Patterns in taxonomic and functional diversity highlight the “mediterraneization” of macroinvertebrate communities in recently intermittent Alpine watercourses

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Abstract

1. Mountain streams are currently shifting from perennial to intermittent regime due to the combined effect of climate change and local anthropogenic pressures. Studies on the effect of flow intermittency on macroinvertebrates are as yet inconclusive due to the large heterogeneity in geographical areas and spatial scales at which this relationship has been investigated. Given that flow intermittency is a recently documented phenomenon in the Alps, only few studies have investigated functional and taxonomical diversity of benthic invertebrate communities in recently intermittent Alpine streams.

2. We used a hierarchical sampling design to investigate patterns in taxonomic and functional diversity of benthic invertebrate communities in 13 recently intermittent Alpine streams in NW-Italy. In each of these streams, we sampled in April 2017 benthic communities in two reaches with different hydrological conditions: a control reach, with permanent flow, and an intermittent reach, which recently experienced non-flow periods in summer.
3. We tested for the response of taxonomic richness at multiple spatial scales by partitioning total diversity into the average richness of local communities and the richness due to variation among local communities both within and among reaches. By partitioning total diversity ($\gamma$) into its local ($\alpha$) and turnover ($\beta$) components we showed a decrease in local and regional species richness both within and among reaches, whereas variation among communities was significantly lower in intermittent reaches only at the reach scale.

4. The analysis of the multidimensional trait space of macroinvertebrates in reaches with different hydrological conditions further revealed a significant reduction of functional diversity, dispersion, and evenness in intermittent reaches. There was trait overdispersion in intermittent reaches, as these hosted both typical Alpine taxa and organisms adapted to flow intermittency. In particular, we observed the replacement of taxa with aquatic respiration and preferring medium- to fast-flowing oligotrophic waters by taxa adapted to lentic habitats and with larval dormancy phases.

5. These results provide strong support that recent flow intermittency determines drastic changes of benthic invertebrate communities in Alpine streams which are facing a “mediterraneization” process. Our work highlights the importance of considering multiple spatial scales and of integrating taxonomic and functional diversity to thoroughly assess the impacts of flow intermittency.

Keywords: flow intermittency, drying, diversity partitioning, nestedness, functional traits
INTRODUCTION

Stream ecosystems are particularly sensitive to climate change, as changes in precipitations, temperature and evaporation direct translate in shifts in river flow regimes and their physical conditions (Lake, 2003). The raising of water temperatures and the disruption of hydrologic cycles, further exacerbated by anthropogenic activities (e.g. deforestation, water capture, streambed alterations; Wohl, 2006) can lead, in turn, to stochastic events (Scheffer et al., 2001), like floods and droughts. The observed and predicted climate warming is also increasingly associated to changes in distribution and phenology of biota (Heino et al., 2009; Whitehead et al., 2009).

Among flow-related disturbances, water scarcity, and consequent riverbed desiccation, is one of the most pressing environmental issues related to climate change, with expected drastic consequences on biodiversity (Lake, 2003). The necessity of understanding such complex dynamics have recently pushed more and more researchers to study the effect of flow intermittency on the taxonomic richness of benthic invertebrate communities. Yet contrasting results have so far been obtained, as either negative relationships (Datry et al., 2014; Leigh & Datry, 2017; Datry et al. 2016a), no relationship (Bonada et al., 2007; Bogan et al. 2015; Datry et al., 2016b; Leigh et al., 2016) or fluctuating relationships (Bogan et al. 2017) were observed. This heterogeneity of results suggests that the effect of flow intermittency may strongly depend on the scale at which this trend is studied. Specifically, in streams experiencing flow intermittency, we can observe the local extinction of species lacking traits conferring resistance (e.g. desiccation-resistant stages) or resilience (e.g. plurivoltine taxa). This may cause a shift in community composition, whereby either only a subset of tolerant species from the original pool will persist in situ or less adapted species will be replaced by a new set of species more tolerant to flow intermittency. These two processes, often acting hand-in-hand, determine variations in nestedness and turnover components of $\beta$-diversity, respectively, and may ultimately result in biotic homogenisation of intermittent reaches and a reduction of the total taxonomic diversity at the regional scale (Socolar et al. 2016).

