Taxonomic and functional homogenisation of macroinvertebrate communities in recently intermittent Alpine watercourses

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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.

25   3. We tested for the response of taxonomic richness at multiple spatial scales by partitioning total

26   diversity into the average richness of local communities and the richness due to variation

27   among local communities both within and among reaches. By partitioning total diversity (γ) into

28   its local (α) and turnover (β) components we showed a decrease in local and regional species

29   richness both within and among reaches, whereas variation among communities was significantly

30   lower in intermittent reaches only at the reach scale.

31   4. The analysis of the multidimensional trait space of macroinvertebrates in reaches with different

32   hydrological conditions further revealed a significant reduction of functional diversity, dispersion,

33   and evenness in intermittent reaches. There was trait overdispersion in intermittent reaches, as

34   these hosted both typical Alpine taxa and organisms adapted to flow intermittency. In particular,

35   we observed the replacement of taxa with aquatic respiration and preferring medium- to fast-

36   flowing oligotrophic waters by taxa adapted to lentic habitats and with larval dormancy phases.

37   5. These results provide strong support that recent flow intermittency determines drastic changes of

38   benthic invertebrate communities in Alpine streams which are facing a "mediterraneization"

39   process. Our work highlights the importance of considering multiple spatial scales and of

40   integrating taxonomic and functional diversity to throughly assess the impacts of flow

41   intermittency.

42   **Keywords:** flow intermittency, drying, diversity partitioning, nestedness, functional traits

43

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INTRODUCTION

45

46   Stream ecosystems are particularly sensitive to climate change, as changes in precipitations,

47   temperature and evaporation direct translate in shifts in river flow regimes and their physical conditions

48   (Lake, 2003). The raising of water temperatures and the disruption of hydrologic cycles, further

49   exacerbated by anthropogenic activities (e.g. deforestation, water capture, streambed alterations; Wohl,

50   2006) can lead, in turn, to stochastic events (Scheffer et al., 2001), like floods and droughts. The observed

51   and predicted climate warming is also increasingly associated to changes in distribution and phenology of

52   biota (Heino et al., 2009; Whitehead et al., 2009).

53   Among flow-related disturbances, water scarcity, and consequent riverbed desiccation, is one of

54   the most pressing environmental issues related to climate change, with expected drastic consequences on

55   biodiversity (Lake, 2003). The necessity of understanding such complex dynamics have recently pushed

56   more and more researchers to study the effect of flow intermittency on the taxonomic richness of benthic

57   invertebrate communities. Yet contrasting results have so far been obtained, as either negative

58   relationships (Datry et al., 2014; Leigh & Datry, 2017; Datry et al. 2016a), no relationship (Bonada et al.,

59   2007; Bogan et al. 2015; Datry et al., 2016b; Leigh et al., 2016) or fluctuating relationships (Bogan et al.

60   2017) were observed. This heterogeneity of results suggests that the effect of flow intermittency may

61   strongly depend on the scale at which this trend is studied. Specifically, in streams experiencing flow

62   intermittency, we can observe the local extinction of species lacking traits conferring resistance (e.g.

63   desiccation-resistant stages) or resilience (e.g. plurivoltine taxa). This may cause a shift in community

64   composition, whereby either only a subset of tolerant species from the original pool will persist *in situ* or

65   less adapted species will be replaced by a new set of species more tolerant to flow intermittency. These

66   two processes, often acting hand-in-hand, determine variations in nestedness and turnover components of

67   *β*-diversity, respectively, and may ultimately result in biotic homogenisation of intermittent reaches and a

68   reduction of the total taxonomic diversity at the regional scale (Socolar et al. 2016).

69   As anticipated, variations in taxonomic composition further reflect changes in the organization of functional

70   space [functional diversity (FD); Petchey & Gaston, 2002], due to the selection of species based on their

71 biological traits related to physiology, behaviour, feeding strategy or dispersal (MacLean & Beissinger,

72 2017). A reduction in total diversity of traits and their dispersion should translate in a functional

73 homogenization of aquatic communities due to the depletion of less tolerant species, i.e. environmental

74 filtering (e.g. Elias et al., 2015; Leigh et al., 2016; Brown et al., 2018). Therefore, a nuanced understanding

75 of the underlying mechanisms shaping the structure of lotic communities in a flow intermittency regime is

76 best achieved by simultaneously looking at multiple spatial scales and diversity measures (Bruno et al.,

77 2019; Van Looy et al., 2019). While similar comprehensive studies have been performed in Mediterranean

78 streams, where flow intermittency is part of their natural hydrological cycle (García-Roger et al., 2013),

79 little is known in other biogeographic areas where streams are changing from perennial to intermittent

80 regimes as a result of anthropogenic activities, therefore generating novel community trajectories (Bogan

81 et al. 2015).

