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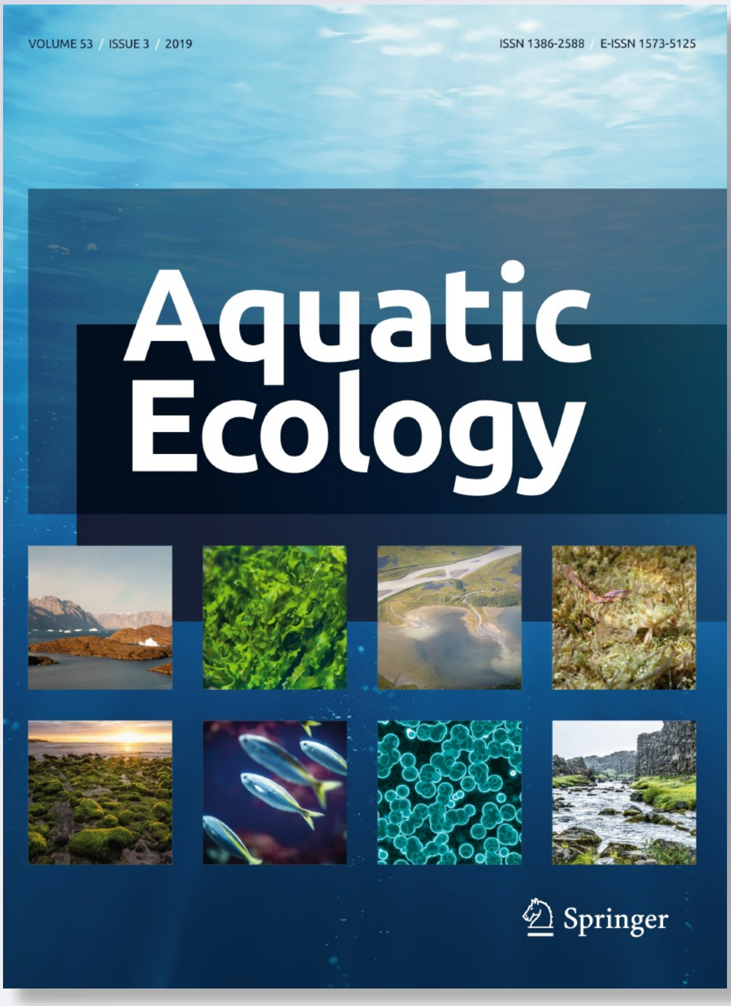

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Effect of shifts in habitats and flow regime associated to water diversion for agriculture on the macroinvertebrate community of a small watershed

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Abstract Water abstraction for irrigation has an important effect on stream organisms in general and aquatic macroinvertebrates in particular. The alteration of flow modifies the habitat conditions and creates important ecological constraints for many of these animals, so shaping the communities and affecting their diversity. With the aim to assess the impact of flow and habitat changes due to water abstraction for agriculture on the macroinvertebrate community of a Mediterranean stream, we characterized physicochemically three sampling sites representing three habitat types and collected the macroinvertebrate assemblage of each one. The three sites were a spring, an irrigation ditch 90 m downstream from the spring that diverge all the water from the natural channel and return it downstream, and a site after an area of agriculture 500 m downstream of the spring. Our hypothesis was that the highest diversity would be found in the irrigation ditch, where conditions were more constant along the year and that could act as a refuge for some organisms, followed by the spring and, afterwards, the downstream site, which would have a very poor community. Nonetheless, although our results showed that the irrigation ditch had the highest values of diversity, the spring and the downstream site did not differ significantly. When analysing the effect of the measured physicochemical parameters on macroinvertebrate communities, the most important was discharge. Thus, our study underlines the effect that water diversion may have on the macroinvertebrate communities even at a small watershed scale.

Keywords Stream · Macroinvertebrates · Agriculture · Water abstraction · Habitat

Introduction

Human alteration of ecosystems is a growing problem in the current Anthropocene. Some of the most impacted environments on Earth are aquatic, as human being has established villages and towns at the shore of the main rivers and water supply points since the beginning of civilizations. Vörösmarty et al. (2010), in a global analysis, reported that 65% of global river

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discharge, and the aquatic habitat supported by this water, is under moderate to high threat. Much of these continental aquatic systems, besides natural drivers, are now also controlled by societal and economic drivers (Meybeck 2003) and are being transformed through, among others, land cover change, urbanization, reservoirs and irrigation schemes (Vörösmarty et al. 2010). In a broader sense, climate change also impacts the global water cycle (Vörösmarty et al. 2004) and so has a direct effect on running waters. This is particularly important in Mediterranean regions, where drought and torrential precipitation episodes are predicted to increase in a near future. For instance, Döll and Schmied (2012) predicted, under certain possible future scenarios, flow regime shifts from perennial to transitional or intermittent, and a decrease in summer discharge. Though many of these stressors are global or at least act at a regional scale, protecting the world's freshwater resources requires diagnosing threats over a broad range of scales, from global to local (Vörösmarty et al. 2010).

Flow is an essential driver of stream ecosystems. In fact, it can be considered as one of the most important variables regulating the ecological integrity of flowing water systems and limits the distribution and abundance of fluvial species, as it is strongly correlated with physicochemical characteristics such as water temperature, channel geomorphology and habitat diversity (Poff et al. 1997). For instance, alterations in flow may modify the processes of transport and deposit of particles in the stream, and this can originate less suitable habitats for particular taxa of the macroinvertebrate community. At the local scale, land-use activities, such as timber harvest, livestock grazing, agriculture and urbanization, are the primary causes of altered flow regimes (Poff et al. 1997). In relation to them, many schemes used to provide water for irrigation and agriculture during dry periods have important consequences on flow regime, and so stream ecosystems functioning. Understanding the responses of freshwater biodiversity to hydrological variation is key to predicting the consequences of changing hydrology due to human water use and climate change, though relationships between hydrology and freshwater biodiversity are not yet clear (Rolls et al. 2018). What is evident is that freshwater ecosystems need enough water, of sufficient quality and at the right time, to provide economically valuable commodities and services to society, as well as to conserve the ecosystem functioning (Allan and Castillo 2007).

In recent years, much effort has been focused on the effect of flow alterations on macroinvertebrate assemblages (e.g. Chessman et al. 2010; Santos and Stevenson 2011; Li et al. 2012; Storey 2016; Calapez et al. 2017; Salmaso et al. 2018; White et al. 2018; Piano et al. 2019). Several of these flow alterations are natural, as occur in intermittent or seasonal streams from Mediterranean regions (Hershkovitz and Gasith 2013), but many others are human-induced due to water abstraction. As noted by Chessman et al. (2010) supporting previous findings of Sheldon and Thoms (2006), to assess these effects on macroinvertebrates, multi-period sampling is recommended in order to capture the replacement of species through time. Moreover, the main effects that have been detected on macroinvertebrate assemblages are not homogeneous (e.g. Clarke et al. 2010; Storey 2016; Gerth et al. 2017), and in many cases the effect of different environmental stressors exacerbates or is masked due to low flows (Rolls et al. 2012).