As anticipated, variations in taxonomic composition further reflect changes in the organization of functional space [functional diversity (FD); Petchey & Gaston, 2002], due to the selection of species based on their
biological traits related to physiology, behaviour, feeding strategy or dispersal (MacLean & Beissinger, 2017). A reduction in total diversity of traits and their dispersion should translate in a functional homogenization of aquatic communities due to the depletion of less tolerant species, i.e. environmental filtering (e.g. Elias et al., 2015; Leigh et al., 2016; Brown et al., 2018). Therefore, a nuanced understanding of the underlying mechanisms shaping the structure of lotic communities in a flow intermittency regime is best achieved by simultaneously looking at multiple spatial scales and diversity measures (Bruno et al., 2019; Van Looy et al., 2019). While similar comprehensive studies have been performed in Mediterranean streams, where flow intermittency is part of their natural hydrological cycle (García-Roger et al., 2013), little is known in other biogeographic areas where streams are changing from perennial to intermittent regimes as a result of anthropogenic activities, therefore generating novel community trajectories (Bogan et al., 2015).

Alpine streams are expected to be particularly affected by flow intermittency, because they are fed at least partially by snowfields and glaciers, which are rapidly disappearing (Gobiet et al., 2014). Moreover, they host specialized organisms adapted to a narrow range of environmental conditions (McGregor et al., 1995). In these lotic systems, summer flow cessation periods are increasing, especially at the mid-latitudes, as they are warming at a rate two to three times faster than the global average (Hansen et al., 2005; Pederson et al., 2011). The rapid shrinkage of glaciers and snowpack (Rauscher et al., 2008) anticipates the hydrological peak in spring and induces modifications in both thermal and hydrological regimes as well as in sediment transport (Brighenti et al., 2019). Moreover, mountainous streams are suffering the heavy flow regulation, hydropower and increased water abstraction aimed to fulfill local human needs e.g., agricultural use, drinking water and energy production (Wohl, 2006; López-Rodríguez et al., 2019). The combined effects of both climate change and anthropogenic disturbance are changing mountain streams from perennial to temporary systems (Bruno et al., 2019), alternating non-flow events and rewetting phases (Fenoglio et al., 2010), bringing new challenges to the conservation and management of these ecosystems. In this context, periodic flow intermittency represents a major threat for stream macroinvertebrates in mountainous lotic ecosystems (e.g. Doretto et al., 2018; Piano et al., 2019a; Piano et al., 2019b), whose resistance and resilience abilities are expected to be limited compared to their counterpart in the
Mediterranean region, where the drying phase is a natural part of the stream hydrological cycle (Leigh et al., 2016; Vannucchi et al., 2013). Investigating the effects of flow intermittency on Alpine benthic invertebrate communities is therefore pivotal to understand how this phenomenon is changing their communities and to predict potential threats in the near future.

We here investigated the effects of flow intermittency on Alpine benthic invertebrate communities using a hierarchical sampling design, wherein a permanent reach, located upstream, and intermittent reach, located downstream, were selected within 13 Alpine streams in SW-Alps recently facing summer flow intermittency. Within each reach we sampled macroinvertebrates in seven patches differing in near-bed conditions. Following modern standards in community ecology analyses (e.g. Pavoine et al., 2011; Jarzyna & Jetz, 2018), we estimate multiple facets of taxonomic and functional diversity at both reach and basin scale to evaluate how hydrological disturbance affects the assemblage composition of benthic invertebrate communities. Specifically, we tested whether: (i) flow intermittency causes taxa loss at local and/or at regional scale; (ii) communities in intermittent sites represent either a subset of taxa usually inhabiting permanent reaches or they are composed by different taxa; and (iii) changes in taxonomic composition are mirrored in the functional diversity due to the filtering of taxa with particular combinations of traits. By assuming that flow intermittency is a major cause of species loss (e.g. Datry et al., 2017; Samways et al., 2020) and considering that the overall pool of species in Alpine streams is expected to be mainly composed by taxa not adapted to drying (e.g. Doretto et al., 2018, 2020a; Piano et al., 2019a), we made the following hypotheses: (i) taxonomic diversity should decrease in intermittent sites at both reach and basin; (ii) variation in taxa composition among permanent and intermittent reaches is best explained by taxa loss (i.e., nestedness) than by taxa replacement (i.e. turnover); and (iii) loss of taxa displaying particular trait combinations should directly lead to a reduction of functional diversity and a general rearrangement of the total functional space.

