at perennial sites (mean inverse

277 Simpson's diversity value 31 ± 0.11) compared to non-perennial sites (21 ± 0.21).

278 Taxonomic richness and functional diversity varied as a function of flow permanence

279 regime and the interaction of flow permanence regime and site but not by the

280 individual influence of site and season or the interaction of any other factors (all

281 p >0.05; Table 2).

282 Site explained the greatest amount of variation in taxonomic community composition,

283 whereas the interaction of site and flow permanence regime was the most influential

284 factor for functional community composition, as indicated by the greater r^2 and F

285 values (Table 3). Both taxonomic and functional communities differed as a function

286 of site, season and flow permanence regime and the interaction of flow regime and

287 site (Table 3). There was no interaction between season and either site or flow

288 permanence regime.

289 Indicator analysis (Table 4) determined that non-perennial sites were characterised

290 by taxa with resistance forms including cocoons and housing against desiccation,

291 such as Lymnaeidae, the strongest indicator taxon. These sites also supported taxa

292 that temporarily attach to substrates and surface swimmers (e.g. Dytiscidae). The

293 family Lumbricidae was also a strong indicator of non-perennial sites. Perennial sites

294 supported large taxa (>8 cm, e.g. Erpobdellidae), taxa with <1 brood per year, those

295 with parasitic and carnivorous feeding modes (e.g. Erpobdellidae, Glossiphoniidae),

296 and those permanently attached to substrates (e.g. Hydrobiidae). Indicator values

297 were generally higher for traits than for taxa and at perennial sites than non-

298 perennial sites (Table 4), and taxonomic indicator values were higher in spring

12

299   compared to autumn (Tables S3 and S4). Indicators were generally comparable

300   between seasons with a few exceptions at non-perennial sites, notably Corixidae,

301   Hydroptilidae, Crangonyctidae in autumn and Lumbricidae in spring. Taxa with

302   passive aquatic dispersal, small body size (>1-2 cm and <0.25 cm) and with adult

303   aquatic stage characterised autumn samples whilst spring supported taxa that

304   temporally attach to substrates or which have an aquatic nymph stage.

305   *3.2 Heterogeneity in macroinvertebrate community composition in perennial*

306   *and non-perennial river reaches*

307   Taxonomic communities demonstrated greater temporal dispersion (mean distance

308   of all communities: 0.369) than functional communities (0.084). Heterogeneity was

309   higher at non-perennial sites compared to perennial sites for taxonomic and

310   functional communities regardless of season (Table 5). Both taxonomic and

311   functional communities differed in heterogeneity between perennial and non-

312   perennial sites within the global and spring models, whereas only functional

313   communities differed in autumn (Table 6). Heterogeneity within perennial or non-

314   perennial groups was comparable between seasons (all $p > 0.05$), but was generally

315   lower in autumn at non-perennial sites, in both taxonomic and functional community

316   composition.

317   *3.3 Perennial and non-perennial macroinvertebrate community associations*

318   *with environment variables*

319   All RDA models were highly significant (all $p = 0.001$), with functional communities

320   demonstrating a stronger association with the measured environmental parameters

321   and the models accounting for greater variance on the first two axes, especially in

322   spring (range 27.77-18.92%; Table S5). In all instances, hydrological metrics and

13

323 wetted channel characteristics influenced functional and taxonomic community

324 composition. Width and depth were correlated with taxonomic and functional

325 communities in both spring and autumn, with one exception (between depth and

326 functional communities in spring; Table 7). Both taxonomic and functional

327 communities were strongly correlated with a greater number of hydrological metrics

328 in spring, whereas substrate characteristics were most important in autumn. The %

329 of S1 zero-flow days had the greatest influence on both taxonomic and functional

330 community composition in spring (as denoted by the greatest F value), whereas in

331 autumn the % of silt and gravel exerted the greatest influence on taxonomic and

332 functional communities, respectively (Table 7). Functional communities

333 demonstrated a greater association with hydrological metrics in both spring and

334 autumn (as indicated by the greater F values for % zero-flow days).

335 Perennial and non-perennial taxonomic communities separated along RDA axis 1 in

336 spring, which was correlated with hydrological metrics describing flow permanence

337 (i.e. % S1 zero-flow days and mean S1; Figure 3a). Variation in community

338 composition within non-perennial sites was associated with substrate conditions (%

339 gravel) and wetted channel characteristics (width, depth), as reflected by separation

340 along RDA axis 2. The two RDA axes accounted for 14.13% of variance and were

341 highly significant ($p < 0.05$; Table 7). There was some evidence of a perennial and

342 non-perennial site separation along RDA axis 1 which represented mean winter

343 discharge, mean summer discharge and depth for autumn taxonomic communities

344 (Figure 3b; RDA axes accounted for 13.86% of variance; Table 7). Variation within

345 flow permanence regimes was correlated with % pebbles. In both seasons,

346 community variability associated with the environmental parameters was greatest in

347 perennial sites.

14

348

349 Separation of perennial and non-perennial sites was evident in spring functional

350 communities along RDA axis 1, which was associated with descriptors of flow

351 permanence regimes (% S1 - zero-flow days for the previous summer) and wetted

352 width (Figure 3c). Positioning of assemblages along RDA axis 2 reflected within flow

353 regime variation in community composition associated with mean winter discharge

354 (mean W1) and the two axes collectively explained 27.77% of the variation in

355 community composition. In contrast, separation of perennial and non-perennial

356 functional assemblages was not evident in autumn (Figure 3d; RDA axes accounted

357 for 18.92% of variance; Table 7). In both seasons, variability in communities

358 associated with the environmental parameters was greatest in non-perennial sites.

