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Diversity mediates the responses of invertebrate density to duration and frequency of rivers' annual drying regime

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Predicting the impacts of global change on highly dynamic ecosystems requires a better understanding of how communities respond to disturbance duration, frequency and timing. Intermittent rivers and ephemeral streams are dynamic ecosystems that are recognized as the most common fluvial ecosystem globally. The complexity of the drying process can give rise to different annual and antecedent hydrological conditions, but their effect on aquatic communities remains unclear. Here, using aquatic invertebrates from 33 streams across a flow-intermittence gradient, we assessed how annual (drying duration and frequency) and recent drying characteristics (duration of the last dry period and flowing duration since the last rewetting) affect the density and diversity metrics of communities and trophic groups while controlling for other key abiotic factors (dissolved oxygen and altitude). We characterized invertebrate communities using taxonomy and functional traits to capture biological features that increase vulnerability to drying. In addition, using structural equation modelling (SEM), we evaluated pathways by which drying characteristics directly impact invertebrate density and whether diversity indirectly mediates such relationships. We show that drying frequency drove reductions in diversity at the community level and within trophic groups, whereas both the drying duration and frequency had a negative influence on density metrics. Reductions in taxonomic richness were linked to increased annual drying duration, whereas functional diversity declined in response to annual drying frequency. Filterer, predator and shredder trophic groups exhibited the strongest negative responses to drying. Recent drying characteristics had a minor effect on density and diversity metrics. Our SEM results demonstrated that diversity mediates the negative impacts of annual drying duration and frequency on invertebrate density through reductions in their taxonomic richness and functional diversity. Our results underscore the importance of considering multiple drying characteristics together with the interdependence of density and diversity to better anticipate drying responses in freshwater ecosystems.

Keywords: biodiversity–ecosystem functioning, drying, flow intermittence, functional traits, intermittent rivers and ephemeral streams, Mediterranean streams

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Introduction

Predicting global change impacts on highly dynamic ecosystems requires a better understanding of how organisms respond to disturbance duration, frequency and timing (Tonkin et al. 2017, Soria et al. 2020). Long-term exposure to predictable and recurrent disturbances, such as diel tidal cycles in coastal systems or fire and aridity in climates where the dry season is highly predictable, has promoted the evolution and acquisition of functional traits that allow the development of life cycles under these stressful and varying conditions (Lytle 2001, Lytle and Poff 2004, Tapias et al. 2004, Bowman et al. 2009). As a result, communities tend to show a marked spatial and temporal turnover of organisms (Tonkin et al. 2017, Crabot et al. 2020), which depend on disturbance aspects operating at both annual and recent scales. However, it is still unclear how the local variety of organisms and functional traits respond to joint variations in disturbance duration, frequency and timing and the degree to which these biotic and abiotic changes simultaneously influence the functioning of highly dynamic ecosystems.

A well-known, global example of a dynamic ecosystems are streams that naturally and eventually experience periods of complete flow cessation in time or space (Datry et al. 2016). Intermittent rivers and ephemeral streams (IRES) are recognized as the most common fluvial ecosystem globally (Messager et al. 2021), and their spatial extent is increasing as a result of climate change and water extraction (Döll and Schmied 2012, Scheider et al. 2017). Consequently, flow regimes around the globe are shifting, exhibiting wide variation in the frequency, timing and duration of surface flow and drying. Characterizing these critical aspects of flow variation is thus a key step towards understanding how freshwater biodiversity and ecosystem functioning respond to global change.

Although all IRES have complete temporal or spatial cessation of flow in common, the complexity of the drying process can give rise to different annual and recent hydrological conditions, which influence community composition and diversity (Aspin et al. 2019, Crabot et al. 2020, Sarremejane et al. 2020). The duration and frequency of drying events filter out intolerant organisms by shortening the window for growth and reproduction (Ledger et al. 2011, Aspin et al. 2019). However, these two characteristics of drying could select for different functional traits, as they represent contrasting limitations for aquatic biota. For example, organisms able to thrive in a stream that experiences an annual drying duration of three months should be able to grow and reproduce when conditions are favourable (Galatowitsch and McIntosh 2016) or resist desiccation through specialized strategies (Stubbington and Datry 2013, Sarremejane et al. 2020). However, these life histories should be different depending on the drying frequency. Thus, organisms with multivoltine cycles or higher dispersal capacity can be favoured in streams with multiple drying periods respect to those with a single drying event because of the limited windows for growth and reproduction. In addition, the characteristics of recent drying

events, such as their duration or the time since the last rewetting, can help to explain spatiotemporal variation in aquatic communities, as these recent characteristics determine when organisms can colonize a given habitat (Bogan et al. 2015, Stubbington et al. 2016). Although the characterization of annual (drying duration and frequency) and recent drying characteristics (event duration and flowing duration since last rewetting) can help to better explain community and ecosystem changes in IRES, the comparison of their effects and relative importance remains poorly explored.

Functional diversity is a promising tool to understand the ecological causes and consequences of environmental change through the study of the range and variability of functional traits (McLean et al. 2019). When applied to IRES, taxonomic and functional diversity are complementary approaches that help to understand whether drying characteristics extirpate taxa with similar functional traits (similar niches) (Aspin et al. 2019, Sarremejane et al. 2021). Compared with taxonomic information, functional diversity also enhances the understanding of biodiversity effects on ecosystem functioning because functional traits better represent organic matter production, consumption or cycling processes than species identities (Cadotte et al. 2011, Carrara et al. 2015, Lefcheck and Duffy 2015).