82   Alpine streams are expected to be particularly affected by flow intermittency, because they are fed

83 at least partially by snowfields and glaciers, which are rapidly disappearing (Gobiet et al., 2014). Moreover,

84 they host specialized organisms adapted to a narrow range of environmental conditions (McGregor et al.

85 1995). In these lotic systems, summer flow cessation periods are increasing, especially at the mid-latitudes,

86 as they are warming at a rate two to three times faster than the global average (Hansen et al., 2005;

87 Pederson et al., 2011). The rapid shrinkage of glaciers and snowpack (Rauscher et al., 2008) anticipates the

88 hydrological peak in spring and induces modifications in both thermal and hydrological regimes as well as in

89 sediment transport (Brighenti et al., 2019). Moreover, mountainous streams are suffering the heavy flow

90 regulation, hydropower and increased water abstraction aimed to fulfill local human needs e.g., agricultural

91 use, drinking water and energy production (Wohl, 2006; López-Rodríguez et al., 2019). The combined

92 effects of both climate change and anthropogenic disturbance are changing mountain streams from

93 perennial to temporary systems (Bruno et al., 2019), alternating non-flow events and rewetting phases

94 (Fenoglio et al., 2010), bringing new challenges to the conservation and management of these ecosystems.

95 In this context, periodic flow intermittency represents a major threat for stream macroinvertebrates in

96 mountainous lotic ecosystems (e.g. Doretto et al., 2018; Piano et al., 2019a; Piano et al., 2019b), whose

97 resistance and resilience abilities are expected to be limited compared to their counterpart in the

98   Mediterranean region, where the drying phase is a natural part of the stream hydrological cycle (Leigh et

99   al., 2016; Vannucchi et al., 2013). Investigating the effects of flow intermittency on Alpine benthic

100 invertebrate communities is therefore pivotal to understand how this phenomenon is changing their

101 communities and to predict potential threats in the near future.

102 We here investigated the effects of flow intermittency on Alpine benthic invertebrate communities

103 using a hierarchical sampling design, wherein a permanent reach, located upstream, and intermittent

104 reach, located downstream, were selected within 13 Alpine streams in SW-Alps recently facing summer

105 flow intermittency. Within each reach we sampled macroinvertebrates in seven patches differing in near-

106 bed conditions. Following modern standards in community ecology analyses (e.g. Pavoine et al., 2011;

107 Jarzyna & Jetz, 2018), we estimate multiple facets of taxonomic and functional diversity at both reach and

108 basin scale to evaluate how hydrological disturbance affects the assemblage composition of benthic

109 invertebrate communities. Specifically, we tested whether: (i) flow intermittency causes taxa loss at local

110 and/or at regional scale; (ii) communities in intermittent sites represent either a subset of taxa usually

111 inhabiting permanent reaches or they are composed by different taxa; and (iii) changes in taxonomic

112 composition are mirrored in the functional diversity due to the filtering of taxa with particular combinations

113 of traits. By assuming that flow intermittency is a major cause of species loss (e.g. Datry et al., 2017;

114 Samways et al., 2020) and considering that the overall pool of species in Alpine streams is expected to be

115 mainly composed by taxa not adapted to drying (e.g. Doretto et al., 2018, 2020a; Piano et al., 2019a), we

116 made the following hypotheses: (i) taxonomic diversity should decrease in intermittent sites at both reach

117 and basin; (ii) variation in taxa composition among permanent and intermittent reaches is best explained

118 by taxa loss (i.e., nestedness) than by taxa replacement (i.e. turnover); and (iii) loss of taxa displaying

119 particular trait combinations should directly lead to a reduction of functional diversity and a general

120 rearrangement of the total functional space.

