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RESPONSE OF BENTHIC COMMUNITIES AND RIVER ECOSYSTEM PROCESSES TO CLIMATE CHANGE

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Foreword

This PhD thesis is organized as a collection of scientific papers which have the overall aim of studying the effects of climate change on benthic communities and river ecosystem processes in Alpine streams. The results presented here regard not only stream macroinvertebrates, but also microbial communities and instream allochthonous organic material decomposition that is widely used to assess river functionality. The introduction serves as a broad overview on climate change and its main effects, focusing on river ecosystems. The state of the art regarding leaf litter decomposition studies is also presented in this section, which can help understanding the importance of investigating this functional process in Alpine streams, which are recently facing intermittence and dry periods. At the beginning of my PhD program, I focused on stream macroinvertebrates response to riverbed drying and flow intermittence, studying the communities collected in the previous years from fourteen Alpine streams that showed a shift from perennial to intermittent flow regime. Meanwhile, I carried out my field experiment, focused on the study of leaf litter decomposition in three Alpine streams belonging to the set of the fourteen ones facing dry events. During my period abroad I focused on the microbial community present in the leaf litter bags, performing 16S rRNA and ITS rRNA extraction, amplification and sequencing at Benbow Lab, Michigan State University. Afterwards, I compared field results regarding coarse particulate organic matter decomposition (hereafter CPOM) with the ones obtained using open-air mesocosms under controlled conditions. The collection of papers presents a broad range of results regarding the effects of global climate change on these river ecosystems: functional processes altered by riverbed drying, macroinvertebrates community composition and niches, comparison between field and mesocosms experiments and microbial community composition and role in perennial and intermittent reaches. The conclusion section presents the main findings regarding what happens to the Alpine streams functional processes and main biological communities in an increasing climate change scenario.

Introduction

1. Climate change and running waters

Due to the evidence of climate change at a global scale, a great number of predictions have been made in the last decades, and nowadays the increasing interest towards this phenomenon and its possible consequences has led to a wide variety of studies, with the contribution of academics, experts, and public administrations. Among all the sources of data and predictions, the Intergovernmental Panel on Climate Change (IPCC) stands out: this is the United Nations body for assessing the science related to climate change and provides a huge number of reports and insights regarding this topic, together with the predictions of different future scenarios on the basis of emissions of greenhouse gases, pollution and so forth. The 2007 IPCC report, states that mean global temperatures had risen by about 0.7°C and further warming was expected in the next 100 years: at that time, the Earth's mean temperature was projected to rise from 1.8 to 4.0°C by the end of the twenty-first century (IPCC, 2007). In a special IPCC report of October 2018, a detailed prediction has been made regarding Global Warming of 1.5°C: this means a world in which warming has been limited to 1.5°C relative to pre-industrial levels. Maintaining the rising temperatures inside this range is difficult, because it all depends on the emissions and the related global responses to the problem, in order to adapt to a sustainable development. Rising temperatures has already resulted in deep alterations to human and natural systems, i.e. increase in droughts, flood, extreme weather, sea level rise and biodiversity loss;

this threatens vulnerable populations at different levels (IPCC, 2012 and 2014; Mysiak et al., 2016; Piano et al., 2019; Doretto et al., 2020), for example in food security, which is strictly linked to increasing migration and poverty. Moreover, numerous unique ecosystems will be at high risk: warm-water tropical reefs and Arctic ecosystems at first (IPCC, 2014). In 2021, IPCC published the Sixth Assessment Report (AR6) on the physical science basis of climate change. In this document, it is clearly stated that human influence has warmed the planet, causing rapid changes in atmosphere, ocean, cryosphere and biosphere. With the observed increase in well-mixed greenhouse gas (i.e. GHG, such as CO_2, CH_4 and N_2O) concentrations, each of the last four decades has been warmer than the previous one since 1850 and the first two decades of the 21st century were characterized by global surface temperatures 0.99°C higher than 1850-1900 (IPCC, 2021). Moreover, this document highlights that human influence is very likely the main driver of the glaciers' global retreat since the 1990s and also of all the climatic extreme events that are occurring since AR5: heatwaves, heavy precipitation, droughts, tropical cyclones and so forth. This interesting and accurate document also provides five new illustrative scenarios, useful to explore the climate response to emissions, land use and air pollutants in the near-term (2021-2040), mid-term (2041-2060) and long-term (2081-2100). As previously mentioned (IPCC, 2018), global warming of 1.5°C (referred to 1850-1900) will be exceeded during the 21st century under the intermediate, high and very high scenarios, but what worries most is that it could increase to 2°C and exceed them under the cited scenarios if deep reductions in CO_2 and other greenhouse gas emissions will not occur in the coming decades.

Regarding this, it is important to remember that global surface temperature can vary above or below the long-term human induced trend, due to natural variability; in these terms, the occurrence of temperature change above a certain level, does not necessarily imply that this global level has been reached (IPCC, 2021). Another important document has been redacted by the IPCC in 2008 regarding Climate Change and Water (Bates et al., 2008). Regarding precipitation, the document states that it is *very likely* that heavy precipitation events will become more frequent, especially in tropical and high-latitude areas; also a tendency for drying in mid-continental areas during summer is addressed, indicating a greater risk of droughts in these regions. The results obtained from global coupled models show that there is an increasing likelihood of summer drying in the mid-latitudes, with an associated risk of drought and heatwaves.

In the previously mentioned technical report, an interdisciplinary writing team was selected to address the subject, dealing only with freshwater.

