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Increased depth to the water table during river drying decreases the resilience of *Gammarus pulex* and alters ecosystem function

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ABSTRACT

River drying has drastic immediate effects on benthic invertebrates, but their high resilience reduces long-term effects on biodiversity and ecosystem functions (e.g. leaf litter decomposition). The hyporheic zone (saturated sediments below the riverbed) can be a refuge for invertebrates in dry rivers and a primary source of colonists supporting population resilience following flow resumption. However, factors such as the depth to the water table below the riverbed, which could determine the quality of the hyporheic refuge, are not well understood. We explored how depth to the water table (control, −5 cm, −30 cm and completely dry) during a 1-week drying event affected the survival and return to the substrate surface (%RTS) of *Gammarus pulex* (Crustacea : Amphipoda) using laboratory mesocosms. We measured litter decomposition and *Gammarus* energy stores to examine effects on ecosystem function and energetic costs for organisms crawling deeper into the hyporheic zone. Two populations, collected from intermittent and perennial rivers, were used to evaluate inter-population variability in response to drying. Survival and %RTS were reduced by ≤39% and 52%, respectively, in the −30 cm and dry treatments, and this had consequences for decomposition (≤46% reduction). Differences between populations in %RTS were high across treatments but did not generally affect survival and decomposition. Our results suggest that increases in depth to the water table during river drying, which often result from longer drying duration and water abstraction, could reduce invertebrate resilience and litter decomposition by diminishing the role of the hyporheic zone as a source of colonists.

KEY WORDS hyporheic zone; recovery; intermittent rivers; alluvial rivers; leaf litter decomposition; mesocosms; climate change; water abstraction

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INTRODUCTION

In many regions, intermittent rivers (i.e. those that cease to flow and experience periodic loss of surface water) comprise the majority of river networks (Acuña *et al.*, 2014; Datry *et al.*, 2014a). Moreover, global climate change and anthropogenic pressures (e.g. water abstraction) are increasing the frequency and duration of drying events and can even lead to drying of perennial rivers (Gleick, 2003; Döll and Schmied, 2012; Jaeger *et al.*, 2014). River drying is an important driver of benthic invertebrate population dynamics and can lead to the immediate loss of species richness and changes in species composition (Bogan and Lytle, 2011; Datry *et al.*, 2014b) and subsequent decreases in key ecosystem functions such as leaf litter decomposition (LLD; Langhans and Tockner, 2006; Corti *et al.*, 2011; Datry *et al.*, 2011). However, resilience (i.e. capacity to return to pre-disturbance levels) of invertebrate populations following flow resumption can be high, reducing long-term effects of river drying on biodiversity and ecosystem functioning (Fritz and Dodds, 2004; Leigh *et al.*, 2015; Vander Vorste *et al.*, 2015a). For example, invertebrate taxonomic richness and functional diversity in intermittent alluvial rivers typically recover less than 1 month after flow resumption (Fowler, 2004; Vander Vorste *et al.*, 2015a). Yet, in other cases, the resilience of populations can be low (e.g. Wood and Armitage, 2004), and this can have negative effects on ecosystem function by altering rates of LLD (e.g. Datry *et al.*, 2011). Understanding the processes that promote population resilience has become a major research focus of freshwater ecology because it can lead directly to management measures and restoration

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approaches in river systems that maximize their resilience to future environmental change (Lake *et al.*, 2007; Palmer *et al.*, 2008).

The vertical migration of benthic invertebrates into and from the hyporheic zone (i.e. the saturated sediments below and adjacent to the riverbed; White, 1993) can promote population resilience in rivers (Williams and Hynes, 1976; Dole-Olivier, 2011; Vander Vorste *et al.*, 2015b). During drying events, invertebrates avoid high water temperatures and intraspecific competition (e.g. Vander Vorste *et al.*, 2015c) and desiccation (e.g. Vadher *et al.,* 2015) by migrating into the hyporheic zone. When flow resumes, the hyporheic zone can then be the primary source of colonists promoting population resilience (Vander Vorste *et al.*, 2015b). However, the potential of the hyporheic zone to promote resilience varies with its accessibility to invertebrates. For instance, fine sediments can prevent invertebrate migration into the hyporheic zone (Navel *et al.*, 2010; Descloux *et al.*, 2013; Vadher *et al.,* 2015). Consequently, LLD is reduced when invertebrate detritivores are unable to migrate into or return from the hyporheic zone, as shown in mesocosm experiments (Navel *et al.*, 2010; Vander Vorste *et al.*, 2015c). Identifying other factors (e.g. depth to the water table) that limit vertical migration of invertebrates is important to quantify the potential of the hyporheic zone to mitigate the effects of river drying (Dole-Olivier, 2011; Stubbington, 2012).