MATERIALS AND METHODS

Sampling design
This study was conducted in 13 low to mid-order streams, experiencing dewatering events since 2011 in multiple reaches. The study streams were selected based on our previous knowledge (Fenoglio et al., 2007) integrated with available historical hydrological data provided by the local Environmental Protection Agency (ARPA). The study streams are all located in the hydroecoregion of SW-Alps (HER 4, Piemonte, NW Italy; Wasson et al. 2007), thus they are characterized by comparable geology, climate, topology and altitude (Moog et al., 2004). In each stream, we selected two 30 m long sampling sections, differing in their hydrological regime: i) an upstream reach, with permanent water throughout the year (hereinafter UP); and ii) a downstream reach, experiencing dewatering events during summer (hereinafter DOWN). In the study area, flow intermittency events lasted on average two months since 2011, with the riverbed almost completely dry for several kilometres (ARPA, 2013; personal observations). Stream drying was caused by the reduction in precipitations, and consequently of snow accumulation, and the parallel increase in water abstraction for human needs. UP and DOWN reaches were located within 10 km, in order to reduce variations in environmental parameters (as demonstrated in Piano et al., 2019) and changes in community composition attributable to geographical distance rather than differences in flow regimes. Both permanent and intermittent sites were identified in the bottom of the valleys. Sampling site elevation was on average 489 m a.s.l., ranging from 307 and 656 m, and permanent and intermittent reaches within the same stream differed on average of 70 m in their elevation (min = 19 m; max = 155 m). Sampling was performed in April 2017 under moderate flow conditions ($Q_{\text{mean}} = 3.98 \pm 4.56$ m$^3$s$^{-1}$) occurring in both reaches, approximately six months after water flow resumption in the DOWN reach (Hydrological bulletins, www.arpa.piemonte.it).

Data collection

In each sampling site, we selected seven sampling patches representative of the different conditions of flow velocity, water depth and substrate composition occurring within each section (7 samples x 2 reaches x 13 streams = 182 samples). In each patch, we measured flow velocity at 0.05 m from the bottom and water depth with a current meter (Hydro-bios Kiel). We used a gravelometer to estimate percentages of different substratum sizes based on the Wentworth’s grain size classification (Wentworth,
1922): boulders (> 256 mm), cobbles (64–256 mm), gravel (2–64 mm), and fine sediment (< 2 mm). One
benthic sample was collected in each sampling patch, using a Surber sampler (250 μm mesh size; 0.062 m²
area), and preserved in 75% ethanol (see Doretto et al., 2020a).

In the laboratory, macroinvertebrates were sorted from the substrate, counted and identified to
the family or genus level according to Campaioli et al. (1994, 1999). Each taxon was then assigned to: i) a
functional feeding group (FFG) based on its morpho-behavioral mechanisms of food acquisition (shredders,
scrapers, predators, collector-gatherers, and filterers); ii) a biological group based on its life-history traits
(size, life-cycle, respiration, reproduction, and locomotion); and iii) an ecological group based on its
ecological requirements (transversal and longitudinal distribution, preferred substrate and current
velocity). FFGs were assigned based on the classification provided by Merritt et al. (2017), whereas
biological and ecological groups were assigned based on the classification proposed by Usseglio-Polatera et
al. (2000), who grouped together taxa showing similar combinations of life-history traits (biological groups)
and ecological preferences (ecological groups). We preferred this approach as it allowed us to synthesize all
the most important life-history and ecological traits within few categories.

Data analysis

We conducted all analyses in R (R Core Team, 2019).

Taxonomic diversity. In order to test our first hypothesis (H1), we partitioned the total taxonomic richness
into its underlying components both within and among reaches. First, we decomposed the total diversity of
each stream (γw) into its within-reach diversity components (hereafter “reach scale”), namely average local
taxa richness of the seven patches within a reach (αw) and the variation among these communities (βw) (Fig.
1). We therefore obtained 13 values of each diversity component, one for each stream. In a second step,
we calculated the among-reach diversity components (hereafter “basin scale”), namely: i) average local
diversity (αo), obtained by averaging the total taxa richness of each reach; ii) total diversity (γo), calculated
as the total number of taxa observed at the basin scale; and iii) the variation in taxa richness (βo) among
reaches (Fig. 1). We therefore obtained a single value for each diversity component. Variation in taxa
composition among local communities (θ-diversity) was calculated with the additive formula as the
difference between $\gamma$- and $\alpha$-diversity (Anderson et al., 2011). Therefore, variation in taxa composition within reaches ($\beta_w$) is calculated as the difference between $\gamma_w$ and $\alpha_w$, while variation in taxa composition among reaches ($\beta_a$) is calculated as the difference between $\gamma$ and $\alpha$. Being calculated with this formula, $\beta$-diversity expresses the number of species that accumulates at regional level as a result of differences between sites at local level. Differences in taxa richness among permanent and intermittent reaches at the reach scale was tested with a paired t-test. On the other hand, differences in taxa richness at basin scale were tested with null modelling, by permuting samples over the two hydrological categories as only a single value for permanent and intermittent reaches was produced. Significant differences between streams can thus be obtained by comparing the observed value of the taxa richness to the null expectation where hydrological categories (permanent vs intermittent) are randomly permuted across the sampling sites (Legendre & Legendre, 1998).