Agriculture, in a broad sense, uses to have severe impacts on aquatic ecosystems (Karaouzas et al. 2007). As pointed out previously, some of these effects are due to water abstraction and are related to flow alterations, but there are other associated consequences of this kind of land use. Some of these are increasing inputs of organic residues, nutrients, pesticides and elimination of the natural protection provided by the riparian vegetation (Hepp et al. 2010). Intensification of agriculture worldwide has increased fine sediment loading to rivers (Naden et al. 2016), which may occlude interstitial spaces in the riverbed, with negative consequences for stream organisms (Doretto et al. 2018a). As pointed out by Genito et al. (2002), a high percentage of agricultural land cover reduced the number of sensitive mayfly and caddisfly taxa and produced a macroinvertebrate community composition that reflected altered stream habitat. At the catchment scale, Richards et al. (1993) found differences in the benthic communities of the most agricultural zones in relation to less intensive zones, mainly due to the variation in the proportions of stoneflies and mayflies, many of which taxa are sensitive to habitat alterations. The effect of agriculture on the distribution and abundance of macroinvertebrate and, particularly, of sensitive taxa of EPT

(Ephemeroptera, Plecoptera and Trichoptera) was also demonstrated by Al-Shami et al. (2011) in Malaysia. These authors detected three main levels of stresses in the analysed streams, the harshest of which was due to agricultural, industrial and municipal discharges. Hepp et al. (2010) underlined similar impacts of urban and agricultural practices on water quality and aquatic diversity. These are only some examples that have demonstrated worldwide the direct and indirect effects of agriculture on aquatic biota [other examples of recent studies analysing this are Egler et al. (2012), Kavanagh and Harrison (2014), Fournier et al. (2018) or Solis et al. (2018)].

Our study aimed to identify diversity changes (both in space and time) in the macroinvertebrate community of three non-distant reaches along a same stream, two of them suffering different impacts related to flow alteration due to agricultural practices. These sites were a source, an irrigation ditch and an artificially intermittent reach due to water abstraction for agriculture. Our hypothesis was that diversity would be maximum at the irrigation ditch, because it can provide habitat stability and permanent flow during the harshest months of the summer, while diversity would be slightly lower in the source, where habitat variability would be slightly greater as a consequence of flow variations, and would be minimum in the downstream site due to accumulative effects of stressors from upper reaches. We also hypothesized that time passed after alteration of the flow would have a synergistic effect on diversity.

Methods

Our study system was the Beas stream (Sierra de Huétor, Granada, Spain), the main fluvial axis of a relatively small watershed (10.54 km^2). It is a tributary of the Darro River, belonging to the Genil basin, and belongs to a Mediterranean climate region. The stream was monitored monthly during a whole year, from May 2017 to April 2018. This year was rainy in the region where the study area is located, with torrential precipitation events occurring in autumn (particularly in November) and spring (in March). Three sampling sites very close to each other were visited and sampled monthly (Fig. 1): a wellspring where the water flows from a little cave in the rock (hereafter, “Source”; coordinates: 37°13′54″N 3°28′24″W; 1130 m a.s.l.), a small channel used to derive water for irrigation (“Irrigation ditch”) at approximately 90 m downstream of the “Source” (37°13′52″N 3°28′24″W; 1120 m a.s.l.) and a reach downstream of a farming area (“Downstream”) at about 500 m downstream of the “Source” (37°13′40″N 3°28′31″W; 1030 m a.s.l.). This last sampling site was dry due to water abstraction for irrigation from June to November. Upstream of the “Source” the stream receives water from three *ramblas* but that section uses to be dry most of the year. It is from the “Source” from where the water flows permanently. The “Source” has a mean width of 0.46 m in the studied period and a mean depth of 0.06 m. The granulometric composition of the substrate was visually estimated in the three sites, and particularly in “Source”, sands (0.006–0.2 mm), gravels (0.2–20 mm) and little silt (< 0.006 mm) were the main components. In the upper part of the reach, there are some mosses and the water is almost stagnant. The “Irrigation ditch” is a small channel of approximately 0.3 m mean width and 0.06 m mean depth. The substrate is composed mainly by gravel and sand, with a few, very small, patches of mosses. All the water from the stream channel is derived to this ditch, so the natural course of the stream remains dry all the year (except during punctual torrential precipitation events that, in our studied year, occurred only once). The “Downstream” sampling site is around 0.62 m mean width (although in some months can reach more than 1 m width) and 0.06 m mean depth during the months in which it has flow. The substrate is composed by gravel, sand and silt, and there are great packs of leaves fallen from the surrounding vegetation, mainly cultivated *Populus* sp. Between “Irrigation ditch” and “Downstream”, there are several plantations of olive trees, almond trees and small orchards for self-supply (Fig. 1). The total area of these crops is 0.07 km^2.

The main physicochemical characteristics of each sampling site were recorded monthly in situ and appear in Table 1. Conductivity and pH were recorded with a VWR sympHony multiparameter probe (VWR International Eurolab S.L., Spain), oxygen was measured with an Eutech DO 450 oximeter (Eutech Instruments Pte. Ltd., Singapore), and flow was determined with a Global Water digital flowmeter (Global Water Instrumentation, USA). This latter variable was afterwards used to calculate discharge in each reach (Fig. 2). Temperature was recorded hourly

