1   Extra terrestrials: drought creates niche space for rare invertebrates in a large-

2   scale and long-term field experiment

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4   Thomas W.H. Aspin[1,2]*, Kieran Khamis[1], Thomas J. Matthews[1,3,4], Gavin M.D. Williams[1], Fredric M.

5   Windsor[5], Guy Woodward[6], Mark E. Ledger[1]

6   [1]School of Geography, Earth and Environmental Sciences, University of Birmingham, UK

7   [2]Environment Agency, Bristol, UK

8   [3]Birmingham Institute of Forest Research, University of Birmingham, Birmingham, B15 2TT, UK.

9   [4]CE3C – Centre for Ecology, Evolution and Environmental Changes/Azorean Biodiversity Group /

10   CHANGE – Global Change and Sustainability Institute and Universidade dos Açores – Faculty of

11   Agricultural Sciences and Environment, PT-9700-042, Angra do Heroísmo, Açores, Portugal.

12   [5]School of Biosciences, Cardiff University, UK

13   [6]Georgina Mace Centre for the Living Planet, Department of Life Sciences, Imperial College London,

14   Silwood Park, Ascot, Berkshire, UK SL5 7PY

15   *Email: twhaspin@gmail.com ORCID ID: 0000-0003-1599-1532

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22 **Abstract**

23 Freshwater habitats are drying more frequently and for longer under the combined pressures of

24 climate change and overabstraction. Unsurprisingly, many aquatic species decline or become locally

25 extinct as their benthic habitat is lost during stream droughts, but less is known about the potential

26 'winners'– those terrestrial species that may exploit emerging niches in drying riverbeds. In

27 particular, we do not know how these transient ecotones will respond as droughts become more

28 extreme in the future. To find out we used a large-scale, long-term mesocosm experiment spanning

29 a wide gradient of drought intensity, from permanent flows to full streambed dewatering, and

30 analysed terrestrial invertebrate community assembly after one year. Droughts that caused stream

31 fragmentation gave rise to the most diverse terrestrial invertebrate assemblages, including 10

32 species with UK conservation designations, and high species turnover between experimental

33 channels. Droughts that caused streambed dewatering produced lower terrestrial invertebrate

34 richness, suggesting that the persistence of instream pools may benefit these taxa as well as aquatic

35 biota. Particularly intense droughts may therefore yield relatively few 'winners' among either

36 aquatic or terrestrial species, indicating that the threat to riverine biodiversity from future drought

37 intensification could be more pervasive than widely acknowledged.

38

39 **1. Background**

40 Climate change and overabstraction of water are leading to increased occurrence of droughts in

41 rivers and streams [1]. As wetted habitat shrinks, aquatic invertebrate species are lost [2], and the

42 reciprocal expansion of dry streambed leads to an increase in terrestrial invertebrate biodiversity [3].

43 However, these initial terrestrial species gains may be reversed as a drought further intensifies, as

44 riverbeds become inhospitable and relict aquatic resources are exhausted [3]. Our currently limited

45 understanding of these dynamics relies on observational data from seasonally dry streams, so we

46   know little of how terrestrial invertebrate communities might develop during the extreme,

47   prolonged droughts set to become increasingly common [4].

48

49   In theory, particularly intense drying should expose species to harsher environmental conditions and

50   more severe food resource shortages, thus eroding terrestrial biodiversity, consistent with the

51   intermediate disturbance hypothesis [5]. Conversely, an increase in alpha diversity would be

52   predicted by the species–area relationship as more terrestrial habitat becomes available [6], and

53   would additionally be expected as plant succession creates greater niche space for invertebrates. It

54   is also unclear how the trajectory of terrestrial community development may vary in space during

55   prolonged drying, hampering our ability to predict the impacts of droughts on biodiversity patterns

56   among reaches (i.e., beta diversity). Beta diversity may increase as different reaches follow different

57   successional trajectories, or decline as complex wetland community types are replaced by a more

58   uniform, dry channel. As future drought intensification gives rise to novel river- and reach-specific

59   drying regimes, evidence of how terrestrial alpha and beta diversity respond will increasingly be

60   required to inform adaptive river management and conservation.

61

62   Experiments are needed to expose ecological communities to these possible future conditions [7] in

63   isolation from the confounding environmental gradients that beset field survey data [8]. We

64   therefore simulated year-long (i.e., supraseasonal) droughts of varying intensity using artificial

65   stream channels (mesocosms), and characterised the terrestrial invertebrate assemblages that

66   developed. Drought treatments ranged from flowing streams retaining connectivity among riffles

67   and pools, through to the disconnection of these habitats and, ultimately, to complete streambed

68   drying. We analysed differences in invertebrate alpha and beta diversity between these treatments,

69   thus exploring the potential impacts of drought intensification on terrestrial community assembly at

70   local and network scales.