359 **4. Discussion**

360 Characterising broad-scale macroinvertebrate responses to hydrological variability

361 and flow permanence is challenging, because interacting local conditions such as

362 antecedent hydrological conditions, the core taxa present, and habitat characteristics

363 simultaneously influence ecological responses (Chakona, Phiri, Magadza, &

364 Brendonck, 2008; Leigh et al., 2016). Tackling this ecological complexity, our 26-

365 year study of taxonomic and functional macroinvertebrate communities

366 demonstrated that the dynamic, heterogeneous communities of non-perennial

367 streams can be captured effectively using paired biological-hydrological data

368 collected over multi-decade timescales. Flow permanence, wetted width and depth

369 were primary drivers of macroinvertebrate community composition, and the

370 importance of substrate conditions increased in autumn.

371 ***4.1 Macroinvertebrate responses within perennial and non-perennial river***

372 ***reaches***

373   Our research, conducted in a wet, cool temperate region, builds on short-term

374   oceanic-climate studies which have found that perennial and non-perennial river

375   reaches support discrete taxonomic macroinvertebrate assemblages (Wood et al.,

376   2005; Stubbington et al., 2011; White et al., 2018). We found that these differences

377   persisted long term (26 years) despite evident intra-annual variability in hydrological

378   conditions and low spatial / temporal extent of flow permanence in oceanic regions

379   compared to dryland regions, including the close proximity of perennial reaches.

380

381   Other studies conducted over shorter time periods have reported that taxonomic

382   community composition at perennial and non-perennial sites may converge if

383   sampling is conducted after several months of continuous flow, reflecting

384   recolonisation by taxa persisting in refuges. However, our clear separation of sites

385   highlights the importance of long-term studies that represent the range and dominant

386   hydrological conditions experienced at a location (Delucchi, 1998; Wood et al., 2005;

387   Sheldon et al., 2010). We found differences in the composition and alpha diversity of

388   taxonomic and functional communities in perennial and non-perennial river reaches.

389   Flow permanence regime was the primary driver of diversity differences, with

390   perennial sites supporting higher values than non-perennial sites regardless of

391   season (Bonada et al., 2007; Datry, 2012; Schriever et al., 2015; Suárez et al.,

392   2017).

393   We did, however, note subtle differences between the two methodological

394   approaches, with functional communities being more strongly influenced by flow

395   permanence regime (and the interaction with site) whilst taxonomic communities

396   demonstrated greater differences among sites. Communities were likely responding

397   to habitat-specific flow velocities, hydraulic habitats and substrate conditions (Lane

398   Pasternack, & Sandoval-Solis, 2018; White et al., 2019). Functional responses may

399   be less sensitive to small-scale fluctuations in hydrological conditions due to their

400   high stability in space and time (Statzner & Bêche, 2010), meaning that extreme

401   conditions may be required to eliminate enough taxa to cause detectable

402   compositional shifts (Schriever et al., 2015). Alpha diversity also demonstrated a

403   significant interaction between site and flow permanence regime, indicating that site-

404   specific environmental controls may play a more important role in the structuring of

405   communities compared to general hydrological patterns (Bonada et al., 2007).

406

407   Broad patterns of compositional differences among non-perennial and perennial river

408   reaches reflected the distributions of taxa adapted to the prevailing hydrological

409   conditions. Non-perennial river reaches were characterised by taxa with traits

410   promoting resistance to drying events, i.e. cocoons and housing against desiccation

411   (Bogan et al., 2014; Leigh et al., 2016). For example, some Lymnaeidae species are

412   characteristic of non-perennial waterbodies and are a desiccation-tolerant

413   component of the aquatic invertebrate 'seedbank' (Stubbington, Gunn, Little, Worrall,

414   & Wood, 2016). *Galba truncatula* was the dominant lymnaeid in our study stream, is

415   capable of air-breathing, and has previously been identified as indicative of

416   intermittent stream reaches (Stubbington et al., 2009; White et al., 2018). Surface

417   swimmers were found to indicative of non-perennial sites reflecting the presence of

418   lentic habitats when flow ceases (Hill and Milner, 2019). Coleopterans of the family

419   Dytiscidae were characteristic of non-perennial sites, possessing adaptations which

420   enable dry-phase persistence, such as air-breathing, an impermeable cuticle that

421   reduces water loss (Holdgate, 1956), and overland flight which enables dispersal

422   between disconnected waterbodies (Jackson, 1952). Lumbricidae were also

17

423   indicative of these sites and likely included aquatic, semi-aquatic and terrestrial

424   species (Stubbington et al., 2016).

425   Taxa characterising the perennial reaches had traits reliant on continuous water

426   availability, such as feeding on aquatic prey (e.g. Erpobdellidae, Glossiphoniidae)

427   and permanent attachment to a substrate (e.g. Hydrobiidae). Although predators can

428   thrive during flow recession due to the concentration of prey within a declining

429   habitat area (Acuña et al., 2005), their typically large body sizes can prevent access

430   to saturated refuges during dry phases, causing population densities to decline

431   (Ledger, Edwards, Brown, Milner, & Woodward, 2011). Perennial reaches were also

432   characterised by K-selected traits, with the prevalence of large-bodied and long-lived

433   taxa which exhibit low voltinism typically increasing with the hydroperiod (wet-phase

434   duration; Corti, Kohler, & Sparks, 1996; García-Roger et al., 2013).