In order to test our second hypothesis (H2), we decomposed total beta diversity, here intended as the dissimilarity between communities in UP and DOWN sites ($\beta_{total}$) into its turnover ($\beta_{repl}$) and nestedness ($\beta_{rich}$) components with the function beta in the BAT package (Cardoso et al., 2020). We decomposed beta diversity within UP and DOWN reaches and we thus obtained 13 values of total beta diversity, turnover and nestedness for UP and DOWN sites, respectively, and we tested for differences between UP and DOWN sites with a paired t-test.

**Functional diversity.** In order to test our third hypothesis, we examined variation in functional diversity between benthic invertebrate communities in UP vs DOWN using functional $n$-dimensional hypervolumes (Mammola & Cardoso, 2020) as implemented in the BAT package (Cardoso et al., 2020). We constructed the community functional hypervolume for each reach with the hypervolume package (Blonder et al., 2014; Blonder, 2019). Since functional traits of benthic invertebrates here considered are categorical, we used the approach proposed by Carvalho & Cardoso (2018) to incorporate categorical variables in the hypervolume estimation procedure. We applied a Gower dissimilarity measure to the complete trait matrix and extracted orthogonal morphological axes through Principal Coordinate Analysis (PCoA). We retained the first three PCoA axes (43% Variance explained) to construct hypervolumes using a gaussian kernel estimator (Blonder et al., 2018) and a default bandwidth for each axis. In turn, we estimated functional $\alpha$
diversity, functional dispersion and functional evenness based on the approach by Mammola & Cardoso (2020). To further inspect functional turnovers among permanent and intermittent reaches, we estimated functional β-diversity following Carvalho & Cardoso (2018). This approach computes a pairwise decomposition of the overall differentiation among kernel hypervolumes into two components: the replacement of space between hypervolumes (β-replacement) and net differences between the amount of space enclosed by each hypervolume (β-richness) (see Cardoso et al., 2014 and Mammola & Cardoso, 2020 for further details). As we had seven values for each reach within each flow category, we tested for significant differences between UP and DOWN reaches using linear mixed models (LMMs), with the flow intermittency category (UP vs DOWN) as independent variable and stream identity as a random factor. We included the latter to account for spatial dependence of the data (sampling reaches nested within streams) (Zuur et al., 2009).

To investigate which combination of traits is selected in UP and DOWN sites, we evaluated the functional contribution of each taxon (observation) within communities to the functional space and originality of each taxon within both UP and DOWN reaches. Taxa with high values of contribution provide rare combinations of traits within the community thus contributing the most to the overall functional diversity of the community (Pavoine et al., 2005; Violle et al., 2017), whereas taxa with high originality values are the ones contributing the most in determining the functional dispersion of the community (Mammola & Cardoso, 2020).

RESULTS

Taxonomic diversity

We observed a significant decline in taxa diversity from UP to DOWN reaches both within and among reaches (Fig. 2a). When considering diversity within reaches, we could detect a significant decrease in all diversity components, indicating that flow intermittency reduces both local (αw: t = 4.85, P < 0.001) and total (γw: t = 6.06, P < 0.001) diversity within reaches from UP to DOWN sites, as well as taxa variation among habitat patches within reaches (βw: t = 4.26, P = 0.001). When considering diversity among reaches,
we could detect a significant decline in both local ($\alpha$: $P < 0.001$) and total ($\gamma$: $P < 0.001$) diversity components from UP to DOWN sites, but not in terms of taxa variation ($\theta_0$: $P = 0.333$).

