



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

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58 25 heterogeneity, flow permanence, hydrology.
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Abstract

Temporary streams constitute a significant proportion of rivers globally and are common in wet, cool, temperate regions. These heterogeneous ecosystems harbour high biodiversity associated with the dynamic turnover of taxa. Despite flow permanence being widely recognised as an important environmental control, few studies have characterised biotic responses to long-term hydrological variability in temporary streams. We examined taxonomic and functional macroinvertebrate communities of perennial and non-perennial river reaches over a 26-year period. Flow permanence resulted in spatial variation in taxonomic and functional macroinvertebrate communities. Non-perennial river reaches, which were characterised by dynamic habitat provision (lotic, lentic and dry states) over the study period, supported more heterogeneous communities than perennial river reaches. Hydrological variables, in particular wetted width, water depth and zero-flow states, were instrumental in structuring taxonomic and functional communities, although the importance of substrate conditions increased in autumn. Hydrological conditions resulted in separation of perennial and non-perennial taxonomic communities regardless of season, whereas functional communities differed only in spring. Our results emphasise that understanding of community responses to hydrological variability is enhanced by analyses that concurrently explore taxonomic and functional responses to long-term intra- and inter-annual hydrological variability. Moreover functional responses represents a robust method to test ecological responses to hydrological drivers. Further research that builds on our work is needed to inform the protection of both perennial and non-perennial streams as they adapt to ongoing environmental change.

51 **1. Introduction**

52 Hydrological variability and in particular flow permanence (Poff & Ward, 1989) are
53 primary determinants of aquatic community composition in lotic ecosystems (Smith &
54 Wood, 2002; Datry et al., 2014a). Non-perennial streams are defined by flow
55 cessation, often experience partial or complete surface drying, and are characterised
56 by high temporal and spatial variability (Leigh et al., 2015; Datry, Larned, & Tockner,
57 2014b; Cid et al., 2017). The frequency, magnitude, duration and predictability of
58 transitions between lotic, lentic and dry states influence the diversity and connectivity
59 of habitat patches and, in turn, biotic responses (Bogan & Lytle, 2007; Leigh & Datry,
60 2017). As such, non-perennial reaches often support high biodiversity as
61 communities vary in space and time in response to changing habitat availability
62 (Bogan, Boersma, & Lytle, 2013; Rolls, Heino, & Chessman, 2016; Tonkin et al.,
63 2017).

64 Historically, most research considering macroinvertebrate responses to flow
65 permanence has focused on taxonomic changes. As discharge declines, flowing
66 habitats are typically lost and rivers become dominated by connected pools inhabited
67 by both lotic refugees and lentic colonists (Hill & Milner, 2018). Over time, pools
68 become isolated and community composition continues to diverge from the lotic
69 community as lentic colonists become increasingly dominant (Bonada, Rieradevall,
70 Prat, & Resh, 2006). Flow resumption allows rapid recolonisation by resistant and
71 resilient lotic species (Rolls et al., 2016; Vander Vorste, Corti, Sagouis, & Datry,
72 2016). Communities within sites with contrasting flow permanence regimes may
73 therefore converge as flowing phase durations increase (Wood, Gunn, Smith, &
74 Abas-Kutty, 2005; Vander Vorste et al., 2016). However, studies of biotic responses
75 to flow permanence are typically short-term (months to years), leaving long-term

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3 76 (decadal) patterns poorly characterised (but see Bêche, Connors, Resh, &
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5 77 Merenlender, 2009; Stubbington, Wood, & Boulton, 2009; Chessman, 2015; Leigh &
6
7 78 Datry, 2017; Wilding et al., 2018).

9
10
11 79 In addition to taxonomic responses, biological traits based on life history, behaviour
12
13 80 morphology and physiology may be more informative when investigating community
14
15 81 responses to hydrological variability and flow permanence (Menezes, Baird, &
16
17 82 Soares, 2010; Schriever et al., 2015). Understanding life-history traits (such as body
18
19 83 size, morphology, lifecycle) associated with the habitat template via functional trait
20
21 84 analysis has been cited as strong basis for the theoretical development of ecological
22
23 85 indicators (Statzner & Bêche, 2010). Macroinvertebrates within non-perennial
24
25 86 streams may exhibit *r*-selected traits such as high dispersal, short life histories, rapid
26
27 87 growth and opportunistic feeding modalities that promote resilience to drying and
28
29 88 enable persistence in dynamic environments (Williams, 1996; García-Roger et al.,
30
31 89 2013; Giam et al., 2017). Taxa may also possess traits promoting resistance to
32
33 90 drying, such as desiccation-tolerant forms or respiration systems which permit air
34
35 91 breathing (Leigh et al., 2016; Datry et al., 2017a). The prevalence of taxa possessing
36
37 92 resistance traits may result in functional redundancy increasing with intermittence
38
39 93 (García-Roger et al., 2013; Boersma, Bogan, Henrichs, & Lytle, 2014). In turn,
40
41 94 functional redundancy can stabilise community trait composition over time (Bêche,
42
43 95 McElravy, & Resh, 2006), potentially facilitating clearer discrimination between
44
45 96 assemblages from sites with contrasting flow permanence compared to taxonomic
46
47 97 approaches (Cid et al., 2016; García-Roger, 2017). Communities in reaches which
48
49 98 dry infrequently or unpredictably may be decoupled from such adaptations, and may
50
51 99 therefore exhibit stronger taxonomic and functional responses (Bêche et al., 2009;
52
53 100 Chessman, 2015).

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3 101 Recognition of their extensive and increasing occurrence, biodiversity, and
4
5 102 ecosystem service provision has resulted in increased research examining
6
7 103 temporary streams (Leigh et al., 2015; Datry et al., 2018). Despite this increase,
8
9 104 most ecological studies are from Mediterranean and dryland climates (e.g. Bonada,
10
11 105 Rieradevall, & Prat, 2007; Bogan et al., 2015; Cid et al., 2016; Leigh et al., 2016),
12
13 106 whereas research in cooler, wetter temperate climates remains restricted to a few
14
15 107 well-studied rivers including groundwater-dominated streams (Wood & Petts, 1999;
16
17 108 Hill & Milner, 2017; White et al., 2018). Such systems support high biodiversity
18
19 109 including rare species but are particularly sensitive to hydrological alterations, which
20
21 110 may manifest instream as reduced discharge in perennial reaches, as flow cessation
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23 111 or drying in near-perennial reaches, and as prolonged ponded or dry states in non-
24
25 112 perennial reaches. This spatial variability makes groundwater-dominated streams
26
27 113 useful systems in which to examine ecological responses to hydrological variability
28
29 114 (Wright, 1992).

