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

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Abstract

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

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

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

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

MATERIALS AND METHODS

Sampling design

This study was conducted in fourteen low order streams located in the hydroecoregion of SW Alps (HER 4, Piemonte, NW Italy; Wasson et al., 2007), characterized by similar geology, climate and altitude. Study streams were selected based on our experience and available historical data on their hydrology (ARPA – local Environmental Protection Agency). In each stream, we selected two sampling reaches differing in their hydrological regime: i) a control section, with permanent water during the whole year (hereinafter PS); ii) an intermittent section, which experiences drying events during summer (hereinafter IS) caused by factors acting at both global (i.e. climate change) and local (i.e. water abstraction) scales. In particular, ISs have already been facing summer drying lasting on average two months since 2011, with the riverbed almost completely dry for several kilometers (ARPA, 2013). PSs were located within 10 km upstream of the ISs to reduce environmental variation between the two reaches. 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 m and 656 m, and permanent and intermittent reaches within the same stream differed on average of 70.2 m in their elevation (min = 19 m; max = 155 m). We performed our sampling campaign in April 2017, before summer snow-melt (6 months after the drying period), under moderate flow (Q_{mean} = 3.98 ± 4.56 m^3/s) occurring in both sections, which allowed us to sample shredders at their latest instars and in the period of their maximum biomass. Water flow in ISs had resumed in November 2016 after a heavy rain event, ending the dry period (Hydrological bulletins, www.arpa.piemonte.it).

In each reach we selected seven independent sampling patches within 30 m of longitudinal distribution, spaced of at least 5 m, which were randomly selected in order to cover different conditions of flow velocity, water depth and substrate composition (7 samples x 2 reaches x 14 streams = 196 samples). In each patch, which consisted of a surface of 0.062 m^2, i.e. the area of the Surber sampler, we measured flow velocity (0.05 m from the bottom) and water depth with a current meter (Hydro-bios Kiel) and we visually estimated percentages of different substratum sizes measured with a gravelometer following the classification of Wentworth, namely boulders (> 256 mm), cobbles (64-256 mm), gravel (2-64 mm) and fine sediment (< 2 mm). One sample was collected in each sampling patch, by using a Surber sampler (250 μm mesh size; 0.062 m^2 area) and we collected both the retained CPOM and macroinvertebrates (Doretto et al., 2020). Collected samples were preserved to plastic jars with 75% ethanol. In the laboratory, CPOM was washed through a 250 μm mesh sieve to eliminate mineral detritus and subsequently separated from macroinvertebrates. The material was subsequently air dried for 24 h, oven dried (105 °C) for 24 h, and then weighed with an electronic balance (accuracy 0.001 g). The CPOM mass was then considered a proxy of food availability in subsequent analyses. In the laboratory, all benthic invertebrates were identified according to Campaioli et al. (1994, 1999) to the family or genus level and counted. Only data referred to shredder taxa were considered for further analysis (see Tab. S1 and Fig. S1 for a complete checklist of shredders collected in this study). The numbers of individuals of the three genera belonging to Nemouridae, i.e. *Nemoura*, *Protonemura* and *Amphinemura*, represent our target variables, whereas the abundance of the other shredder taxa was considered as a proxy for competition.

Statistical analyses

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

Wilcoxon test

In a first step, we checked whether environmental and biotic parameters, namely Froude number, SI, CPOM mass and the number of other shredder taxa (hereinafter Competition), and abundances of the three examined genera differed between PSs and ISs with a non-parametric Wilcoxon test.