435   Notable seasonal differences in indicator taxa within non-perennial reaches included

436   taxa with smaller body sizes, aquatic passive dispersal and adult aquatic stages in

437   autumn. Taxa possessing such traits include Crangonyctidae and Corixidae, which

438   were characteristic of non-perennial reaches. These taxa are associated with slow-

439   flowing and/or lentic waters (Jass & Klausmeier, 2003; Van de Meutter, Stoks, & De

440   Meester, 2006; Wiberg-Larsen, 2008), and may have inhabited pools and ponded

441   habitats (Hill & Milner, 2018). Habitat complexity may have enabled their localised

442   persistence after flow resumption in autumn (Lane et al., 2018), prior to likely

443   displacement by high winter flows. Indicator traits unique to spring included nymph

444   life stages and temporary attachment to substrates. Such taxa are likely to be insects

445   with seasonal life cycle dynamics, for example Simuliidae, whose abundance peaked

446   in spring with temporary attachment enabling rapid migration to refuges as drying

447 commences. That such taxa were not non-perennial indicators suggests non-

448 discriminant seasonal colonisation of reaches with contrasting flow permanence

449 regimes.

450 Indicator values were generally higher for functional traits than taxa, highlighting the

451 potential of traits as ecological indicators of environmental conditions (Sarremejane

452 et al., 2017a). Functional responses may facilitate clearer observations of responses

453 to changing environmental conditions (such as hydrology) as there is less 'noise' in

454 datasets, due to functional redundancy (García-Roger, 2017), and some traits such

455 as resistance forms may enable the persistence of multiple taxa at non-perennial

456 sites (Boersma et al., 2014). Including trait-based assessments in ecological

457 monitoring may therefore enhance fundamental understanding of the links between

458 biodiversity and ecosystem function (Monk et al., 2018; Reiss, Bridle, Montoya, &

459 Woodward, 2009).

460 *4.2 Heterogeneity in macroinvertebrate community composition of perennial*

461 *and non-perennial river reaches*

462 Taxonomic and functional communities inhabiting non-perennial sites were

463 characterised by greater heterogeneity than perennial river reaches. Such variability

464 may reflect higher temporal and spatial habitat heterogeneity (Bonada et al., 2007;

465 Datry et al., 2014b; Cid et al., 2017), which allows a diverse range of aquatic taxa to

466 sequentially inhabit lotic and lentic instream habitats (Bogan & Lytle, 2007; Munné &

467 Prat, 2011; Giam et al., 2017; Tonkin et al., 2017). Overall, functional assemblage

468 compositions were temporally and spatially stable in this study (demonstrated by the

469 significantly lower heterogeneity values compared to taxonomic communities),

470 despite hydrological variability which encompassed drought events and periods of

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471   above-average precipitation, suggesting their potential as stable indicator (Bêche et

472   al., 2006; Statzner & Bêche, 2010; Dolédec, Tilbian & Bonada, 2017).

473   Some evidence of seasonal effects was detected in community heterogeneity, with

474   non-perennial sites demonstrating reduced heterogeneity in autumn in both

475   taxonomic and functional communities, whereas perennial reaches did not differ in

476   heterogeneity among seasons. Autumn is typically when the influence of flow

477   permanence and low flows on aquatic communities is most evident in temperate-

478   zone streams, due to recent flow resumption. Non-perennial sites may be more

479   temporally heterogeneous in spring dependent on dispersal of recolonists from

480   refuges following flow resumption; these processes are likely to be highly variable

481   (Cañedo-Argüelles et al., 2015; Sarremejane, Mykrä, Bonada, Aroviita, & Muotka,

482   2017b). For example, Perrow, Leeming, England, & Tomlinson (2007) noted specific

483   flowing-water species at some Misbourne sites after flow resumption following the

484   1996/1997 drought that had not been recorded elsewhere in the river, thereby

485   increasing heterogeneity.

4.3 Perennial and non-perennial macroinvertebrate community associations

with environmental variables

488   Our results indicate that hydrological metrics were the primary factors structuring

489   functional and taxonomic macroinvertebrate communities, most notably in spring,

490   and for functional communities. Trait-based approaches may provide a robust means

491   of assessing ecological responses to hydrology across regions with different taxon

492   pools (White et al., 2017a). The high temporal stability in functional communities can

493   help facilitate discrimination of spatial environmental differences in assemblage

494   composition (Cid et al., 2016; García-Roger 2017) and may explain why spring

495 functional communities demonstrated the strongest association to a measured

496 environmental parameter.

497 The % of zero-flow days in the previous summer (April–September) was of greatest

498 importance in structuring spring communities. Wetted channel characteristics were

499 also significant drivers, with width demonstrating a significant correlation in all four

500 models and depth in all but one. Autumn communities were more strongly correlated

501 with substrate conditions (% silt, pebble or gravel) that characterise the legacy of

502 hydraulic conditions. These results provide further evidence that localised hydraulic

503 observations may improve the characterisation of forces that biota are responding to

504 at the time of sampling compared to the use of discharge-related statistics alone

505 (Malcolm et al., 2012b; Monk et al., 2018; White et al., 2019)

506 Functional redundancy may promote community resilience to environmental variation

507 (Schriever et al., 2015; Oliver et al., 2015) and may explain the lack of separation

508 between functional communities in perennial and non-perennial river reaches in the

509 autumn. Non-perennial communities in autumn may represent a subset of those from

510 perennial reaches (Arscott et al., 2010; Datry, 2012; Datry et al., 2014a), comprising

511 resistant representatives that are able to persist in moist habitats/substrates (e.g.

