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Flow intermittency negatively affects three phylogenetically related shredder stoneflies by reducing CPOM availability in recently intermittent Alpine streams in SW-Italian Alps

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1 **The ecological niche of shredders in recently intermittent Alpine streams: a case study in SW-**
2 **Italian Alps**

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9 **Abstract**

10 Several Alpine streams are currently facing recurrent summer drying events with detrimental
11 consequences on stream detritivores, i.e. shredders, due to the negative effects on the organic matter
12 (CPOM) availability. We examined the ecological niche of three phylogenetically related shredder
13 genera belonging to the family of Nemouridae (Plecoptera), namely Nemoura, Protonemura and
14 Amphinemura, in 14 Alpine streams recently facing recurrent summer flow intermittency. We
15 evaluated the overlap among their ecological niches measured in terms of hydraulic stress, substrate
16 composition, food resource availability and competition with other shredder taxa and we examined
17 potential changes in their ecological niches between permanent and intermittent sites. Our results
18 showed that CPOM availability decreases in intermittent sites and the three genera are all negatively
19 affected by flow intermittency. We then observed a broad overlap of the ecological niches of
20 Protonemura and Amphinemura, but not with Nemoura. Finally, the three genera showed a
21 consistent preference for microhabitat with high food availability and low competition in
22 intermittent sites, possibly due to food limitation. Overall, our results emphasize how the negative
23 effect of flow intermittency on shredders in Alpine streams is mainly due to the decrease in CPOM
24 availability, with consequent potential cascade effects on stream ecosystem functionality.

25 **Keywords:** CPOM, ecological niche, hypervolume, Nemouridae, stream detritivores

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INTRODUCTION

Mountainous low-order lotic systems have always been characterized by highly predictable natural hydrological and geomorphological **dynamics**, with an increase in water flow and fine sediments in summer, during snow melting, and a minimum discharge in winter (McGregor et al., 1995). **The recurrent occurrence** of flow intermittency is currently becoming one of the most dramatic threats to mountainous streams (Fenoglio et al., 2010; Brighenti et al., 2019), which are changing from perennial to temporary systems. **These newly temporary streams are characterized with recurrent non-flow events, occurring in summer, followed by rewetting phases in late autumn** (Fenoglio et al., 2010) due to the interactive effects of both climate change and anthropogenic disturbance (Belmar et al., 2019; Bruno et al., 2019). These recurrent **drying** events are expected to alter the distribution of lotic biota by influencing physical conditions and distribution of trophic resources (e.g. Calapez et al., 2014; Elias et al., 2015; Milner et al., 2017; Doretto et al., 2018; Falasco et al., 2018; Doretto et al., 2019; Piano et al., 2019a).

Stream detritivores, which feed on fragments of leaf litter and other plant detritus (i.e. shredders, sensu Merritt et al. 2017), represent a key component in the lotic food web of mountainous streams (Boyero et al., 2012). In fact, **small, upland, snow-melt driven streams in temperate regions** are mainly heterotrophic ecosystems, as most of the energetic support is allochthonous (Vannote et al., 1980) and originate from terrestrial plant organic matter, such as dead leaves introduced during autumn abscission in forested areas (e.g. Petersen & Cummins, 1974; Vannote et al., 1980; Merritt et al., 2017) or grass fragments in arctic-alpine areas (Fenoglio et al., 2014; Taylor & Andrushchenko, 2014). **In particular, detritivores have evolved to take advantage of pulsed organic matter inputs (Benstead & Huryn, 2011), having their life cycles synchronized with the autumnal litter fall (Fenoglio et al., 2005). Early instars take advantage of the organic matter that enters the streams and feed on CPOM until individuals are ready to emerge as winged adults in spring/early summer (Cummins et al., 1989; Bo et al., 2013; Ferreira et al., 2013). This enables**

63 shredder biomass and body size to increase throughout the winter and to reach a maximum in early
64 spring, just before the adult insects emerge from the water, while being at a minimum in late spring
65 and early summer, just after emergence (Fenoglio et al., 2005; González & Graça, 2003; Tierno de
66 Figueroa et al., 2009; Bo et al., 2013)