The depth to the water table below the substrate surface (i.e. the thickness of the vadose zone between the riverbed surface and the saturated hyporheic zone) could influence whether the hyporheic zone is used as a refuge during drying events. The depth to the water table generally remains shallow in river reaches with upwelling conditions (gaining reaches); whereas, depth can gradually increase during drying in reaches with downwelling conditions (losing reaches) because of transmission loss of surface water into the substrate (Boulton, 2003; Larned *et al.*, 2011). In the latter case, organisms will be forced to burrow or crawl further into the hyporheic zone, which will require energy (Shepard *et al.*, 2013) and increase the risk of becoming stranded in dry substrate (Stumpp and Hose, 2013). It is still unclear how depth to the water table influences the subsequent return of invertebrates to the substrate surface and if there are negative effects on LLD, which depends on invertebrate resilience in intermittent rivers. As more perennial rivers become intermittent because of climate change and surface and groundwater abstraction (Gleick, 2003; Döll and Schmied, 2012; Jaeger *et al.*, 2014), understanding how increasing depth to the water table influences populations and ecosystem function is necessary to predict the effects of increased drying.

The shift of perennial to intermittent flow regimes in many regions has also sparked the need to consider the population-level variability in the response of aquatic organisms to drying. Local adaptation, the process in which traits have evolved differently among populations (i.e. divergent selection), could cause one population to outperform (e.g. higher survival) another population if both populations are exposed to identical conditions (Kawecki and Ebert, 2004). For example, populations of adult *Galba truncatulata*, a freshwater snail, collected from intermittent rivers were more tolerant to desiccation than populations from perennial rivers (Chapuis and Ferdy, 2012). This suggests that a similar response of populations originating from intermittent and perennial rivers to drying cannot be assumed.

In this study, we explored how water level during a 1-week drying event influenced the resilience, measured as survival and % of individuals returned to the substrate surface (%RTS), of benthic invertebrate populations and LLD following re-inundation. We tested this using *Gammarus pulex* (Crustacea : Amphipoda), a common and important invertebrate detritivore that inhabits both intermittent and perennial European rivers. We hypothesized that lowering the water level below the substrate surface would reduce population resilience following re-inundation because organisms would face a higher risk of desiccation from having to crawl further into the hyporheic zone. In turn, we hypothesized that lowering water levels would reduce LLD through its effects on population resilience. Lastly, we hypothesized that glycogen energy stores of *G. pulex* would be reduced when levels were lowered as a result of the increased energetic costs of migrating further into the hyporheic zone to find saturated conditions. We tested for homogeneity in the response to drying and hyporheic zone use of two populations of *G. pulex*, one collected from an intermittent river and one from a perennial river, to evaluate the potential influence of local adaptation.

METHODS

Study organism and collection sites

Gammarus pulex was used to test our hypotheses about the effects of lowering water levels below the substrate surface on population resilience, LLD and energy stores because it is a common species across European streams and plays an important role in LLD (Mathews, 1967; Dangles and Malmqvist, 2004; Handa *et al.*, 2014). Two populations of *G. pulex* were collected within 1 week of each other (April 2015) from small tributaries within the Rhône River Valley, France. Population I (PopI) was collected from the intermittent Seguissous River near Bouquet, France (04°16′20.4″E, 44°10′06.1″N). Seguissous is a third-order river (width = 3 m, depth = 0.5 m; average at sampling location) with coarse gravel substrate that dried during the summer months of the sampling year (re-visited August

2015) like most rivers of this size in the Mediterranean region of France (Snelder *et al.*, 2013). Population P (PopP) was collected from a tributary to the Suzon River near Dijon, France (04°52′57″E, 47°24′13″N). This small, first-order tributary (width = 1.5 m, depth = 0.3 m: average at sampling location) also has coarse gravel substrate but flows perennially, as confirmed by previous visits (February–July 2014; Vander Vorste *et al.*, 2015c).