121 **MATERIALS AND METHODS**

122 **Sampling design**

123   This study was conducted in 13 low to mid-order streams, experiencing dewatering events since

124   2011 in multiple reaches. The study streams were selected based on our previous knowledge (Fenoglio et

125   al., 2007) integrated with available historical hydrological data provided by the local Environmental

126   Protection Agency (ARPA). The study streams are all located in the hydroecoregion of SW-Alps (HER 4,

127   Piemonte, NW Italy; Wasson et al. 2007), thus they are characterized by comparable geology, climate,

128   topology and altitude (Moog et al., 2004). In each stream, we selected two 30 m long sampling sections,

129   differing in their hydrological regime: i) an upstream reach, with permanent water throughout the year

130   (hereinafter UP); and ii) a downstream reach, experiencing dewatering events during summer (hereinafter

131   DOWN). In the study area, flow intermittency events lasted on average two months since 2011, with the

132   riverbed almost completely dry for several kilometres (ARPA, 2013; personal observations). Stream drying

133   was caused by the reduction in precipitations, and consequently of snow accumulation, and the parallel

134   increase in water abstraction for human needs. UP and DOWN reaches were located within 10 km, in order

135   to reduce variations in environmental parameters (as demonstrated in Piano et al., 2019) and changes in

136   community composition attributable to geographical distance rather than differences in flow regimes. Both

137   permanent and intermittent sites were identified in the bottom of the valleys. Sampling site elevation was

138   on average 489 m a.s.l., ranging from 307 and 656 m, and permanent and intermittent reaches within the

139   same stream differed on average of 70 m in their elevation (min = 19 m; max = 155 m). Sampling was

140   performed in April 2017 under moderate flow conditions (Q_{mean} = 3.98 ± 4.56 m^{3}s^{-1}) occurring in both

141   reaches, approximately six months after water flow resumption in the DOWN reach (Hydrological bulletins,

142   www.arpa.piemonte.it).

143   **Data collection**

144   In each sampling site, we selected seven sampling patches representative of the different

145   conditions of flow velocity, water depth and substrate composition occurring within each section (7

146   samples x 2 reaches x 13 streams = 182 samples). In each patch, we measured flow velocity at 0.05 m from

147   the bottom and water depth with a current meter (Hydro-bios Kiel). We used a gravelometer to estimate

148   percentages of different substratum sizes based on the Wentworth's grain size classification (Wentworth,

149   1922): boulders (> 256 mm), cobbles (64–256 mm), gravel (2–64 mm), and fine sediment (< 2 mm). One

150   benthic sample was collected in each sampling patch, using a Surber sampler (250 μm mesh size; $0.062m^2$

151   area), and preserved in 75% ethanol (see Doretto et al., 2020a).

152   In the laboratory, macroinvertebrates were sorted from the substrate, counted and identified to

153   the family or genus level according to Campaioli et al. (1994, 1999). Each taxon was then assigned to: i) a

154   functional feeding group (FFG) based on its morpho-behavioral mechanisms of food acquisition (shredders,

155   scrapers, predators, collector-gatherers, and filterers); ii) a biological group based on its life-history traits

156   (size, life-cycle, respiration, reproduction, and locomotion); and iii) an ecological group based on its

157   ecological requirements (transversal and longitudinal distribution, preferred substrate and current

158   velocity). FFGs were assigned based on the classification provided by Merritt et al. (2017), whereas

159   biological and ecological groups were assigned based on the classification proposed by Usseglio-Polatera et

160   al. (2000), who grouped together taxa showing similar combinations of life-history traits (biological groups)

161   and ecological preferences (ecological groups). We preferred this approach as it allowed us to synthesize all

162   the most important life-history and ecological traits within few categories.

163   **Data analysis**

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

165   <u>Taxonomic diversity.</u> In order to test our first hypothesis (H1), we partitioned the total taxonomic richness

166   into its underlying components both within and among reaches. First, we decomposed the total diversity of

167   each stream (γ_w) into its within-reach diversity components (hereafter "reach scale"), namely average local

168   taxa richness of the seven patches within a reach (α_w) and the variation among these communities (β_w) (Fig.