71

2. Methods

(a) Drought experiment and data collection

We used outdoor mesocosms to replicate conditions in perennial headwater streams, in Hampshire, UK [further details given in 8]. Of the 21 channels in the experiment, we used 18 for this study, with the remainder unable to be assigned to a particular treatment as their riffle habitat was partially but not fully submerged. All channels had gravel beds with riffle-pool sequences (four per channel), analogous to their natural counterparts. The channels were fed by borehole water and seeded with a "common garden" of water crowfoot (*Ranunculus penicillatus* subsp. *pseudofluitans*), algae, and aquatic invertebrates from the adjacent chalk stream. Following six months of aquatic community establishment under ambient flow, we left three channels as controls and adjusted flows across the remainder to create a gradient of drought intensity. This gradient spanned three characteristic habitat states [2]: (i) flowing channels, with no dry substratum (connected [*CON*]; n = six channels); (ii) fragmented channels, with approximately 50 % dry substratum and isolation of pool habitats (fragmented [*FRAG*]; nine channels); and (iii) dewatered streambeds, with 95-99% dry substratum (dry [*DRY*]; three channels). These treatments were designed to capture a broad spectrum of hydrological states, from stable flows through to the harsh, patchy conditions, including prolonged ponding and drying, associated with supraseasonal drought [9,10].

After one year of drought, channels from each treatment had developed plant communities representative of the major hydrological classification groups of ephemeral chalk stream macrophytes [10], driven by wind dispersal of seeds (see supplementary material, Figure S1). There was a shift from fully aquatic taxa such as water crowfoot and water parsnip (*Berula erecta*) in connected streams to emergent (e.g. watercress; *Nasturtium officinale*) and wetland species (e.g.

95   reed canary grass; *Phalaris arundinacea*) in fragmented channels. The riffle habitats of fragmented

96   channels had largely terrestrialised and so these channels also supported many of the non-aquatic

97   herbs that dominated dry streams, such as willowherbs (*Epilobium* spp.), mayweed

98   (*Tripleurospermum inodorum*) and nettle (*Urtica urens*). As plant growth is a key driver of terrestrial

99   invertebrate diversity in riverine environments [11], we estimated the total volume of terrestrial

100   vegetation in each channel. The percentage cover of plants was estimated across the top three

101   riffles and three pools per channel at the end of the experiment. The volume (m^3) of each plant

102   taxon was calculated from its areal coverage (m^2) multiplied by plant stand height (m). We then

103   sampled terrestrial invertebrates through exhaustive (i.e. until no further individuals could be found)

104   sweep netting and hand searching [see 12] of one randomly selected riffle-pool pair (1.5 m^2) to yield

105   a single invertebrate sample for each channel. Invertebrates were collected from dry gravels,

106   emergent and terrestrial plants and, in connected channels, from emergent fronds of water

107   crowfoot. Invertebrate specimens were identified to species wherever possible, with aphids

108   (Aphidae), chalcid wasps (Chalcidoidea), springtails (Collembola), vinegar flies (Drosophilidae) and

109   non-biting midges (Chironomidae) identified to family level.

110

111   **(b) Statistical analyses**

112   All analyses were conducted in R (version 4.2.2) [13,14]. We quantified differences in invertebrate

113   community composition between treatments using non-metric multidimensional scaling (NMDS) and

114   then tested for significant differences in alpha diversity. To account for the underlying influence of

115   relative abundance on species detection success (and thus diversity estimation), we equalised

116   samples by adjusting for sample coverage, which allows for fairer comparisons of diversity estimates

117   drawn from communities with greater or lesser proportions of rare species [see 15]. We compared

118   samples at 90% coverage (i.e. the level of sample completeness giving a 10% probability that the

119   next recorded individual will belong to a previously undetected species), dropping a single sample

120   from all further analyses as it exhibited a particularly low coverage value (60%), and was therefore

121   not deemed to be representative of the community in the (connected) channel from which it was

122   collected. We then calculated alpha diversity as Hill-Shannon diversity to afford similar sensitivity to

123   rare and common species and retain intuitive scaling behaviour (i.e. proportional to changes in

124   richness [15]). We compared Hill-Shannon diversities using a Kruskal-Wallis one-way analysis of

125   variance to account for different numbers of replicates per group. Following rejection of the null

126   hypothesis (no significant difference between treatments), we conducted Conover-Imam tests to

127   determine which treatments differed significantly in alpha diversity, controlling for the false

128   discovery rate using the Benjamini-Hochberg procedure [16].