512 *Galba truncatula*; Stubbington & Datry, 2013). In contrast, spring communities also

513 include taxa which may have recolonised non-perennial reaches from perennial

514 sources upstream or persisted in-situ following flow resumption. The composition of

515 functional processes may therefore have been altered resulting in separation of the

516 two functional flow permanence communities during spring. In contrast taxonomic

517 composition demonstrated some overlap in perennial and non-perennial river

518 reaches but differences in composition were evident in both spring and autumn.

5. Conclusion

520 As global recognition of the biodiversity value and ecosystem service provision of

521 temporary streams increases (Acuña et al., 2017; Datry et al., 2017b), so does their

522 extent, in response to interacting climate change and water resource pressures.

523 Ecologically robust conservation and management strategies are therefore required,

524 and will be most effective if informed by analyses that interpret both taxonomic and

525 functional responses to flow permanence in the context of long-term intra- and inter-

526 annual hydrological variability (Döll & Schmied, 2012; Watts et al., 2015). Our results

527 suggest that functional approaches could be a robust means of assessing ecological

528 responses to hydrological drivers. We call for future research that builds on our work

529 to further inform the protection of both perennial and non-perennial streams as they

530 adapt to ongoing environmental change.

541 **Data availability:** The daily mean discharge data for sites 1 and 6 can be obtained

542 freely from the National River Flow Archive at https://nrfa.ceh.ac.uk/data using the

543 station codes of 39127 and 39102. Biological data for all sites and spot gauged data

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3   544   (sites 2-5) are available on request from the Environment Agency via
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5   545   http://apps.environment-agency.gov.uk/contact/.
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11
12   547   **List of Figures**
13
14
15   548   **Figure 1.** Locations of biotic and abiotic parameters used in the study and the flow
16
17   549   permanence regime of the River Misbourne, UK.
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19
20   550   **Figure 2.** Typical hydrographs of discharge regimes at: (a) non-perennial and; (b)
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22   551   perennial river reaches on the River Misbourne during the study period. Note:
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24   552   gauging records were not present at sites 1-4 until 1994. Site 3 (non-perennial) and
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26   553   site 6 (perennial) are shown.
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30   554   **Figure 3.** Redundancy analysis (RDA) of: (a) spring taxonomic; (b) autumn
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32   555   taxonomic; (c) spring functional and; (d) autumn functional macroinvertebrate
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34   556   community composition on the River Misbourne. Perennial sites = grey; non-
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36   557   perennial sites = black. Only significant environmental parameters influencing faunal
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38   558   data are presented. % W1 zero-flow days = % of winter zero-flow days; % S1 zero-
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40   559   flow days and % S2 zero-flow days = % of summer zero-flow days; mean W1 =
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42   560   mean winter discharge; mean S1 and S2 = mean summer discharge. Two years of
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44   561   antecedent summer (April–September) and one year of winter (October–March) data
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46   562   were used. Note the difference in scale between panes a-b and c-d.
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Tables

Table 1. Summary of abiotic characteristics (mean values) for each of the six River Misbourne sites. Values represent the mean values recorded from three seasons (spring, summer and autumn) during a representative year (2005) as undertaken by the Environment Agency.

Site	Latitude	Longitude	Distance from source (km)	Altitude (m a.s.l.)	Local gradient (m km^{-1})	Width (m)	Depth (cm)	Discharge (m^3 s^{-1})	Flow permanence regime	Mean number of no flow days per year	Predictably of dry phase (Sd6)
1	51°40′59 N	00°40′12 W	4.9	107	3.6	9.0	50.0	0.06	Non-perennial	80.68	0.73
2	51°40′19 N	00°37′53 W	7.8	98	2.8	4.0	25.0	0.14	Perennial	44.84	1.00
3	51°38′54 N	00°34′49 W	12.7	78	3.1	2.4	11.0	0.08	Non-perennial	111.52	0.38
4	51°36′36 N	00°33′25 W	18.1	60	3.8	1.8	24.0	0.06	Non-perennial	201.88	0.41
5	51°35′05 N	00°32′21 W	21.6	49	4.5	3.9	20.7	0.18	Perennial	0.00	1.00
6	51°34′41 N	00°30′58 W	23.8	40	4.2	5.3	7.3	0.26	Perennial	0.00	1.00

Table 2. Summary of linear mixed-effects models associated with flow regime, season, site and the interaction of these factors. Significant results (p <0.05) are emboldened.

Factor	Taxonomic richness		Functional diversity	
	F	p	F	p
Regime	20.19	**<0.001**	17.65	**<0.001**
Season	0.42	0.519	0.79	0.373
Site	0.57	0.452	0.70	0.403
Regime × season	0.50	0.478	0.42	0.519
Regime × site	8.06	**0.004**	7.94	**0.005**
Season × site	0.04	0.835	0.28	0.600
Regime × season × site	0.21	0.645	0.21	0.645
Marginal r^2	**20.73**		**14.16**	
Conditional r^2	**23.04**		**17.90**	

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Table 3. Summary of PERMANOVA output assessing the relative importance of the different environmental controls on taxonomic and functional communities. Significant results (p <0.05) are emboldened.