Freshwater invertebrates are good candidates to investigate how these drying characteristics affect freshwater ecosystems because these organisms show contrasting responses to drying (Chessman 2015) and provide essential contributions to organic matter processing and transfer to vertebrate predator populations (Cardinale et al. 2002, Gessner et al. 2010, Gutiérrez-Cánovas et al. 2021). Previous studies have found that increasing drying duration causes strong changes in invertebrate communities, including reduced density and diversity (Bogan et al. 2013, Lancaster and Ledger 2015, Sarremejane et al. 2021) and shifts in functional trait diversity and composition (Aspin et al. 2019, Belmar et al. 2019, Crabot et al. 2020). Drying duration can also produce changes in food webs, but trophic group vulnerability depends on how food resources respond to drying (McIntosh et al. 2017). For example, drying events can directly limit feeding strategies based on coarse organic matter palatability (Arias-Real et al. 2018), the flux of fine particulate organic matter carried by water (von Schiller et al. 2017) and prey density (Ledger et al. 2013). Thus, invertebrate shredders, filterers or predators tend to be more vulnerable to drying than grazers or gathering collectors (Ledger et al. 2011, Arias-Real et al. 2018, Soria et al. 2020) because their food (algae and benthic detritus, respectively) is less affected by flow intermittence and drying events (Martínez et al. 1998) or shows rapid recovery after flow resumption (Timoner et al. 2012). Only a few studies have explored the simultaneous effects of annual and recent drying characteristics on taxonomic richness (Leigh and Datry 2017), and sometimes using multiple river locations from the same basin (Crabot et al. 2020, Sarremejane et al. 2020). Therefore, there is still a need to explore their effect and importance across a wider range of community characteristics, including functional diversity

and trophic groups, and using data from independent rivers at large spatial scales.

In light of the growing awareness regarding freshwater invertebrate declines and increasing flow intermittence (Döll and Schmied 2012, Sánchez-Bayo and Wyckhuys 2019), it is urgent to understand which hydrological and ecological mechanisms drive drying impacts on invertebrate communities. However, our current knowledge is limited by an incomplete view of the ecological mechanisms through which invertebrate communities and their functions respond to drying characteristics. Negative responses of invertebrate density to drying have been frequently attributed to the direct effects of abiotic stress on their fitness (growth, reproduction and survival) (Ledger et al. 2011, Galatowitsch and McIntosh 2016) or colonization opportunities (sites with increased drying duration or frequency are more difficult to colonize; Cañedo-Argüelles et al. 2020, Crabot et al. 2020). However, density can also decline in response to abiotically driven reductions in taxonomic or functional diversity (Lefcheck and Duffy 2015), because abiotic filtering could cancel out the positive effects of invertebrate diversity on density (Mittelbach et al. 2001, Cardinale et al. 2002, 2006). For example, increased facilitation, niche complementarity and/ or an increased probability of retaining highly performing taxa in more diverse communities favour the efficient conversion of resources to individuals and biomass (Cardinale et al. 2006, Lefcheck and Duffy 2015, van der Plas 2019, Arias-Real et al. 2021a), reduce competition (Carrara et al. 2015) and increase the capacity to cope with and recover from disturbances (McLean et al. 2019, Arias-Real et al. 2021). However, responses of density and diversity to drying are usually assessed independently, overlooking the potential indirect effects of abiotic stress on density through diversity (Cardinale et al. 2006, Steudel et al. 2012, Beaumelle et al. 2020). Revealing how of biodiversity–ecosystem functioning relationships respond to drying stress will help anticipate the functional consequences of invertebrate losses.

In this study, using invertebrate communities from 33 streams across a wide flow-intermittence gradient, we assessed how annual and recent drying characteristics influence invertebrate diversity, density and trophic groups by testing the following predictions. First, the density and diversity of invertebrate communities and trophic groups are better explained by a combination of drying characteristics rather than by a single factor (e.g. annual drying duration). Second, shredder, filterer and predatory invertebrates exhibit stronger negative responses to drying than grazers, gathering–collectors or omnivorous taxa because their food resources (prey, terrestrial leaves or suspended fine organic matter) are more vulnerable to drying. Third, changes in invertebrate density are better explained by variation in invertebrate diversity than by the direct effects of drying; this leads to a novel indirect mechanism by which diversity mediates drying effects on invertebrate density. To test these predictions, 1) we characterized the annual characteristics of drying (drying duration and frequency) and recent drying conditions (duration of the recent drying event and flowing duration since the last rewetting).

Next, 2) using linear regressions and multimodel inference, we determined the effect and importance of these drying characteristics on invertebrate diversity (taxonomic richness and functional diversity) and the density of communities and trophic groups while controlling for other key abiotic factors (dissolved oxygen and altitude). Finally, 3) using structural equation modelling, we evaluated the pathways by which drying characteristics directly influence invertebrate density and how diversity indirectly mediates such relationships.

Methods

Study site

This study was conducted at 33 independent streams located in nine river basins with low human impact across Catalonia (NE Spain) (supporting information). The primary land uses in the riparian zone were forest, scrubland, grassland and extensive agriculture (mainly olive groves and vineyards) (based on Corine Land Cover 2006 data in a buffer area of 1 km around each sampling site). The stream order of the sites ranged from two to four. The climate is typically Mediterranean with dry and warm summers, and precipitation mainly occurs during spring and autumn. See the Supporting information for more details regarding the study sites.