67 As a consequence, flow intermittency is expected to impact shredders not only due to
68 hydrological and geomorphological alterations but also because recurrent drying events
69 significantly alter organic matter processing, as already observed in Mediterranean streams (e.g.
70 Abril et al., 2016). For instance, surface flow disappearance and increased water temperature
71 usually reduce the decomposition rate especially in dry streambed sediments, where the activity of
72 Ingoldian fungi, bacteria and invertebrates are inhibited by emersion (Corti et al., 2011; Receveur et
73 al., 2020), while increased water temperatures will likely cause an alteration in organic input
74 decomposition (Boulton et al., 2008). In addition, lower flow velocity reduces the removal of fine
75 sediments, with consequent high fine sediment deposition, which can alter the quality and quantity
76 of energy inputs, in terms of both in-stream production (Henley et al., 2000; Bona et al., 2016) and
77 allochthonous coarse organic matter availability (Doretto et al., 2016). In particular, the burial of
78 leaf litter by sediments reduces availability, quality and palatability of this resource, with
79 consequent alterations of both the microbial community and the growth rate of invertebrate
80 shredders (Danger et al., 2012). On top of that, such dramatic consequences on river food webs
81 might persist even after several months following flow resuming, because of the so-called “drying
82 memory” (Datry et al., 2011; Pinna et al., 2016; Piano et al., 2019a). Physical alterations and
83 consequent changes in food resources induced by flow intermittency thus likely represent strong
84 selective pressures that may influence the ecological niche of shredders. Although some studies
85 investigated the response of this trophic group to flow intermittency in Alpine streams (e.g.
86 Fenoglio et al., 2007; Doretto et al., 2018; Piano et al., 2019a), little is known about the role of
87 these events in shaping the ecological niches of shredder taxa.

88 We here investigated the influence of flow intermittency, CPOM availability,
89 hydromorphological parameters and competition on the distribution and the ecological niche of
90 three phylogenetically-related shredder genera, namely *Nemoura*, *Protonemura* and *Amphinemura*,
91 belonging to the family of Nemouridae (Plecoptera). We focused our attention on these genera as
92 they are expected to be particularly sensitive to flow intermittency due to their life-history and
93 ecological traits, as they are medium-sized, monovoltine crawlers, with aquatic respiration,
94 preferring fast flowing waters and coarse substrates (Usseglio-Polatera et al., 2000; Tachet et al.,
95 2010). We conducted our study in fourteen streams in Italian SW-Alps experiencing summer flow
96 intermittency since 2011, where we evaluated the distribution of our focal genera during base flow
97 conditions in April 2017, when shredder larvae reach their maximum density and size. This work is
98 part of the research project NOACQUA dedicated to the investigation of the effect of flow
99 intermittency on the biodiversity and ecosystem processes in mountainous Italian streams, which
100 has already published data (see Piano et al., 2019b in this journal). We hypothesized that: i) flow
101 intermittency negatively affects the abundances of the examined genera; ii) their ecological niches
102 overlap, thus suggesting possible exploitative competition; and iii) recurrent drying events change
103 their ecological niches.

104

105 MATERIALS AND METHODS

106 Sampling design

107 This study was conducted in fourteen low order streams located in the hydroecoregion of
108 SW Alps (HER 4, Piemonte, NW Italy; Wasson et al., 2007), characterized by similar geology,
109 climate and altitude. Study streams were selected based on our experience and available historical
110 data on their hydrology (ARPA – local Environmental Protection Agency). In each stream, we
111 selected two sampling reaches differing in their hydrological regime: i) a control section, with
112 permanent water during the whole year (hereinafter PS); ii) an intermittent section, which