129

130   Beta diversity, interpreted here as the dissimilarity in species composition among the communities

131   of two (i.e. pairwise dissimilarity) or more (i.e. multiple site dissimilarity) channels [17], was

132   calculated and decomposed into turnover (species replacement) and nestedness-resultant (species

133   gain/loss; hereafter NRD) components using the partitioning methods of Baselga [18]. Under this

134   framework, total beta diversity is calculated as Sorensen dissimilarity (β_{sor}), turnover as Simpson

135   dissimilarity (β_{sim}) and NRD as the difference between these ($\beta_{sor} - \beta_{sim} = \beta_{nes}$). High turnover would

136   imply the presence of many species unique to certain channels; whereas high NRD would signify (i) a

137   nesting of species-poor assemblages within richer ones, and thus (ii) greater overlap in species'

138   identities among channels [18].

139

140   We calculated (1) pairwise measures of beta diversity (β_{sor}, β_{sim} and β_{nes}), to analyse turnover and

141   NRD between the pooled communities of each of the three treatments; and (2) multiple site

142   dissimilarity (β_{SOR}, β_{SIM} and β_{NES}) to compare the communities of all channels within each treatment

143   [18]. As each treatment contained a different number of channels, with a minimum of three (dry),

144   we accounted for a sampling effort effect by conducting analyses on random subsets of three

145 channels. We calculated beta diversity for 100 combinations of paired treatment subsample pools

146 (pairwise measures) and for 100 combinations of treatment-specific subsamples (multiple site

147 measures). The final results were obtained by taking the mean and 95% confidence intervals of these

148 100 repeats.

149

150 **3. Results**

151 We recorded 166 terrestrial invertebrate taxa, 158 of which were found in fragmented and dry

152 streams, and 131 of which were unique to these channels (full taxa list in supplementary material,

153 Table S1). Taxa from connected channels were predominantly dipterans with an aquatic larval phase

154 (but collected in their adult form so considered here as terrestrial invertebrates; e.g., non-biting

155 midges, shore flies (Ephydridae) and dagger flies (Empididae)), while dry channels were associated

156 with numerous species of arachnid and hymenopteran (Figure 1). Assemblages in fragmented

157 channels were not simply intermediate combinations of those found in the other treatments but

158 were instead diverse and distinct, comprising beetles, true bugs, dipterans and arachnids (Figure 1),

159 reflecting high terrestrial plant coverage (see supplementary material, Figure S2). These channels

160 harboured nine nationally scare species (i.e. those with species quality scores (SQS) of 4 in Pantheon

161 (https://pantheon.brc.ac.uk/lexicon/sqs)) while a further species of conservation note, the UK

162 Biodiversity Action Plan (BAP) moth *Scotopteryx chenopodiata*, was recorded in both fragmented

163 and dry channels. Hill-Shannon diversity differed between treatments (Kruskal-Wallis $\chi^2(2)$ = 13.2, *p*

164 = 0.001), being significantly greater in fragmented channels (mean = 21 ± 4) than in both connected

165 (5 ± 3; Conover-Imam t(12) = 7.91, *p* < 0.001) and dry streams (11 ± 2; t(10) = 3.97, *p* = 0.001). The

166 latter also contained significantly richer communities than connected channels (t(6) = 2.42, *p* =

167 0.015).

168

169   Communities of connected channels differed substantially from those of both fragmented (β_{sor} =

170   0.73) and dry (β_{sor} = 0.66) streams. This was driven primarily by NRD (β_{nes} = 0.41) and turnover (β_{sim} =

171   0.49) respectively (Figure 2a). Fragmented and dry channel communities were relatively similar to

172   each other (β_{sor} = 0.53), with turnover the dominant component (β_{sim} = 0.32). There were also large

173   differences among channels within each treatment (connected β_{SOR} = 0.63; fragmented β_{SOR} = 0.64;

174   dry β_{SOR} = 0.65; Figure 1b). Connected channel communities differed from one another due to both

175   turnover (β_{SIM} = 0.30) and NRD (β_{NES} = 0.33), whereas differences among fragmented and to a slightly

176   lesser extent dry channel communities were largely attributable to turnover (β_{SIM} = 0.60 and 0.54

177   respectively).