Characterization of drying and non-hydrological abiotic factors

To characterize drying metrics, in February 2016, we placed Leveloggers (Solinst Levelogger Edge, full-scale reading precision of 0.05%) in the streambed in lotic and lentic habitats for water level and temperature recordings, which allowed us to infer water presence (and drying) during the 12 month preceding biological sampling. The Leveloggers were recorded at hourly intervals for one year (from February 2016 until February 2017). The recorded data were corrected with barometric pressure variations using data from Barologgers installed in the riparian area to measure the atmospheric pressure changes (Solinst Barologger, full-scale reading precision of 0.05%) and data from meteorological stations near each site (Supporting information). Using these temperature and water level data loggers, we measured two annual and two recent drying characteristics. Annual drying characteristics included information on the total number of days with zero flow (zero flow total; ZFT) and their annual frequency (i.e. the number of zero flow periods; ZFP). Recent drying characteristics included the number of days with zero flow in the last zero-flow period (zero-flow last; ZFL) and the number of days since the last rewetting event (rewetting; RE) (Fig. 1) (Arias-Real et al. 2020). The total precipitation during the 12 months preceding biological sampling represented the average conditions of the last 20 years (Cañedo-Argüelles et al. 2020; Servei Meteorològic de Catalunya, <www.meteocat.es>).
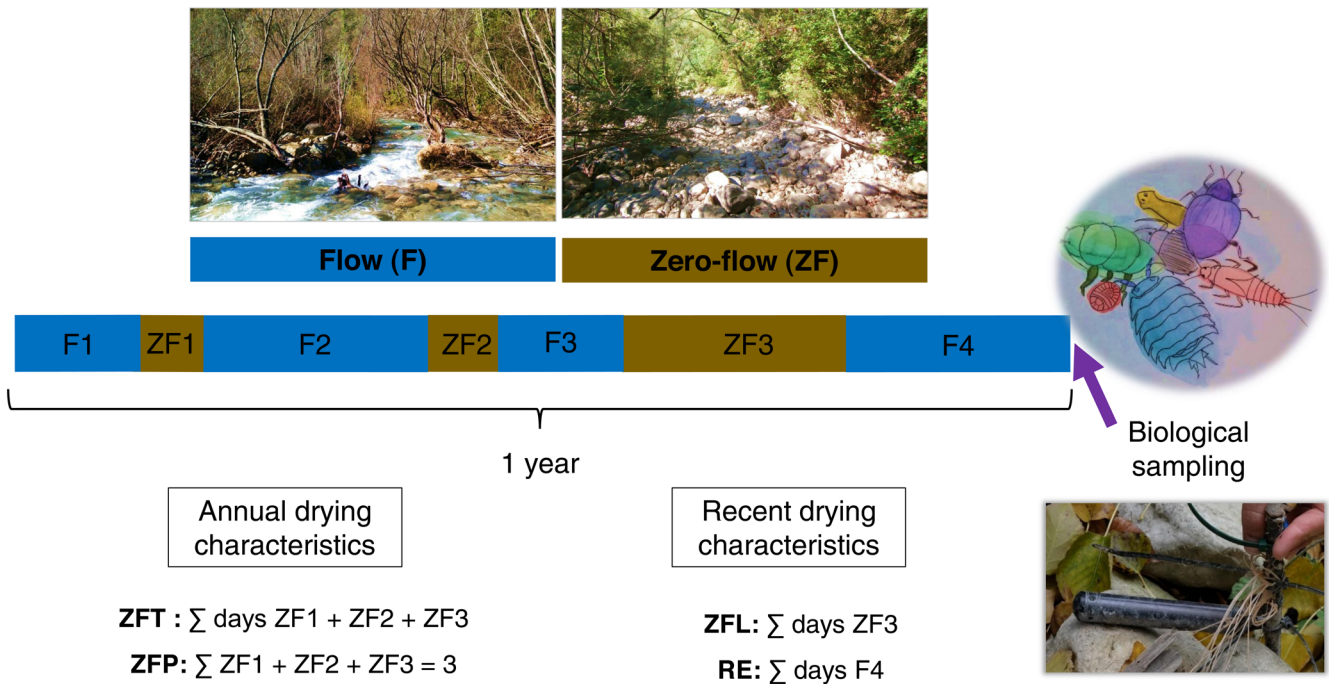
Figure 1. Illustration of the calculation of annual and recent drying characteristics: ZFT is the annual number of total zero-flow days (drying duration), ZFP is the annual number of zero-flow periods (drying frequency), ZFL is the number of days in the recent zero-flow period (duration of the last drying period) and RE is the number of days between flow resumption and sampling (time since the last rewetting) (modified from Arias-Real et al. 2021a).

For each stream, we characterized climatic, geomorphologic, land use and water chemistry variables (Supporting information).

Invertebrate data collection and functional traits

At each stream site, we collected invertebrate samples just after the rainy season (February 2017) to ensure that all streams were in the flowing phase, i.e. all mesohabitats were available and connected. Sampling methods for river invertebrates typically display a tradeoff between the capacity to capture taxon richness or taxon density. As we aimed to characterize both richness and density-based metrics, we used two methods that are effective in representing either taxon richness (kick-net) or density (Surber).

To characterize the total invertebrate density (and related measures), we collected three quantitative Surber samples (area: 400 cm², mesh size: 250 µm) in riffle areas. To characterize invertebrate richness, we used kick-net samples (mesh size: 500 µm) through a multihabitat standardized protocol with a sampling effort proportional to each habitat occurrence (Jáimez-cuéllar et al. 2002) and a duration of five minutes. All samples were preserved in formalin (4%). Specimens were counted and identified in the laboratory to the genus level for most taxa or to the family level in a few cases (e.g. Diptera), resulting in a total of 73 invertebrate taxa.

To characterize the functional features of the invertebrate taxa, we compiled a database containing 11 functional traits (and 64 categories), including maximum body size, life cycle duration, number of generations per year, aquatic stage, reproduction, dispersal mode, resistance strategy, respiration, locomotion,

food resources (Tachet et al. 2002, Bonada and Dolédec 2011) and trophic preferences (<www.freshwaterecology.info>; Moog 2002, Schmidt-kloiber and Hering 2015). These traits are potentially related to resilience and resistance to drying and resource utilization in the stream habitat (Supporting information for full details about expected trait responses to drying; Vázquez and Simberloff 2002, Bonada et al. 2007, Díaz et al. 2008, Ledger et al. 2011, Chessman 2015, Schriever and Lytle 2016, Vadher et al. 2017, Aspin et al. 2019, Belmar et al. 2019, Bruno et al. 2019). Trophic preferences support key ecosystem processes such as energy and organic matter consumption and transfer to higher trophic levels, nutrient cycles and secondary production (Wallace and Webster 1996, De Crespin De Billy et al. 2002, Arias-Real et al. 2018). The traits were fuzzy coded, i.e. for each invertebrate taxon, a degree of affinity (ranging from 0 to 10) was assigned to each trait category according to the frequency of occurrence within the genus. Prior to analysis, fuzzy coded data were converted into percentages of affinity for each trait.

Based on trophic preferences, we defined six trophic groups that included taxa showing at least 50% trophic specialization for a given feeding mode: filterers, gathering-collectors, grazers, predators and shredders. We classified omnivores as those taxa not showing a sufficient degree of specialization (less than 50%) for any feeding mode.

Density and diversity metrics

We calculated a total of 15 density (seven) and diversity (eight) metrics. Density metrics included the total density

and the density of each of the six trophic groups (e.g. predator density) based on quantitative Surber samples. Diversity metrics included community taxonomic richness (based on kick-net samples), community functional diversity (Surber samples) and functional diversity for each of the six trophic groups (Surber samples). To calculate the functional diversity measures, we used functional dispersion (Laliberté and Legendre 2010), which represents the diversity of trait values with respect to community or trophic group mean trait profiles (i.e. the average distance of occurring taxa to community or trophic group centroids in the functional space). The distances to centroids were weighted by log-transformed taxon density to account for differences in taxon relative densities. Community functional diversity represented the mean of the standardized functional diversity for the six trophic groups. To avoid circularity in the estimation of functional diversity for trophic groups and community means, we focused on ten out of 11 traits, excluding trophic strategies because trophic groups were defined based on trophic preferences. We used a Gower index adapted to fuzzy-coding traits to build a reduced trait space where functional diversity metrics were calculated (Pavoine et al. 2009, Maire et al. 2015). Methodological details regarding trait-based metric calculations are available in supporting information.