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36 115 This study examined taxonomic and functional macroinvertebrate assemblage
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38 116 responses to antecedent hydrological variability (including flow cessation and drying
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40 117 events) at sites with contrasting flow permanence regimes in a UK groundwater-
41
42 118 dominated stream. We analysed a long-term dataset consisting of macroinvertebrate
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44 119 samples and continuous, site-specific hydrological measurements. Hydrological data
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46 120 were supplemented by observations of flow states aiding discrimination between
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48 121 lentic conditions and streambed drying events. The following research questions
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50 122 were addressed:

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54 123 (i) Do perennial and non-perennial river reaches support distinct taxonomic
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56 124 and functional macroinvertebrate assemblages?
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3 125 (ii) Does heterogeneity of taxonomic and functional macroinvertebrate
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5 126 assemblages differ between perennial and non-perennial river reaches?
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8 127 (iii) Which environmental parameters are influential in structuring the
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10 128 composition of taxonomic and functional macroinvertebrate assemblages
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12 129 in perennial and non-perennial river reaches?
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14 130 **2. Methods**

15 131 **2.1 Study sites**

16
17 132 The River Misbourne is a small chalk stream in southern England which flows for
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19 133 approximately 27 km to its confluence with the River Colne (Figure 1). Land use
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21 134 within the 94.8 km² catchment is predominately arable (34.07%) with some grassland
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23 135 (28.33%), mixed woodland (21.50%) and urban land cover (16.67%); NRFA, 2017).
24
25

26 136 The Misbourne experiences predictable annual discharge variation and loses water
27
28 137 from its upper reaches and the middle section upstream of Chalfont St. Peter (Figure
29
30 138 1). Natural variation in water loss is exacerbated by anthropogenic influences (e.g.,
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32 139 channel realignment to provide water to historic mills and instream channel
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34 140 modifications).
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41 141 Six sampling sites (1-6 from upstream to downstream) with contrasting flow
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43 142 permanence were examined during the 26-year study period (1990–2016; Table 1;
44
45 143 Figure 1). For the purposes of this study, sites were categorised as ‘non-perennial’
46
47 144 being those that dry regularly (sites 1, 3 and 4), or ‘perennial’ being those that never
48
49 145 cease to flow or maintain flow except during extreme drought events (sites 2, 5 and
50
51 146 6.) Table 1 summarises the physical characteristics of each site and the flow
52
53 147 permanence regime according to two hydrological metrics: the six-month
54
55 148 predictability of dry periods (Sd6; which ranges from 0 - no predictability to 1 - total
56
57 149 predictability) and the mean number of days with zero flow (Gallart et al., 2012).
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3 150 Hydrological variability was evident within and among the six sampling sites during
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5 151 the study period. Droughts occurred in England in 1997-1998, 2006-2007 and 2011-
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7 152 2012, and wet phases in 2001 and 2014 (Marsh, Cole, & Wilby, 2007; Marsh et al.,
8
9 153 2013).

13 154 **2.2 Macroinvertebrate data**

15 155 Benthic macroinvertebrate samples were collected by the Environment Agency of
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17 156 England using their standard sampling protocol for statutory monitoring purposes: a
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19 157 3-minute kick sample (1-mm mesh size) encompassing all habitats in proportion to
20
21 158 their occurrence, followed by a 1-minute hand search (Murray-Bligh, 1999).
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23 159 Sampling was conducted in spring (March – May) and autumn (September –
24
25 160 November; UKTAG, 2014) and began between 1990 and 1998 (dependent on site)
26
27 161 and continued until 2016. For sites surveyed more than once in a single sampling
28
29 162 season, the most recent replicate was retained to ensure independence of samples,
30
31 163 leaving a total of 180 samples. Taxa were mainly identified to family level, except
32
33 164 Hydracarina and Collembola, which were recorded as such. Due to the semi-
34
35 165 quantitative technique, abundance data were standardised into ordinal classes (1 =
36
37 166 1–9, 2 = 10–32, 3 = 33–99, 4 = 100–332, 5 = 333–999, 6 = ≥1000 individuals per
38
39 167 sample; Durance & Ormerod, 2009; Mathers et al., 2016).
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48 169 The functional composition of macroinvertebrate communities was characterised by
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50 170 assigning taxa into 11 categories comprising 61 biological traits from the Tachet,
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52 171 Bournaud, Richoux, & Usseglio-Polatera (2010) database (Table S1). Traits were
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54 172 assigned to genera based on a fuzzy-coding approach with scores ranging from zero
55
56 173 (indicating no affinity) to five (the strongest affinity based on available literature;
57
58 174 Chevene, Dolédec, & Chessel, 1994). Because we worked at the family level, we
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1
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3 175 averaged the affinities of all genera recorded within a family and these affinity scores
4
5 176 were rescaled as proportions for each category (sum=1) for each taxon (*sensu*
6
7 177 Gayraud et al., 2003). To produce a trait abundance matrix, taxon-trait categories
8
9 178 were multiplied by the ordinal abundances and subsequently scaled such that each
10
11 179 trait equalled one (Larsen & Ormerod, 2010a; Descloux, Datry, & Usseglio-Polatera,
12
13 180 2014; White et al., 2017a). Trait abundance and diversity can be achieved at the
14
15 181 family level (i.e.) regardless of spatial scales (Gayraud et al., 2003; García-Roger et
16
17 182 al., 2013). Chironomidae and taxa recorded at a coarser resolution than family level
18
19 183 were excluded, because these taxa are likely to include multiple representatives with
20
21 184 highly variable responses (Monk et al., 2012; Cañedo-Argüelles, Bogan, Lytle, &
22
23 185 Prat, 2016) Of the 90 taxa recorded, 78 were assigned functional traits. Alpha
24
25 186 diversity of each sample was determined via taxa richness and the inverse
26
27 187 Simpson's diversity value (Oksanen et al., 2015), which accounts for the fixed
28
29 188 number of traits and the lack of independence between traits (Larsen & Ormerod,
30
31 189 2010b; White et al., 2017b).