Environmental factor	Taxonomic			Functional		
	F	r^2	p	F	r^2	p
Regime	9.04	0.043	**0.001**	11.96	0.057	**0.001**
Season	3.96	0.019	**0.001**	3.98	0.019	**0.001**
Site	13.26	0.063	**0.001**	3.42	0.016	**0.002**
Regime × season	1.35	0.006	0.169	1.90	0.009	0.054
Regime × site	8.79	0.042	**0.001**	13.86	0.066	**0.001**
Season × site	0.83	0.004	0.620	0.58	0.002	0.805
Regime × season × site	0.85	0.004	0.612	1.21	0.006	0.259

Table 4. Summary of the top ten indicator taxa and traits for non-perennial and perennial flow regimes on global (spring and autumn) dataset.

Taxonomic			Functional		
Taxa	Indicator value	*p*-value	Trait	Indicator value	*p*-value
Non-perennial *			**Non-perennial**		
Lymnaeidae	0.773	0.001	Resistance form: cocoons	0.780	0.001
Lumbricidae	0.621	0.011	Resistance form: housing against desiccation	0.780	0.001
Physidae	0.534	0.049	Locomotion: surface swimmer	0.770	0.001
Dytiscidae	0.505	0.049	Respiration: tegument	0.737	0.001
Hydroptilidae	0.505	0.003	Locomotion: temporarily attached	0.731	0.006
Crangonyctidae	0.474	0.004	Feeding group: scraper	0.729	0.001
			Reproduction strategy: clutches, cemented eggs	0.729	0.001
Perennial			Aquatic stage: adult	0.729	0.003
Elmidae	0.811	0.001	Food consumed: living microphytes	0.728	0.004
Gammaridae	0.772	0.001	Voltinism: 1	0.721	0.002
Sericostomatidae	0.728	0.001			
Baetidae	0.697	0.039	**Perennial**		
Ephemeridae	0.690	0.001	Voltinism: <1	0.776	0.001
Erpobdellidae	0.684	0.001	Maximal potential size: >8cm	0.775	0.001
Hydrobiidae	0.658	0.001	Respiration: plastron	0.768	0.001
Hydropsychidae	0.653	0.002	Locomotion: burrower	0.761	0.001
Goeridae	0.615	0.001	Reproduction strategy: isolated eggs, cemented	0.759	0.001
Glossiphoniidae	0.609	0.030	Feeding group: predator	0.751	0.001
			Locomotion: permanently attached	0.736	0.001
			Reproduction strategy: clutches, free	0.736	0.032
			Respiration mode: gill	0.735	0.001
			Dispersal strategy: aerial passive	0.730	0.001

* N.B. only six taxa were identified for this flow regime

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Table 5. Summary of multivariate dispersion distance by flow regime for taxonomic and functional communities on all (global), spring (March – May) and autumn (September – November) samples.

	Taxonomic			Functional		
	Global	Spring	Autumn	Global	Spring	Autumn
Perennial	0.340	0.330	0.340	0.075	0.074	0.074
Non-perennial	0.378	0.378	0.364	0.105	0.111	0.096

Table 6. Summary of ANOVA permutation dispersion tests between perennial and non-perennial sites for the global, spring and autumn datasets. Significant (p <0.05) results are emboldened.

	df	F	p
Taxonomic			
Global	1,178	14.447	**<0.001**
Spring	1,88	10.368	**0.002**
Autumn	1,88	2.749	0.101
Functional			
Global	1,178	34.07	**<0.001**
Spring	1,88	19.07	**<0.001**
Autumn	1,88	14.771	**<0.001**

Table 7. Summary of significant variables influencing macroinvertebrate community composition as determined by redundancy analyses. % W1 zero-flow days = % of winter zero-flow days; % S1 zero-flow days and % S2 zero-flow days = % of summer zero-flow days; mean W1= mean winter discharge; mean S1 and S2 = mean summer discharge. Two years of antecedent summer (April–September) and one year of winter (October–March) data were used respectively.

| Variable	F	p	Variable	F	p
Taxonomic spring			*Functional spring*		
% S1 zero-flow days	7.36	0.005	% S1 zero-flow days	23.6	0.005
Depth	2.63	0.005	Mean W1	2.30	0.040
Width	3.65	0.005	Width	2.08	0.040
Mean W1	2.72	0.005			
Gravel	2.25	0.010			
Mean S1	1.59	0.040			
Taxonomic autumn			*Functional autumn*		
Silt	6.09	0.005	Gravel	7.18	0.005
Pebble	4.87	0.005	% S2 zero-flow days	4.50	0.005
% W1 zero-flow days	2.50	0.005	Cobble	2.85	0.010
Depth	2.27	0.010	Width	2.13	0.040
Width	2.50	0.005	Depth	3.32	0.005
Mean S1	1.89	0.010			