We confirmed the species identity of each population through morphology and molecular analysis. A subsample of individuals ($n = 100$) from each population was identified using a dissecting microscope and regional taxonomic key (Piscart and Bollache, 2012). Following the experiment, molecular analysis was performed on ten individuals from PopI and seven individuals from PopP (previously been confirmed from this sampling location; Foucreau *et al.*, 2013). For this analysis, DNA [cytochrome c oxidase subunit I (COI)] was extracted (Walsh *et al.*, 1991; Lagrue *et al.*, 2014), amplified (LCO1490 and HCO2198; Folmer *et al.*, 1994) and sequenced (Sanger *et al.*, 1977) to allow comparisons of patristic genetic distances (Lefébure *et al.*, 2006) to be made with known haplotypes (Lagrue *et al.*, 2014). These comparisons confirmed the morphological identification of *G. pulex*, except for one individual from PopP that corresponded to *Gammarus fossarum.* Of the individuals identified as *G. pulex,* corresponding to Group A in Lagrue *et al.* (2014), there was ≤12% patristic distance between the two populations, which is below the threshold (16%) identified by Lefébure *et al.* (2006) for distinct crustacean species. Nevertheless, phylogenetic reconstruction indicated the presence of two clades and therefore potential differences in the response of the two populations to water level treatments because of local adaption to environmental conditions.

Before the experiment, populations were kept in separate aquaria (40 × 22 × 25 cm) in a temperature-controlled (15 ± 2 °C) room and allowed to acclimatize to laboratory temperature, water quality and food source for a 7- to 14-day period (Navel *et al.*, 2010). Dechlorinated tap water was kept at a constant temperature (15 ± 2 °C) using a thermostatic water pump (TECO, Ravena, Italy), and oxygen concentrations were maintained near saturation in aquaria. Alder leaves (*Alnus glutinosa*) were provided as a food source after immersion in river water, pre-filtered to remove any invertebrates, in the laboratory for 10 days to allow for microbial colonization and therefore improve leaf palatability (Graça *et al.*, 1993).

Mesocosm description

Thirty two mesocosms were constructed from opaque PVC tubing (70-cm length × 25-cm diameter, 2-mm thickness) with a PVC end cap, forming a vertical column (Vander Vorste *et al.*, 2015c). Each mesocosm had two sections, a 30-cm surface section and a 40-cm hyporheic section, to allow separation and enumeration of %RTS following drying events. These two sections were fixed during the experiment using PVC flanges (25-cm diameter) that allowed quick separation at the end of the experiment. Coarse gravel (10–14 mm) was extracted from Rhône River, France, to provide a realistic substrate that, based on porosity, would not limit the vertical migration of *G. pulex* (Navel *et al.*, 2010). Gravel was washed and dried to remove organic material before use. Water was continuously pumped from a 1000-l tank into the bottom of the mesocosms using peristaltic pumps at a rate of $1.6\,\mathrm{l\,h^{-1}}$ (one renewal of water volume/24 h), creating a slightly positive vertical hydraulic gradient (i.e. upwelling movement of water). Water exited mesocosms through a hole (2-cm diameter) located 5 cm below the top of each column, which was screened (500-µm mesh) to prevent invertebrates from escaping. Surface water was aerated to keep dissolved oxygen (O_2) concentrations between 8.5 and $9.5\,\mathrm{mg\,l^{-1}}$. A 12:12-h light : dark cycle was applied using Grolux (35 W, 8500 K, Sylvania Inc., Noida, India) aquarium lights above mesocosms. More details on the mesocosms are provided in Vander Vorste *et al.* (2015c), and a short video explaining construction of mesocosms is available (Vander Vorste *et al.*, 2015d).

Experimental design

To explore the influence of lowering water levels below the substrate surface on the resilience of *G. pulex* following re-inundation, a factorial experimental design was used to test the effects of four water level treatments across the two populations of *G. pulex* (four replicate mesocosms per water level × population treatment combination). Water level treatments were control (i.e. no drying), −5 cm and −30 cm below the substrate surface and dry (Figure 1). At the start of the experiment, 120 individuals of *G. pulex* (7- to 10-mm body length; mean dry weight = 7.5 ± 0.9 mg) were placed in each mesocosm using a small hand-net and left to acclimatize for 24 h. Mesocosms were then dried for 1 week by removing a plug from the pre-installed drain at the respective location on each column to allow water to slowly seep out of the mesocosm over a 48-h period. In the −5 cm and −30 cm water level treatments, water was continuously pumped into the hyporheic zone to avoid stagnation after water level treatments were reached. In the dry treatment, water was completely absent from the mesocosms; however, substrate remained moist during the drying event. Moisture content was measured by weighing substrates, collected after 1 week of drying, from two additional mesocosms before and after oven drying for 24 h at 60 °C. In the dry treatment, moisture content during the final day of drying was 0.8 ± 0.1% (Mean ± S.D.) in the upper 10 cm of substrate and 1.8 ± 0.1% in the bottom 10 cm of substrate.