169   1). We therefore obtained 13 values of each diversity component, one for each stream. In a second step,

170   we calculated the among-reach diversity components (hereafter "basin scale"), namely: i) average local

171   diversity (α_a), obtained by averaging the total taxa richness of each reach; ii) total diversity (γ_a), calculated

172   as the total number of taxa observed at the basin scale; and iii) the variation in taxa richness (β_a) among

173   reaches (Fig. 1). We therefore obtained a single value for each diversity component. Variation in taxa

174   composition among local communities (β-diversity) was calculated with the additive formula as the

175 difference between γ- and α-diversity (Anderson et al., 2011). Therefore, variation in taxa composition

176 within reaches (β_w) is calculated as the difference between γ_w and α_w, while variation in taxa composition

177 among reaches (β_a) is calculated as the difference between γ_a and α_a. Being calculated with this formula, β-

178 diversity expresses the number of species that accumulates at regional level as a result of differences

179 between sites at local level. Differences in taxa richness among permanent and intermittent reaches at the

180 reach scale was tested with a paired t-test. On the other hand, differences in taxa richness at basin scale

181 were tested with null modelling, by permuting samples over the two hydrological categories as only a single

182 value for permanent and intermittent reaches was produced. Significant differences between streams can

183 thus be obtained by comparing the observed value of the taxa richness to the null expectation where

184 hydrological categories (permanent vs intermittent) are randomly permuted across the sampling sites

185 (Legendre & Legendre, 1998).

186 In order to test our second hypothesis (H2), we decomposed total beta diversity, here intended as the

187 dissimilarity between communities in UP and DOWN sites (β_{total}) into its turnover (β_{repl}) and nestedness

188 (β_{rich}) components with the function *beta* in the BAT package (Cardoso et al., 2020). We decomposed beta

189 diversity within UP and DOWN reaches and we thus obtained 13 values of total beta diversity, turnover and

190 nestedness for UP and DOWN sites, respectively, and we tested for differences between UP and DOWN

191 sites with a paired t-test.

192   <u>Functional diversity.</u> In order to test our third hypothesis, we examined variation in functional

193 diversity between benthic invertebrate communities in UP vs DOWN using functional *n*-dimensional

194 hypervolumes (Mammola & Cardoso, 2020) as implemented in the BAT package (Cardoso et al., 2020). We

195 constructed the community functional hypervolume for each reach with the *hypervolume* package (Blonder

196 et al., 2014; Blonder, 2019). Since functional traits of benthic invertebrates here considered are categorical,

197 we used the approach proposed by Carvalho & Cardoso (2018) to incorporate categorical variables in the

198 hypervolume estimation procedure. We applied a Gower dissimilarity measure to the complete trait matrix

199 and extracted orthogonal morphological axes through Principal Coordinate Analysis (PCoA). We retained

200 the first three PCoA axes (43% Variance explained) to construct hypervolumes using a gaussian kernel

201 estimator (Blonder et al., 2018) and a default bandwidth for each axis. In turn, we estimated functional α

202   diversity, functional dispersion and functional evenness based on the approach by Mammola & Cardoso

203   (2020). To further inspect functional turnovers among permanent and intermittent reaches, we estimated

204   functional β-diversity following Carvalho & Cardoso (2018). This approach computes a pairwise

205   decomposition of the overall differentiation among kernel hypervolumes into two components: the

206   replacement of space between hypervolumes (β-replacement) and net differences between the amount of

207   space enclosed by each hypervolume (β-richness) (see Cardoso et al., 2014 and Mammola & Cardoso, 2020

208   for further details). As we had seven values for each reach within each flow category, we tested for

209   significant differences between UP and DOWN reaches using linear mixed models (LMMs), with the flow

210   intermittency category (UP vs DOWN) as independent variable and stream identity as a random factor. We

211   included the latter to account for spatial dependence of the data (sampling reaches nested within streams)

212   (Zuur et al., 2009).

213   To investigate which combination of traits is selected in UP and DOWN sites, we evaluated the

214   functional contribution of each taxon (observation) within communities to the functional space and

215   originality of each taxon within both UP and DOWN reaches. Taxa with high values of contribution provide

216   rare combinations of traits within the community thus contributing the most to the overall functional

217   diversity of the community (Pavoine et al., 2005; Violle et al., 2017), whereas taxa with high originality

218   values are the ones contributing the most in determining the functional dispersion of the community

219   (Mammola & Cardoso, 2020).