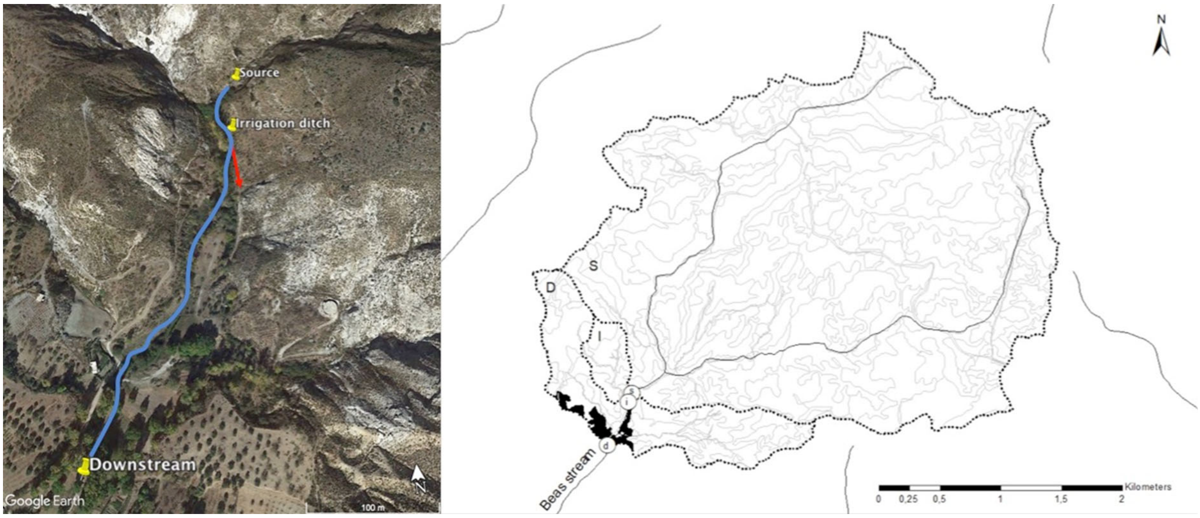


Fig. 1 Map of the study area of the Beas stream. Left: location of the three sampling sites (line in blue indicates de natural channel of the stream; arrow in red indicates the site in which water is derived through an irrigation ditch). Right: Limits of the catchment of each sampling site ("S" marks the catchment of "Source", "I" the catchment of the "Irrigation ditch" and "D" the catchment of "Downstream") with the area occupied by agriculture coloured in black. Within each catchment, lower case letters indicate the sampling sites ("s": source; "i": irrigation ditch; "d": downstream). (Color figure online)

Table 1 Physicochemical characteristics of the sampling sites during the study period

	Source					Irrigation ditch					Downstream				
	N	Mean	SD	Min	Max	*N*	Mean	SD	Min	Max	*N*	Mean	SD	Min	Max
pH	12	8.5	0.2	7.9	8.7	12	8.8	0.2	8.5	9.3	7	9.0	0.4	8.2	9.3
Conductivity (µS/cm)	12	327.9	15.3	288.8	343.0	12	330.7	24.9	256.8	355.0	7	321.4	12.0	299.0	336.0
Mean daily temperature (°C)	248	12.5	0.3	12.0	13.0	244	12.0	1.3	9.9	14.1	151	12.0	2.0	10.2	18.0
Dissolved oxygen (%)	12	98.0	1.2	95.1	99.2	12	88.4	3.1	84.2	92.2	7	94.3	2.2	90.3	97.0
Oxygen concentration (mg/l)	12	10.3	0.1	10.0	10.6	12	9.4	0.4	8.9	10.7	7	10.3	0.5	9.5	10.9
Depth (m)	12	0.1	0.0	0.1	0.1	12	0.1	0.0	0.1	0.1	7	0.1	0.0	0.1	0.1
Velocity (m/s)	12	0.2	0.1	0.1	0.4	12	0.2	0.1	0.0	0.5	7	0.4	0.1	0.2	0.5
Width (m)	12	0.5	0.4	0.1	0.9	12	0.3	0.0	0.3	0.3	7	0.6	0.3	0.3	1.1
Discharge (dm^3/s)	12	3.6	2.2	0.5	6.7	12	3.5	2.5	0.3	9.0	7	15.3	13.6	6.5	40.3

with one datalogger IBcod (Alpha Mach, Inc., Canada) placed under a stone in the middle of the streambed of each site during the study period. Unfortunately, some of these dataloggers were lost during the study, so we missed data of several days.

Quantitative samples of macroinvertebrates were collected using a Surber sampler (0.1 m^2 area, 250 µm mesh size). Three replicates were taken each month in each site. In "Irrigation ditch" due to the Surber sampler did not fit in it, we used a kick sampler (250 µm mesh size) and delimited a similar, rectangular area in front of it (0.1 m^2) to make samples comparable among sites. No macroinvertebrate samples were collected in "Downstream" during the dry period. All organisms were preserved in 70% ethanol and brought to the laboratory for their sorting and identification. Macroinvertebrates were identified at the genus level, when achievable, or at the maximum possible taxonomic level and counted. A list of all the taxa captured during the sampling period in each site can be consulted in Electronic Supplementary Material.

The macroinvertebrate community in each site and date was analysed in terms of composition and structure using the three collected replicates each time. α-diversity was calculated by means of the Shannon–Wiener index [$H' = -\sum (p_i \operatorname{Ln} p_i)$] and the Simpson index [$D = \sum (p_i^2)$]. As the latter, D, is a dominance index, $1 - D$ was used as a diversity index. We used both at the beginning to compare their results, as the former weigh more rare taxa and the latter is more influenced by common taxa (Krebs 1999). A Spearman test was employed to assess for a possible correlation between values of both indexes in order to proceed with subsequent analyses with only one measure of diversity (if they were correlated) or with both.

A two-way ANOVA was used to test differences among sites (factor "Site"), sampling dates (factor "Time") and to try to reveal a possible interaction between both factors. Type III sum of squares was used for this analysis because the design was unbalanced, as in "Downstream" there were no data during 5 months of the year, when it was dry. Before performing the analysis, assumptions of normality of residuals and homoscedasticity were tested using Shapiro–Wilk and Levene tests. After the ANOVA, a pairwise *t* test with pooled SD was used to make comparisons among the levels of the significant factors.

To study the communities in terms of β-diversity, i.e. the taxa turnover among communities, we performed a permutational multivariate analysis of variance (PERMANOVA) with Bray–Curtis distance matrices and applying 9999 permutations. We did it using the adonis2 function from the vegan package in *R* (Oksanen et al. 2018; R Core Team 2018). Because this function allows adjustment of semiparametric MANOVAs, residuals of the model were not evaluated. For those factors that were statistically significant, pairwise PERMANOVAs applying the Bonferroni's *p* adjustment after 9999 permutations were used on distance matrices. Afterwards, in order to find the average contribution of each taxon to the Bray–Curtis distance among communities, i.e. to identify which taxa contributed the most to the differences among them, we used the similarity percentages procedure (SIMPER; Clarke 1993) to make pairwise comparisons of the studied communities. Comparisons were made for those pair of levels of each factor that were significant in the PERMANOVA analysis. We also applied 9999 permutations to get more robust *p* values.