Data analysis

First, to select the most influential non-hydrological factors, we performed Pearson correlations between richness, density and functional diversity (i.e. community metrics) and climatic (annual precipitation), geographical and basin characteristics (altitude of river, catchment area and river order), land use (percentages of natural, agricultural and urban uses), water temperature and water chemistry variables (nutrients, dissolved oxygen and pH). We chose altitude and dissolved oxygen for richness and functional diversity because they showed the highest correlation coefficients with total density, taxon richness and functional diversity (Supporting information). In addition, they strongly influence the distribution and life histories of stream invertebrates (Díaz et al. 2008, Verberk et al. 2011).

To analyse the effects of the drying characteristics and non-hydrological factors on the density and diversity metrics, we used linear regression models and a multimodel inference approach (Burnham and Anderson 2002). First, we built nine linear regression models (LMs) for each density and diversity metric, including different combinations of drying characteristics and non-hydrological factors as predictors (Supporting information). All these models contained dissolved oxygen and altitude to quantify the effect of non-hydrological environmental variation among sites in combination with single (models 1–4) or combined drying characteristics (models 5–9). We avoided models that simultaneously included both the annual drying duration and the duration of the recent drying event because they were highly correlated ($r = 0.72$, Supporting information). As a result, models included predictor combinations showing variance inflation factors < 2,

suggesting an acceptable degree of collinearity (Zuur et al. 2009a). Second, to quantify regression coefficients, statistical support and the importance of the drying characteristics and non-hydrological factors, we adopted a multimodel inference approach (Burnham et al. 2011) using the function *model. sel*() from the MuMIn R package (Bartoń 2020). Based on the second-order Akaike information criterion (AICc) for small sample sizes, we ranked the nine alternative models according to their AICc values and retained those with an AICc difference ≤ 2 with respect to the highest-ranking model. We also derived the model explained variance (R^2) and Akaike weights to determine the explanatory capacity and the relative likelihood of each model (statistical support), respectively. For each density and diversity metric, based on model's Akaike weights, we obtained the mean-weighted partitioned variance for each predictor (Hoffman and Schadt 2016). To visualize the overall response of these models and using model's Akaike weights, we conducted a weighted-average of their standardized regression coefficients and predictions across the retained models (ΔAICc ≤ 2).

Finally, to quantify the direct and indirect (via diversity) effects of drying duration and frequency on density, represented by total density, we used a structural equation modelling (SEM) approach (piecewiseSEM package, Lefcheck 2016). SEM is a causal inference tool that is used to examine the complex networks in natural ecosystems because several influences and responses can be analysed simultaneously. Therefore, our SEM allows us to investigate whether density responses to drying are mediated by invertebrate diversity, which is represented by taxon richness or functional diversity. Using a multimodel inference approach (based on AICc) (Shipley 2013), we evaluated 14 SEM structures, which included SEMs in which density and diversity respond independently (drying→diversity and drying→density), SEMs that considered only indirect drying effects on density mediated by diversity (drying→diversity→density), and SEMs that included both direct and indirect drying effects on density via diversity (drying→diversity→density and drying→density). We evaluated the statistical support for the models based on their AICc values (lower values indicate higher explanatory capacity) and Akaike model weights (likelihood of being the best model). These models tested different combinations of annual drying duration and frequency to predict diversity metrics and density. Recent drying variables were not used in these models because of their lower predictive capacity. All models included dissolved oxygen as a predictor of diversity, whereas altitude was not considered as its inclusion was not statistically supported (increased AICc values). These models included three to five (direct and indirect) causal relationships. This ensured that we tested SEM structures with more than five observations per free parameter (33: 6 = 5.5), which is sufficient to run robust SEMs (Wolf et al. 2013, Grace et al. 2015). The overall model fit was assessed using Fisher's C-test, in which small and nonsignificant values (p-value > 0.05) indicate a good fit of the model (Shipley 2013).

To reduce distribution skewness, before the analyses, the drying frequency and richness were squared-root-transformed, and the duration of the recent drying event and time since the last rewetting were fourth-root-transformed. In addition, the total density and multitrophic and trophic group densities were log-transformed. All variables were z-standardized (mean = 0, SD = 1) to allow the comparison of model coefficients. In all cases, model residuals were visually assessed to verify linear model assumptions (Zuur et al. 2009b). All statistical analyses were performed using R ver. 3.4.1 (<www.r-project.org>).

Results

General description of hydrological, non-hydrological and biotic metrics

The streams studied covered a steep gradient of drying, ranging from streams of permanent flow to others with long dry periods (ZFT, 0–257 days, median = 76; Supporting information). These rivers experienced varying frequencies of drying, ranging from 0 to 8 dry events (ZFP median = 1 event). The duration of the recent drying event ranged from 0 to 246 days (ZFL, median = 18 days), and the time since the last rewetting varied from 6 to 336 days (RE, median = 57 days). Dissolved oxygen ranged from 4.5 to 9.2 mg l^{-1} over

an altitudinal range of 81–920 m a.s.l. Over these drying and non-hydrological abiotic gradients, invertebrate communities varied in their taxonomic richness (7–42 taxa), total density (750–212 825 ind. m^{-2}) and density per trophic group (150–37 300 ind. m^{-2}). Overall, the five most abundant taxa were Chironomidae (524 250 ind. m^{-2}), Lumbriculidae (229 725 ind. m^{-2}), Ceratopogonidae (171 325 ind. m^{-2}), Lymnaeidae (150 725 ind. m^{-2}) and Gammaridae (108 375 ind. m^{-2}). With the exception of Gammaridae (30% occurrence), these taxa were also common (occurrences between 69 and 100%) in streams with more than 100 dry days. Nine taxa were exclusive to streams showing up to 100 dry days: the caddisflies *Limnephilus*, *Micrasema* and *Rhyacophila*; the diperan Culicidae; the mayfly *Ephemerella*; the stoneflies *Capnia* and *Brachyptera*; the tricladid *Dugesia* and the water beetle *Hydraena*.