By partitioning total $\beta$-diversity, here intended as the dissimilarity among community matrices, into its turnover and nestedness components, we observed a higher contribution of nestedness than turnover in both UP and DOWN sites (UP: $\beta_{\text{total}} = 0.69$, $\beta_{\text{repl}} = 0.19$, $\beta_{\text{rich}} = 0.50$; DOWN: $\beta_{\text{total}} = 0.66$, $\beta_{\text{repl}} = 0.21$, $\beta_{\text{rich}} = 0.45$), although no significant differences between UP and DOWN sites were observed (turnover: $t = -0.507$, $P = 0.621$; nestedness: $t = 1.05$, $P = 0.313$). This suggests that the contribution of the two components is equal in the two hydrological levels (Fig. 2b).

Functional diversity

Results of the LMMs showed that all functional metrics significantly differ between UP and DOWN reaches (Tab. 1). $\alpha$-diversity, dispersion and evenness were significantly lower in DOWN than in UP reaches (Fig. 3). When considering $\beta$-diversity, we observed significantly higher values in pairwise comparisons between communities in DOWN reaches, whereas communities in UP reaches were in general more similar to one another, suggesting trait overdispersion in sites experiencing flow intermittency. In DOWN reaches the $\beta$-richness component was more preponderant than in UP reaches, whereas the $\beta$-replacement was higher in UP reaches. When evaluating the contribution of each taxon, we inferred that Tipulidae (Diptera) was the taxon contributing the most to the functional space in UP reaches, and Perla (Plecoptera, Perlidae) in DOWN reaches. In other words, Tipulidae contributed with unique traits in UP sites, while Perla contributed with unique traits in DOWN sites.

DISCUSSION

According to our results, flow intermittency is causing a decline in macroinvertebrate taxonomic richness at both reach and basin scale, suggesting that this phenomenon, which is expected to increase in frequency in the near future in Alpine streams (Brighenti et al., 2019), will represent a threat for Alpine stream biodiversity.
Decomposing the total diversity into its taxonomic components allowed us to obtain a more detailed view on which of the two main mechanisms, i.e. species filtering (alpha diversity) and biotic homogenization (beta diversity components) (Socolar et al. 2016), and at which spatial scale, is affecting macroinvertebrate assemblages. We could highlight that the overall decline in macroinvertebrate richness is mainly due to local diversity reduction among and within reaches. In particular, at the reach scale, local macroinvertebrate communities are impoverished and become more homogeneous. Conversely, we did not record a significant difference in taxonomic variation among reaches between permanent and intermittent sites. These results are partially supported by García-Roger (2013), who found that variation in intermittent streams during the wet season is accounted by both the microhabitat (in systems with pools during the dry season) and the stream (in systems where the riverbed completely dries during the dry season) scale. The lack of taxonomic variation among reaches observed in our study might indicate that the biotic homogenization of macroinvertebrate communities caused by flow intermittency, which is a relatively new phenomenon in the investigated streams, has not become pervasive yet. However, this result should be interpreted with caution as we here identified our taxa at the family to genus levels, preventing to infer whether a homogenization process is ongoing at species level too.

When analysing the contribution of nestedness and turnover components, we could detect that variation among local communities is mainly due to richness difference in both permanent and intermittent reaches, while taxa replacement seemingly played a minor role, in accordance with the fact we sampled during the base flow regime. In fact, β-diversity should be low during non-drought phases and dominated by nestedness as organisms experience favourable high hydrological connectivity between sites (Larned et al., 2010). Conversely, the turnover component of dissimilarity increases with increasing intermittency (Tornés & Ruhi, 2013; Piano et al., 2017; Aspin et al., 2018). Although we did not record significant differences in the relative contribution of these two components in permanent and intermittent reaches, we observed a slightly higher contribution of turnover in intermittent than permanent sites. Despite some contrasting results found in literature (Datry et al., 2014), this is in agreement with Rolls et al. (2016) who showed a dominance of turnover processes in the dissimilarity partitioning on macroinvertebrates and fish subjected to intermittent flow. This may be due to the environmental filter imposed by physical and
chemical alterations associated with stream drying—lentification with the creation of isolated pools, where organisms experience oxygen reduction and increase in nutrient concentration, fine sediment deposition and biotic interactions (Lake, 2003)—that causes the replacement of the most sensitive taxa by tolerant ones (Dolédec et al., 2017).