Freshwater ecosystems represent only 0.01% of the total water volume of the planet, but they provide a broad range of ecosystem services along with their important role in nutrient cycling and as a source of biodiversity. However, climate change has altered these ecosystems on both supply and demand sides, because of the changes in temperatures, precipitations and water flow regimes (Carpenter et al., 2011; Pham et al., 2019). This phenomenon is causing shifts in river discharge, widespread melting of snow and ice and increasing magnitude and frequency of extreme events, including heavy rainfalls, floods and droughts (Kundzewicz et al., 2008; Coppola et al., 2014). Moreover, given that temperature and precipitation patterns affect nutrient cycling and thermal niches of organisms, ongoing climate change can further affect freshwater quality: for example, higher temperatures can increase the metabolic rate and decrease oxygen solubility, leading to eutrophication (Schiedek et al., 2007). Climate change also affects groundwater recharge rates: at high latitudes, for example, thawing of permafrost leads to changes in level and quality of groundwater, because of the coupling with surface waters. Precipitation variability can also result in exceeding infiltration capacity of the soil, or in increasing groundwater recharge due to the heavy rainfalls which allow water to infiltrate enough before evaporating (IPCC, 2008). Aquatic species will be damaged too (Döll & Zhang, 2010): stream fragmentation and the decrease in river discharge can reduce the genetic flow of aquatic biota, along with the pauperization of freshwater communities and loss of specialist taxa. Additionally, local human impact, such as water abstraction for hydropower, industrial, agricultural or domestic purposes and operation of dams, can further exacerbate the effects of climate change. In normal conditions, seasonal pattern changes in temperatures and flow conditions make rivers a predictable habitat in which river biota have evolved and thrived (Poff et al., 1997); hence, the greater the deviation in seasonal conditions, the greater the expected alteration to biological communities, riverscape and overall ecosystem stability (O’ Briain, 2019).

2. Effects of flow intermittency on benthic communities and decomposition processes

The vulnerability of river biota to climate change is related to the limited dispersion ability and tolerance to flow and temperature changes of many species (Folke, 2006; Domisch et al., 2017). In these conditions, community instability increases as species turnover accelerates, causing a shift to a new community composition. For example, where climate change leads to a shift from a perennial regime to seasonally drying conditions, there will likely be radical consequences for the biological communities present in the habitat. If disturbances do not cause substantial habitat alteration, species plasticity and recolonization ability will lead community composition (Death, 2008); at the same time, if habitat is significantly altered, biota recovery can be drastically retarded or arrested (Garner et al., 2015; Piniewski et al., 2017). Tridimensional in-stream connection can also be affected in a changing climate scenario: this phenomenon can further impact the stability of benthic communities, robustness and resistance to allochthonous species invasion (Baiser et al., 2010; Strona & Lafferty, 2016). Rheophilic taxa, for example, adapted to fast-flowing habitats, are among the most sensitive organisms during low flow conditions (Brooks et al., 2011): if river connectivity is lost followed by shrinking or disappearance of habitats, natural selection will facilitate species able to continue the recruitment process in such conditions (Kannan et al, 2018). In case of reduced streamflow velocity, sediment and particulate organic matter deposition is enhanced (Acuña et al., 2007), negatively affecting stream organisms, such as macroinvertebrates and fishes (Hakala & Hartman, 2004; Kemp et al., 2011).

Moreover, if streamflow ceases completely, algal growth can be promoted because of stagnation (Caramujo et al., 2008) and dissolved organic carbon is drastically reduced (i.e. DOC; Everard, 1995) due to the acidic conditions and the inhibition of microbial communities caused by the drought (Scott et al., 1998). Due to the increase of mean temperatures all river ecosystems are facing these consequences: perennial, intermittent and ephemeral ones across the world. In naturally intermittent and ephemeral streams species have already evolved adaptations to survive dry periods, even if longer, more frequent and more extreme droughts can negatively affect these communities too (Bêche et al., 2009).

According to the U.S. Environmental Protection Agency definition, an intermittent stream is any river that flows only during certain times of the year, and may not have any flowing surface water during the dry season. For this reason, flow intermittency has gained more and more attention from the stream ecology researchers, because understanding what happens in these ecosystems is crucial in a climate change scenario, that will exacerbate the timing, the duration and the effects of drying events. In literature, many studies can be found regarding flow intermittency in Mediterranean and semi-arid ecosystems, where this phenomenon is naturally present due to high temporal climate variation, with decreasing precipitations during summer (Skoulikidis et al., 2017). In fact, in Mediterranean regions, non-perennial rivers and streams are the dominant freshwater type (Bonada & Resh, 2013) and the so called “dry rivers" are well known in the culture of these areas; this region is considered a global hotspot of biodiversity and endemisms (Bonada et al., 2007; Tierno de Figueroa et al., 2013), but unfortunately it also contains a high proportion of the most endangered species worldwide (Myers et al., 2000; Darwall et al., 2014). In these ecosystems, natural seasonal dryness is exacerbated by human activities, for example an extensive use of water for agricultural irrigation, making possible the definition of “artificially dry” or “artificially intermittent” streams and rivers (Skoulikidis et al., 2011). This artificial intermittence can lead to a decline of freshwater species richness and abundances and to a loss of migratory pathways for many fish species (Larned et al., 2010) together with a marked sensitivity towards anthropogenic water quality pressures, i.e. eutrophication, hypoxia and contaminants concentration (López-Doval et al., 2013). Many of these studies focus on leaf litter decomposition processes, which have been proposed as a tool to assess river functionality and ecosystem integrity (Gessner & Chauvet, 2002). In river ecosystems, allochthonous organic matter (mainly leaf litter falling from the trees in the riparian zone) represents the major fraction of energetic input, since the detritus chain is fundamental in the energetic pathways of these ecosystems (Cummins, 1979). The degradation of this organic matter is complex and led by physico-chemical and biological processes. At first, the leaching phenomenon occurs, where physical abrasion breaks down a fraction (20-25% maximum) of the allochthonous material which will end up as dissolved organic matter. Then, the conditioning phenomenon occurs, where microbial community plays a key role (Rossi et al., 1983): the colonization of leaf litter made by bacteria and fungi can enhance the decomposition process, producing nutrients and cellulolytic enzymes, making also the organic material more palatable for further consumers, such as those shredder macroinvertebrates which mainly feeds on CPOM (Hieber & Gessner, 2002). Stream macroinvertebrates activity is fundamental in this process, because their action turns coarse detritus in pellets and orts, which will be carried downstream as fine particulate organic matter (FPOM) and exploited by other consumers (Fenoglio et al., 2019).