113 experiences **drying** events during summer (hereinafter IS) caused by factors acting at both global
114 (i.e. climate change) and local (i.e. water abstraction) scales. In particular, **ISs have already been**
115 facing summer **drying** lasting on average two months since 2011, with the riverbed almost
116 completely dry **for several kilometers** (ARPA, 2013). PSs were located within 10 km upstream of
117 the ISs to reduce environmental variation between the two **reaches**. **Both permanent and intermittent**
118 **sites were identified in the bottom of the valleys**. Sampling site elevation was on average 489 m
119 **a.s.l., ranging from 307 m and 656 m, and permanent and intermittent reaches within the same**
120 **stream differed on average of 70.2 m in their elevation (min = 19 m; max = 155 m)**. We performed
121 our sampling campaign in April 2017, **before summer snow-melt** (6 months after the drying period),
122 under moderate flow ($Q_{\text{mean}} = 3.98 \pm 4.56 \text{ m}^3/\text{s}$) occurring in both sections, which allowed us to
123 sample shredders at their latest instars and in the period of their maximum biomass. Water flow in
124 ISs had resumed in November 2016 after a heavy rain event, **ending the dry** period (Hydrological
125 bulletins, www.arpa.piemonte.it).

126 **In each reach we selected seven independent sampling patches within 30 m of longitudinal**
127 **distribution, spaced of at least 5 m**, which were randomly selected in order to cover different
128 conditions of flow velocity, water depth and substrate composition (7 samples x 2 **reaches** x 14
129 streams = 196 samples). **In each patch, which consisted of a surface of 0.062 m², i.e. the area of the**
130 **Surber sampler**, we measured flow velocity (0.05 m from the bottom) and water depth with a
131 current meter (Hydro-bios Kiel) and we visually estimated percentages of different substratum sizes
132 measured with a gravelometer following the classification of Wentworth, namely boulders (> 256
133 mm), cobbles (64-256 mm), gravel (2-64 mm) and fine sediment (< 2 mm). One sample was
134 collected in each sampling patch, by using a Surber sampler (250 μm mesh size; 0.062 m² area) and
135 **we collected both the retained CPOM and macroinvertebrates** (Doretto et al., 2020). Collected
136 samples were **preserved to** plastic jars with 75% ethanol. In the laboratory, CPOM was washed
137 through a 250 μm mesh sieve **to eliminate mineral detritus and subsequently separated from**

138 **macroinvertebrates**. The material was subsequently air dried for 24 h, oven dried (105 °C) for 24 h,
139 and then weighed with an electronic balance (accuracy 0.001 g). The CPOM **mass** was then
140 considered a proxy of food availability in subsequent analyses. In the laboratory, all benthic
141 invertebrates were identified according to Campaioli et al. (1994, 1999) to the family or genus level
142 and counted. Only data referred to shredder taxa were considered for further analysis (see Tab. S1
143 and Fig. S1 for a complete checklist of shredders collected in this study). The numbers of
144 individuals of the three genera belonging to Nemouridae, i.e. *Nemoura*, *Protonemura* and
145 *Amphinemura*, represent our target variables, whereas the abundance of the other shredder taxa was
146 considered as a proxy for competition.

147 **Statistical analyses**

148 All statistical analyses were performed with R software (R Core Team, 2019). In a
149 preliminary step, we calculated the Froude number (Gordon et al., 1992) and the Substrate Index
150 (SI, modified by Quinn & Hickey, 1994 after Jowett et al., 1991) in order to obtain synthetic
151 measures of hydraulic stress and substrate composition. The Froude number is a measure of
152 hydraulic turbulence, hence high values correspond to erosive microhabitats. It is calculated as:
153 $v/\sqrt{d \cdot g}$, where v = flow velocity, measured as m/s, d = water depth, measured as m, and g is the
154 gravity acceleration. The SI quantifies the coarseness of the substrate composition, with high values
155 corresponding to coarse substrates and it is calculated as: $0.8 \cdot \%Rocks + 0.7 \cdot \%Boulders +$
156 $0.6 \cdot \%Cobbles + 0.5 \cdot \%Gravel + 0.4 \cdot \%Sand$. We focused our attention on these parameters since
157 they have already been successfully used to describe the physical niche of benthic invertebrates
158 (e.g. Lamouroux et al., 2004; Mesa, 2010; Bo et al., 2017; Piano et al., 2019b).