Effects and importance of drying characteristics on density and diversity

Annual drying duration (mean standardized coefficient ± SE: −0.36 ± 0.03) and frequency (−0.07 ± 0.03) had consistent negative effects on invertebrate density metrics (Fig. 2a, Supporting information). Drying frequency had a stronger negative effect on diversity metrics (−0.22 ± 0.05) than drying duration (−0.08 ± 0.06). A consistent positive effect was observed for the time since the last rewetting (0.06 ± 0.03) but
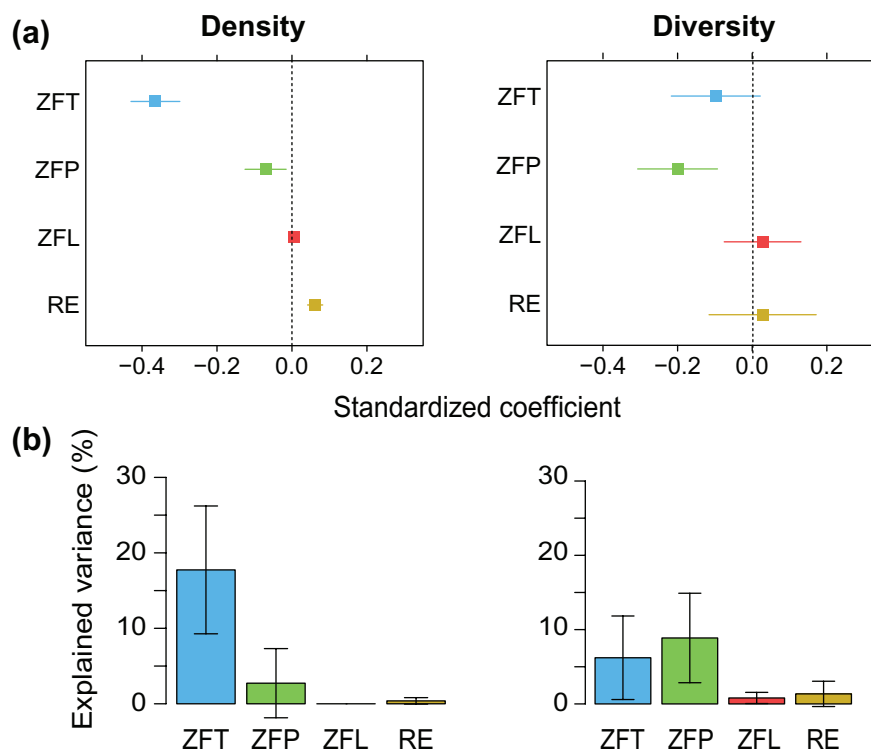

Figure 2. The weighted mean standardized coefficients (a) and explained variance (b) of density and diversity metrics. Standardized coefficients and explained variance represent the weighted mean values of retained models (ΔAICc ≤ 2) (Supporting information). ZFT, annual number of total zero-flow days (annual drying duration); ZFP, annual number of zero-flow periods (annual drying frequency); ZFL, duration of recent dry period; and RE, time since last rewetting.

the recent drying duration had a weak influence on density and diversity metrics (the mean effect size overlapped with zero).

Drying duration was generally a better predictor of density metrics (mean $R^2 = 17.7\%$, range = 0.0–31.3%) than drying frequency (2.7%, range = 0.0–16.6%), time since the last rewetting (0.4%, range = 0.0–1.6%) and recent drying duration (0.0%; it was not included in any retained model) (Fig. 2b). For the diversity metrics, both the annual drying frequency (mean = 9.4%, range = 0.0–21.6%) and duration (mean = 5.2%, range = 0.0–16.1%) were the most explanatory characteristics. The relative contribution of annual drying duration and frequency to each metric varied considerably across density and diversity metrics (Fig. 2b). For taxonomic richness, annual drying duration (20.3%) was more influential than annual drying frequency (5.7%). Drying characteristics collectively explained a higher proportion of the variance for density (22.3%) relative to dissolved oxygen and elevation (9.8%), whereas both categories of variables made roughly similar contributions to explaining diversity (drying characteristics: 18.6%; non-hydrological factors: 22.7%, Fig. 3).

Shredders, filterers and predators tend to show stronger negative associations to annual drying duration (mean standardized coefficient ± SE: −0.24 ± 0.02) and frequency (−0.22 ± 0.04) than grazers, gathering collectors and omnivorous taxa (duration, −0.18 ± 0.07; frequency, −0.05 ± 0.05) (Fig. 4, Supporting information). Similarly, drying characteristics explained more of the variance in the diversity and density of shredders, filterers and predators (24.2%) than that observed for other trophic groups (9.7%).