3. Experimental methods: leaf litter bags and field/mesocosms studies

Leaf litter bags experiments were also carried out in order to investigate which macroinvertebrates colonized these substrata (without looking at the decomposition rate) and if there were differences between this technique and Surber samples (Peralta-Maraver et al., 2011). In this experiment, carried out in Fardes Stream (Granada, Spain), two different leaf species were used, one native and one introduced; no significant differences were found when looking at macroinvertebrates colonization between the two leaf types, instead both substrata attracted a rich and diverse community. Interestingly, when comparing Surber data with the leaf litter ones, Peralta-Maraver and colleagues observed that some groups seemed to be attracted by leaf bags, reaching very high densities: predators because of the higher amount of prey and shredders because of the high CPOM content. For its complexity (multiple factors occurring) and its importance in assessing river functionality, leaf litter decomposition studies have been used to investigate the effects of flow intermittency looking at in-stream functional processes. In Pinna & Basset, 2004 for example, leaf litter decomposition process was investigated in three sub-basins of the river Tirso (Sardinia, Italy) with 4 different stream orders: the strongest effects of summer drying events occurred at the lowest order branches, suggesting that resilience to this disturbance, in Mediterranean climates, increases with stream order. This work also highlights that the disturbance of stream communities in Mediterranean eco-regions can reduce site-to-site variability, as a result of the patchiness of these communities. Another interesting work was carried out in a former perennial river of Central Appennines by Di Sabatino et al. (2021), who investigated leaf litter breakdown and benthic macroinvertebrates in: i) a perennial site; ii) a site which experienced irregular patterns of intermittency during the previous years. Di Sabatino and colleagues found out that the low rate of detritus breakdown in the previously intermittent site was mainly due to a significant decrease in shredder macroinvertebrates, rather than to CPOM availability.

The leaf litter bags technique is used to assess allochthonous CPOM decomposition: fresh or dry leaves are weighted and enclosed in bags made of coarse or fine mesh; the mesh size is selected in order to investigate decomposition including or excluding stream macroinvertebrates from the process, respectively (Mora-Gómez et al., 2015). Using fine mesh bags, only the microbial community (which play an important role in the process) is investigated. The leaf bags are placed instream and then removed at selected intervals: dry weight alone or coupled with ash free dry mass are performed, to assess how much leaf material have been lost during days/weeks. This method helps the researchers to understand the decomposition process by looking at leaf mass loss and the community of macro- and/or micro-consumers involved. In literature, many experiments have been conducted using dry leaves collected after the abscission. The choice of leaf type is crucial in those experiments: some authors have chosen tree species naturally present along the riverbed (Dangles et al., 2001), others invasive species that have colonized the selected area (Wasserman et al., 2021). For example, in Di Sabatino et al. 2020, two different leaf types were used for the experiment: *Populus nigra,* commonly present in the selected study area and *Phragmites australis,* an invasive species. The results of this experiment carried out in a carbonatic rheocrene spring located in a protected area in the Appennines, report that the overall breakdown rate of *P. nigra* was twice as fast as *P. australis;* a higher decay rate for native litter detritus was demonstrated, but no significant differences were found regarding macroinvertebrates colonizing these two leaf types, suggesting that a faster breakdown of native detritus may not necessarily be related to differences in structure and functional organization of colonizing assemblages. Litter quality is also extremely important, because its nutrient content and its texture (though or softer leaves) strongly influence the timing of decomposition processes (Simon et al., 2018). Leaf litter bags can be filled with only one leaf species per bag (Tonin et al., 2014) or with a mixture of different ones (Santonja et al, 2019). Interestingly, Di Sabatino and colleagues also developed a new technique to perform litter decomposition experiments, revisiting the original leaf litter bags existing method (Boulton & Boon, 1991). The Leaf-Nets (LN; Di Sabatino et al., 2016) consist in a single layer of previously air-dried leaves of choice included between two 10 × 10 cm PVC nets, with 1 cm mesh size; the two nets are laterally joined by a short plastic-coated steel wire, forming a sampling unit. A variable number of these sampling units (according to the selected surface to cover) are assembled one above the other and laterally anchored with a plastic-coated steel wire leaving a 1–3-cm space between each unit. These Leaf-Nets were at first proposed to investigate macroinvertebrates community and leaf decomposition in non-wadeable rivers (Di Sabatino et al., 2016) with good results, then used to investigate community composition and river functionality in spring habitats (Di Sabatino et al., 2018; Cristiano et al., 2019). This newly proposed sampling method proved useful to assess community composition and leaf litter decomposition in different river ecosystems, without using invasive methods (such as massive surber sampling) and considerably reducing the costs when compared to other techniques.

Other materials used to assess organic matter decomposition are also wooden sticks and cotton strips placed in the streambed (Smeti et al., 2018; Colas et al., 2019).