220

221   **RESULTS**

222   **Taxonomic diversity**

223   We observed a significant decline in taxa diversity from UP to DOWN reaches both within and

224   among reaches (Fig. 2a). When considering diversity within reaches, we could detect a significant decrease

225   in all diversity components, indicating that flow intermittency reduces both local (α_w: t = 4.85, P < 0.001)

226   and total (γ_w: t = 6.06, P < 0.001) diversity within reaches from UP to DOWN sites, as well as taxa variation

227   among habitat patches within reaches (β_w: t = 4.26, P = 0.001). When considering diversity among reaches,

228   we could detect a significant decline in both local (α_a: $P < 0.001$) and total (γ_a: $P < 0.001$) diversity

229   components from UP to DOWN sites, but not in terms of taxa variation (β_a: $P = 0.333$).

230   By partitioning total β-diversity, here intended as the dissimilarity among community matrices,

231   into its turnover and nestedness components, we observed a higher contribution of nestedness than

232   turnover in both UP and DOWN sites (UP: $\beta_{total} = 0.69$, $\beta_{repl} = 0.19$, $\beta_{rich} = 0.50$; DOWN: $\beta_{total} = 0.66$, $\beta_{repl} =$

233   0.21, $\beta_{rich} = 0.45$), although no significant differences between UP and DOWN sites were observed

234   (turnover: $t = -0.507$, $P = 0.621$; nestedness: $t = 1.05$, $P = 0.313$). This suggests that the contribution of the

235   two components is equal in the two hydrological levels (Fig. 2b).

236

237   **Functional diversity**

238   Results of the LMMs showed that all functional metrics significantly differ between UP and DOWN

239   reaches (Tab. 1). α-diversity, dispersion and evenness were significantly lower in DOWN than in UP reaches

240   (Fig. 3). When considering β-diversity, we observed significantly higher values in pairwise comparisons

241   between communities in DOWN reaches, whereas communities in UP reaches were in general more similar

242   to one another, suggesting trait overdispersion in sites experiencing flow intermittency. In DOWN reaches

243   the β-richness component was more preponderant than in UP reaches, whereas the β-replacement was

244   higher in UP reaches. When evaluating the contribution of each taxon, we inferred that Tipulidae (Diptera)

245   was the taxon contributing the most to the functional space in UP reaches, and *Perla* (Plecoptera, Perlidae)

246   in DOWN reaches. In other words, Tipulidae contributed with unique traits in UP sites, while *Perla*

247   contributed with unique traits in DOWN sites.

248

249   **DISCUSSION**

250   According to our results, flow intermittency is causing a decline in macroinvertebrate taxonomic

251   richness at both reach and basin scale, suggesting that this phenomenon, which is expected to increase in

252   frequency in the near future in Alpine streams (Brighenti et al., 2019), will represent a threat for Alpine

253   stream biodiversity.

254   Decomposing the total diversity into its taxonomic components allowed us to obtain a more

255   detailed view on which of the two main mechanisms, i.e. species filtering (alpha diversity) and biotic

256   homogenization (beta diversity components) (Socolar et al. 2016), and at which spatial scale, is affecting

257   macroinvertebrate assemblages. We could highlight that the overall decline in macroinvertebrate richness

258   is mainly due to local diversity reduction among and within reaches. In particular, at the reach scale, local

259   macroinvertebrate communities are impoverished and become more homogeneous. Conversely, we did

260   not record a significant difference in taxonomic variation among reaches between permanent and

261   intermittent sites. These results are partially supported by García-Roger (2013), who found that variation in

262   intermittent streams during the wet season is accounted by both the microhabitat (in systems with pools

263   during the dry season) and the stream (in systems where the riverbed completely dries during the dry

264   season) scale. The lack of taxonomic variation among reaches observed in our study might indicate that the

265   biotic homogenization of macroinvertebrate communities caused by flow intermittency, which is a

266   relatively new phenomenon in the investigated streams, has not become pervasive yet. However, this

267   result should be interpreted with caution as we here identified our taxa at the family to genus levels,

268   preventing to infer whether a homogenization process is ongoing at species level too.