178

179   **4. Discussion**

180   This study has shown that streams exposed to supraseasonal drying can support diverse and distinct

181   terrestrial invertebrate communities and provide refuges for rare species. However, dry streambeds

182   did not support the most diverse or notable species assemblages, demonstrating that the

183   persistence of instream pools through droughts, which are crucial for aquatic biota [19], could also

184   be invaluable for terrestrial fauna. We therefore present rare experimental evidence that future

185   drought intensification could threaten terrestrial as well as aquatic biodiversity across impacted

186   riverscapes.

187

188   Some differences in invertebrate community composition were apparent between our channels and

189   that typical of riparian habitats and seasonally dry streams. Notable early riparian colonists of dry

190   streambeds, such as ground beetles (Carabidae) [11], were absent from our samples, but the

191   widespread presence of other common inhabitants of riparian zones, such as rove beetles

192   (Staphylinidae) and money spiders (Linyphiidae), suggests that this is unlikely to reflect the lack of

193   riparian habitat in the mesocosms. It could instead point towards a key difference between the

194   effects of seasonal drying and prolonged drought on terrestrial species composition, with

195   adaptations that allow rapid colonisation of newly dry streambeds, such as inundation tolerance and

196   strong flight among some carabids [11,20], becoming significantly less advantageous over longer dry

197   periods. Further studies of prolonged stream droughts are needed to explore this.

198

199   The absence of some seasonally dry streambed specialists did not prevent the emergence of high

200   species diversity in fragmented channels, which partly reflected a prevalence of phytophagous

201   insects including leaf beetles (Chrysomelidae), mirid bugs (Miridae) and weevils (Curculionidae),

202   consistent with high terrestrial plant coverage. Several of the nationally scarce species we recorded

203   have close associations with specific plants, such as *Gymnetron veronicae* with speedwells (*Veronica*

204   spp.) and *Drupenatus nasturtii* with cresses (*Nasturtium* spp.). As riparian plants close to a stream

205   can differ markedly from those further away, reflecting differences in subsurface moisture and

206   humidity [11], so those of fragmented streams would also seem to vary analogously between the

207   centre of an exposed riffle and its margins. Pool and riffle interfaces were colonised by wetland

208   plants (e.g., *V. anagallis-aquatica*), contrasting with the more terrestrial species (e.g., *T. inodorum*

209   and *U. urens*) found in drier gravels. In fragmented streams, as in riparian zones, this patchiness

210   would appear to produce high invertebrate richness due to the niche space afforded to

211   monophagous taxa (see supplementary material, Figure S3).

212

213   Patchiness and host specificity could be strong drivers of the high species turnover we observed

214   between different fragmented and dry channels, which arose despite their close spatial proximity

215   (see supplementary material, Figure S4). This contrasted with the high nestedness observed

216   between connected channels, which itself could reflect differences in the timing of insect emergence

217   between streams. In fragmented channels turnover appeared to be driven partly by shifts in habitat

availability between treatments (e.g. semi-aquatic beetles present in fragmented but not dry

channels) but to a greater extent by patch dynamics, with fragmentation generating discrete areas

of streambed with different successional trajectories. For instance, while some fragmented pools

were dominated by emergent plants (e.g. *V. anagallis-aquatica*, *N. officinale*), others retained a

sizeable coverage of *R. penicillatus* in varying growth forms (see supplementary material, Figure S5).

Supraseasonal drought may therefore produce high terrestrial biodiversity among fragmented and

dry reaches at the stream network (i.e. metacommunity) scale, a pattern that contrasts with the loss

of beta diversity widely observed among insect communities in response to climate and land-use

change [21].

Our study provides rare experimental evidence of a mechanistic relationship between drought

intensity and riverine terrestrial biodiversity. Its findings suggest that drying stream channels can

provide important habitat for rare and threatened species, a particularly notable observation amid

growing concerns over the impacts of natural habitat loss and other pressures on terrestrial insect

populations [22]. The difference in alpha diversity between fragmented and dry streams reported

here nonetheless highlights the importance of drought-resilient surface water refuges in adaptive

river management and conservation.

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293 **Figure 1** Non-metric multidimensional scaling output (stress = 0.1) illustrating differences in

294 assemblage composition between treatments, with species scores displayed by taxonomic order.

295 Shaded polygons are the minimum convex hulls that encompass all the channels in each treatment

296 and photos depict an example channel from each treatment (clockwise from bottom left: connected,

297 fragmented and dry). Labelled species are those designated nationally scarce in the UK.

298 **Figure 2** Pairwise Sorensen dissimilarities between the pooled communities of connected,

299 fragmented and dry treatments, partitioned into turnover (β_{sim}) and nestedness-resultant (β_{nes})

300 components (top) and multiple site dissimilarities among channels within each treatment (bottom).

301 Error bars in both panels display +95% CI.

302