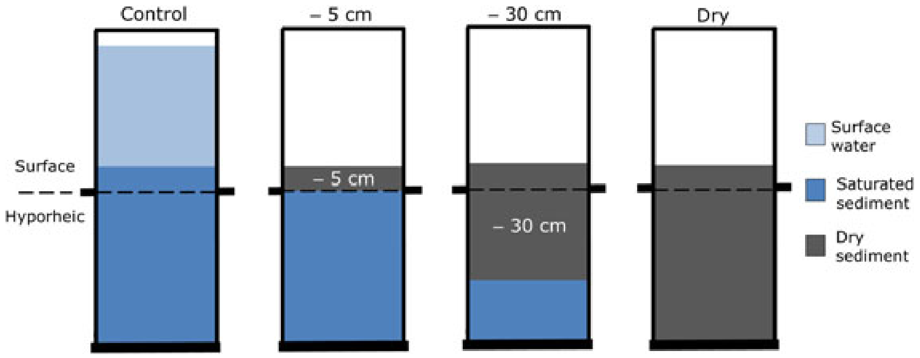

Figure 1. Experimental design used in mesocosms ($n = 32$), showing the four water level treatments (control, −5 cm, −30 cm and dry) with their respective levels of surface water, saturated and dry substrate.

After drying events, drain plugs were reinstalled, and columns were allowed to fill with water to the pre-drying level within 6 h. After 1 week of re-inundation, to allow invertebrates to return from the hyporheic zone to the surface (Vander Vorste *et al.*, 2015b), all individuals were collected from the surface and hyporheic zones of the mesocosms.

Individuals were collected at the end of the experiment by placing mesocosms into a 60 × 80 × 40 cm basin, with care taken to avoid agitation of the surface water that may cause organisms to redistribute vertically. The hyporheic section was then separated from the surface section by removing the stainless steel bolts that attached the two sections of the mesocosm and rapidly sliding the surface zone into the large basin, leaving the hyporheic section of the mesocosm undisturbed. During this process, the water level in the mesocosms was maintained until sections were separated to avoid migration of organisms into the hyporheic section. Finally, substrate from each section was sieved (500 μm) to recover all individuals from their respective section to calculate the proportion that returned to the surface (Vander Vorste *et al.*, 2015c, d). %RTS was calculated based on the number of individuals that survived the experiment.

Survival of individuals

Upon collection, all individuals were placed in sorting trays and inspected for any movement to assess the proportion of individuals that survived the experiment (% survival). Individuals that did not survive were counted and separated from living individuals so they were not used for glycogen assays (see section on Measuring Glycogen Contents). Because *G. pulex* is known to feed on its conspecifics (MacNeil *et al.*, 1997), individuals not found at the end of the experiment (32 ± 17%) were presumed to be dead and consumed.

Leaf litter decomposition

To assess the effect of lowering water levels on LLD, 4.0 ± 0.1 g of alder leaves, with primary veins removed and dried at 60 °C for 24 h, were enclosed in 15 × 6 cm plastic mesh (10-mm diameter) bags. Mesh bags allowed *G. pulex* to enter freely and decompose leaf litter. Leaf litter was preconditioned as described previously. After conditioning, one leaf litter bag was placed on the substrate surface of each mesocosm just before re-inundation. Following the experiment, leaves were collected, dried at 60 °C for 24 h and re-weighed. LLD was calculated as the difference between initial and final leaf weight in grammes.

Measuring glycogen contents

To assess the effect of lowering water levels on energetic costs, all surviving individuals from the specific mesocosm ($n = 32$) were combined, freeze-dried, weighed (±0.1 mg) and ground into powder with a small mortar in pre-weighed glass tubes. Glycogen (μmol glycosyl g^{-1} dry mass) was extracted using standard enzymatic methods with prepared solutions (Sigma-Aldrich, Saint-Quentin Fallavier, France) described in further detail in Salin *et al.* (2010). Assays were made using an Aquamate spectrophotometer (Thermo Scientific Inc., Waltham, MA, USA) at 25 °C (Vander Vorste *et al.*, 2015c).