Direct and indirect effects of drying characteristics on density

The four models that ranked highest based on AICc (lowest AICc values; cumulative model weight = 0.93) included significant indirect negative effects of annual drying duration or frequency on total density, which were mediated by significant reductions in functional diversity or taxonomic richness (Fig. 5a, Supporting information). The two top-ranked models ranked based on AICc considered only significant indirect effects of annual drying frequency on total density (via diversity), followed by two other SEMs that included both significant indirect and direct effects of annual drying duration on total density. Models using functional diversity ranked higher based on AICc values and had greater statistical support (cumulative weight = 0.56) than those using taxonomic richness (cumulative weight = 0.37). Among the evaluated SEMs, the four models including independent responses of density and diversity to drying showed the poorest fit (cumulative weight = 0, Fisher's test p-values ranged from 0.012 to 0.061). The direct effects of annual drying duration were less negative in the SEMs, including mediating diversity effects (−0.34 ± 0.05), than in previous linear models considering independent responses (−0.60 ± 0.10). Functional diversity (−0.54 ± 0.10) and taxonomic richness (−0.56 ± 0.10) had
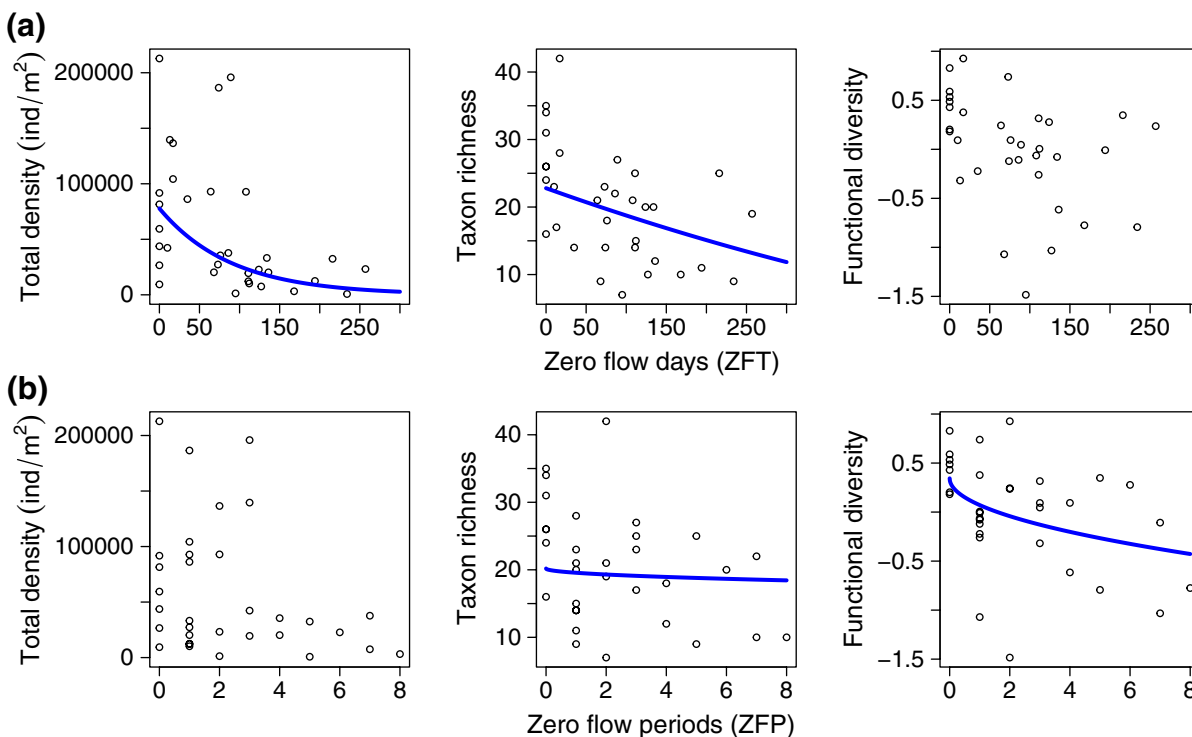

Figure 3. Plots showing responses of total density, taxonomic richness and functional diversity to annual drying duration (zero flow days, (a) and annual drying frequency (zero flow periods, (b). Fitted values represent weighted mean responses of retained models (ΔAICc ≤ 2) (Supporting information).
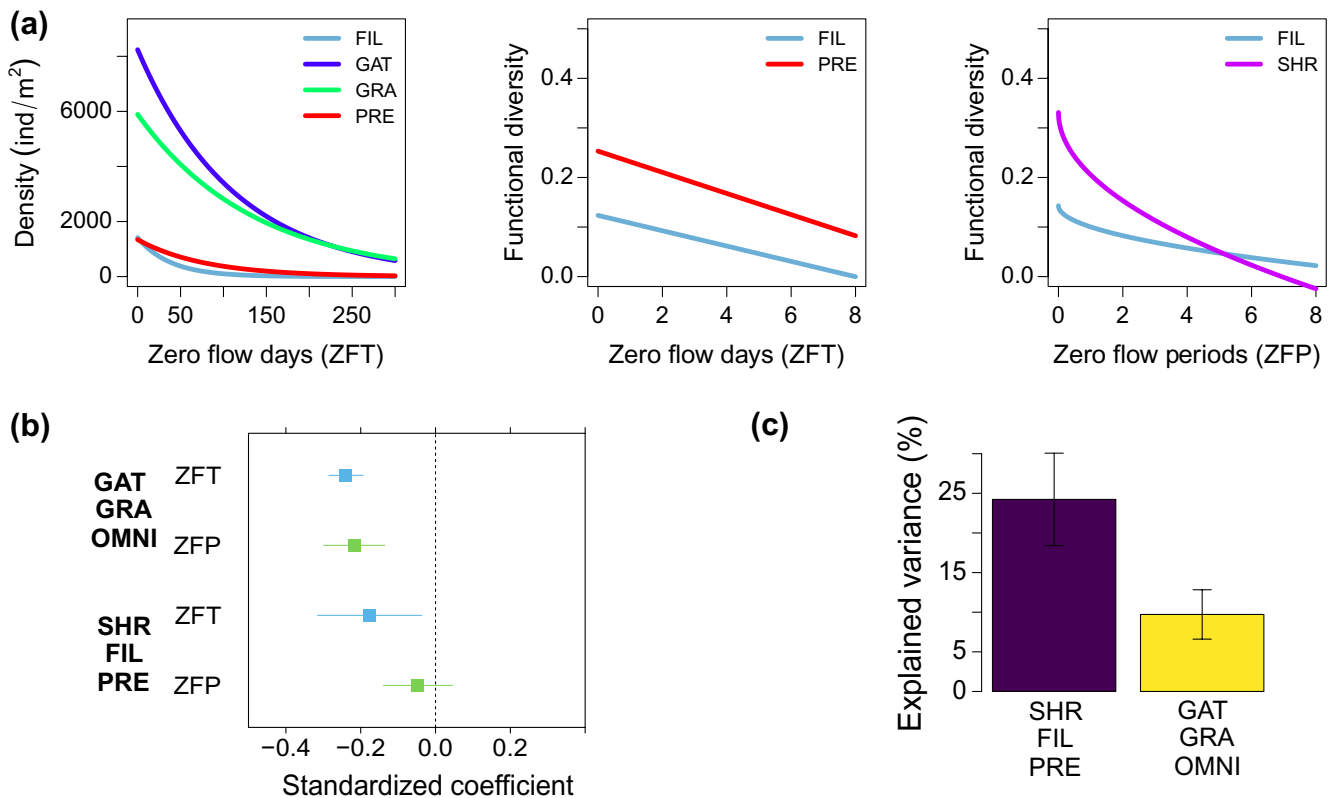
Figure 4. Plots showing responses of trophic group density and functional diversity to annual drying duration (zero flow days) and annual drying frequency (zero flow periods (a)). Fitted values represent weighted mean responses of retained models (ΔAICc ≤ 2) (Supporting information). Trophic group labels: FIL, filterers; GAT, gathering collectors; GRA, grazers; PRE, predators; and OMNI, omnivores. We also show the comparison of mean standardized coefficients (b) and the variance explained by drying characteristics (c) for shredders, filterers and predators in comparison with other trophic groups.

higher absolute effects on density than the direct effects of annual drying (Fig. 5b). Models considering single direct effects of annual drying duration were less explanatory (30%) than those including the direct effects of diversity or both diversity and drying duration (44%) (Fig. 5b).