269   When analysing the contribution of nestedness and turnover components, we could detect that

270   variation among local communities is mainly due to richness difference in both permanent and intermittent

271   reaches, while taxa replacement seemingly played a minor role, in accordance with the fact we sampled

272   during the base flow regime. In fact, β-diversity should be low during non-drought phases and dominated

273   by nestedness as organisms experience favourable high hydrological connectivity between sites (Larned et

274   al., 2010). Conversely, the turnover component of dissimilarity increases with increasing intermittency (

275   Tornés & Ruhì, 2013; Piano et al., 2017; Aspin et al., 2018). Although we did not record significant

276   differences in the relative contribution of these two components in permanent and intermittent reaches,

277   we observed a slightly higher contribution of turnover in intermittent than permanent sites. Despite some

278   contrasting results found in literature (Datry et al., 2014), this is in agreement with Rolls et al. (2016) who

279   showed a dominance of turnover processes in the dissimilarity partitioning on macroinvertebrates and fish

280   subjected to intermittent flow. This may be due to the environmental filter imposed by physical and

281   chemical alterations associated with stream drying—lentification with the creation of isolated pools, where

282   organisms experience oxygen reduction and increase in nutrient concentration, fine sediment deposition

283   and biotic interactions (Lake, 2003)—that causes the replacement of the most sensitive taxa by tolerant

284   ones (Dolédec et al., 2017).

285   Functional diversity patterns showed that reaches experiencing flow intermittency are less

286   functionally diverse than permanent ones, with communities in intermittent sites showing lower diversity,

287   variability and a less homogeneous distribution of functional traits. Opposite results were obtained by

288   Belmar et al. (2019) in Mediterranean streams, where they did not observe any significant effect of flow

289   intermittence on functional diversity. These different outcomes may indicate that flow intermittency exerts

290   a stronger environmental pressure on benthic invertebrate communities in mountainous lotic ecosystems

291   than in Mediterranean streams. In fact, the overall pool of macroinvertebrates in Alpine areas lacks

292   functional traits to cope with flow intermittency, which are present in their counterpart in the

293   Mediterranean region, where the drying phase is a natural part of the stream hydrological cycle (Leigh et

294   al., 2016; Vannucchi et al., 2013). On the other hand, the analysis of functional β-diversity showed how

295   intermittent reaches experience trait overdispersion, and this is mainly due to a net difference in the

296   amount of space enclosed by the trait space rather than replacements of traits. In other words,

297   communities in intermittent streams are composed by taxa that show a subset of the entire pool of

298   functional traits, but this subset is different depending on the reach considered. Similar results were

299   obtained by Dolédec et al. (2017), as they observed that Mediterranean taxa, which have to cope with

300   higher hydrological variability, may perform more diverse functional roles than temperate taxa and

301   intermittent streams show higher functional variability than permanent systems within the Mediterranean

302   area. This may be due to the fact that intermittent sites are colonized by species with traits adapted to flow

303   intermittence even during baseflow conditions leading to the coexistence of taxa displaying a wide range of

304   traits. The variation in trait composition among reaches can be explained by the high variability of lotic

305   systems experiencing flow intermittency, which in turn affect the community assemblages through

306   stochastic extinctions and colonization (Datry et al., 2016).

307   The analysis of taxa contribution to the functional diversity allowed us to highlight which trait

308   combination is less typical in permanent and intermittent reaches. We observed that Tipulidae shows

309   unique traits for the environmental conditions in permanent sites, whereas *Perla* shows unique traits for

310   environmental conditions in intermittent sites, confirming the results that we obtained in related studies

311   (Doretto et al., 2018; Doretto et al., 2020b; Piano et al., 2019a). In other words, Tipulidae and *Perla* traits

312   are atypical (and possibly maladaptive) for permanent and intermittent stretches, respectively. Tipulidae is

313   a monovoltine, crawler taxon, living in slow flowing waters, such as side-arms or near the banks, whose

314   larvae have a dormancy phase, whereas *Perla* is a large-sized, monovoltine, crawler taxon, with aquatic

315   respiration, preferring oligotrophic, cold, fast-flowing waters (Usseglio-Polatera et al., 2000; Merritt et al.,

316   2017). Large semivoltine taxa with long larval life are expected to be unable to survive in periodically dry

317   environments, while fast growing, plurivoltine organisms are adapted to complete their life cycle in less

318   than one year, emerging just before the loss of surface water (Corbet et al., 2006; López-Rodríguez et al.,

319   2009a, b; Bonada & Dolédec, 2018). Small body size is another trait that increases the survival in

320   intermittent reaches as reduced dimensions allow fast development and population growth (Bonada et al.