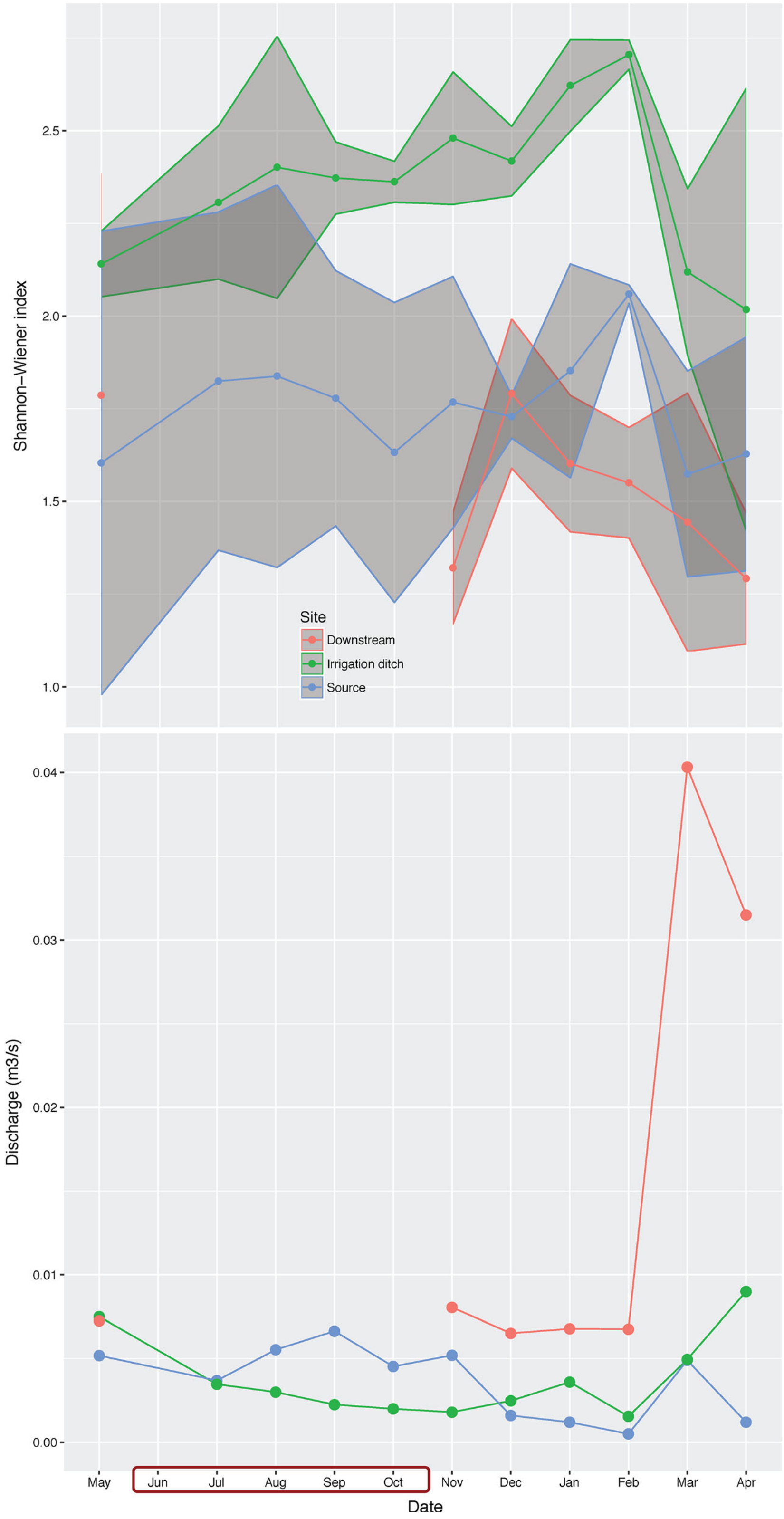
Finally, to relate the macroinvertebrate community of each site and date with the abiotic variables of its habitat, we performed a non-metric multidimensional scaling (NMDS) using the metaMDS function in the vegan package (Oksanen et al. 2018). For this analysis, the Wisconsin square root standardization was applied to data and dissimilarities among sites were calculated using the Bray–Curtis distance. Together with sites, the main environmental variables registered in situ each date (except for temperature, in which case we used the mean value of the whole day in which the sampling was carried out) that were statistically significant were first represented as vectors in the two-dimensional space obtained. As the response of taxa, and so communities, is not linear to those environmental parameters, smooth surfaces were calculated for each of them and fitted to ordinations with the ordisurf function from the vegan package (Oksanen et al. 2018). The values of stress and of non-metric R^2 were also calculated.

Results

After the whole sampling period, 44 taxa were recorded in "Source", 58 in "Irrigation ditch" and 38 in "Downstream", of which we collected 10,817, 15,423 and 1717 individuals, respectively (Electronic Supplementary Material).

The analysis of the α-diversity of the macroinvertebrate community in each site throughout the studied year showed the highest overall values of both H' and $1 - D$ in "Irrigation ditch". Particularly, the Shannon–Wiener index (H') ranged from 0.89 to 2.41 (mean = 1.75 ± 0.34; $N = 36$) in "Source", from 1.33 to 2.81 (mean = 2.35 ± 0.28; $N = 36$) in "Irrigation ditch" and from 1.10 to 2.21 (mean = 1.54 ± 0.32; $N = 21$) in "Downstream". On the other hand, the Simpson diversity index ($1 - D$) ranged from 0.33

Fig. 2 Top: Mean values of the Shannon–Wiener diversity index (dotted lines) in each month and for each site throughout the study period. Grey areas represent the standard deviation. Down: Discharge (m^3/s) recorded in each sampling site during the sampling period. Brown rectangle in the *X* axis represents the dry period in "Downstream". (Color figure online)



to 0.87 (mean = 0.71 ± 0.12; $N = 36$) in “Source”, from 0.72 to 0.92 (mean = 0.85 ± 0.04; $N = 36$) in “Irrigation ditch” and from 0.47 to 0.85 (mean = 0.72 ± 0.09; $N = 21$) in “Downstream”. As both indexes were highly correlated (Spearman correlation $R = 0.96$, $p < 0.05$), only H' was used for subsequent analyses. This index showed variations along the year (Fig. 2). The two-way ANOVA only showed significant differences among sites ($F_{2,62} = 54.58$, $p < 0.05$), but not among dates. Neither an interaction between sites and time was detected. The post hoc analyses resulted in significant differences between “Source” and “Irrigation ditch” and between “Irrigation ditch” and “Downstream” ($p < 0.05$), but not between “Source” and “Downstream” (though $p = 0.05$, so the result should be considered marginal).

On the other hand, the PERMANOVA test showed significant effect of factor “Site” and “Time” (factor “Site” $F_{2,62} = 16.92$, $R^2 = 0.21$, $p < 0.05$; factor “Time” $F_{11,62} = 2.89$, $R^2 = 0.19$, $p < 0.05$), as well as an interaction between both factors ($F_{17,62} = 2.02$, $R^2 = 0.21$, $p < 0.05$). The post hoc analysis (pairwise PERMANOVA) showed that for factor “Site”, significant differences were found among the three pairwise comparisons, i.e. among the three sites ($p < 0.05$), while for factor “Time”, only marginal significant differences ($p = 0.046$) were found between May and March. The SIMPER analysis used to determine which taxa contributed the most to differences among the three sites, on one side, and between May and March, on the other, showed that *Baetis* sp., *Polycelis* sp. and *Hydrocyphon* sp., significantly contributed to differences between “Source” and “Irrigation ditch”; *Protonemura* sp., *Leuctra* sp., *Agapetus* sp., Orthocladiinae and Tanypodinae to differences between “Source” and “Downstream”; and *Baetis* sp., *Polycelis* sp., *Hydrocyphon* sp. and Chironominae to differences between “Irrigation ditch” and “Downstream”. For factor “Time”, the taxa that significantly contributed the most to the differences between the communities in May and March were *Agapetus* sp. and Ceratopogoninae. For each factor, several other taxa significantly contributed to differences, but their contribution was very small and has not been enumerated.