159 *Wilcoxon test*

160 In a first step, we checked whether environmental and biotic parameters, namely Froude
161 number, SI, CPOM mass and the number of other shredder taxa (hereinafter Competition), and

162 abundances of the three examined genera differed between PSs and ISs with a non-parametric
163 Wilcoxon test.

164 *Niche hypervolume*

165 In a second step, we compared the overall ecological niches of the examined genera based
166 on abundance data in both PS and IS sites to investigate whether their ecological requirements
167 overlap, thus suggesting possible exploitative competition. To perform this, we calculated their
168 ecological niche as their multidimensional Hutchinsonian hypervolume with a kernel density
169 estimation (KDE) procedure via the *hypervolume* R package (Blonder, 2015) based on the Froude
170 number, SI, CPOM mass and the number of other shredder taxa measured at each Surber sample.
171 All variables were standardized before this analysis in order to achieve the same dimensionality for
172 all axes and the hypervolume was calculated with the *hypervolume_gaussian* R command (Blonder,
173 2015), which constructs a hypervolume based on a Gaussian kernel density estimate. We
174 standardized the choice of bandwidth for each variable through a Silverman estimator, and we set a
175 threshold that included 100% of the total probability density. We then calculated the intersection
176 between the hypervolumes and their overlap statistics for each pair of genera via the
177 *hypervolume_set* and *hypervolume_overlap_statistics* R commands respectively (Blonder, 2015).
178 Overlap statistics include the Jaccard and Sorensen similarity indices, which range from 0 to 1 (0 =
179 no overlap; 1 = complete overlap).

180 *Outlying Mean Index (OMI)*

181 In a third step, we assessed whether flow intermittency affects the ecological niche of the
182 three examined genera by means of the Outlying Mean Index (OMI) analysis performed on PS and
183 IS sites separately. The OMI is a two-table ordination technique that can be implemented even with
184 low number of individuals, such as those observed in our work. It positions the sampling units in a
185 multidimensional space as a function of environmental parameters and the distribution of species in

186 this space represents their realized niches (Dolédec et al., 2000). It is based on the concept of
187 marginality, i.e. the distance between the mean habitat conditions observed in the sampling sites
188 where the taxon is present and the mean habitat conditions across the study area. Taxa with high
189 marginality occur in atypical habitats within the study area, while those with low marginality occur
190 in typical habitats within the study area. Besides the marginality, the OMI analysis also calculates
191 the tolerance of each taxon, which is calculated as the niche breadth, namely the amplitude in the
192 distribution of each species along the sampled environmental gradients. Low tolerance values mean
193 that a species is distributed across a limited range of conditions (specialist species), while high
194 tolerance values imply that a species is distributed across habitats with widely varying
195 environmental conditions (generalist species). The OMI analysis were performed via the function
196 “niche” in the package *ade4* (Chessel et al., 2004; Dray & Dufour, 2007; Dray et al., 2007) for the
197 R software (R Core Team, 2019).

198 **RESULTS**

199 *Wilcoxon test*

200 The Wilcoxon test highlighted significant differences among PS and IS sites in terms of
201 Substrate Index, CPOM **mass**, and Competition, while no differences were observed for the Froude
202 number (Tab. 1). In particular, our results highlight how PS sites have significant higher values of
203 SI, CPOM and Competition than IS sites.