321   2007). The shift from rheophilous to lentic taxa has also been recorded in literature (e.g. Acuña et al., 2005;

322   Pace et al., 2013; Chessmann, 2015), as flow intermittency in usually associated with lentification and

323   consequent oxygen reduction (Lake, 2003), which may compromise the survival of many taxa, especially in

324   mountainous lotic ecosystems where organisms have to be adapted to high flow velocities and the

325   pronounced oligotrophy (Fenoglio et al., 2010). In addition, it should be noted that, compared to other

326   exclusively lotic organisms, Tipulidae larvae can survive even in terrestrial habitats if characterized by

327   sufficient moisture (Pritchard, 1983), thus being naturally tolerant to intermittent regimes.

328   Overall, our results demonstrate that benthic invertebrate biodiversity is threatened at both local

329   and regional scale, whereas the biotic homogenization process is still ongoing. We can therefore suggest

330   that macroinvertebrate communities are currently facing an ongoing "mediterraneization process", which

331   is still in progress, and the results of the analyses of functional diversity support this hypothesis. In fact, a

332   more in-depth analysis of their functional diversity revealed that Alpine streams are currently facing the

333   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.

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.

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564 **Tables**

565 Table 1. Mean values with standard deviation in brackets of functional diversity measures in

566 permanent (UP) and intermittent (DOWN) sites, and estimated parameters (Est), with standard errors (*SE*)

567 in brackets, and p-values (*P*) of LMMs performed on functional metrics (FD = functional α-diversity; Fdis =

568 functional dispersion; Feve = functional evenness) to test for differences between UP and DOWN sites. All

569 comparisons are significant.

Functional measures	*UP*	*DOWN*	*B*Est ± *SE*	*P*
FD	0.20 (±0.10)	0.13 (±0.13)	−0.071 (±0.014)	< 0.001
Fdis	0.53 (±0.04)	0.49 (±0.06)	−0.053 (±0.006)	< 0.001
Feve	0.17 (±0.06)	0.13 (±0.09)	−0.039 (±0.014)	0.007
β-diversity (β-replacement + β-richness)	0.63 (±0.14)	0.74 (±0.15)	0.119 (±0.003)	< 0.001
β-replacement	0.25 (±0.16)	0.19 (±0.18)	−0.062 (±0.004)	< 0.001
β-richness	0.38 (±0.24)	0.56 (±0.29)	0.181 (±0.006)	< 0.001

570

571   **Figure captions**

572   Figure 1. Schematic overview of the calculated diversity components (α_w = local diversity within

573   reaches; α_a = local diversity among reaches; β_w = variation among local communities within reaches; β_a =

574   variation among local communities among reaches; γ_w = total diversity within reaches; γ_a = total diversity

575   among reaches) to test the effect of flow intermittency (UP = orange; DOWN = light blue) within- (reach

576   scale) and among stretches (basin scale).

577   Figure 2. a) diversity partitioning of total diversity in the study area into its within- and among

578   reaches components in UP and DOWN reaches (α_w = dark grey; β_w = medium grey; β_a = light grey); b)

579   decomposition of β-diversity into its nestedness (dark grey) and turnover (light grey) components in in UP

580   and DOWN reaches.

581   Figure 3. a–c) Functional richness (a), dispersion (b) and evenness (c) of benthic invertebrate

582   communities in permanent (UP) and intermittent (DOWN) reaches; d–f) Density of functional β-diversity

583   values for pairwise comparison of communities in permanent (UP) and intermittent (DOWN) reaches. Total

584   functional β-diversity (d) is split in two components: β-replacement (e) is turnover in functional

585   composition explained by replacement of space between hypervolumes, and β-richness (f) is the turnover

586   in functional composition explained by net differences between the amount of space enclosed by each

587   hypervolume (Carvalho & Cardoso, 2018; Mammola & Cardoso, 2020).