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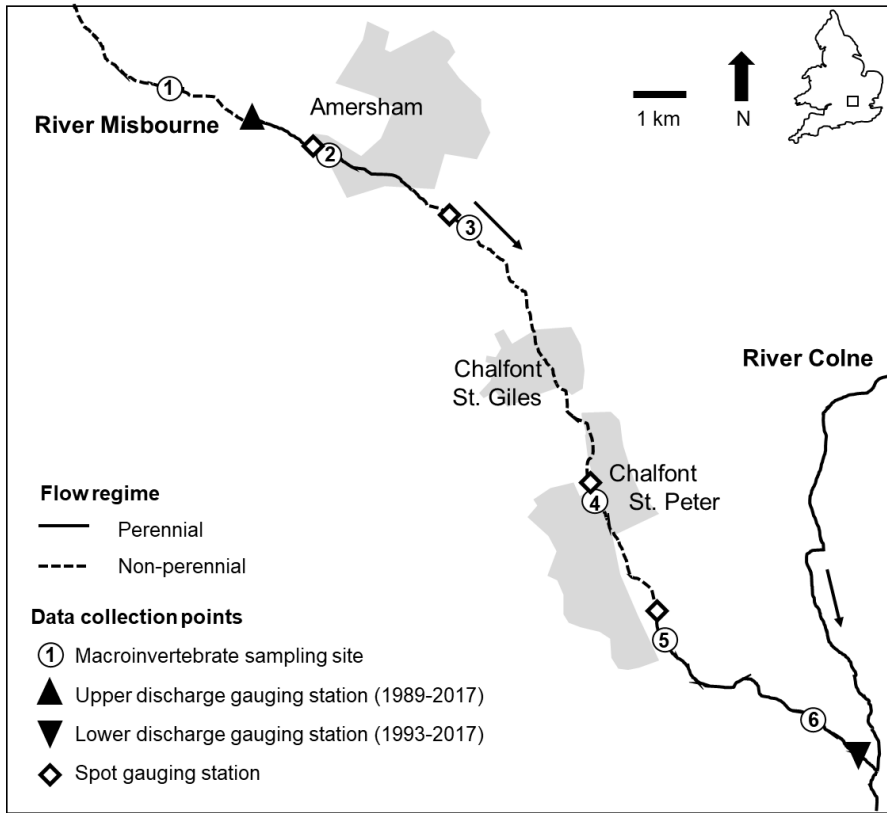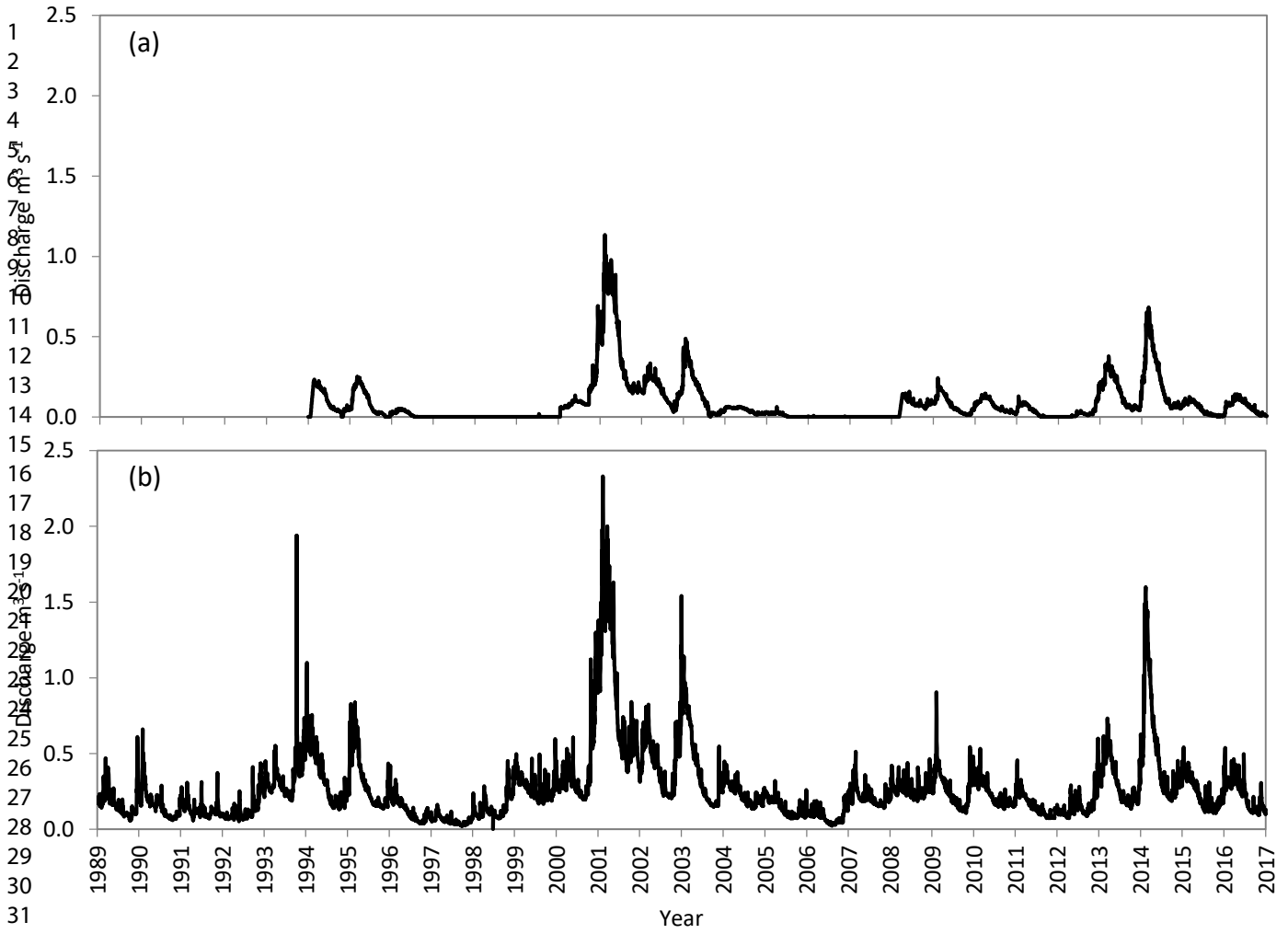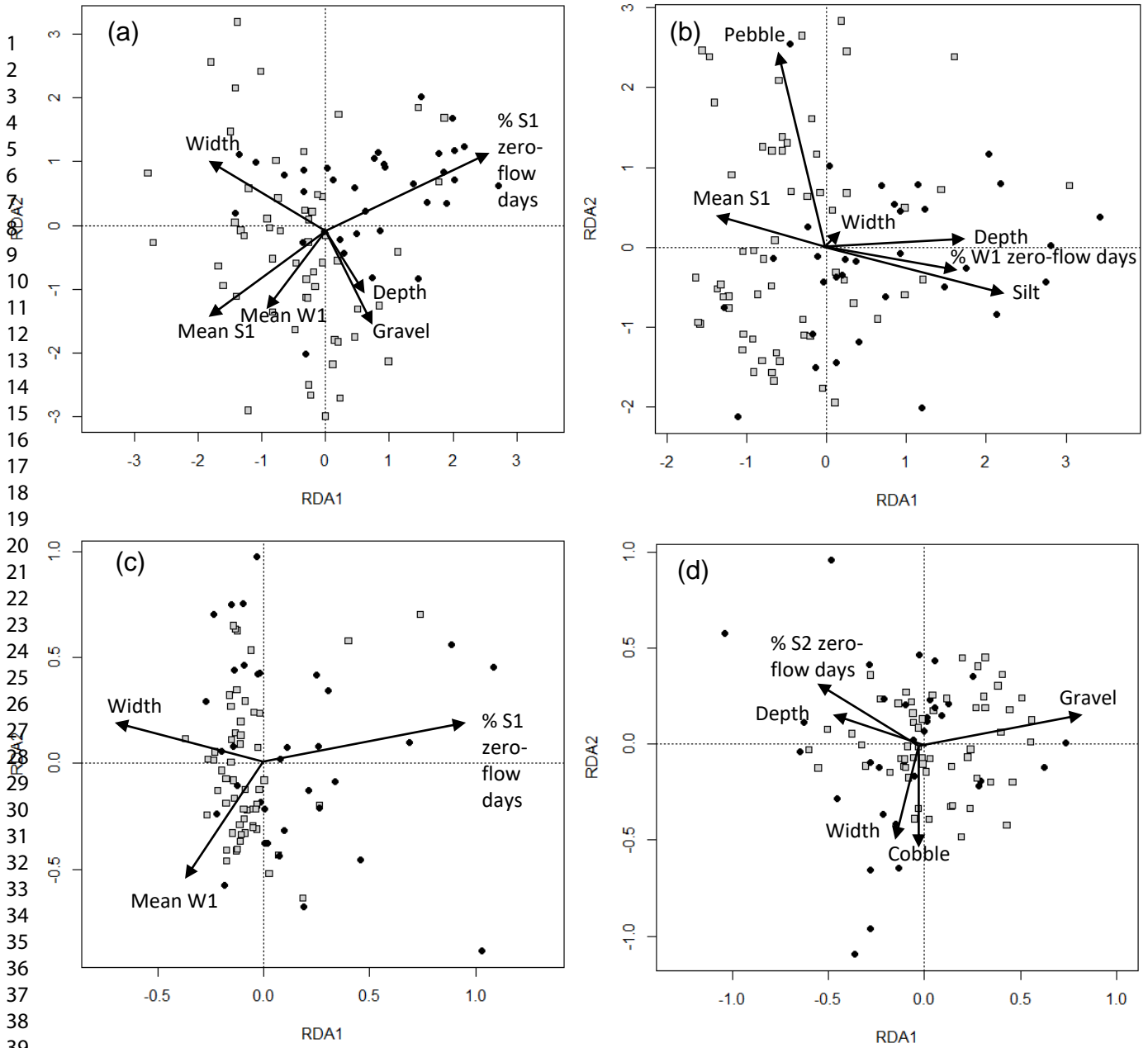
Figure 1.