204 We collected 492 Nemouridae, among which 450 individuals in PS sites and 72 individuals
205 in IS sites. Among the three genera, *Protonemura* resulted the most abundant and widely distributed
206 genus, with 264 individuals recorded in 40 samples, with higher occurrence and abundance in PS
207 (Occurrence = 26 samples; Abundance = 225 individuals) than IS sites (Occurrence = 14 samples;
208 Abundance = 39 individuals). *Amphinemura* was slightly less abundant than *Protonemura*, with a
209 total of 236 individuals of *Amphinemura*, among which 205 in PS sites and 31 in IS sites recorded

210 in 41 samples and 15 samples respectively. *Nemoura* showed the lowest occurrence and abundance
211 as we collected 22 individuals, among which 20 in PS sites and 2 in IS sites, recorded in 10 and 2
212 samples respectively. Of the 196 investigated patches, 118 had no Nemouridae, 50 had only one
213 genus, 26 hosted 2 genera and only 2 patches accounted for the three examined genera. The
214 Wilcoxon test highlighted significant differences among PS and IS sites for all the three genera with
215 higher abundance values in PS than in IS sites (Tab. 1).

216 *Niche hypervolume*

217 Among the three examined genera, *Protonemura* showed the highest dimension of the four-
218 dimensional hypervolume (559.4), followed by *Amphinemura* (353.0) and *Nemoura* (248.8). The
219 three hypervolumes partially overlap (Fig. 1) as demonstrated by the Jaccard and Sorensen
220 similarity indices (Tab. 2). The highest overlap value is observed between the ecological niches of
221 *Amphinemura* and *Protonemura* (Tab. 2). Although *Nemoura* has the smallest hypervolume, about
222 50% of its ecological niche is unique and does not overlap with that of *Protonemura* and
223 *Amphinemura* (Unique 1 values at the first and second line respectively in Tab. 2). When examining
224 more in detail the overlap between ecological niches (Fig. 1), we can observe how the high unique
225 portion of *Nemoura* niche is mainly determined by its wider distribution when other shredder taxa
226 are present (Competition), whereas it seems limited by the amount of organic matter (CPOM).
227 *Amphinemura* and *Protonemura* show an opposite pattern compared to *Nemoura*, as they occupy all
228 ecological niches determined by CPOM while they are limited by the competition with other
229 shredder taxa (Fig. 1). When considering the Froude number and the Substrate Index, the ecological
230 niches of the three genera broadly overlap, suggesting that they have similar requirements in terms
231 of hydro-morphological conditions (Fig. 1).

232 *Outlying Mean Index (OMI)*

233 The first two axes of the Outlying Mean Index (OMI) analysis were selected and they
234 accounted for the 99.9% and 95.2% of total explained variance in PS and IS sites respectively.

235 In PS sites, *Amphinemura* showed the highest marginality and tolerance values, thus being
236 the genus that occupies the most atypical microhabitats, whereas *Nemoura* showed the lowest
237 marginality, which means that it occupies the most typical microhabitats in permanent sites (Tab.
238 3). When considering tolerance, *Amphinemoura* showed the highest values, thus having the widest
239 niche, while *Protonemura* showed the lowest tolerance values, thus being the genus with the
240 narrowest niche in permanent sites (Tab. 3). In IS sites, *Nemoura* has the highest marginality, thus
241 occupying the most atypical microhabitats in intermittent sites, whereas *Protonemura* has the
242 lowest marginality value, thus occurring in the most typical microhabitats of intermittent sites (Tab.
243 3). When considering niche width, *Amphinemura* resulted the genus with the widest niche, as it had
244 the highest tolerance value, whereas *Protonemura* has the narrowest niche as demonstrated by the
245 fact that it has the lowest tolerance value (Tab. 3).

246 In PS sites, the first axis contributed for the 92.0%, thus explaining most of the variance of
247 our samples, and it represents a gradient of increasing competition and decreasing coarseness of the
248 substrate, whereas the second axis contributed for the 7.82% to the total explained variance and it is
249 a gradient of decreasing CPOM mass and increasing hydraulic stress (Tab. 4). In IS sites, the first
250 axis alone contributed for the 82.7%, again explaining most of the variance of our samples, and it
251 represents a gradient of decreasing CPOM mass and SI, while the second axis contributed for the
252 12.5% to the total explained variance and it represents a gradient of decreasing SI and increasing
253 CPOM mass (Tab. 4).