This type of studies carried out in Mediterranean, tropical and semi-arid streams and rivers were mainly focused on the effects of flow intermittency on leaf litter decomposition. In Monroy et al. 2016, for example, the decomposition of *Alnus glutinosa* (black alder, highly palatable) and *Quercus robur* (oak, recalcitrant) was studied using both fine and coarse mesh bags placed in the Ebro basin, Spain, where dry events occurred during the experiment. They found out that, where drought events were more intense, total decomposition was negatively affected and macroinvertebrates took longer to recover after drying, while microbial communities recovered more quickly after flow resumption. Moreover, flow intermittency impacted more on high quality leaf litter than on low quality one. In Abril et al., 2016 black alder leaves (*Populus nigra*) were used to assess organic matter decomposition in the Iberian Peninsula, looking at sites with running waters, isolated pools and moist or dry sediments. Decomposition rates turned out to be more pronounced in isolated pools and moist sediments, along with fungal and microbial biomass. When looking only at aquatic habitats (excluding sediments), no significant differences were found in total macroinvertebrate density, even if total abundance varied between pools, mainly because of the differences in oxygen availability between sites. This work highlights what is reported in other studies, i.e. that in temporary streams decomposition processes tend to be slowed down (Herbst & Reice, 1982), because processing efficiency is reduced during drought events (Maamri et al, 1997). At the same time, flow fragmentation can produce spatial and temporal heterogeneity, creating different ecological conditions which can result in a highly heterogeneous decomposition process. Another interesting study was focused on substrate quality, an important factor implied in decomposition processes along with the influence of temperature. Martínez et al. (2016) found out that, in temperate streams, alder, oak and beech leaves decomposed differently because of their different quality and that temperature had a major influence in litter of poor quality when compared to high quality one. Alder leaf litter showed higher decomposition rates in both coarse and fine mesh bags, due to higher microbial metabolism and colonization by invertebrates, which confirms that stream biota has a clear preference for high quality substrate. Oak and beech, instead, were more sensitive to temperature variation because of their texture and intrinsic quality and showed to be strongly influenced by dissolved nutrients concentration during their decomposition process (Pérez et al., 2014). As shown by these reported experiments, investigating litter decomposition processes is quite complicated, because of the high number of factors involved: intrinsic factors such as leaf litter type and quality, biotic communities (macroinvertebrates, bacteria and fungi) and extrinsic ones, such as temperature, dissolved nutrients, physical abrasion and others. Correlating this phenomenon to climate change is even more complicated: when looking at CPOM decomposition in river ecosystems that are facing dry periods, it is difficult to predict when the drought will occur and how long it will last in field conditions, together with the influence of anthropogenic pressure which can exacerbate the effects of climate change itself, adding a set of confounding factors. For this reason, some studies have been carried out in meso and microcosms, in order to control all the variables in laboratory or in open-air stream mesocosms. In Aspin et al., 2019, for example, open-air metal flumes were used to look at how stream macroinvertebrate communities react to severe droughts, without the interference of confounding factors that could have been found in the field; individual traits such as dispersal, movement, life cycle etc. played a very important role in shaping macroinvertebrate communities during and after drought events. Another interesting experiment was carried out using stream mesocosms to test the effect of recurrent droughts on macroinvertebrate consumer assemblages (composition and secondary production) and functional groups (Ledger et al., 2011); using stream mesocosms, they were able to study macroinvertebrate community and biomass production without further confounding factors and they found out that the production of shredders was strongly reduced by drought events, along with the suppression of large engulfing predators and contrasting results regarding the piercer ones. A further remarkable aspect is that the effects of drying events differed among functional feeding groups, affecting organic matter processing, food web structure and energy flow distribution. Of course, studying the effects of climate change in controlled conditions (in-laboratory microcosms, open-air mesocosms and so forth) could be very useful, but also neglects a series of factors that are inevitably involved in the response of river ecosystem processes and communities.

4. Effects of climate change in mountain stream ecosystems

Unfortunately, even if a huge number of studies have been made regarding the effects of climate change on river ecosystems, the majority of them have been conducted in those areas that naturally face flow intermittence and droughts. The widespread effects of this phenomenon have led to an increase of these consequences also in those streams and rivers that were previously considered perennial, especially in glacial and mountain areas. In the last decade, some studies have been carried out addressing these ecosystems, trying to understand the actual situation and the future scenarios due to global warming. Outcomes of the variation in the cryosphere have been well documented: runoff (Box et al., 2006), changing hazard conditions (Haeberli & Burn, 2002) and ocean freshening (Bindoff et al., 2007), with demonstration that they generally are a response to the reduction of snow and ice masses due to enhanced warming (Bates et al., 2008). Evidence of increased runoff in recent decades due to enhanced glacier melt has already been detected in the tropical Andes and in the Alps. Glacier retreat causes deep changes in the landscape, affecting the living conditions and local tourism in many mountain regions around the world (Watson and Haeberli, 2004; Mölg et al., 2005).