Data analysis

Differences in the % survival, %RTS (calculated as explained earlier), LLD and glycogen contents were tested using a two-way analysis of variance (ANOVA). The factorial design was a 4 (water level: control, − 5 cm, − 30 cm and dry) × 2 (population: PopI and PopP) with interactions. Post hoc Tukey's honest significant difference multiple comparisons were used to compare mean levels within water level and population treatment factors. Residual variances and Levene's test were used to check for homogeneity of variance and normality, and subsequently, all percentages were arc($\sqrt{}$-x)-transformed, and LLD and glycogen content values were $\log_{10}(x)$-transformed to meet these assumptions. LLD was further tested using linear regression for the relationship between decomposition and the % survival. All analyses were made using R (version 3.1.1; R Project for Statistical Computing, Vienna, Austria).

RESULTS

Survival of G. pulex

Survival of *G. pulex* differed among the water level treatments and populations, and there was a significant water level × population interaction effect indicating differences in survival between populations, which were not consistent across water level treatments (Table I, Figure 2a). In both populations, there was no difference between the −5 cm and control treatment (Tukey's test, $P > 0.05$; Figure 2a). Reductions of 29% and 39% in survival occurred when water level was lowered to −30 cm (Tukey's tests; PopI, $P = 0.009$; PopP, $P < 0.001$) and in the dry treatment (Tukey's tests; PopI, $P < 0.001$; PopP, $P = 0.005$), respectively, compared with the control treatment (Table I, Figure 2a). The comparison of the two populations showed that survival in PopP was twofold higher than in PopI in the dry treatment (Tukey's test, $P < 0.001$), whereas survival did not differ between populations in the other water level treatments (Tukey's tests, $P > 0.05$; Figure 2a).

Proportion of individuals returned to the surface

Of the individuals that survived the experiment, %RTS differed among water level treatments and populations, and there was significant water level × population interaction effect, indicating differences between populations in the %RTS were not consistent across water level treatments (Table I, Figure 2b). In PopI, reductions of 20–24% in the %RTS occurred in −5 cm, −30 cm and completely dry treatments compared with the control treatment (Tukey's tests, $P = 0.007$, $P = 0.003$, $P < 0.001$, respectively; Figure 2b). In PopP, the %RTS was not reduced in the −5 cm treatment nor in the completely dry treatment, compared with the control (Tukey's tests, $P > 0.05$; Figure 2b). There was a reduction of 52% in the −30 cm treatment compared with the control treatment (Tukey's test, $P < 0.001$). Between the two populations, PopP had a lower %RTS compared with PopI in all water level treatments (Tukey's tests, $P < 0.01$, for all).

Leaf litter decomposition

Water level treatments had significantly different effects on LLD, but there were no differences in LLD between populations and no water level × population interaction indicating that the feeding activities of the two populations

Table I. Results from the two-way analysis of variance testing the effects of water level treatment (control, −5 cm, −30 cm and dry), population (PopI and PopP) and the water level × population interaction on % survival and % of individuals returned to the surface (% RTS) of *Gammarus pulex* following 1 week of drying and 1 week of re-inundation.

	Water level				Population				Water level × population			
Dependent variable	*df*	*SS*	*F*	*P*	*df*	*SS*	*F*	*P*	*df*	*SS*	*F*	*P*
% Survival	3	0.415	23.659	<0.001	1	0.047	8.085	0.009	3	0.053	3.026	0.050
% Returned to surface	3	0.176	11.274	<0.001	1	0.396	76.119	<0.001	3	0.074	4.744	0.010

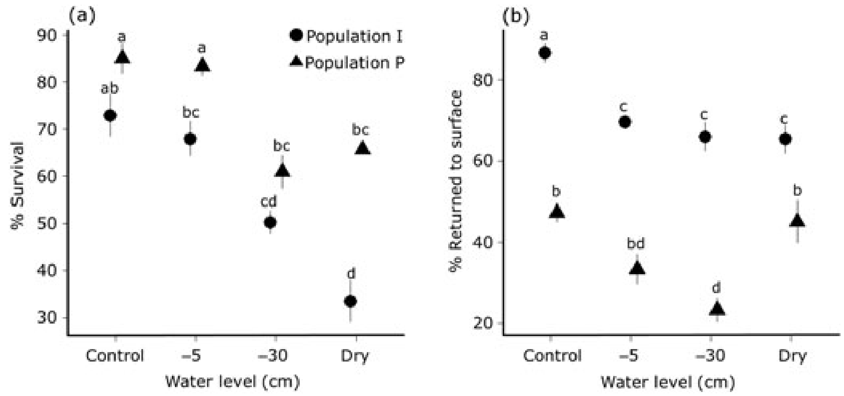

Figure 2. Mean ± S.E. of % survival (a) and % returned to the surface (%RTS) (b) following 1 week of drying and 1 week of re-inundation. Treatments with the same letters are not significantly different (post hoc Tukey's test, $P > 0.05$).