Discussion

Using a dataset covering a large spatial scale and multiple independent streams, we demonstrated that invertebrate responses to drying are better predicted by combinations of drying patterns, mainly by annual drying duration and frequency. Our data suggest that annual metrics are more important in explaining invertebrate responses compared with those describing characteristics of recent drying. Shredders, filterers and predators tend to be the most responsive trophic groups. We also found that the density decline was better explained by indirect reductions in invertebrate diversity than by direct negative effects of annual drying duration or frequency. Taken together, our results underscore the importance of considering multiple drying characteristics and the interdependence of density and diversity responses to better anticipate the effects of drying on freshwater biodiversity.

Consistent with our first prediction, density and diversity were better explained by combinations of various drying patterns. The prominent role of annual drying duration and frequency in our models suggests that the distribution of dry days and periods at an annual scale controls the variety of life forms that are able to colonize and thrive in IRES (Crabot et al. 2020, Sarremejane et al. 2020). Increased annual drying duration or frequency can limit the occurrence of certain taxa that are unable to complete their life cycles in short periods (Chessman 2015, Aspin et al. 2019) or because of their low tolerance to chemical stress or desiccation (Stubbington and Datry 2013, Granados et al. 2020). However, our data indicate that the annual drying duration and frequency appear to exert different direct effects on invertebrate communities, suggesting that both characteristics should be considered to better predict community dynamics in IRES. When not considering diversity effects on density, drying duration was the dominant hydrological driver of density, on which non-hydrological factors had a lower influence. Reductions in invertebrate density with increasing annual drying duration can reflect detrimental effects on growth rates, body size and reproductive capacity (Ledger et al. 2011, Galatowitsch and McIntosh 2016), which constrain secondary production (Patrick et al. 2019). Reductions in taxonomic richness were explained by annual
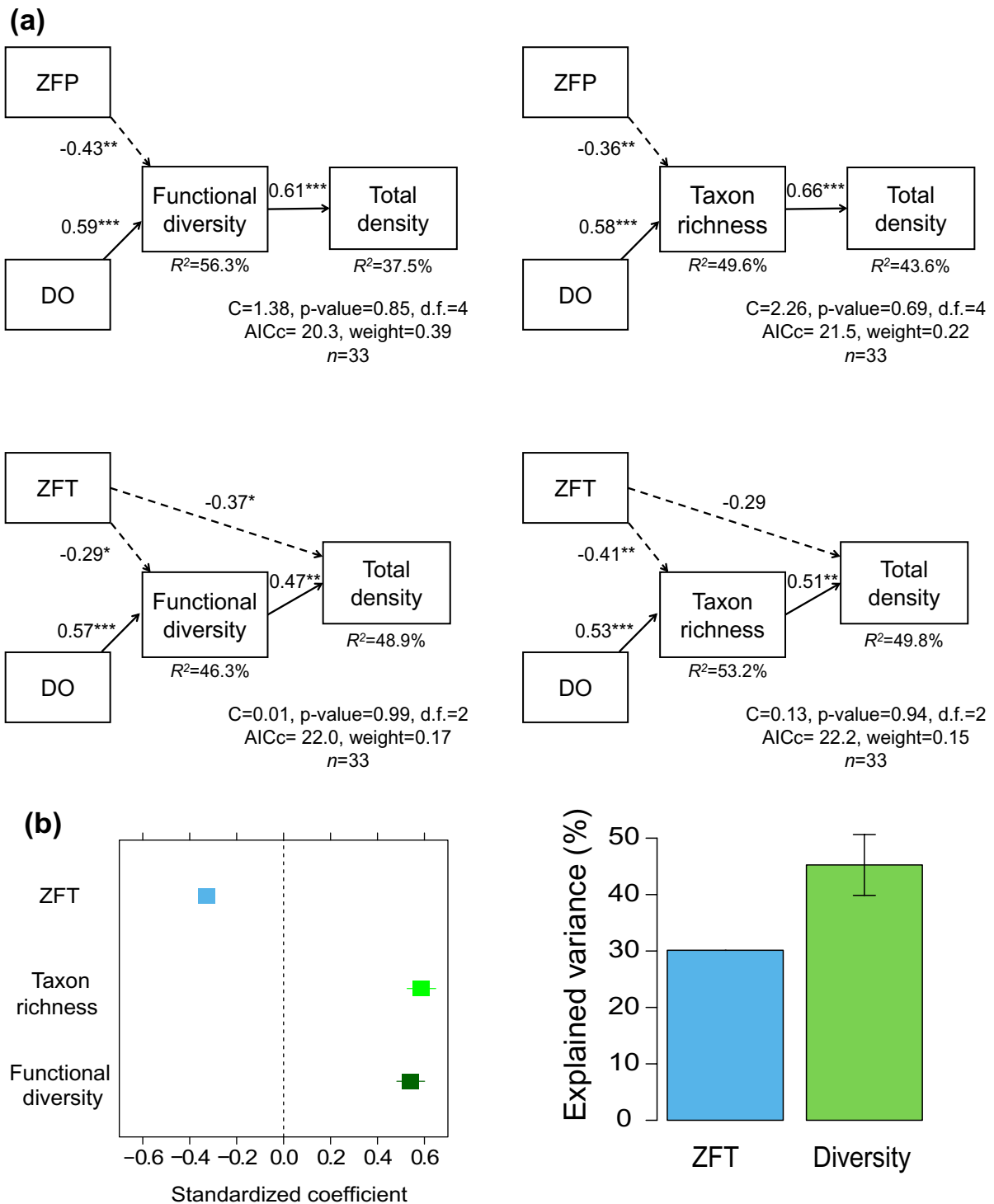
Figure 5. Highest ranked SEMs showing direct and indirect effects (via diversity: taxon richness or functional diversity) of drying characteristics (annual drying duration: ZFT, annual drying frequency: ZFP) and dissolved oxygen (DO) on total density (a). Solid black arrows represent significant positive relationships, and broken black arrows represent significant negative effects. R^2 denotes the proportion of the variance explained for each response variable, appearing below. Significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. We also show the mean standardized coefficients and explained variance (b) of the direct effects of ZFT and diversity (taxon richness and functional diversity) on total density.