32 33 34 35 36 37 38 190 **2.3 Hydrological data**

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40 191 Discharge has been gauged continuously in the lower Misbourne (51°40' N, 00°39'
41
42 192 W) since 1985 and in the upper river (51°34' N, 00°29' W) since 1993 (Figure 1) with
43
44 193 additional spot discharge measurements taken along the river under varying flow
45
46 194 conditions (Figure 1). Table S2 shows the time periods and number of spot gauge
47
48 195 measurements used to establish flow relationships at each site. Daily mean
49
50 196 discharge data provided by the Environment Agency of England (available from
51
52 197 NRFA, 2017) and spot-gauging measurements closest to the biological monitoring
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54 198 sites were used to generate site-specific discharge time series using linear
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56 199 regressions (see Gordon, McMahon, Finlayson, Gippel, & Nathan 2004). This
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3 200 approach provides sufficient representation of hydrological conditions at a site, gives
4
5 201 a good description of relative patterns but may lack precision for low flows (Malcolm
6
7 202 et al., 2012a), and has been used successfully in hydroecological assessments
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9
10 203 (Gordon et al., 2004; Westwood et al., 2017). For sites 2-5, between one and four
11
12 204 linear regressions were constructed reflecting different hydrological periods
13
14 205 associated with discharge levels (Table S2). Figure 2 provides hydrographs for
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16
17 206 typical perennial and non-perennial river reaches.

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19 207
20
21 208 Using the derived daily mean discharge data, we calculated antecedent hydrological
22
23 209 metrics previously shown to be relevant when examining macroinvertebrate
24
25 210 response to flows (Dunbar et al. 2010a, 2010b). We divided the year into two periods
26
27 211 which we termed summer flows (April–September) and winter flows (October–
28
29 212 March). Each flow period for each site thus provides a series of blocks of discharge
30
31 213 data, each of 183 days for summer and 182/183 days for winter. For each block, two
32
33 214 statistics were calculated: the % of zero-flow days for the previous year (% of winter
34
35 215 zero-flow days – % W1 zero-flow days; % of summer zero-flow days – % S1 zero-
36
37 216 flow days) and two characterising mean discharge (mean winter discharge – Mean
38
39 217 W1; mean summer discharge – Mean S1). In addition, mean discharge (Mean S2)
40
41 218 and % of zero-flow days (% S2 zero-flow days) were calculated for the summer
42
43 219 before S1, resulting in six hydrological statistics.

50 220 **2.4 Data analysis**

51 221 *2.4.1 Macroinvertebrate responses in perennial and non-perennial river reaches*

52 222 All analyses were conducted in R version 3.4.3 (R Development Core Team, 2017).

53 223 To assess whether alpha diversity varied in association with flow permanence

54 224 regime (perennial or non-perennial), season, site and the interaction of these factors,

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2
3 225 differences in alpha diversity were tested via a linear mixed-effects model using the
4
5 226 'lme' function in the 'nlme' package (Pinheiro et al., 2016). Season, site and flow
6
7 227 permanence regime were fitted as fixed interacting effects and year as a random
8
9 228 effect to account for community compositions from individual sites being potentially
10
11 229 correlated over time. Significance values were obtained using Type II analysis of
12
13 230 variance (ANOVA) in the 'car' package (Fox & Weisberg, 2011). Marginal r^2
14
15 231 (proportion of variance explained by the fixed effects alone) and conditional r^2
16
17 232 (proportion of variance explained by the fixed and random effects) values were
18
19 233 extracted using the 'MuMIn' package (Bartoń, 2016).
20
21
22
23
24 234 Differences in community composition were explored as a function of the interactive
25
26 235 explanatory factors of site, flow permanence regime and season via a permutational
27
28 236 multivariate ANOVA (PERMANOVA) using the 'adonis' function in the 'vegan'
29
30 237 package (Oksanen et al., 2015). Indicator taxa and traits were identified for perennial
31
32 238 and non-perennial sites using the 'multipatt' function within the 'indicspecies'
33
34 239 package (De Cáceres & Jansen, 2016). An indicator value of >0.25 was accepted as
35
36 240 ecologically relevant (Dufrêne & Legendre, 1997), and all significant indicators with a
37
38 241 fidelity value of <0.25 removed to exclude rare taxa and traits (De Cáceres,
39
40 242 Legendre, Wiser, & Brotons, 2012; Trivellone et al., 2014). Indicator values were
41
42 243 assigned based on global, spring and autumn datasets to assess variation between
43
44 244 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

1
2
3 250 'vegan'. Tests were run on flow permanence regime (perennial and non-perennial)
4
5 251 on global, spring and autumn datasets. Statistical differences in multivariate
6
7 252 dispersion between the flow permanence groups and within flow permanence regime
8
9 253 by season were tested using one-way ANOVA. Heterogeneity of all taxonomic and
10
11 254 functional samples (regardless of factors) were determined to establish overall
12
13 255 temporal variability of the two approaches and tested using ANOVA.
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17

18 256 *2.4.3 Perennial and non-perennial macroinvertebrate community associations with* 19 20 257 *environmental variables*

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22 258 Redundancy analyses (RDA) were performed using the 'ordistep' function in 'vegan'
23
24 259 to examine relationships between taxonomic / functional composition and 13
25
26 260 environmental parameters, specifically six hydrological metrics (described above),
27
28 261 five substrate characteristics assessed via visual estimates (% boulders, cobbles,
29
30 262 pebbles, gravel, silt), and two mean wetted channel dimensions (width, depth;
31
32 263 Murray-Bligh, 1999). Prior to analysis, a Hellinger transformation was applied to the
33
34 264 taxonomic data. A stepwise (forward and backward) selection procedure using
35
36 265 permutational-based significance tests (999 permutations) was used to identify
37
38 266 factors that influenced assemblages, with only significant variables included in the
39
40 267 final model. Final variables were checked for collinearity using the 'vif' function in the
41
42 268 'car' package to ensure that all 'variance inflation factors' were <3 (Zuur et al., 2010).
43
44 269 Analyses were conducted on spring and autumn datasets for taxonomic and
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46 270 functional communities.
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53 271 **3. Results**

54 272 ***3.1 Macroinvertebrate responses in perennial and non-perennial river reaches***