on surface were similarly affected by water level treatments (Table II, Figure 3a). LLD was not reduced compared with the control in the −5 cm treatment (Tukey's test, $P > 0.05$), but there was a 38% and 46% decrease in leaf mass consumed in the −30 cm treatment (Tukey's test, $P = 0.001$) and the completely dry treatment, respectively (Tukey's test, $P < 0.001$; Table II, Figure 3a). LLD was strongly and positively correlated with the % survival of *G. pulex* in the experiment ($R^2 = 0.38$, $P < 0.001$, $n = 31$), whereas no correlation was observed between LLD and %RTS ($R^2 = -0.01$, $P = 0.442$, $n = 31$; Figure 3b).

Glycogen content of G. pulex

In the individuals that survived the experiment, there were differences in mean glycogen content between water level treatments but not between populations, and there was no water level × population interaction effect. This indicates that energy stores of the two populations were similarly affected by water level treatments (Table II, Figure 4). Mean glycogen content was not reduced in the −5 and −30 cm treatments compared with the control (Tukey's tests, $P > 0.05$; Figure 4). In the dry treatment, there was a 25% reduction in mean glycogen content compared with the control treatment (Tukey's test, $P < 0.001$; Figure 4).

DISCUSSION

Effect of depth to the water table below the riverbed during drying events on resilience

In agreement with our first hypothesis, the resilience of *G. pulex*, measured as % survival and %RTS, to drying was negatively affected by lowering the water level below the substrate surface. There were reductions in % survival (up to 29%) and %RTS (up to 52%) for treatments with a water level of −30 cm indicating that depth to the water table during drying is an important consideration when studying the resilience of invertebrate populations in intermittent rivers. The likely mechanism behind the decrease in resilience is that *G. pulex* was forced to crawl further into the substrate to reach saturated conditions. Considering the hyporheic zone presents a maze-like corridor of interstices, it is likely that organisms faced a high risk of becoming stranded in dry substrate during drying events (Stumpp and Hose, 2013). Although it has been previously shown that fine sediments reduce the capacity of the hyporheic zone to provide refuge to benthic invertebrates (Navel *et al.*, 2010; Descloux *et al.*, 2013; Vadher *et al.,* 2015), we showed that lowering the water level from −5 to −30 cm also reduced this capacity, despite the use of coarse gravels (10–14 mm). Future studies should aim to see how increased water table

Table II. Results from the two-way analysis of variance testing the effects of water level treatment (control, −5 cm, −30 cm and dry), population (PopI and PopP) and the water level × population interaction on the leaf mass consumed and glycogen content of *Gammarus pulex* following 1 week of drying and 1 week of re-inundation.

	Water level				Population				Water level × population			
Dependent variable	*df*	*SS*	*F*	*P*	*df*	*SS*	*F*	*P*	*df*	*SS*	*F*	*P*
Leaf mass consumed	3	2.383	13.214	<0.001	1	0.020	0.326	0.573	3	0.175	0.969	0.424
Glycogen content	3	0.415	15.528	<0.001	1	0.001	0.137	0.715	3	0.063	2.368	0.097

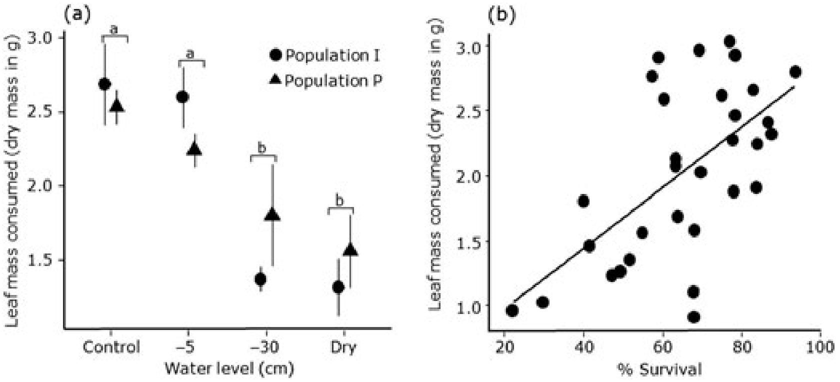

Figure 3. Mean ± S.E. of leaf mass consumed following 1 week of re-inundation (a). Linear correlation between leaf litter decomposition and the % survival of both populations of *Gammarus pulex* ($R^2 = 0.38$, $P < 0.001$) (b). Treatments with the same letters are not significantly different (post hoc Tukey's test, $P > 0.05$).