Generally, warming causes a major spring–summer melting of glaciers, particularly in areas of ablation, which results in loss of seasonal snow, increasing the exposure of surface crevasses and the anticipation in terms of 1-2 weeks of spring peak river flows. Glacier melting can also cause discharge variability, affecting ecological processes in alpine rivers, highly sensitive to hydrological and thermal fluctuations (Poff et al., 1996; Poff & Zimmermann, 2010). One of the main issues is to understand how glacier shrinking will impact on biological communities in response to changes in glacier runoff: annual glacial runoff will decrease, increasing the frequency of low-flow periods until the complete loss of glacier outflow (Baraer et al., 2012). Regarding mountain streams and rivers, the situation is becoming worse and worse: perennial watercourses that have to face drying events and intermittence, biological communities strongly impacted and the entire ecosystem is at risk because of supra-seasonal changes in functionality, precipitation, temperatures and so forth. In this changing climate scenario, alpine stream organisms will likely be affected at both micro- and macroscopic level, from genes to communities (Hotaling et al., 2017). Species at risk usually have three options to persist: migration to a more suitable habitat, plastic response to environmental changes or adaptation (Pauls et al., 2013). Some of those alpine taxa cannot use migration as a solution to changing habitat conditions, because they are caught in the so called “summit traps” at the upper, isolated end of available habitat (Pertoldi & Bach, 2007; Sheldon, 2012) and the adaptation phenomenon is strictly dependent by genetic variation (Hohenlohe et al., 2010); for all these reasons, plastic life history responses are considered as the most common persistence mechanism (Lencioni & Bernabò, 2015).

The Alps, because of their geographical location, are subjected to the large-scale influences of different climate regimes such as Atlantic, Mediterranean, Polar, Continental and African, coupled with local features such as topography and proximity to the sea; all of these characteristics result in large climatic variability which enhances biodiversity (Alpine Ecological Network, 2018). Between the late 19th and early 21st century, in the framework of changing climate, this area encountered an increase of 2 °C in average annual temperature, more than twice the global and European averages (EEA, 2009). Alpine glaciers retreat and permafrost loss are coupled, because the majority are located close to the melting point, being particularly sensitive to increases in atmospheric temperature: this could lead to increased occurrence and magnitude of debris flows, rockfalls and rockslides and enhancing creeping speed of active rock glaciers (Kellerer-Pirklbauer et al., 2011). The seasonal melting of glaciers and snow are the main hydrology drivers in the Alpine areas: water discharge can thus be highly variable. However, in order to evaluate the hydrological shifts caused by climate change in alpine environments, it is important to consider the interactions between snowmelt, ice melt and groundwater (Milner et al., 2009). Because of the occurrence of glaciers retreat and disappearance and the premature seasonal snowmelt, the role of snowpack as natural water reservoir is reduced, along with the importance of air warming; this leads to the occurrence of dry summers also in Alpine streams, especially in warm and dry years (Zierl and Bugmann, 2005). Habitat conditions in those streams are mainly due to water origin, making possible the identification of three stream types: kryal, krenal and rhithral (Ward, 1994). The first streamtype is characterized by the fact of being glacier-fed, the second fed by groundwater and the last by snowmelt and/or precipitation, leading to different conditions regarding water temperature, pH, turbidity, electrical conductivity, discharge, biofilm and sediments (Fegel et al., 2016). Furthermore, energy inputs, treeline shifts and organic carbon availability can be affected by seasonality, varying with the magnitude of glacial discharge (Fenoglio et al., 2015). In Brighenti et al., 2019, a complete review about the alpine area has been made, taking into account glaciers and permafrost situation at that time and looking at the possible future implications of climate change in such a sensitive ecosystem.

In fact, as previously mentioned, Alpine streams host a very high biodiversity, because of their environmental heterogeneity and relative isolation, limiting the distribution of specialized taxa and promoting endemisms (Füreder, 2007). Life histories of alpine macroinvertebrates, for example, are poorly understood, but these traits are very important to understand population persistence during environmental changes, i.e. development rate, size at maturity, reproductive and dispersal traits. These traits are usually highly plastic in aquatic insects, at least at certain bounds (Vannote & Sweeney, 1980; Newbold et al., 1994) but could also be adaptive and respond rapidly to natural selection (Poff et al., 2006); therefore, stream macroinvertebrates can respond to habitat variation through phenotypic plasticity, adaptation or both (Stearns, 1989; Lytle et al., 2008). Another important aspect is the monitoring of feeding traits: together with the climate warming, basal food resources shift from mainly autochthonous to mainly allochthonous (Hauer et al, 1997); strong shifts in food sources can rapidly change the structural and functional alpine community composition. In Hotaling et al. (2017), for the first time since Ward (1994), research regarding alpine streams including or not the presence of glaciers, has been widely investigated in its current state and future perspectives. Another interesting paper concerning these topics is the one published in 2016 by Cauvy-Fraunié et al. Here, a field experiment is coupled with a study in mesocosms, with the aim of assessing the response of benthic algal and animal communities to flow reduction and evaluate the ability of benthic communities to return to their initial structure after flow disturbance cease (i.e. resilience, see Nimmo et al., 2015). The results showed that meltwater flow reduction affects the benthic community, causing an increase in biomass of benthic algae and macroinvertebrate herbivores; those changes after flow reduction are 30 times faster than the time required to return to pre-disturbance state after flow recovery (Cauvy-Fraunié et al., 2016). The reduced contribution of glacial influence will cause an increase in water temperature and conductivity, coupled with a decrease in turbidity (Jacobsen et al., 2012); this imply an increased overall productivity, enhancing benthic algae biomass and therefore herbivore densities (Dewson et al., 2007). Since benthic communities are so important to establish which changes are occurring to the environmental conditions, in this work it is confirmed that they could be used as key indicators to identify premature signals of ecosystem damaging, such as slowing return rates from perturbation. An interesting experiment was carried out by Papadaki et al. (2016) in the south-western part of the Balkan Peninsula, an area characterized by habitat heterogeneity which supports high species richness. The main aim of the study was to assess climate change impacts on hydrological patterns and fish habitats in the Acheloos mountain river system, Greece: different emission scenarios from the IPCC were used to estimate potential impacts on flow regime and biota using a hydrological model. Simulating the streamflow could have been challenging in mountain rivers, because of their topography and hydrological processes (Rahman et al., 2013); moreover, in less developed regions, meteorological information are lacking, being confined at coastal locations (Soulis, 2015). In this study, the detected habitat alterations were able to cause geomorphic simplification, floodplain disconnection and disruption of connectivity, affecting the native biota and making an adaptation process difficult (Poff et al., 2007). Furthermore, climate change coupled with inadequate water management and conservation policies are threatening mountain streams, causing alterations to ecological processes and services provided (Postel and Richter, 2003). In addition, negative impacts of climate change are likely to be more pronounced in those pristine, high-elevation and headwater streams (Battin et al., 2007) where the increased frequency of low flows can drastically reduce habitat quality and quantity.