55
56 273 Taxa richness for the 26-year study period was higher at perennial sites (mean \pm SE
57
58 274 34 ± 0.56 , range 9-39) compared to non-perennial sites (18 ± 0.81 , range 4-30) with
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3 275 15 families being unique to perennial river reaches (most notably Odontoceridae and
4
5 276 Psychomyiidae). Functional diversity was higher at perennial sites (mean inverse
6
7 277 Simpson's diversity value 31 ± 0.11) compared to non-perennial sites (21 ± 0.21).
8
9
10 278 Taxonomic richness and functional diversity varied as a function of flow permanence
11
12 279 regime and the interaction of flow permanence regime and site but not by the
13
14 280 individual influence of site and season or the interaction of any other factors (all
15
16
17 281 $p > 0.05$; Table 2).
18
19
20 282 Site explained the greatest amount of variation in taxonomic community composition,
21
22 283 whereas the interaction of site and flow permanence regime was the most influential
23
24 284 factor for functional community composition, as indicated by the greater r^2 and F
25
26 285 values (Table 3). Both taxonomic and functional communities differed as a function
27
28 286 of site, season and flow permanence regime and the interaction of flow regime and
29
30 287 site (Table 3). There was no interaction between season and either site or flow
31
32
33 288 permanence regime.
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36
37 289 Indicator analysis (Table 4) determined that non-perennial sites were characterised
38
39 290 by taxa with resistance forms including cocoons and housing against desiccation,
40
41 291 such as Lymnaeidae, the strongest indicator taxon. These sites also supported taxa
42
43 292 that temporarily attach to substrates and surface swimmers (e.g. Dytiscidae). The
44
45 293 family Lumbricidae was also a strong indicator of non-perennial sites. Perennial sites
46
47 294 supported large taxa (>8 cm, e.g. Erpobdellidae), taxa with <1 brood per year, those
48
49 295 with parasitic and carnivorous feeding modes (e.g. Erpobdellidae, Glossiphoniidae),
50
51 296 and those permanently attached to substrates (e.g. Hydrobiidae). Indicator values
52
53 297 were generally higher for traits than for taxa and at perennial sites than non-
54
55 298 perennial sites (Table 4), and taxonomic indicator values were higher in spring
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3 299 compared to autumn (Tables S3 and S4). Indicators were generally comparable
4
5 300 between seasons with a few exceptions at non-perennial sites, notably Corixidae,
6
7 301 Hydroptilidae, Crangonyctidae in autumn and Lumbricidae in spring. Taxa with
8
9
10 302 passive aquatic dispersal, small body size (>1-2 cm and <0.25 cm) and with adult
11
12 303 aquatic stage characterised autumn samples whilst spring supported taxa that
13
14
15 304 temporally attach to substrates or which have an aquatic nymph stage.
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17

18 305 **3.2 Heterogeneity in macroinvertebrate community composition in perennial** 19 20 306 **and non-perennial river reaches**

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22
23 307 Taxonomic communities demonstrated greater temporal dispersion (mean distance
24
25 308 of all communities: 0.369) than functional communities (0.084). Heterogeneity was
26
27 309 higher at non-perennial sites compared to perennial sites for taxonomic and
28
29 310 functional communities regardless of season (Table 5). Both taxonomic and
30
31 311 functional communities differed in heterogeneity between perennial and non-
32
33 312 perennial sites within the global and spring models, whereas only functional
34
35 313 communities differed in autumn (Table 6). Heterogeneity within perennial or non-
36
37 314 perennial groups was comparable between seasons (all $p > 0.05$), but was generally
38
39 315 lower in autumn at non-perennial sites, in both taxonomic and functional community
40
41 316 composition.
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46 317 **3.3 Perennial and non-perennial macroinvertebrate community associations** 47 48 318 **with environment variables**

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51 319 All RDA models were highly significant (all $p = 0.001$), with functional communities
52
53 320 demonstrating a stronger association with the measured environmental parameters
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55 321 and the models accounting for greater variance on the first two axes, especially in
56
57 322 spring (range 27.77-18.92%; Table S5). In all instances, hydrological metrics and
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3 323 wetted channel characteristics influenced functional and taxonomic community
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5 324 composition. Width and depth were correlated with taxonomic and functional
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7 325 communities in both spring and autumn, with one exception (between depth and
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9 326 functional communities in spring; Table 7). Both taxonomic and functional
10
11 327 communities were strongly correlated with a greater number of hydrological metrics
12
13 328 in spring, whereas substrate characteristics were most important in autumn. The %
14
15 329 of S1 zero-flow days had the greatest influence on both taxonomic and functional
16
17 330 community composition in spring (as denoted by the greatest F value), whereas in
18
19 331 autumn the % of silt and gravel exerted the greatest influence on taxonomic and
20
21 332 functional communities, respectively (Table 7). Functional communities
22
23 333 demonstrated a greater association with hydrological metrics in both spring and
24
25 334 autumn (as indicated by the greater F values for % zero-flow days).
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30
31 335 Perennial and non-perennial taxonomic communities separated along RDA axis 1 in
32
33 336 spring, which was correlated with hydrological metrics describing flow permanence
34
35 337 (i.e. % S1 zero-flow days and mean S1; Figure 3a). Variation in community
36
37 338 composition within non-perennial sites was associated with substrate conditions (%
38
39 339 gravel) and wetted channel characteristics (width, depth), as reflected by separation
40
41 340 along RDA axis 2. The two RDA axes accounted for 14.13% of variance and were
42
43 341 highly significant ($p < 0.05$; Table 7). There was some evidence of a perennial and
44
45 342 non-perennial site separation along RDA axis 1 which represented mean winter
46
47 343 discharge, mean summer discharge and depth for autumn taxonomic communities
48
49 344 (Figure 3b; RDA axes accounted for 13.86% of variance; Table 7). Variation within
50
51 345 flow permanence regimes was correlated with % pebbles. In both seasons,
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53 346 community variability associated with the environmental parameters was greatest in
54
55 347 perennial sites.
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5 349 Separation of perennial and non-perennial sites was evident in spring functional
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7 350 communities along RDA axis 1, which was associated with descriptors of flow
8
9 351 permanence regimes (% S1 - zero-flow days for the previous summer) and wetted
10
11 352 width (Figure 3c). Positioning of assemblages along RDA axis 2 reflected within flow
12
13 353 regime variation in community composition associated with mean winter discharge
14
15 354 (mean W1) and the two axes collectively explained 27.77% of the variation in
16
17 355 community composition. In contrast, separation of perennial and non-perennial
18
19 356 functional assemblages was not evident in autumn (Figure 3d; RDA axes accounted
20
21 357 for 18.92% of variance; Table 7). In both seasons, variability in communities
22
23 358 associated with the environmental parameters was greatest in non-perennial sites.

28 359 **4. Discussion**

30 360 Characterising broad-scale macroinvertebrate responses to hydrological variability
31
32 361 and flow permanence is challenging, because interacting local conditions such as
33
34 362 antecedent hydrological conditions, the core taxa present, and habitat characteristics
35
36 363 simultaneously influence ecological responses (Chakona, Phiri, Magadza, &
37
38 364 Brendonck, 2008; Leigh et al., 2016). Tackling this ecological complexity, our 26-
39
40 365 year study of taxonomic and functional macroinvertebrate communities
41
42 366 demonstrated that the dynamic, heterogeneous communities of non-perennial
43
44 367 streams can be captured effectively using paired biological-hydrological data
45
46 368 collected over multi-decade timescales. Flow permanence, wetted width and depth
47
48 369 were primary drivers of macroinvertebrate community composition, and the
49
50 370 importance of substrate conditions increased in autumn.