Functional diversity patterns showed that reaches experiencing flow intermittency are less functionally diverse than permanent ones, with communities in intermittent sites showing lower diversity, variability and a less homogeneous distribution of functional traits. Opposite results were obtained by Belmar et al. (2019) in Mediterranean streams, where they did not observe any significant effect of flow intermittence on functional diversity. These different outcomes may indicate that flow intermittency exerts a stronger environmental pressure on benthic invertebrate communities in mountainous lotic ecosystems than in Mediterranean streams. In fact, the overall pool of macroinvertebrates in Alpine areas lacks functional traits to cope with flow intermittency, which are present in their counterpart in the Mediterranean region, where the drying phase is a natural part of the stream hydrological cycle (Leigh et al., 2016; Vannucchi et al., 2013). On the other hand, the analysis of functional β-diversity showed how intermittent reaches experience trait overdispersion, and this is mainly due to a net difference in the amount of space enclosed by the trait space rather than replacements of traits. In other words, communities in intermittent streams are composed by taxa that show a subset of the entire pool of functional traits, but this subset is different depending on the reach considered. Similar results were obtained by Dolédec et al. (2017), as they observed that Mediterranean taxa, which have to cope with higher hydrological variability, may perform more diverse functional roles than temperate taxa and intermittent streams show higher functional variability than permanent systems within the Mediterranean area. This may be due to the fact that intermittent sites are colonized by species with traits adapted to flow intermittence even during baseflow conditions leading to the coexistence of taxa displaying a wide range of traits. The variation in trait composition among reaches can be explained by the high variability of lotic systems experiencing flow intermittency, which in turn affect the community assemblages through stochastic extinctions and colonization (Datry et al., 2016).
The analysis of taxa contribution to the functional diversity allowed us to highlight which trait combination is less typical in permanent and intermittent reaches. We observed that Tipulidae shows unique traits for the environmental conditions in permanent sites, whereas *Perla* shows unique traits for environmental conditions in intermittent sites, confirming the results that we obtained in related studies (Doretto et al., 2018; Doretto et al., 2020b; Piano et al., 2019a). In other words, Tipulidae and *Perla* traits are atypical (and possibly maladaptive) for permanent and intermittent stretches, respectively. Tipulidae is a monovoltine, crawler taxon, living in slow flowing waters, such as side-arms or near the banks, whose larvae have a dormancy phase, whereas *Perla* is a large-sized, monovoltine, crawler taxon, with aquatic respiration, preferring oligotrophic, cold, fast-flowing waters (Usseglio-Polatera et al., 2000; Merritt et al., 2017). Large semivoltine taxa with long larval life are expected to be unable to survive in periodically dry environments, while fast growing, plurivoltine organisms are adapted to complete their life cycle in less than one year, emerging just before the loss of surface water (Corbet et al., 2006; López-Rodríguez et al., 2009a, b; Bonada & Dolédec, 2018). Small body size is another trait that increases the survival in intermittent reaches as reduced dimensions allow fast development and population growth (Bonada et al. 2007). The shift from rheophilous to lentic taxa has also been recorded in literature (e.g. Acuña et al., 2005; Pace et al., 2013; Chessmann, 2015), as flow intermittency in usually associated with lentification and consequent oxygen reduction (Lake, 2003), which may compromise the survival of many taxa, especially in mountainous lotic ecosystems where organisms have to be adapted to high flow velocities and the pronounced oligotrophy (Fenoglio et al., 2010). In addition, it should be noted that, compared to other exclusively lotic organisms, Tipulidae larvae can survive even in terrestrial habitats if characterized by sufficient moisture (Pritchard, 1983), thus being naturally tolerant to intermittent regimes.

Overall, our results demonstrate that benthic invertebrate biodiversity is threatened at both local and regional scale, whereas the biotic homogenization process is still ongoing. We can therefore suggest that macroinvertebrate communities are currently facing an ongoing “mediterraneization process”, which is still in progress, and the results of the analyses of functional diversity support this hypothesis. In fact, a more in-depth analysis of their functional diversity revealed that Alpine streams are currently facing the extinction of rheophilous organisms, typical of fast-flowing, well-oxygenated waters, which are gradually
replaced by lentic taxa, with life-traits that confer adaptations to flow intermittency. As the frequency and magnitude of anthropogenic and climatic droughts in the mountain areas are predicted to increase (Brighenti et al., 2019), further studies should be performed in the next future to prevent the loss of specialized benthic species, their functional diversity, and the key role they play in the riverine ecological network.