When looking at experiments conducted using the leaf litter bags technique to investigate river functionality, the majority of the publications are focused on the process itself and the various factors involved. A few field works were performed in alpine/mountain streams using this technique to assess the effects of global climate change, mainly because this phenomenon is hitting those areas too only recently. For example, in an experiment carried out in the high Andes of Ecuador (Andino et al., 2021), detritus decomposition was coupled to the investigation of macroinvertebrates community, functional feeding groups (FFGs) and microbial role in decomposition processes focusing on glacial meltwater influence. The results showed that glacial influence acts directly on FFGs and decomposition rates of detritus: generalist functional feeding groups dominated when glacial influence is high and decomposition rate turned out to be hump-shaped in relation to that. Furthermore, this experiment suggests that tropical glacier loss will not change the fundamental role that microbial community has in detritus decomposition. This study was based on the investigation of the effects of melting glaciers water on mountain streams, one of the main effects of global climate change on these ecosystems. However, some mountain streams are also experiencing real drying events, with loss of surface water and disruption of their tridimensional connections. Another experiment was carried out along the Fersina stream, Trento, NE Italy, but using artificial flumes (i.e. open-air mesocosms) in order to conduct a manipulative experiment, without the influence of many of the confounding factors that can occur (see Doretto et al., 2018 and Gruppuso et al., 2021). However, there are few experiments conducted in the field, regarding CPOM decomposition and macroinvertebrates and microbial communities directly coupled with the occurrence of dry events in alpine streams. For this reason, we wanted to further investigate this topic, using leaf litter decomposition to assess Alpine rivers functionality from the perspective of global climate change. The field experiment began placing a total of 960 leaf litter bags (480 containing *Quercus robur* and 480

Castanea sativa) made of coarse mesh in 3 Alpine streams: Po, Pellice and Varaita rivers. The sampling campaign began on December 13th 2018 and ended on April 19th 2019, for a total of six sampling dates, interspersed with 21 days. The leaf bags collected were used to asses: i) leaf litter decomposition (leaf mass remaining after performing dry weight); ii) C:N content; iii) macroinvertebrates community composition; iv) microbial community (using high-throughput sequencing techniques). Environmental variables were measured during the whole experiment with dataloggers in order to assess water temperature in continuum and with a multiparametric probe at each sampling date.

This thesis collects all the obtained results regarding CPOM decomposition processes in Alpine streams facing intermittency, focusing on macroinvertebrates and microbial communities and also comparing these findings with the ones conducted in artificial flumes’ controlled conditions.

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CHAPTER 1: MACROINVERTEBRATES AND RIVERBED DRYING

IF ALPINE STREAM RUN DRY: THE DROUGHT MEMORY OF BENTHIC COMMUNITIES

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Abstract

Several mountain streams are currently changing from perennial to temporary regimes due to increasing water abstraction and global climate change with expected detrimental effects on stream biodiversity and functionality. We here examined whether macroinvertebrates and diatoms, experiencing recurring non-flow periods, showed alterations even after complete flow resumption in 13 mountain streams in SW Italian Alps. Benthic communities were sampled after complete flow resumption in April 2017 in a control section, with permanent flow, and in an intermittent section, which experiences recurrent non-flow periods during summer, in each stream. We tested for differences in terms of taxonomic composition, diversity and functional groups between permanent and temporary sections. Our results showed a significant alteration of benthic invertebrate, but not diatom communities in temporary sections. Different species composition and low diversity values in temporary sections were due to the replacement of monovoltine taxa, with aquatic respiration, preferring medium to fast flowing, oligotrophic waters by plurivoltine taxa, with aerial respiration preferring lentic habitats. Such results provide some insights into the mechanisms by which non-flow periods impact Alpine streams, and further investigations in mountain areas are required in the future to better unravel the repercussions on stream ecosystem processes.

Keywords: Diatoms; Macroinvertebrates; Hydrological intermittency; Functional traits