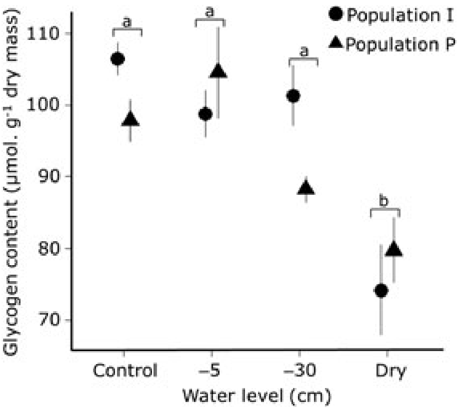

Figure 4. Mean ± S.E. of glycogen content measured in *Gammarus pulex* following 1 week of re-inundation. Treatment groups (brackets) with the same letters are not significantly different (post hoc Tukey's test, $P > 0.05$).

depth may influence survival and return to the surface of other taxa that serve important functional roles in intermittent rivers. For taxa that seek the hyporheic zone as a refuge during drying, we expect that the depth to the water table and substrate size are two dominant factors that influence survival and %RTS of these populations.

Effects on leaf litter decomposition and G. pulex energy stores

Leaf litter decomposition was reduced by 38% and 46% when the water level was lowered to −30 cm and in the dry treatments, respectively, likely because of lower survival and %RTS. This demonstrates that a reduction in invertebrate resilience can reduce LLD, a primary ecosystem function. This could have important implications in intermittent rivers considering that *Gammarus* spp. contributes greatly to LLD (Mathews, 1967; Dangles and Malmqvist, 2004; Handa *et al.*, 2014). For example, *Gammarus* spp. composed 83 ± 13% of detritivore abundance in a temperate river (Handa *et al.*, 2014). Lower survival and %RTS of *Gammarus* spp. following flow resumption could mean that other detritivores will face less competition for food resources and could compensate for LLD (i.e. insurance hypothesis; Yachi and Loreau, 1999). However, few other detritivores are as efficient at LLD (Piscart *et al.*, 2011a) or reach comparable abundances to *Gammarus* spp. (Dangles and Malmqvist, 2004). Furthermore, the survival and %RTS of other taxa could also be reduced by increasing depth to the water table during drying events, especially alluvial rivers where the hyporheic zone constitutes a primary source of colonists (Vander Vorste *et al.*, 2015b). The negative effects of increased depth to the water table on LLD may continue downstream because LLD constitutes a major source of fine particulate organic matter (FPOM) to downstream communities (e.g. filter feeders; Cuffney *et al.*, 1990). For example, reduced richness and abundance of detritivores caused by an insecticide disturbance resulted in a 33% decrease in the annual FPOM transported downstream of a headwater stream (Cuffney *et al.*, 1990). At larger scales, a reduction in LLD (e.g. Langhans and Tockner, 2006; Datry *et al.*, 2011; Corti *et al.*, 2011) could significantly influence biogeochemical cycles considering intermittent rivers comprise an estimated 50% of the global river network (Datry *et al.*, 2014a). However, large-scale effects of river drying on carbon and nutrient cycles are only beginning to be estimated (von Schiller *et al.*, 2014).

Glycogen energy stores consumed because of energy costs associated with migration and lack of leaf litter in the hyporheic zone during the drying event were restored within 1 week of re-inundation, except in the completely dry treatment. This confirms a previous study indicating that *Gammarus* spp. energy stores can recover from short-term starvation (28 days for *G. fossarum*, Hervant *et al.*, 1999) but shows that short-term desiccation in the dry treatment posed a greater stress on organisms (i.e. more energy consumption) because energy stores could not be fully restored within 1 week of re-inundation. For benthic invertebrates, short-term starvation in the hyporheic zone is likely because leaf litter conditioning by aquatic hyphomycetes is greatly reduced compared with that on the surface (Cornut *et al.*, 2014), and buried leaf litter is inherently less accessible (Piscart *et al.*, 2011b). Future experiments that quantify the duration that different invertebrate taxa can survive in the hyporheic zone are needed to better understand how longer drying events (e.g. 1–3 months) will affect population resilience.