Acknowledgements

We thank M. Apostolo, R. Bolpagni, M. C. Bruno, G. Burgazzi, C. Garetto, A. Laini, D. Melchio, D. Morandini, D. Nizzoli and B. Palmia for their assistance and contribution during the field and laboratory activities. Marco Baltieri (ATAAI - Associazione Tutela Ambienti Acquatici e Ittiofauna) is greatly acknowledged for his help in the identification of temporary sampling stretches. This work was realized within the framework of the PRIN NOACQUA “Risposte di comuNità e processi ecOsistemici in corsi d’ACQUA soggetti a intermittenza idrologica” - code 2O1572HW8F, funded by the Italian Ministry of Education, University and Research and is part of the research fellowship “Aquatic invertebrate communities as sentinels of climate change in Italian Alpine streams” funded by Fondazione CRT and of the activities of ALPSTREAM, a research center financed by FESR, Interreg Alcotra 2014-2020, EcO Project of the Piter Terres Monviso.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of Interest Statement

The authors declare no conflict of interest.
REFERENCES


Table 1. Mean values with standard deviation in brackets of functional diversity measures in permanent (UP) and intermittent (DOWN) sites, and estimated parameters (Est), with standard errors (SE) in brackets, and p-values (P) of LMMs performed on functional metrics (FD = functional α-diversity; Fdis = functional dispersion; Feve = functional evenness) to test for differences between UP and DOWN sites. All comparisons are significant.

<table>
<thead>
<tr>
<th>Functional measures</th>
<th>UP</th>
<th>DOWN</th>
<th>BEst ± SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>FD</td>
<td>0.20 (±0.10)</td>
<td>0.13 (±0.13)</td>
<td>-0.071 (±0.014)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Fdis</td>
<td>0.53 (±0.04)</td>
<td>0.49 (±0.06)</td>
<td>-0.053 (±0.006)</td>
<td>&lt; 0.001</td>
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<tr>
<td>Feve</td>
<td>0.17 (±0.06)</td>
<td>0.13 (±0.09)</td>
<td>-0.039 (±0.014)</td>
<td>0.007</td>
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<tr>
<td>β-diversity</td>
<td></td>
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<tr>
<td>(β-replacement + β-</td>
<td>0.63 (±0.14)</td>
<td>0.74 (±0.15)</td>
<td>0.119 (±0.003)</td>
<td>&lt; 0.001</td>
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<td>richness)</td>
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<tr>
<td>β-replacement</td>
<td>0.25 (±0.16)</td>
<td>0.19 (±0.18)</td>
<td>-0.062 (±0.004)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>β-richness</td>
<td>0.38 (±0.24)</td>
<td>0.56 (±0.29)</td>
<td>0.181 (±0.006)</td>
<td>&lt; 0.001</td>
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Figure captions

Figure 1. Schematic overview of the calculated diversity components ($\alpha_w =$ local diversity within reaches; $\alpha_a =$ local diversity among reaches; $\beta_w =$ variation among local communities within reaches; $\beta_a =$ variation among local communities among reaches; $\gamma_w =$ total diversity within reaches; $\gamma_a =$ total diversity among reaches) to test the effect of flow intermittency (UP = orange; DOWN = light blue) within- (reach scale) and among stretches (basin scale).

Figure 2. a) diversity partitioning of total diversity in the study area into its within- and among reaches components in UP and DOWN reaches ($\alpha_w =$ dark grey; $\beta_w =$ medium grey; $\beta_a =$ light grey); b) decomposition of $\beta$-diversity into its nestedness (dark grey) and turnover (light grey) components in in UP and DOWN reaches.

Figure 3. a–c) Functional richness (a), dispersion (b) and evenness (c) of benthic invertebrate communities in permanent (UP) and intermittent (DOWN) reaches; d–f) Density of functional $\beta$-diversity values for pairwise comparison of communities in permanent (UP) and intermittent (DOWN) reaches. Total functional $\beta$-diversity (d) is split in two components: $\beta$-replacement (e) is turnover in functional composition explained by replacement of space between hypervolumes, and $\beta$-richness (f) is the turnover in functional composition explained by net differences between the amount of space enclosed by each hypervolume (Carvalho & Cardoso, 2018; Mammola & Cardoso, 2020).