57 371 ***4.1 Macroinvertebrate responses within perennial and non-perennial river*** 58 59 372 ***reaches***

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3 373 Our research, conducted in a wet, cool temperate region, builds on short-term
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5 374 oceanic-climate studies which have found that perennial and non-perennial river
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7 375 reaches support discrete taxonomic macroinvertebrate assemblages (Wood et al.,
8
9 376 2005; Stubbington et al., 2011; White et al., 2018). We found that these differences
10
11 377 persisted long term (26 years) despite evident intra-annual variability in hydrological
12
13 378 conditions and low spatial / temporal extent of flow permanence in oceanic regions
14
15 379 compared to dryland regions, including the close proximity of perennial reaches.
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21 381 Other studies conducted over shorter time periods have reported that taxonomic
22
23 382 community composition at perennial and non-perennial sites may converge if
24
25 383 sampling is conducted after several months of continuous flow, reflecting
26
27 384 recolonisation by taxa persisting in refuges. However, our clear separation of sites
28
29 385 highlights the importance of long-term studies that represent the range and dominant
30
31 386 hydrological conditions experienced at a location (Delucchi, 1998; Wood et al., 2005;
32
33 387 Sheldon et al., 2010). We found differences in the composition and alpha diversity of
34
35 388 taxonomic and functional communities in perennial and non-perennial river reaches.
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38 389 Flow permanence regime was the primary driver of diversity differences, with
39
40 390 perennial sites supporting higher values than non-perennial sites regardless of
41
42 391 season (Bonada et al., 2007; Datry, 2012; Schriever et al., 2015; Suárez et al.,
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44 392 2017).
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49
50 393 We did, however, note subtle differences between the two methodological
51
52 394 approaches, with functional communities being more strongly influenced by flow
53
54 395 permanence regime (and the interaction with site) whilst taxonomic communities
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56 396 demonstrated greater differences among sites. Communities were likely responding
57
58 397 to habitat-specific flow velocities, hydraulic habitats and substrate conditions (Lane
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3 398 Pasternack, & Sandoval-Solis, 2018; White et al., 2019). Functional responses may
4
5 399 be less sensitive to small-scale fluctuations in hydrological conditions due to their
6
7 400 high stability in space and time (Statzner & Bêche, 2010), meaning that extreme
8
9 401 conditions may be required to eliminate enough taxa to cause detectable
10
11 402 compositional shifts (Schriever et al., 2015). Alpha diversity also demonstrated a
12
13 403 significant interaction between site and flow permanence regime, indicating that site-
14
15 404 specific environmental controls may play a more important role in the structuring of
16
17 405 communities compared to general hydrological patterns (Bonada et al., 2007).
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23 407 Broad patterns of compositional differences among non-perennial and perennial river
24
25 408 reaches reflected the distributions of taxa adapted to the prevailing hydrological
26
27 409 conditions. Non-perennial river reaches were characterised by taxa with traits
28
29 410 promoting resistance to drying events, i.e. cocoons and housing against desiccation
30
31 411 (Bogan et al., 2014; Leigh et al., 2016). For example, some Lymnaeidae species are
32
33 412 characteristic of non-perennial waterbodies and are a desiccation-tolerant
34
35 413 component of the aquatic invertebrate 'seedbank' (Stubbington, Gunn, Little, Worrall,
36
37 414 & Wood, 2016). *Galba truncatula* was the dominant lymnaeid in our study stream, is
38
39 415 capable of air-breathing, and has previously been identified as indicative of
40
41 416 intermittent stream reaches (Stubbington et al., 2009; White et al., 2018). Surface
42
43 417 swimmers were found to indicative of non-perennial sites reflecting the presence of
44
45 418 lentic habitats when flow ceases (Hill and Milner, 2019). Coleopterans of the family
46
47 419 Dytiscidae were characteristic of non-perennial sites, possessing adaptations which
48
49 420 enable dry-phase persistence, such as air-breathing, an impermeable cuticle that
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51 421 reduces water loss (Holdgate, 1956), and overland flight which enables dispersal
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53 422 between disconnected waterbodies (Jackson, 1952). Lumbricidae were also
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3 423 indicative of these sites and likely included aquatic, semi-aquatic and terrestrial
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5 424 species (Stubbington et al., 2016).
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8 425 Taxa characterising the perennial reaches had traits reliant on continuous water
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10 426 availability, such as feeding on aquatic prey (e.g. Erpobdellidae, Glossiphoniidae)
11
12 427 and permanent attachment to a substrate (e.g. Hydrobiidae). Although predators can
13
14 428 thrive during flow recession due to the concentration of prey within a declining
15
16 429 habitat area (Acuña et al., 2005), their typically large body sizes can prevent access
17
18 430 to saturated refuges during dry phases, causing population densities to decline
19
20 431 (Ledger, Edwards, Brown, Milner, & Woodward, 2011). Perennial reaches were also
21
22 432 characterised by *K*-selected traits, with the prevalence of large-bodied and long-lived
23
24 433 taxa which exhibit low voltinism typically increasing with the hydroperiod (wet-phase
25
26 434 duration; Corti, Kohler, & Sparks, 1996; García-Roger et al., 2013).
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31
32 435 Notable seasonal differences in indicator taxa within non-perennial reaches included
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34 436 taxa with smaller body sizes, aquatic passive dispersal and adult aquatic stages in
35
36 437 autumn. Taxa possessing such traits include Crangonyctidae and Corixidae, which
37
38 438 were characteristic of non-perennial reaches. These taxa are associated with slow-
39
40 439 flowing and/or lentic waters (Jass & Klausmeier, 2003; Van de Meutter, Stoks, & De
41
42 440 Meester, 2006; Wiberg-Larsen, 2008), and may have inhabited pools and ponded
43
44 441 habitats (Hill & Milner, 2018). Habitat complexity may have enabled their localised
45
46 442 persistence after flow resumption in autumn (Lane et al., 2018), prior to likely
47
48 443 displacement by high winter flows. Indicator traits unique to spring included nymph
49
50 444 life stages and temporary attachment to substrates. Such taxa are likely to be insects
51
52 445 with seasonal life cycle dynamics, for example Simuliidae, whose abundance peaked
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54 446 in spring with temporary attachment enabling rapid migration to refuges as drying
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3 447 commences. That such taxa were not non-perennial indicators suggests non-
4
5 448 discriminant seasonal colonisation of reaches with contrasting flow permanence
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8 449 regimes.