Figure 2.

Figure 3.

Supporting information for: Structural and functional responses of
macroinvertebrate assemblages to long-term flow variability and drying at perennial
and non-perennial sites

Kate L. Mathers, Rachel Stubbington, Judy England, David Leeming, Christian G. Westwood

Table S1. Trait categories and modalities used to characterise functional responses of River Misbourne
macroinvertebrate communities (adapted from Tachet et al., 2010)

Category	Modalities	Category	Trait
Maximal potential size	< 0.25 cm	Respiration	Gill
	> 0.25 - 0.5 cm		Plastron
	> 0.5 - 1 cm		Spiracle
	> 1 - 2 cm		Hydrostatic vesicle
	> 2 - 4 cm		Tegument
	> 4 - 8 cm	Locomotion	Flier
	> 8 cm		Surface swimmer
Life cycle duration	≤ 1 year		Full water swimmer
	> 1 year		Crawler
Voltinism	<1		Burrower
	1		Interstitial
	>1		Temporarily attached
Aquatic stages	Egg		Permanently attached
	Larva	Feeding group	Absorber
	Nymph		Deposit feeder
	Adult		Shredder
Reproduction strategy	Ovoviviparity		Scraper
	Isolated, free eggs		Filter-feeder
	Isolated eggs, cemented		Piercer
	Clutches, cemented eggs		Predator
	Clutches, free		Parasite
	Clutches, in vegetation	Food consumed	Microorganisms
	Asexual		Detritus < 1mm
Dispersal strategy	Aquatic passive		Dead plant ≥ 1 mm
	Aquatic active		Living microphytes
	Aerial passive		Living macrophytes
	Aerial active		Dead animal ≥ 1 mm
Resistance form	Eggs / statoblasts		Living microinvertebrates
	Cocoons		Living macroinvertebrates
	Housings against desiccation		Vertebrates
	Diapause / dormancy		
	None		

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Table S2. Summary of the River Misbourne linear regression analyses. y = flow at the spot gauge site and x = flow at the fixed gauging station.