Finally, the NMDS used to relate communities and environmental factors measured in situ showed a clear grouping of all the macroinvertebrate samples collected in the same site (Figs. 3 and 4; stress = 0.19; non-metric fit $R^2 = 0.96$). The highest dispersion of points corresponds to “Downstream”, and there are also some points from “Irrigation ditch” that separate from the rest. Discharge, pH, dissolved oxygen and temperature are the four environmental parameters that are statistically significant. Nonetheless, the fitting for temperature is near null ($R^2 = 0.08$), while for the other variables is higher ($R^2 = 0.35$ for discharge, $R^2 = 0.30$ for dissolved oxygen and $R^2 = 0.18$ for pH). When represented as vectors (Fig. 3), discharge and temperature (and, to a lesser extent, pH and oxygen) seem to separate communities from “Downstream” from the rest, and oxygen discriminates among communities from “Irrigation ditch” and “Source”. When represented as smooth fitting surface, discharge groups together most communities from “Source” and “Irrigation ditch”, dissolved oxygen shows a growing gradient from “Irrigation ditch” to “Source” first and “Downstream” latter, pH discriminates “Source”, with lower values, from “Downstream”, with the highest, and temperature (though not very informative due to its poor fitting) separates “Downstream” points from the rest.

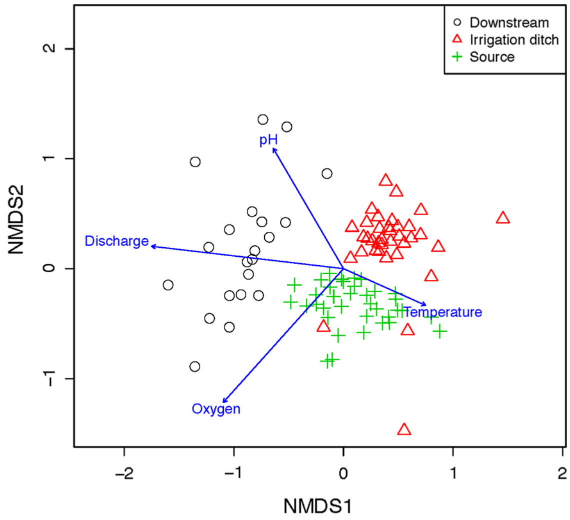


Fig. 3 NMDS ordinations of the communities studied by site and their relation with the environmental parameters analysed represented as linear vectors (NMDS stress = 0.19, non-metric fit $R^2 = 0.96$)

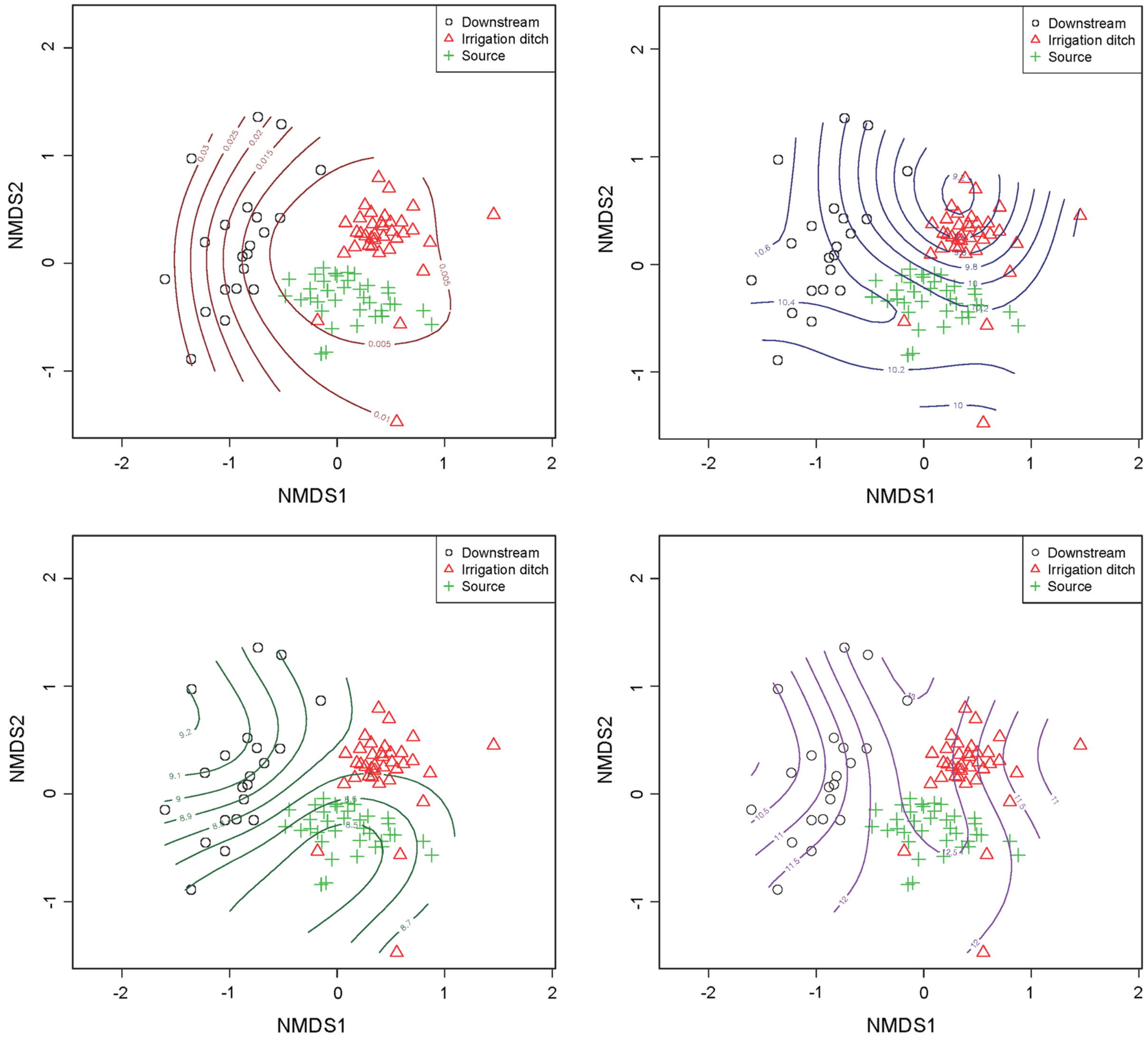


Fig. 4 NMDS ordinations of the communities studied by site and their relation with the environmental parameters analysed represented as smooth surfaces (NMDS stress = 0.19, non-metric $R^2 = 0.96$). Each point represents the community in each of the three Surber samples collected each date at each site. Top-left: surface fitting for discharge (m^3/s; $R^2 = 0.35$; $p < 0.05$); Top-right: surface fitting for dissolved oxygen (mg/l; $R^2 = 0.30$; $p < 0.05$); Bottom-left: surface fitting for pH ($R^2 = 0.18$; $p < 0.05$); Bottom-right: surface fitting for temperature (°C; $R^2 = 0.08$; $p < 0.05$)