254 *Nemoura* is negatively correlated with axis 1 and positively correlated with axis 2 in PS
255 sites, while it is positively correlated with both axes in IS sites (Tab. 4). This genus is positively
256 affected by Froude number (0.31), Competition (0.55), and weakly by CPOM mass (0.10), but
257 negatively by SI (-0.22) in PS sites (Fig. 2a), while it is positively affected by CPOM mass (0.23)

258 and negatively by Competition (-0.28), Froude (-0.76) and SI (-1.06) in IS sites (Fig. 2b). Therefore,
259 *Nemoura* shifts from reophilous microhabitats with finer substrates, high hydraulic stress and
260 competition in permanent sites towards microhabitats with finer substrates, high CPOM availability
261 and low competition and hydraulic stress in intermittent sites.

262 *Protonemura* is positively correlated with both axis 1 and axis 2 in PS sites and negatively
263 correlated with both axes in IS sites (Tab. 4). In PS sites, it is positively affected by Froude number
264 (0.14) and negatively affected by SI (-0.17), while it showed an extremely low influence of CPOM
265 **mass** (-0.06) and Competition (-0.05) (Fig. 2a). In IS sites, it showed a positive relationship with
266 CPOM (0.41) and SI (0.71), whereas it is negatively affected by Competition (-0.11) and Froude (-
267 0.17) (Fig. 2b). Therefore, *Protonemura* prefers microhabitats with finer substrates and high
268 hydraulic stress in permanent sites, whereas it is mainly found in microhabitats with coarser
269 substrates, high CPOM availability and low hydraulic stress and competition in intermittent sites.

270 *Amphinemura* displayed a negative correlation with axis 1, while it positively correlates with
271 axis 2 in PS sites, whereas it is negatively correlated with axis 1, but it positively correlates with
272 axis 2 in IS sites (Tab. 4). In Ps sites, this genus is favored by Competition (0.61), followed by
273 CPOM **mass** (0.45), and SI (0.38), but it is weakly negatively affected by Froude number (-0.14)
274 (Fig. 2a). In IS sites, it is positively correlated with CPOM mass (0.80), Froude (0.12) and SI (0.35),
275 while it is not affected by Competition (-0.01). Therefore, *Amphinemura* selects microhabitats with
276 coarser substrates and high competition and CPOM availability in permanent sites, while it is not
277 affected by competition in intermittent sites.

278

279 **DISCUSSION**

280 We here evaluated the role of flow intermittency in shaping the niche of three coexisting
281 phylogenetically related shredders in mountainous streams recently facing summer seasonal **drying**
282 events. As summer drying may strongly affect the CPOM processing —by altering the

283 decomposition by fungi and bacteria— and availability —**high fine sediment deposition buries**
284 **CPOM and it reduces palatability and quality**— in the subsequent months, we here tested whether
285 three genera belonging to the Nemouridae family, namely *Nemoura*, *Protonemura* and
286 *Amphinemura*, were negatively affected by flow intermittency.

287 We first tested whether recurrent **drying** events caused a reduction in the abundance of the
288 examined genera between permanent and intermittent reaches. In agreement with our hypothesis,
289 the three genera were significantly more abundant in permanent than in intermittent sites, as well as
290 the other shredder taxa. This is in accordance with the results observed in a related study performed
291 in the same study area, where we observed a significant negative effect of flow intermittency on the
292 relative abundance of scrapers taxa (Piano et al. 2019a). This reduction is likely driven by the lower
293 availability of organic matter in intermittent than in permanent sites, which is in turn potentially
294 determined by the lower retention capacity of the riverbed substrate in intermittent sites. In fact,
295 although the flow had recovered since 6 months at the sampling moment, we still observed a
296 significant lower value of the substrate index in intermittent sites, indicating that sites experiencing
297 recurrent **drying** events are characterized with finer substrates than perennial sites. Heavy fine
298 sediment accumulation (i.e., clogging) is strictly associated with flow reduction and droughts,
299 **because lower water velocity prevent the export of fine sediments** (Dewson et al., 2007; Rolls et al.,
300 2012), thus reducing the retention capacity of the substrate. In addition, the high fine sediment
301 deposition can alter the quality and quantity of energy inputs, in terms of both in-stream production
302 (Henley et al., 2000; Bona et al., 2016) and allochthonous coarse organic matter availability in
303 mountainous streams (Doretto et al., 2016; Doretto et al., 2017). In particular, the burial of leaf litter
304 by sediments reduces availability, quality and palatability of this resource, with consequent
305 alterations of both the microbial community (Receveur et al., 2020) and the growth rate of
306 invertebrate shredders (Danger et al., 2012).