Niche hypervolume

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

Outlying Mean Index (OMI)

In a third step, we assessed whether flow intermittency affects the ecological niche of the three examined genera by means of the Outlying Mean Index (OMI) analysis performed on PS and IS sites separately. The OMI is a two-table ordination technique that can be implemented even with low number of individuals, such as those observed in our work. It positions the sampling units in a multidimensional space as a function of environmental parameters and the distribution of species in this space represents their realized niches (Dolédec et al., 2000). It is based on the concept of marginality, i.e. the distance between the mean habitat conditions observed in the sampling sites where the taxon is present and the mean habitat conditions across the study area. Taxa with high marginality occur in atypical habitats within the study area, while those with low marginality occur in typical habitats within the study area. Besides the marginality, the OMI analysis also calculates the tolerance of each taxon, which is calculated as the niche breadth, namely the amplitude in the distribution of each species along the sampled environmental gradients. Low tolerance values mean that a species is distributed across a limited range of conditions (specialist species), while high tolerance values imply that a species is distributed across habitats with widely varying environmental conditions (generalist species). The OMI analysis were performed via the function "niche" in the package *ade4* (Chessel et al., 2004; Dray & Dufour, 2007; Dray et al., 2007) for the R software (R Core Team, 2019).

RESULTS

Wilcoxon test

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

We collected 492 Nemouridae, among which 450 individuals in PS sites and 72 individuals in IS sites. Among the three genera, *Protonemura* resulted the most abundant and widely distributed genus, with 264 individuals recorded in 40 samples, with higher occurrence and abundance in PS (Occurrence = 26 samples; Abundance = 225 individuals) than IS sites (Occurrence = 14 samples; Abundance = 39 individuals). *Amphinemura* was slightly less abundant than *Protonemura*, with a total of 236 individuals of *Amphinemura*, among which 205 in PS sites and 31 in IS sites recorded in 41 samples and 15 samples respectively. *Nemoura* showed the lowest occurrence and abundance as we collected 22 individuals, among which 20 in PS sites and 2 in IS sites, recorded in 10 and 2 samples respectively. Of the 196 investigated patches, 118 had no Nemouridae, 50 had only one genus, 26 hosted 2 genera and only 2 patches accounted for the three examined genera. The Wilcoxon test highlighted significant differences among PS and IS sites for all the three genera with higher abundance values in PS than in IS sites (Tab. 1).

Niche hypervolume

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

Outlying Mean Index (OMI)

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

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

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

Nemoura is negatively correlated with axis 1 and positively correlated with axis 2 in PS sites, while it is positively correlated with both axes in IS sites (Tab. 4). This genus is positively affected by Froude number (0.31), Competition (0.55), and weakly by CPOM mass (0.10), but negatively by SI (-0.22) in PS sites (Fig. 2a), while it is positively affected by CPOM mass (0.23) and negatively by Competition (-0.28), Froude (-0.76) and SI (-1.06) in IS sites (Fig. 2b). Therefore, *Nemoura* shifts from reophilous microhabitats with finer substrates, high hydraulic stress and competition in permanent sites towards microhabitats with finer substrates, high CPOM availability and low competition and hydraulic stress in intermittent sites.

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

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

DISCUSSION

We here evaluated the role of flow intermittency in shaping the niche of three coexisting phylogenetically related shredders in mountainous streams recently facing summer seasonal drying events. As summer drying may strongly affect the CPOM processing —by altering the decomposition by fungi and bacteria— and availability —high fine sediment deposition buries CPOM and it reduces palatability and quality— in the subsequent months, we here tested whether three genera belonging to the Nemouridae family, namely *Nemoura*, *Protonemura* and *Amphinemura*, were negatively affected by flow intermittency.

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

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

Conversely, ecological niches of *Protonemura* and *Amphinemura*, both belonging to the subfamily Amphinemurinae, broadly overlap, suggesting the possibility of exploitative competition. Although having the widest niche, the results of the OMI analysis suggest that *Protonemura* is outcompeted by *Amphinemura*, which has the highest tolerance in both permanent and especially in intermittent sites. Although being negatively affected by flow intermittency, as demonstrated by the lower number of individuals in intermittent than in permanent sites, *Amphinemura* resulted the most tolerant genus to flow intermittency among Nemouridae. This can be due to the lower body size dimensions of this genus compared to *Nemoura* and *Protonemura* (Fochetti et al., 2009) as small body size is also a common attribute of macroinvertebrate taxa living in intermittent streams because smaller individuals have display fast development and population growth, which allow to complete the life-cycle before the water disappears (Bonada et al. 2007).

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

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

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

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

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

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

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Figure captions

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

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