Introduction

Stream ecosystems have been recognized as extremely sensitive to climate change, because of the raising of water temperatures and the disruption of hydrologic cycles (Heino et al. 2009; Whitehead et al. 2009; Fenoglio et al. 2010). Changes in precipitations, temperature and evaporation determine shifts in river flow regimes and groundwater recharge (IPCC 2013), with consequences for the temporal and spatial distribution of biodiversity and ecosystem processes in streams and rivers (Acuña and Tockner 2010; Datry et al. 2016). This climatic alteration can lead to extreme flow events, like floods and non-flow events, which can be exacerbated by anthropogenic activities (e.g. deforestation, water capture, streambed alterations; Fenoglio et al. 2010; Ledger and Milner 2015). Among flow regime alterations, riverbed desiccation is one of the most pressing environmental issues related to climate change (Wilhite 2000). In Mediterranean streams, non-flow events represent natural component of their hydrologic cycle. Therefore, freshwater biota in temporary streams is physically, chemically and biologically shaped by sequential, predictable and seasonal non-flow events (Boulton et al. 2014). Therefore, freshwater biota of periodically temporary environments shows adaptations and strategies to survive dry periods (e.g. desiccation resistant stages or colonization of hyporheic environment; Boulton and Lake 1992; Fenoglio et al. 2006; Bonada et al. 2007a; López-Rodríguez et al. 2009a; Souffreau et al. 2010). This is in accordance with the *natural flow regime paradigm*, which states that the structure and function of a lotic ecosystem, and the adaptation of its constituent aquatic species, are determined by the pattern of temporal variation in river flows (Poff et al. 1997; Lytle and Poff 2004). However, consequences of desiccation on aquatic organisms are expected to be different in temperate streams, where this phenomenon is quite recent and communities are still not adapted (Calapez et al. 2014; Elias et al. 2015). Among these, Alpine and in general mountain streams are becoming increasingly affected by intermittent flow (Beniston 2012; Chiogna et al. 2016). In the Alpine areas, low flows normally occur during winter and are caused by snow accumulation and freezing processes (Vezza et al. 2010; Fenoglio et al. 2015). However, summer non-flow periods are becoming more common since mountainous regions in the mid-latitudes are warming at a rate two to three times faster than the global average (Hansen et al. 2005; Pederson et al. 2011), causing rapid shrinkage of glaciers and snowpack (Hall and Fagre 2003; Rauscher et al. 2008). Moreover, these lotic ecosystems are suffering the heavy flow regulation, hydropower and increased water abstraction aimed to fulfill local human needs (e.g. agricultural use, drinking water, etc). As a consequence, mountain streams are changing from perennial to temporary systems with expected long-term detrimental effects on stream biodiversity and functionality (Durance and Ormerod 2007; Fenoglio et al. 2007; Leigh et al. 2016a; Pinna et al. 2016). From an evolutionary perspective, by influencing mortality rates, desiccation represents a primary selective pressure on aquatic communities (Lytle and Poff 2004). These strong environmental filters are expected to differentially affect species from the regional pool by either favouring species that can tolerate drying conditions and/or disfavouring species lacking resistance or resilience mechanisms to the disturbance, in accordance with the *habitat templet theory* (Southwood 1977, 1988; Townsend and Hildrew 1994). On one hand, in naturally intermittent Mediterranean streams, stretches experiencing drying likely have, on average, less diverse communities than those of nearby perennial sites (e.g. Datry et al. 2014), because of the exclusion of the most sensitive species (but see Bonada et al. 2007a for contrasting results in a large scale analysis). On the other hand, such environmental filtering is expected to cause turnover among perennial and intermittent stretches due to substitution of non-adapted taxa by disturbance-tolerant species (Myers et al. 2015). Both phenomena may result in biotic homogenization of temporary streams due to the loss of rare and specialised species and the gain of tolerant species (Socolar et al. 2016). Selection process due to desiccation is likely to favour particular traits (Webb et al. 2010; Luck et al. 2012) conferring resistance (i.e. set of traits that enable organisms to survive during non-flow periods) and/or resilience (set of traits that enable organisms to recolonize and recruit after non-flow events). A trait is defined as a characteristic that reflects a species adaption to its environment (Menezes et al. 2010) which can be morphological or physiological attributes measured at the individual level (Violle et al. 2007; Webb et al. 2010), or aspects of the realized niche of the species (Bowler et al. 2015). Exploring shifts in the functional profile of a community could therefore shed light into species-disturbance relationships (McGill et al. 2006) but also into their repercussion on the ecosystem functionality. Depending on which traits are extirpated, such trait shifts may, in fact, have severe effects on ecological functions, with a wide range of potential impacts on functional processes (Hooper et al. 2005; Cardinale et al. 2012). Alterations in community diversity and functional traits might be temporary, depending on the recovery capacity of aquatic organisms: species highly resilient or resistant to drying are able to recover within a few weeks or months, therefore limiting long-term effects of desiccation (Rincon and Cressa 2000; Fritz and Dodds 2004; Vander Vorste et al. 2016). However, some taxa might not be able to recover so quickly, with cumulative detrimental effects on diversity and functionality over the years. For instance, Datry et al. (2011), in the Albarine river (France), found persistent differences in shredder density among intermittent and permanent sections long after flow resumption, underlining the so-called “drying memory” of the system. Therefore, the assessment of non-flow effects needs to consider not only immediate but also long-term impacts. We here investigated the long-term effects of drying events (“drying memory”) on benthic organisms, in thirteen Italian Alpine streams, where summer non-flow events, lasting two months, have been recorded since 2011. In particular, we compared the response of two different communities, i.e. macroinvertebrates and diatoms, to highlight potential differences in their recovery capacity, since they are known to exhibit differential responses to stream alteration, like metal pollution (Hirst et al. 2002; De Jonge et al. 2008), physical alterations (Bona et al. 2008) or siltation (Bona et al. 2016). In each of these streams, we sampled and compared an intermittent and a perennial stretch during baseflow conditions to quantify differences as a result of eventual cumulative effects of non-flow events. We here assumed that aquatic communities in intermittent sites would have reached a complete recovery if their composition and diversity was comparable to that of communities sampled in perennial sites. Such comparison allowed us to investigate differences between diatoms and macroinvertebrates as well as underlying selection mechanisms, i.e. species loss versus species replacement, due to filtering of non-adapted species and/or gain in generalist species. In this context, we hypothesized that: (i) recovery capacity differs between benthic invertebrates and diatoms due to their different reproduction rates and dispersal capacities (Elias et al. 2015); (ii) cumulative effects of non-flow events on benthic communities mainly result in species loss processes and (iii) species loss is due to the depletion of non-adapted taxa.