307 Second, we examined whether the examined genera differed in the dimension of their
308 realized ecological niches and whether they overlap in their ecological requirements, thus

309 suggesting potential exploitative competition. Our results displayed only a partial overlap of the
310 niche hypervolumes of the three genera. Although our model organisms were found to co-occur in
311 similar hydro-morphological conditions, they differ in their niche dimension and they occupy
312 different species in terms of food availability and potential competition. In particular, we could
313 observe a clear phylogenetic differentiation in their ecological niches. *Nemoura*, which is the only
314 genus belonging to the subfamily Nemurinae in our study, displayed the smallest niche, but it also
315 showed the highest unique component, which can be due both to competitive exclusion and the
316 capacity to exploit atypical habitats. The results of the OMI analysis likely support this second
317 hypothesis as *Nemoura* occupies the most atypical habitats in intermittent sites. In addition, this
318 genus is also possibly the most negatively affected by flow intermittency as its tolerance decreases
319 from perennial to intermittent sites. We can hypothesize that this can be due to the fact that some
320 species within this genus, such as *Nemoura cinerea*, display semivoltine populations (Fochetti et al.,
321 2009). In fact, voltinism has been recognized as one of the most sensitive traits (Bonada & Doledec,
322 2018) to flow intermittency as shifts from semivoltinism to multivoltinism have been observed from
323 perennial to intermittent hydrological regimes in Mediterranean streams (López-Rodríguez et al.
324 2009a, b).

325 Conversely, ecological niches of *Protonemura* and *Amphinemura*, both belonging to the
326 subfamily Amphinemurinae, broadly overlap, suggesting the possibility of exploitative competition.
327 Although having the widest niche, the results of the OMI analysis suggest that *Protonemura* is
328 outcompeted by *Amphinemura*, which has the highest tolerance in both permanent and especially in
329 intermittent sites. Although being negatively affected by flow intermittency, as demonstrated by the
330 lower number of individuals in intermittent than in permanent sites, *Amphinemura* resulted the most
331 tolerant genus to flow intermittency among Nemouridae. This can be due to the lower body size
332 dimensions of this genus compared to *Nemoura* and *Protonemura* (Fochetti et al., 2009) as small
333 body size is also a common attribute of macroinvertebrate taxa living in intermittent streams

334 because smaller individuals have display fast development and population growth, which allow to
335 complete the life-cycle before the water disappears (Bonada et al. 2007).

336 Third, by examining how the four environmental factors drive the realized niches of
337 examined genera, we could highlight that flow intermittency induces environmental changes, which
338 in turn affect the ecological niches of Nemouridae.

339 The relationship with CPOM is weak in permanent sites, except for *Amphinemura*, but it
340 becomes consistently and highly positive in intermittent sites. Conversely, the presence of other
341 shredders, here considered as a proxy of competition, showed opposite effects on the ecological
342 niches of our examined genera, by positively influencing them in permanent sites, except for
343 *Protonemura*, but having a consistent weak negative effect in intermittent sites. We can hypothesize
344 that in permanent sites, where food availability is high, different shredder taxa can coexist, whereas
345 in intermittent reaches access to trophic resources is dominated by exploitative competition among
346 shredder taxa due to food limitation, with detrimental effects on Nemouridae. In fact, Tierno de
347 Figueroa & Lopez-Rodriguez (2019) reviewed how Nemouridae are highly dependent on CPOM,
348 even if some species can act as collector-gatherers (Lopez-Rodriguez et al., 2010).