drying duration, whereas functional diversity declined at sites exposed to more frequent drying events. This suggests that the studied functional strategies were constrained by the temporal windows for growth and reproduction rather than by the total annual dry days. This is particularly important given that climatic droughts are expected to increase in both duration and frequency (Vicente-Serrano et al. 2020), potentially affecting both the taxonomic and functional diversity of streams. However, a wider range of abiotic drivers appear to determine diversity patterns, as observed by the stronger explanatory power of non-hydrological factors relative to drying characteristics. Although variation in dissolved oxygen was unrelated to drying characteristics in our study ($r = -0.18$ to -0.02), low dissolved oxygen concentrations can limit the occurrence of organisms with aquatic breathing (i.e. gills, tegument and plastron respiration; Verberk et al. 2016). In addition, altitude emerges as an additional factor complementing hydrological variables when predicting spatial variation in invertebrate communities and trophic groups. Altitude is a good surrogate of terrestrial productivity and thermal and hydrological regimes, offering a good means to track spatial changes in biodiversity (Picazo et al. 2020). Although both the duration of the last drying event and the time since the last rewetting have the potential to alter density and diversity (Stubbington et al. 2016), they displayed a low explanatory capacity in our models. One potential reason is that invertebrate communities can recover quickly after flow resumption, and we were unable to track recent drying effects after the rainy season. It is likely that recent metrics could gain importance in predicting community changes when studying the temporal dynamics of IRES, particularly in samples close to flow resumption (Soria et al. 2020).

Consistent with our second prediction, shredders, filterers and predators showed the strongest responses to drying. Although previous results have found similar patterns (Bonada et al. 2007, Ledger et al. 2011, Soria et al. 2020), our study provides novel insights into the vulnerability of trophic groups to different characteristics of drying. In particular, shredders were more sensitive to increasing annual drying frequency, predators responded mainly to annual drying duration and filterers were affected by both annual drying characteristics. Vulnerability to drying characteristics can arise because flow intermittence and drying events directly limit these feeding strategies. For example, increased dewatering can reduce the quantity and quality of coarse organic matter, constraining shredder growth and survival (Sanpera-Calbet et al. 2017, Arias-Real et al. 2018) and leading to reduced rates of organic matter decomposition (Arias-Real et al. 2020, Truchy et al. 2020). Drying can also limit the flux of fine organic matter carried by water (von Schiller et al. 2017), constraining food availability for filterers (Wallace and Webster 1996). In addition, drying stress reduces the overall flow of energy reaching apex positions in the food web through a limited diversity and density of prey, which constrain predator performance (Ledger et al. 2013, McIntosh et al. 2017). Besides trophic preferences, other traits such as body size or respiration mode can also play an important role in regulating the responses of invertebrates to drying (Bonada et al. 2007, Chessman 2015, Aspin et al. 2019). This is evidenced by the density reduction across trophic groups because all of them include organisms with potentially drying-sensitive traits (e.g. aquatic respiration).

Consistent with our third prediction, our results support the positive link between invertebrate diversity and density (Mittelbach et al. 2001, Cardinale et al. 2006), showing that density responded both directly and indirectly to drying stress. By focusing only on direct drying effects, previous studies might have missed this link, potentially overestimating the direct effects of drying duration and underestimating the negative impacts arising from diversity loss (Ledger et al. 2011, Datry et al. 2014, Aspin et al. 2018). Such diversity-mediated effects can arise due to the higher capacity of diversified communities to support higher productivities and in turn larger stocks of individuals (Mittelbach et al. 2001, Cardinale et al. 2006, Lefcheck and Duffy 2015, Steudel et al. 2016, Arias-Real et al. 2021). For example, communities showing a diverse representation of niches and functional traits tend to have a more efficient and complementary use of resources (Jonsson and Malmqvist 2000, Cardinale et al. 2002, Gessner et al. 2010, van der Plas 2019), reduced competence (Carrara et al. 2015) and higher stability in response to environmental change (McLean et al. 2019, Arias-Real et al. 2021a). Higher densities can also be a consequence of diversified communities having a higher probability of containing highly productive species through sampling effects (Cardinale et al. 2006). Although the relationship between diversity and density is well established (Cardinale et al. 2006, Lefcheck and Duffy 2015), future manipulative studies should confirm the causality and strength of this relationship under controlled conditions.

In conclusion, a combination of drying characteristics, such as drying duration and frequency, are needed to explain changes in invertebrate communities and trophic groups. We found that changes in invertebrate density arose from the direct and indirect effects of these drying patterns. These findings answer recent calls to investigate the causes of freshwater population declines (Sánchez-Bayo and Wyckhuys 2019) by identifying that drying-caused losses of aquatic biodiversity are linked to declines in invertebrate density. Our framework, based on multiple drying characteristics and the functional aspects of biodiversity, enables better prediction of how stream communities and ecosystem functioning will respond to global change.

Author contributions

Rebeca Arias-Real and **Cayetano Gutiérrez-Cánovas** contributed equally to this publication. **Rebeca Arias del Real**: Conceptualization (lead); Data curation (lead); Formal analysis (equal); Investigation (lead); Methodology (equal); Validation (lead); Writing – original draft (lead). **Cayetano Gutiérrez-Cánovas**: Conceptualization (lead); Data curation (supporting); Formal analysis (lead); Investigation (equal); Methodology (lead); Validation (equal); Visualization (lead); Writing – original draft (lead). **Margarita Menéndez**: Investigation (equal); Methodology (equal); Project administration (equal); Supervision (equal); Writing – review and editing (equal). **Verónica Granados**: Data curation (equal); Investigation (equal); Methodology (equal); Validation (equal); Writing – review and editing (equal). **Isabel Muñoz**: Conceptualization (equal); Funding acquisition (lead); Investigation (equal); Methodology (equal); Project administration (lead); Supervision (lead); Writing – review and editing (equal).

Data archiving statement

Data are available from the Dryad Digital Repository: (Arias-Real et al. 2021c), and at Github Digital Repository <https://github.com/Arias-Real/diversity_mediates_drying_impact> (Arias-Real et al. 2021b).

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