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11 450 Indicator values were generally higher for functional traits than taxa, highlighting the
12
13 451 potential of traits as ecological indicators of environmental conditions (Sarremejane
14
15 452 et al., 2017a). Functional responses may facilitate clearer observations of responses
16
17 453 to changing environmental conditions (such as hydrology) as there is less 'noise' in
18
19 454 datasets, due to functional redundancy (García-Roger, 2017), and some traits such
20
21 455 as resistance forms may enable the persistence of multiple taxa at non-perennial
22
23 456 sites (Boersma et al., 2014). Including trait-based assessments in ecological
24
25 457 monitoring may therefore enhance fundamental understanding of the links between
26
27 458 biodiversity and ecosystem function (Monk et al., 2018; Reiss, Bridle, Montoya, &
28
29 459 Woodward, 2009).

35 460 ***4.2 Heterogeneity in macroinvertebrate community composition of perennial*** 36 37 461 ***and non-perennial river reaches***

38
39 462 Taxonomic and functional communities inhabiting non-perennial sites were
40
41 463 characterised by greater heterogeneity than perennial river reaches. Such variability
42
43 464 may reflect higher temporal and spatial habitat heterogeneity (Bonada et al., 2007;
44
45 465 Datry et al., 2014b; Cid et al., 2017), which allows a diverse range of aquatic taxa to
46
47 466 sequentially inhabit lotic and lentic instream habitats (Bogan & Lytle, 2007; Munné &
48
49 467 Prat, 2011; Giam et al., 2017; Tonkin et al., 2017). Overall, functional assemblage
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51 468 compositions were temporally and spatially stable in this study (demonstrated by the
52
53 469 significantly lower heterogeneity values compared to taxonomic communities),
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55 470 despite hydrological variability which encompassed drought events and periods of
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3 471 above-average precipitation, suggesting their potential as stable indicator (Bêche et
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5 472 al., 2006; Stutzner & Bêche, 2010; Dolédec, Tilbian & Bonada, 2017).
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8 473 Some evidence of seasonal effects was detected in community heterogeneity, with
9
10 474 non-perennial sites demonstrating reduced heterogeneity in autumn in both
11
12 475 taxonomic and functional communities, whereas perennial reaches did not differ in
13
14 476 heterogeneity among seasons. Autumn is typically when the influence of flow
15
16 477 permanence and low flows on aquatic communities is most evident in temperate-
17
18 478 zone streams, due to recent flow resumption. Non-perennial sites may be more
19
20 479 temporally heterogeneous in spring dependent on dispersal of recolonists from
21
22 480 refuges following flow resumption; these processes are likely to be highly variable
23
24 481 (Cañedo-Argüelles et al., 2015; Sarremejane, Mykrä, Bonada, Aroviita, & Muotka,
25
26 482 2017b). For example, Perrow, Leeming, England, & Tomlinson (2007) noted specific
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28 483 flowing-water species at some Misbourne sites after flow resumption following the
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30 484 1996/1997 drought that had not been recorded elsewhere in the river, thereby
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32 485 increasing heterogeneity.
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39 486 **4.3 Perennial and non-perennial macroinvertebrate community associations** 40 41 487 **with environmental variables**

42
43 488 Our results indicate that hydrological metrics were the primary factors structuring
44
45 489 functional and taxonomic macroinvertebrate communities, most notably in spring,
46
47 490 and for functional communities. Trait-based approaches may provide a robust means
48
49 491 of assessing ecological responses to hydrology across regions with different taxon
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51 492 pools (White et al., 2017a). The high temporal stability in functional communities can
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53 493 help facilitate discrimination of spatial environmental differences in assemblage
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55 494 composition (Cid et al., 2016; García-Roger 2017) and may explain why spring
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3 495 functional communities demonstrated the strongest association to a measured
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5 496 environmental parameter.
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8 497 The % of zero-flow days in the previous summer (April–September) was of greatest
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10 498 importance in structuring spring communities. Wetted channel characteristics were
11
12 499 also significant drivers, with width demonstrating a significant correlation in all four
13
14 500 models and depth in all but one. Autumn communities were more strongly correlated
15
16 501 with substrate conditions (% silt, pebble or gravel) that characterise the legacy of
17
18 502 hydraulic conditions. These results provide further evidence that localised hydraulic
19
20 503 observations may improve the characterisation of forces that biota are responding to
21
22 504 at the time of sampling compared to the use of discharge-related statistics alone
23
24 505 (Malcolm et al., 2012b; Monk et al., 2018; White et al., 2019)
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26
27 506 Functional redundancy may promote community resilience to environmental variation
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29 507 (Schriever et al., 2015; Oliver et al., 2015) and may explain the lack of separation
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31 508 between functional communities in perennial and non-perennial river reaches in the
32
33 509 autumn. Non-perennial communities in autumn may represent a subset of those from
34
35 510 perennial reaches (Arscott et al., 2010; Datry, 2012; Datry et al., 2014a), comprising
36
37 511 resistant representatives that are able to persist in moist habitats/substrates (e.g.
38
39 512 *Galba truncatula*; Stubbington & Datry, 2013). In contrast, spring communities also
40
41 513 include taxa which may have recolonised non-perennial reaches from perennial
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43 514 sources upstream or persisted in-situ following flow resumption. The composition of
44
45 515 functional processes may therefore have been altered resulting in separation of the
46
47 516 two functional flow permanence communities during spring. In contrast taxonomic
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49 517 composition demonstrated some overlap in perennial and non-perennial river
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51 518 reaches but differences in composition were evident in both spring and autumn.
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519 **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.

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540 constructive comments.

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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2
3 544 (sites 2-5) are available on request from the Environment Agency via

4
5 545 <http://apps.environment-agency.gov.uk/contact/>.

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11 547 **List of Figures**

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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)
21
22 551 perennial river reaches on the River Misbourne during the study period. Note:
23
24 552 gauging records were not present at sites 1-4 until 1994. Site 3 (non-perennial) and
25
26 553 site 6 (perennial) are shown.

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29
30 554 **Figure 3.** Redundancy analysis (RDA) of: (a) spring taxonomic; (b) autumn
31
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-
35
36 557 perennial sites = black. Only significant environmental parameters influencing faunal
37
38 558 data are presented. % W1 zero-flow days = % of winter zero-flow days; % S1 zero-
39
40 559 flow days and % S2 zero-flow days = % of summer zero-flow days; mean W1 =
41
42 560 mean winter discharge; mean S1 and S2 = mean summer discharge. Two years of
43
44 561 antecedent summer (April–September) and one year of winter (October–March) data
45
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	<i>p</i>	F	<i>p</i>
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 r²	20.73		14.16	
Conditional r²	23.04		17.90	

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	<i>p</i>	F	r ²	<i>p</i>
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

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For Peer Review

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3 **911** **Table 4.** Summary of the top ten indicator taxa and traits for non-perennial and perennial flow regimes on global (spring and autumn)
4 dataset.