349 On the other hand, the role of the hydro-morphological parameters is controversial. When
350 considering the Froude number, *Nemoura* and *Protonemura* were favored in reophilous
351 microhabitats in permanent but not in intermittent sites, whereas *Amphinemura* resulted weakly
352 affected by this factor. Our sampling sites are located in mountainous streams, where near-bed
353 hydraulic stress is naturally high. In these conditions, CPOM usually accumulates when particles hit
354 an obstruction, such as a rock, log or vegetation, where the hydraulic stress is lower (Hoover et al.,
355 2006; Quinn et al., 2007). Therefore, at microhabitat level, high Froude numbers are expected to
356 negatively affect the occurrence of shredder taxa as often associated with low CPOM retention. We
357 can suggest that in permanent sites, where there is no food limitation, *Nemoura* and *Protonemura*
358 better thrive in fast flowing conditions, which represent optimal habitats for Nemouridae (Usseglio-

359 Polatera et al., 2000), whereas in intermittent sites they select suboptimal habitats, where the CPOM
360 concentration is expected to be higher.

361 Regarding the substrate preferences, the relationship with the Substrate Index indicates that
362 only *Protonemura* differentially selects microhabitats based on this parameter in permanent and
363 intermittent sites. In fact, it is mainly found on finer substrates in permanent sites whereas it shifts
364 on coarser substrates in intermittent sites. This change can again be due to the food limitation in
365 intermittent sites, where *Protonemura* selects microhabitat with coarser substrates that have a
366 higher retention capacity of CPOM. The relationship of *Nemoura* and *Amphinemura* with this
367 parameter corroborates this hypothesis, as *Amphinemura*, which shows the stronger association with
368 CPOM in both permanent and intermittent sites, has a consistent positive relationship with the
369 Substrate Index, whereas *Nemoura*, which is the genus with the lowest association with CPOM in
370 both permanent and intermittent sites, consistently prefers finer substrates.

371 Overall, our results emphasize how stream physical parameters and resource availability
372 play a key role in determining the distribution and the ecological niche of shredders in Alpine
373 streams. Recurrent **drying** events negatively affect the examined genera, which are less represented
374 in ISs than in PSs, and the narrower niches in ISs than in PSs, and their niche shift in ISs furtherly
375 corroborate this hypothesis. According to our results, the negative effect of flow intermittency is
376 mainly due to the reduced availability of CPOM compared to permanent sites confirming previous
377 findings in other temperate geographical areas (Datry et al., 2011; Pinna et al., 2016). Consequently,
378 water flow reduction and recurrent **drying** events are expected to alter the availability of energetic
379 inputs, with potential dramatic effects on stream ecosystem functionality (e.g. Ledger et al., 2008;
380 Datry et al., 2011; Piano et al., 2019b). The expected decrease in allochthonous trophic resources
381 will likely cause a bottom-up effect in the food web, directly influencing the survival, growth and
382 reproduction of invertebrate shredders. As changes in the processing of allochthonous trophic
383 resources may also depend on the microbial and fungal activity involved in this process, further

384 investigations in mountain areas are required in the next future to better unravel the role of flow
385 intermittency on these components of lotic ecosystems.

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576 **Figure captions**

577 Figure 1. Pair plots showing the estimated four-dimensional hypervolumes for the three
578 examined genera. The colored points for each genus reflect the centroids (large points), original
579 observations (intermediate points) and the stochastic points sampled from the inferred hypervolume
580 (small points). All variable are standardized.

581 Figure 2. Projection of environmental variables on the axis of OMI analysis in PS (a) and IS
582 (b) sites and representation of ecological niches of the three examined genera. Values of distances
583 among one square and the other along the two axes are determined by the d value reported in the
584 top right corner of the pictures.

585