Discussion

At a local scale, agriculture has both direct and indirect effects on nearby aquatic ecosystems. Direct effects use to be related with the water abstraction for irrigation and the habitat modification due to the construction of ditches and weirs for that purpose (Salmaso et al. 2018). Indirect effects are typically related with the diffuse contamination of waters by nitrogen and phosphorous compounds, by chemical products used as herbicides or insecticides or due to sediment inputs (Sponseller et al. 2001; Solis et al. 2018). Nonetheless, a limitation to study these effects and their consequences on aquatic biota is that, when the watershed is wide and there are multiple stressors operating simultaneously, it is difficult to identify the origin of these impacts. This is more pronounced if a comparison is established among reaches in a stream or river that are distant from each other because the natural conditions of the lotic system may have changed. In our case, we study three reaches in a stream very close to each other, but with particularities in the habitat conditions either to natural ("Source") or anthropogenic causes ("Irrigation ditch" and "Downstream"). As pointed out by Rolls et al. (2012), generalizing low-flow ecology relationships through time at large spatial scales is likely to be challenging, so these relationships may be more easily identified at regional or context-specific scales.

The comparative analysis of both taxa richness (S) and abundance of macroinvertebrates in the three studied sites shows clear differences among them. The most taxa rich site and where macroinvertebrates were more abundant was "Irrigation ditch", followed by "Source" and "Downstream". The latter is, by long, the poorest site regarding macroinvertebrate assemblages. These observations are confirmed by the analysis of the α-diversity, clearly higher in "Irrigation ditch", while results slightly differ when comparing the other two sites between them with each index. Thus, our initial hypothesis is only partially supported. Applying H', "Source" has a somewhat higher diversity than "Downstream", while with $1 - D$ both sites are, overall, similar in terms of diversity. This is probably related to the (mathematical) nature of each index that, as mentioned in the "Methods" section, weighs differently rare taxa and common taxa (Krebs 1999). In fact, focusing on H', no significant differences were found between "Source" and "Downstream", but the causes of such a low diversity and of the absence of differences are different in each site. Despite "Source" is not under any type of stress, opposite to "Downstream", comparing the months in which both sites have water and so the macroinvertebrate community could be recorded, the similarities in terms of diversity could be a consequence of the particular homogeneous conditions of this kind of habitats. Environmental fluctuations in springs are rare in comparison with headwater streams, so one would expect to find greater importance of predation and competition in regulating invertebrate populations and communities (Thorp 2015), and that this would have an effect on diversity. Our results partially confirm the findings of Barquín and Death (2004), who reported that spring-fed streams from Northern Spain had lower invertebrate diversity and greater invertebrate densities than nearby runoff-fed streams. In the case of "Downstream", the reasons that explain its low diversity are different and related to differences in habitat conditions and to the artificial drought that this reach suffers due to upstream water abstraction for agriculture. This creates a selective filter that many organisms that are found in the nearby upstream reach cannot overpass and so the macroinvertebrate assemblage is impoverished. In fact, stress-tolerant organisms such as Chironomidae, *Baetis* sp. and Lumbriculidae dominate the community in "Downstream". There are also records of sensitive taxa, such as some stoneflies and caddisflies, but these are punctual and probably coming from upstream by drift. The importance of permanent sites acting as refugia for some taxa and of drift in the recovery of communities after drought (Smith and Wood 2002; Rolls et al. 2012; Doretto et al. 2018b; White et al. 2018), as well as the refuge provided by the hyporheic zone (Vander Vorste et al. 2016), has been previously pointed out. James et al. (2008) tested the importance of refugia experimentally modifying stream flows by constructing weirs and diversions simulating short-term low flows. These authors did not detect impacts on the abundance of common macroinvertebrates, suggesting that they are resistant to flow reductions if refugia are available. This fact, together with the drift from upstream, may contribute importantly to the recolonization of sites such as "Downstream", where the unpredictable intermittency of flow creates very harsh conditions and so a great mortality among populations of many organisms.

The comparison of the whole communities through a dissimilarity analysis among sites and sampling dates provides a complementary view. In this case, the three sites are significantly different, and there are also differences among May and March, and interestingly, there exists an interaction between time and sites. Nonetheless, as shown by the values of R^2, their effect on the macroinvertebrate assemblages should be considered low. The existence of interaction supposes that time has a synergistic effect on the macroinvertebrate communities of the three sites, i.e. differences among communities from different sites change with time in a different manner. A possible explanation of this interaction is that, along the year, physicochemical conditions, such as flow, varies more in some sites than in others, so affecting to the macroinvertebrates living in them and increasing the dissimilarities among sites. The independent effect of factor time, as is only marginally significant, should be taken with caution and probably it is only accidental. Taxa that contribute to the differences found among May and March are *Agapetus* sp. and Ceratopogoninae, which in some dates disappear or are very scarce in some communities. On the other hand, differences between "Downstream" and the other two sites are due to great changes in abundances of some taxa, such as *Protonemura* sp., *Leuctra* sp. or *Agapetus* sp., as well as some subfamilies of Chironomidae. Regarding this family of typically stress-tolerant midges, as pointed out earlier, they are proportionally the group better represented in the community of "Downstream", but its abundance is much lower than that found in upper reaches. This supports that conditions in this habitat are quite harsh even for this kind of organisms. Another interesting finding is that crustaceans, in our case gammarids, do not contribute to differences between "Source" and the other two sites, and are very scarce too. Several authors have reported that non-insect taxa, such as amphipods and isopods, are more frequent in springs, while insects rapidly substituted them downstream (e.g. Barquín and Death 2004; Thorp 2015), but this is not the case in our study system. The scarcity of crustaceans in this stream could be related to water chemistry or historical events (a lack of colonization by these organisms in the past).