5 **912**

Taxonomic			Functional		
Taxa	Indicator value	<i>p</i> -value	Trait	Indicator value	<i>p</i> -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			Perennial		
Elmidae	0.811	0.001	Aquatic stage: adult	0.729	0.003
Gammaridae	0.772	0.001	Food consumed: living microphytes	0.728	0.004
Sericostomatidae	0.728	0.001	Voltinism: 1	0.721	0.002
Baetidae	0.697	0.039			
Ephemeraeidae	0.690	0.001	Perennial		
Erpobdellidae	0.684	0.001	Voltinism: <1	0.776	0.001
Hydrobiidae	0.658	0.001	Maximal potential size: >8cm	0.775	0.001
Hydropsychidae	0.653	0.002	Respiration: plastron	0.768	0.001
Goeridae	0.615	0.001	Locomotion: burrower	0.761	0.001
Glossiphoniidae	0.609	0.030	Reproduction strategy: isolated eggs, cemented	0.759	0.001
			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

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33 * 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			

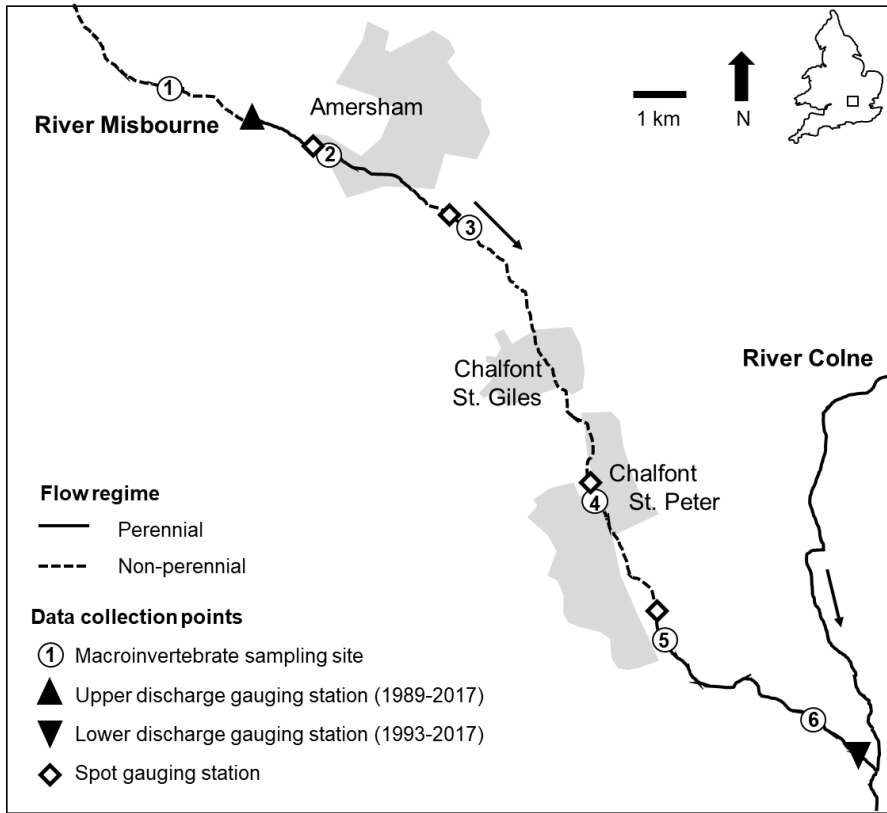


Figure 1.

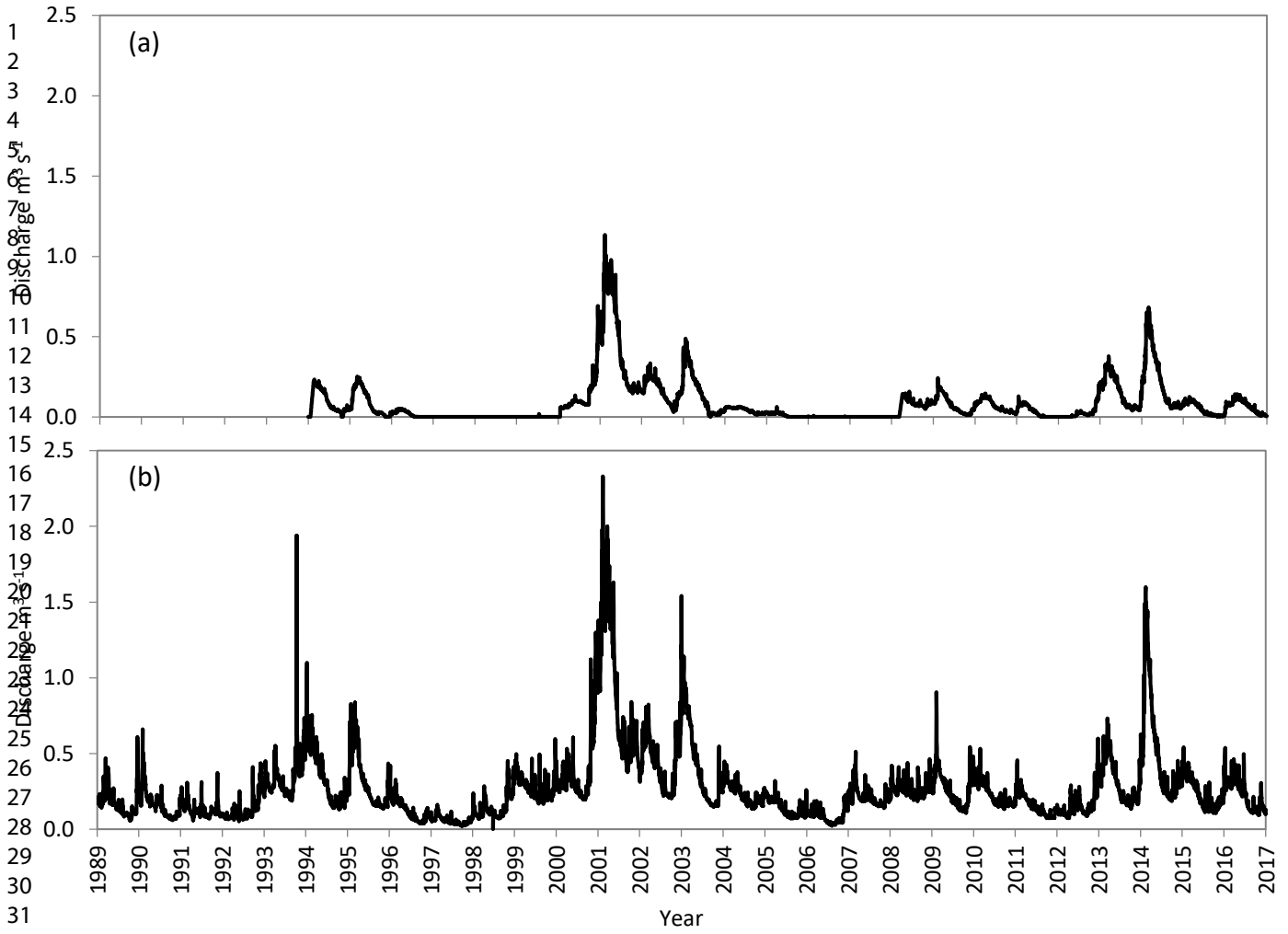


Figure 2.