Population-level variability in hyporheic zone use

Population I and PopP of *G. pulex* did not differ in their survival or LLD following re-inundation in the −5 and −30 cm water level treatments. However, a high proportion (~50%) of the perennial river population inhabiting the hyporheic zone, which could not be attributed to lowering water levels, may have been responsible for the approximately 2× greater survival in completely dry treatment. Migration into the hyporheic zone could lower the risk of individuals from becoming stranded near the substrate surface during the drying event. Individuals stranded near the surface by lowering water levels were potentially less tolerant to desiccation because of a lower moisture content compared with deeper substrates (Stubbington and Datry, 2013; Poznańska *et al.*, 2013). This difference in hyporheic zone use between populations could not have been caused by size, which was controlled for during the experiment, but may be related to other abiotic or biotic conditions that are normally present in their respective habitats. For example, temperature and biotic interactions can serve as environmental cues that initiate invertebrate migration (Vander Vorste *et al.*, 2015c), and without these cues, the population from the intermittent stream may have behaved differently than during a natural drying event. Because this study did not exhaustively test for population-level differences that could indicate local adaptation to river drying, we suggest that future studies could explore these differences using several replicate populations per flow regime (intermittent vs perennial). To this end, DNA analysis must be used to confidently distinguish responses attributable to drying from those caused by genetic differences among populations (Colson-Proch *et al.*, 2009).

Spatio-temporal limitations of the hyporheic zone as a source of colonization

The depth to the water table during drying events in alluvial rivers depends primarily on the regional hydrogeological setting, which controls the level of the regional aquifer below the riverbed and the duration of drying events (Boulton, 2003; Larned *et al.*, 2011). Near geological knickpoints (i.e. sharp change in valley width or channel slope; Stanford and Ward, 1993; Capderrey *et al.*, 2013), water generally remains flowing perennially, but as alluvial rivers become unconfined and water is lost into the subsurface (losing reaches), reaches often dry for periods of several weeks to months during the summer (e.g. Doering *et al.*, 2007; Vander Vorste *et al.*, 2015a). These losing reaches can comprise between 7% and 78% of the river length (e.g. Konrad, 2006) and be as long as 29 km (e.g. Doering *et al.*, 2007). During drying events, the depth to the water table in losing reaches can increase at rates of 5 cm per week (e.g. Clinton *et al.*, 1996) to 9 cm per day (e.g. Stella *et al.*, 2010), depending on substrate porosity. Following these rates, depths to the water table may exceed the ability of invertebrates to colonize within a few weeks to months after surface water dries in losing reaches that are perched above the alluvial aquifer. Therefore, the contribution of the hyporheic zone as a source of colonists following flow resumption will be lowest in perched, losing reaches that experience drying events (Malard *et al.*, 2002; Dole-Olivier, 2011). The relationship between depth to the water table and invertebrate resilience should be tested across multiple taxa that use the hyporheic zone (e.g. Chironomidae, Leptophlebiidae and Leuctridae) during drying events and at greater depths than those used in this study to develop more accurate predictions on the spatio-temporal limitations of the hyporheic zone as a refuge during drying events

Implications for river management and climate change

Climate change and increased water abstraction will increase the extent and duration of drying events at a global scale (Gleick, 2003; Döll and Schmied, 2012; Jaeger *et al.*, 2014), and this poses serious threats to biodiversity and ecosystem functioning (Dudgeon *et al.*, 2006; Handa *et al.*, 2014). Therefore, a major goal of river managers is to develop management measures that will protect and/or restore population resilience in the face of these threats (Lake *et al.*, 2007; Palmer *et al.*, 2008). The hyporheic zone can be a primary source of invertebrate colonists following flow resumption in intermittent rivers (e.g. alluvial rivers) that promotes high population resilience (Williams and Hynes, 1976; Fowler, 2002; Vander Vorste *et al.*, 2015b). However, the contribution of the hyporheic zone to population resilience will vary spatially along riverscapes (Malard *et al.*, 2002; Stubbington and Datry, 2013) as it is closely linked to the hydromorphological characteristics, such as depth to the water table and substrate size (Navel *et al.*, 2010; Descloux *et al.*, 2013; Vadher *et al.,* 2015), which control the vertical migration of invertebrates. To improve river management, empirical evidence gathered from this and the previously listed experiments could be used to identify river reaches where the hyporheic zone will likely contribute to population resilience using recent habitat modelling approaches (e.g. Snelder *et al.*, 2011). This would allow managers to more precisely direct management measures that protect and/or restore vertical connectivity to ensure that the contribution of the hyporheic zone to population resilience is not lost.

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