When analysing how the main physicochemical conditions explain the grouping of the communities, we can observe that temperature is not relevant, probably because due to the proximity of the three sampling sites to the spring of the stream, temperature differences are minimal. Only in "Downstream" there are some notable fluctuations, but due to the null R^2 found in the NMDS analysis this variable should not be considered as determinant. Discharge is the variable with the highest R^2. It groups, on the one hand, all the macroinvertebrate assemblages (from several dates during the study period) from "Source" and "Irrigation ditch", and, on the other, those inhabiting "Downstream". There are 2 months, March and April, in which discharge in "Irrigation ditch" was exceptionally high due to continuous precipitation events in the region, and so these points in the graphical representation are a little disengaged of the rest from this site. During this period, the ditch filled up of sediments brought from upstream, and so this had a detrimental effect on the macroinvertebrates inhabiting there. Due to the direct effect of flow on dissolved oxygen (Calapez et al. 2017, 2018), oxygen also groups macroinvertebrate communities from "Source" and "Irrigation ditch" and separates those from "Downstream". Points representing communities in the latter site are exposed to higher levels of dissolved oxygen than those from the other two sites. Thus, this high oxygen conditions would not prevent to some of the organisms inhabiting higher reaches and more intolerant to oxygen depletion to inhabit this site. This supports that the selection imposed by the absence of surface flow during several months of the year due to water abstraction is strong enough to have a great impact on these communities. Also, pH discriminates "Downstream" communities from the rest. This parameter is slightly higher in this site, what could reflect the effect of the limestone substrate and sediment inputs coming from the surrounding agricultural areas, as the riversides of this reach have not a proper riparian vegetation that could act as buffer (Hunt et al. 2017). In this sense, Sponseller et al. (2001) reported that such inputs are frequent in streams with particular land uses (including agricultural) adjacent to the channel. In our study site, this was evident after the great precipitation events occurring in the area in winter and spring, when the channel was almost clogged with gravels and sands and its depth was greatly reduced.

An interesting result of this study is that, despite the modification of the channel in "Irrigation ditch" and so the alteration of the habitat, this site has higher diversity than the other two studied sites, even "Source". This may be a consequence of permanent conditions in flow regime and good water quality due to its closeness to the spring. Other studies have also pointed out that the particular conditions created by some artificial habitats may increase the density of some macroinvertebrates (e.g. Nakano and Tsuno 2016). In fact, Käiro et al. (2017) pointed out that channelization had no significant effects on physicochemical parameters or on macroinvertebrates when channelization was done decades earlier, as it is our case, though diminished several community parameters, such as diversity, in comparison with natural sites. In our study system, this does not occur, as diversity in "Source" is lower than in "Irrigation ditch" for the reasons mentioned earlier.

Drought generated due to water abstraction in upper reaches of "Downstream", together with punctual floods occurring after intense precipitation events clearly determine the structure of the macroinvertebrate community. Both floods and droughts are the major forms of natural disturbance in running waters (Lake 2000; Stubbington 2012), and also when they have an anthropogenic origin, and so they have important repercussions on stream fauna. In this sense, it is also important to consider the antecedent conditions (Rolls et al. 2012). In our stream, water diversion and low-flow conditions have been occurring for years, and flow conditions in “Downstream” are unpredictable, so this creates an important ecological filter for many macroinvertebrates, mainly sensitive species that disappear from the community rapidly if no refugia are available. The hyporheic zone of this reach could have played an important role as refugia in the past for some taxa, but in some cases rapid onset of drought (or floods) prevents macroinvertebrates to migrate down into this habitat (Stubbington 2012). Many of the responses of macroinvertebrates to these changing conditions are related to particular biological traits, and therefore, it has been proposed to use these traits as indicators of stressor intensities in catchments under agricultural land uses (Lange et al. 2014).

In conclusion, flow seems to be the principal determinant of macroinvertebrate communities in the study site. The relationships between flow regime variation and freshwater biodiversity are key to informing the management of hydrological regimes to protect or restore freshwater biodiversity and ecosystem services (Davies et al. 2014; Rolls et al. 2018). Nonetheless, due to some restoration measures may have negative effects (Dolph et al. 2015), conservation, management and restoration of streams in agricultural areas should be treated and studied at a local scale.

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References

Allan JD, Castillo MM (2007) Stream ecology: structure and function of running waters. Springer, Dordrecht

Al-Shami SA, Rawi CSM, Ahmad AH, Hamid SA, Nor SAM (2011) Influence of agricultural, industrial, and anthropogenic stresses on the distribution and diversity of macroinvertebrates in Juru River Basin, Penang, Malaysia. Ecotoxicol Environ Saf 74:1195–1202

Barquín J, Death RG (2004) Patterns of invertebrate diversity in streams and freshwater springs in Northern Spain. Arch Hydrobiol 161:329–349

Calapez AR, Branco P, Santos JM, Ferreira T, Hein T, Brito AG, Feio MJ (2017) Macroinvertebrate short-term responses to flow variation and oxygen depletion: a mesocosm approach. Sci Total Environ 599–600:1202–1212

Calapez AR, Serra SRQ, Santos JM, Branco P, Ferreira T, Hein T, Brito AG, Feio MJ (2018) The effect of hypoxia and flow decrease in macroinvertebrate functional responses: a trait-based approach to multiple-stressors in mesocosms. Sci Total Environ 637–638:647–656

Chessman BC, Jones HA, Searle NK, Growns IO, Pearson MR (2010) Assessing effects of flow alteration on macroinvertebrate assemblages in Australian dryland rivers. Freshw Biol 55:1780–1800

Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure. Aust J Ecol 18:117–143

Clarke A, Mac Nally R, Bond N, Lake PS (2010) Flow permanence affects aquatic macroinvertebrate diversity and community structure in three headwater streams in a forested catchment. Can J Fish Aquat Sci 67:1649–1657

Davies PM, Naiman RJ, Warfe DM, Pettit NE, Arthington AH, Bunn SE (2014) Flow–ecology relationships: closing the loop on effective environmental flows. Mar Freshw Res 65:133–141

Döll P, Schmied HM (2012) How is the impact of climate change on river flow regimes related to the impact on mean annual runoff? a global-scale analysis. Environ Res Lett 7:014037

Dolph CL, Eggert SL, Magner J, Ferrington LCJ, Vondracek B (2015) Reach-scale stream restoration in agricultural streams of southern Minnesota alters structural and functional responses of macroinvertebrates. Freshw Sci 34:535–546

Doretto A, Piano E, Bona F, Fenoglio S (2018a) How to assess the impact of fine sediments on the macroinvertebrate communities of alpine streams? a selection of the best metrics. Ecol Indic 84:60–69

Doretto A, Piano E, Falasco E, Fenoglio S, Bruno MC, Bona F (2018b) Investigating the role of refuges and drift on the resilience of macroinvertebrate communities to drying conditions: an experiment in artificial streams. River Res Appl 62:245–249

Egler M, Buss DF, Moreira JC, Baptista DF (2012) Influence of agricultural land-use and pesticides on benthic macroinvertebrate assemblages in an agricultural river basin in southeast Brazil. Braz J Biol 72:437–443

Fournier M-L, Echeverría-Sáenz S, Mena F, Arias-Andrés M, de la Cruz E, Ruepert C (2018) Risk assessment of agriculture impact on the Frío River watershed and Caño Negro Ramsar wetland, Costa Rica. Environ Sci Pollut Res 25:13347–13359

Genito D, Gburek WJ, Sharpley AN (2002) Response of stream macroinvertebrates to agricultural land cover in a small watershed. J Freshw Ecol 17:109–119