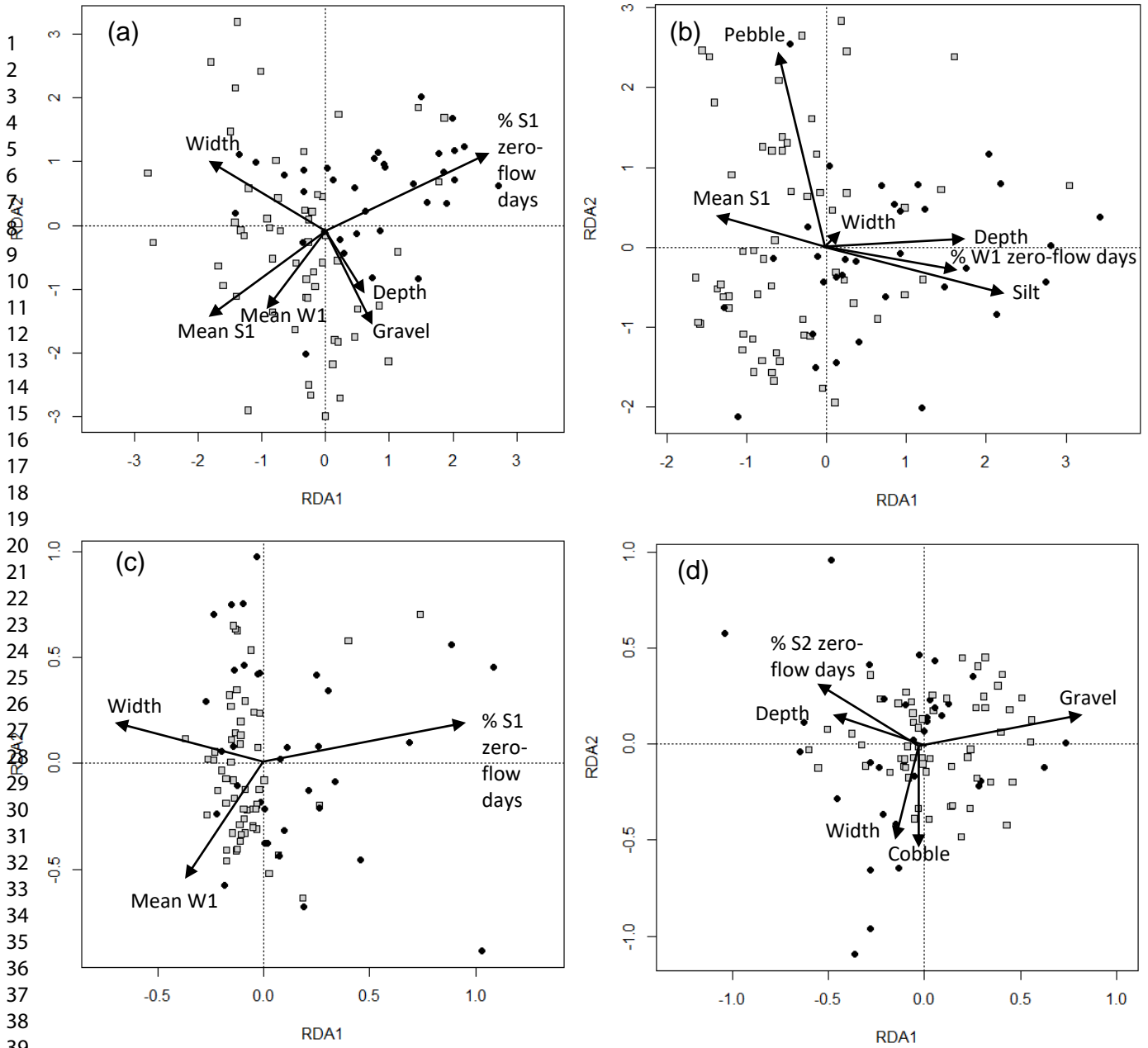


Figure 3.

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

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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	Food consumed		Scraper
Isolated, free eggs	Filter-feeder			
Isolated eggs, cemented	Piercer			
Clutches, cemented eggs	Predator			
Clutches, free	Parasite			
Clutches, in vegetation	Microorganisms			
Dispersal strategy	Asexual		Detritus < 1 mm	
	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			

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.

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	<i>p</i> -value	Trait	Indicator value	<i>p</i> -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
			Respiration: tegument	0.740	0.001
Perennial			Reproduction strategy: clutches, cemented eggs	0.736	0.002
Elmidae	0.796	0.001	Aquatic stage: nymph	0.734	0.045
Gammaridae	0.781	0.001	Feeding group: scraper	0.732	0.001
Sericostomatidae	0.738	0.001	Food consumed: living microphytes	0.726	0.009
Hydropsychidae	0.714	0.002	Voltinism: 1	0.717	0.009
Erpobdellidae	0.697	0.002			
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

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	<i>p</i> -value	Trait	Indicator value	<i>p</i> -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
			Feeding group: scraper	0.726	0.015
Perennial			Maximal potential size: > 1-2cm	0.726	0.008
Elmidae	0.822	0.001	Dispersal strategy: aquatic passive	0.722	0.001
Gammaridae	0.762	0.002	Voltinism: 1	0.722	0.05
Sericostomatidae	0.718	0.001	Maximal potential size: < 0.25cm	0.641	0.006
Ephemeraeidae	0.687	0.007			
Erpobdellidae	0.669	0.001	Perennial		
Simuliidae	0.648	0.016	Respiration: plastron	0.776	0.001
Hydrobiidae	0.647	0.002	Voltinism: < 1	0.775	0.001
Goeridae	0.632	0.002	Maximal potential size: > 8cm	0.768	0.001
Odontoceridae	0.582	0.002	Locomotion: burrower	0.761	0.001
Psychomyiidae	0.537	0.005	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

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

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