	Number of data points	Time period	R^2 value
Site 1 – No regression applied. Direct gauge data used			
Site 2: y = 1.199x – 0.040	224	October 1993 - February 2013	0.887
Site 3: y = 1.229x – 0.583	230	October 1993 – October 2010	0.583
Site 3: y = 1.033x – 0.050	49	November 2010- January 2016	0.901
Site 4: y = 0.672x – 0.092	33	October 1993 - May 2010	0.665
Site 4: y = 2.455x – 0.247	64	November 2000 - January 2008	0.949
Site 4: y = 1.156x – 0.163	14	February 2011 - June 2015	0.789
Site 4: y = 1.652x – 0.144	78	December 2000 - June 2016	0.700
Site 5: y = 0.740x – 0.026	179	February 1993 - April 2008	0.886
Site 6 – No regression applied. Direct gauge data used			

N.B. Sites 3 and 4 have multiple regressions reflecting changes in hydrological conditions; sites 1 and 6 are derived directly from the upper and lower gauging stations respectively.

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Table S3. Summary of the top ten indicator taxa and traits for each flow regime for spring samples (March – May).

Taxonomic			Functional		
Taxa	Indicator value	*p*-value	Trait	Indicator value	*p*-value
Non-perennial *			**Non-perennial**		
Lymnaeidae	0.725	0.001	Resistance form: cocoons	0.792	0.001
Lumbricidae	0.685	0.006	Resistance form: housing against desiccation	0.792	0.001
Dytiscidae	0.604	0.002	Locomotion: surface swimmer	0.766	0.011
			Locomotion: temporarily attached	0.748	0.003
Perennial			Respiration: tegument	0.740	0.001
Elmidae	0.796	0.001	Reproduction strategy: clutches, cemented eggs	0.736	0.002
Gammaridae	0.781	0.001	Aquatic stage: nymph	0.734	0.045
Sericostomatidae	0.738	0.001	Feeding group: scraper	0.732	0.001
Hydropsychidae	0.714	0.002	Food consumed: living microphytes	0.726	0.009
Erpobdellidae	0.697	0.002	Voltinism: 1	0.717	0.009
Hydracarina	0.692	0.006			
Ephemeridae	0.689	0.002	**Perennial**		
Hydrobiidae	0.669	0.001	Maximal potential size: > 8cm	0.776	0.001
Caenidae	0.657	0.043	Reproduction strategy: isolated eggs, cemented	0.775	0.001
Leptoceridae	0.651	0.022	Voltinism: < 1	0.768	0.001
			Food consumed: microorganisms	0.761	0.001
			Locomotion: permanently attached	0.759	0.001
			Locomotion: burrower	0.751	0.001
			Feeding group: predator	0.736	0.001
			Respiration: plastron	0.736	0.032
			Respiration mode: gill	0.735	0.001
			Maximal potential size: > 2-4cm	0.730	0.001

* N.B. only three taxa were identified for this flow permanence regime

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Table S4. Summary of the top ten indicator taxa and traits for each flow regime for autumn samples (September – November).

Taxonomic			Functional		
Taxa	Indicator value	*p*-value	Trait	Indicator value	*p*-value
Non-perennial *			**Non-perennial**		
Lymnaeidae	0.820	0.001	Locomotion: surface swimmer	0.773	0.009
Hydroptilidae	0.557	0.005	Resistance form: cocoons	0.764	0.001
Corixidae	0.482	0.039	Resistance form: housing against desiccation	0.764	0.001
Crangonyctidae	0.479	0.034	Aquatic stage: adult	0.735	0.004
			Respiration: tegument	0.734	0.001
Perennial			Feeding group: scraper	0.726	0.015
Elmidae	0.822	0.001	Maximal potential size: > 1-2cm	0.726	0.008
Gammaridae	0.762	0.002	Dispersal strategy: aquatic passive	0.722	0.001
Sericostomatidae	0.718	0.001	Voltinism: 1	0.722	0.05
Ephemeridae	0.687	0.007	Maximal potential size: < 0.25cm	0.641	0.006
Erpobdellidae	0.669	0.001			
Simuliidae	0.648	0.016	**Perennial**		
Hydrobiidae	0.647	0.002	Respiration: plastron	0.776	0.001
Goeridae	0.632	0.002	Voltinism: < 1	0.775	0.001
Odontoceridae	0.582	0.002	Maximal potential size: > 8cm	0.768	0.001
Psychomyiidae	0.537	0.005	Locomotion: burrower	0.761	0.001
			Reproduction strategy: clutches, free	0.759	0.001
			Feeding group: predator	0.751	0.001
			Maximal potential size: > 0.25 - 0.5cm	0.736	0.001
			Locomotion: flier	0.736	0.032
			Dispersal strategy: aerial passive	0.735	0.001
			Reproduction strategy: isolated eggs, cemented	0.730	0.001

* N.B. only four taxa were identified for this flow regime

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Table S5. Summary of the model outputs from redundancy analyses

Dataset	Principal component (% variance explained)			F	p
	1	2	Total		
Taxonomic spring	9.08	5.05	14.13	2.28	0.001
Taxonomic autumn	8.20	5.66	13.86	2.46	0.001
Functional spring	23.12	4.65	27.77	3.34	0.001
Functional autumn	12.1	6.82	18.92	2.82	0.001