Gerth WJ, Li J, Giannico GR (2017) Agricultural land use and macroinvertebrate assemblages in lowland temporary streams of the Willamette Valley, Oregon, USA. Agric Ecosyst Environ 236:154–165

Hepp LU, Milesi SV, Biasi C, Restello RM (2010) Effects of agricultural and urban impacts on macroinvertebrates assemblages in streams (Rio Grande do Sul, Brazil). Zoologia 27:106–113

Hershkovitz Y, Gasith A (2013) Resistance, resilience, and community dynamics in mediterranean-climate streams. Hydrobiologia 719:59–75

Hunt L, Marrochi N, Bonetto C, Liess M, Buss DF, Vieira da Silva C, Ciu M-C, Resh VH (2017) Do Riparian buffers protect stream invertebrate communities in South American Atlantic forest agricultural areas? Environ Manag 60:1155–1170

James AB, Dewson ZS, Death RG (2008) Do stream macroinvertebrates use instream refugia in response to severe short-term flow reduction in New Zealand streams? Freshw Biol 53:1316–1334

Käiro K, Haldna M, Timm H, Virro T (2017) The effect of channelization on the biological quality of lowland streams using macroinvertebrates as proxies. Hydrobiologia 794:167–177

Karaouzas I, Gritzalis KC, Skoulikidis N (2007) Land use effects on macroinvertebrate assemblages and stream quality along an agricultural river basin. Fresenius Environ Bull 16:645–653

Kavanagh JA, Harrison SSC (2014) The contribution of a drainage network to the spatial and temporal patterns of macroinvertebrate diversity across an agricultural headwater catchment. Biol Environ Proc R Irish Acad 114B:181–197

Krebs CJ (1999) Ecological methodology. Harper and Row, New York

Lake PS (2000) Disturbance, patchiness, and diversity in streams. J N Am Benthol Soc 19:573–592

Lange K, Townsend CR, Matthaei CD (2014) Can biological traits of stream invertebrates help disentangle the effects of multiple stressors in an agricultural catchment? Freshw Biol 59:2431–2446

Li F, Cai Q, Jiang W, Qu X, Jia X (2012) Flow-related disturbances in forested and agricultural rivers: influences on benthic macroinvertebrates. Int Rev Hydrobiol 97:215–232

Meybeck M (2003) Global analysis of river systems: from Earth system controls to Anthropocene syndromes. Philos Trans R Soc Lond Ser B Biol Sci 358:1935–1955

Naden PS, Murphy JF, Old GH, Newman J, Scarlett P, Harman M, Duerdoth CP, Hawczak A, Pretty JL, Arnold A, Laizé C, Hornby DD, Collins AL, Sear DA, Jones JI (2016) Understanding the controls on deposited fine sediment in the streams of agricultural catchments. Sci Total Environ 547:366–381

Nakano D, Tsuno M (2016) Macroinvertebrate assemblages in an artificial habitat—a settling basin of a hydroelectric power plant: comparison with a natural riffle habitat. Limnology 17:201–206

Oksanen J, Guillaume Blanchet F, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H (2018) vegan: Community ecology package. R package version 2.5-2. https://CRAN.R-project.org/package=vegan. Accessed 25 Sept 2018

Piano E, Doretto A, Falasco E, Fenoglio S, Gruppuso L, Nizzoli D, Viaroli P, Bona F (2019) If Alpine streams run dry: the drought memory of benthic communities. Aquat Sci 81:32. https://doi.org/10.1007/s00027-019-0629-0

Poff NL, Allan JD, Bain MB, Karr JR, Prestegaard KL, Richter BD, Sparks RE, Stromberg JC (1997) The natural flow regime. Bioscience 47:769–784

R Core Team (2018) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/. Accessed 25 Sept 2018

Richards C, Host GE, Arthur JW (1993) Identification of predominant environmental factors structuring stream macroinvertebrate communities within a large agricultural catchment. Freshw Biol 29:285–294

Rolls RJ, Leigh C, Sheldon F (2012) Mechanistic effects of low-flow hydrology on riverine ecosystems: ecological principles and consequences of alteration. Freshw Sci 31:1163–1186

Rolls RJ, Heino J, Ryder DS, Chessman BC, Growns IO, Thompson RM, Gido KB (2018) Scaling biodiversity responses to hydrological regimes. Biol Rev 93:971–995

Salmaso F, Crosa G, Espa P, Gentili G, Quadroni S, Zaccara S (2018) Benthic macroinvertebrates response to water management in a lowland river: effects of hydro-power vs irrigation off-stream diversions. Environ Monit Assess 190:33

Santos AN, Stevenson RD (2011) Comparison of macroinvertebrate diversity and community structure among perennial and non-perennial headwater streams. Northeast Nat 18:7–26

Sheldon F, Thoms MC (2006) Relationships between flow variability and macroinvertebrate assemblage composition: data from four Australian dryland rivers. River Res Appl 22:219–238

Smith H, Wood PJ (2002) Flow permanence and macroinvertebrate community variability in limestone spring systems. Hydrobiologia 487:45–58

Solis M, Bonetto C, Marrochi N, Paracampo A, Mugni H (2018) Aquatic macroinvertebrate assemblages are affected by insecticide applications on the Argentine Pampas. Ecotoxicol Environ Saf 148:11–16

Sponseller RA, Benfield EF, Valett HM (2001) Relationships between land use, spatial scale and stream macroinvertebrate communities. Freshw Biol 46:1409–1424

Storey R (2016) Macroinvertebrate community responses to duration, intensity and timing of annual dry events in intermittent forested and pasture streams. Aquat Sci 78:395–414

Stubbington R (2012) The hyporheic zone as an invertebrate refuge: a review of variability in space, time, taxa and behaviour. Mar Freshw Res 63:293–311

Thorp JH (2015) Functional relationships of freshwater invertebrates. In: Thorp JH, Rogers DC (eds) Ecology and general biology. Thorp and Covich's freshwater invertebrates, vol 1, 4th edn. Elsevier, London, pp 65–82

Vander Vorste R, Malard F, Datry T (2016) Is drift the primary process promoting the resilience of river invertebrate communities? a manipulative field experiment in an intermittent alluvial river. Freshw Biol 61:1276–1292

Vörösmarty C, Lettenmaier D, Lévêque C, Meybeck M, Pahl-Wostl C, Alcamo J et al (2004) Humans transforming the global water system. EOS 85:509–520

Vörösmarty CJ, McIntyre PB, Gessner MO, Dudgeon D, Prusevich A, Green P et al (2010) Global threats to human water security and river biodiversity. Nature 467:555–561

White JC, House A, Punchard N, Hannah DM, Wilding NA, Wood PJ (2018) Macroinvertebrate community responses to hydrological controls and groundwater abstraction effects across intermittent and perennial headwater streams. Sci Total Environ 610:1514–1526