Materials and methods

Sampling design

This study was conducted in thirteen low order streams belonging to the SW Alps hydroecoregion (HER 4, Piemonte, NW Italy; Wasson et al. 2007, Fig. 1), therefore comparable in terms of geology, climate and altitude to reduce confounding factors. Such hydroecoregion includes Cottian and Maritime Alps, whose streams are characterized by a nivo-pluvial regime. In consequence, several stretches are already facing seasonal hydrological alterations in summer because of reduction in precipitations and the following increase in water abstraction for human needs. We selected two sampling sections for each stream, with different levels of hydrological disturbance: (i) a control section, with permanent water during the whole year (hereinafter CON section); (ii) an intermittent section, which experiences non-flow periods during summer (hereinafter DIS section). CON sections were consistently located upstream, therefore representing a potential source of organisms for DIS sections after flow resumption. Selected streams were chosen based on interviews to fishermen (Associazione Tutela Ambienti Acquatici e Ittiofauna) and on the data provided by the ARPA - Environmental Protection Agency of Piedmont, according to which these streams have been already facing seasonal summer non-flow events lasting two months since 2011, with the riverbed almost completely dry in their DIS sections, as a combination of climatic factors and anthropogenic pressures (A.A.V.V. 2011; Borroni 2011; ARPA 2013). CON sections were identified within 10 km upstream of the DIS section to reduce environmental variability between the two stretches (Fig. 1). To verify if a "drying memory" phenomenon occurred in benthic invertebrate and/or in diatom communities after the complete flow resumption, we performed our sampling campaign in April 2017 (6 months after the drying period), under moderate flow (Qmean = 3.98 ± 4.56 m3/s) occurring in both sections. Water flow in DIS sections had resumed in November 2016 after a heavy rain event, interrupting the dewatering period of the streams (Hydrological bulletins, http://www.arpa.piemonte.it).

Data collection

In each sampling stretch, we collected a set of 13 local environmental variables. Water physical and chemical parameters (water dissolved oxygen, oxygen saturation, pH, water temperature, conductivity) were measured with a multiparametric probe (Hydrolab mod. Quanta).

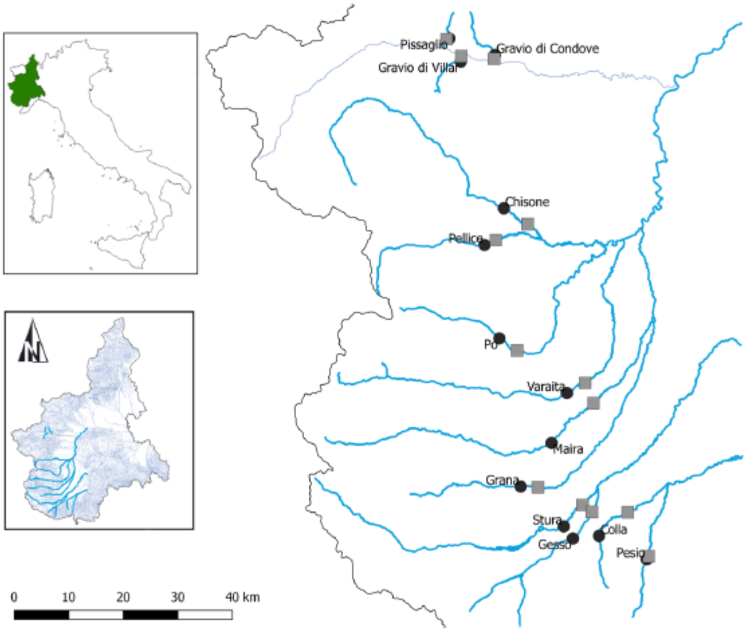


Fig. 1 Map of the thirteen study streams with respective sampling sites (circles = CON sites; squares = DIS sites).

Additionally, to check the water quality, water samples were collected from the middle of the water course in each sampling section. The samples were then analysed with standard spectrophotometric (Perkin Elmer, Lambda 35) methods for soluble reactive and total phosphorus (APHA 1998), ammonium (Koroleff 1970), nitrate (APHA 1998) and total nitrogen (APHA 1998). In each stretch we defined five microhabitats spaced at least 5 m apart, where we measured flow velocity (0.05 m from the bottom) and water depth with a current meter (Hydro-bios Kiel) and we visually estimated the % of six classes of substratum (boulders, cobbles, pebbles, gravels, sand and silt). These microhabitats were selected following the Italian multihabitat sampling method (Buffagni and Erba 2007). One sample of macroinvertebrates was collected in each microhabitat, for a total of five samples in each stretch, using a Surber sampler (250 µm mesh size; 0.062 m2 area). Collected samples were conserved into plastic jars with 75% ethanol. In the laboratory, all benthic invertebrates were identified according to Campaioli et al. (1994, 1999) to the family or genus level and counted. Based on their trophic strategies and their biological and ecological requirements, macroinvertebrates were classified into the Functional Feeding Groups (FFGs—Merritt et al. 2017) and biological and ecological groups (Usseglio-Polatera et al. 2000) respectively. Macroinvertebrates are classified into Functional Feeding Groups based on their morpho-behavioral mechanisms of food acquisition (Merritt et al. 2017). Biological and ecological groups were defined by Usseglio-Polatera et al. (2000) by grouping together taxa displaying similar biological (e.g. size, life-cycle, respiration, reproduction, dispersal, locomotion) and ecological (transversal and longitudinal distribution, preferred substrate and current velocity, trophic status) traits respectively. For benthic diatoms, one sample for each stretch was collected, brushing 3 different cobbles, covering at least an area of 10 cm2 (European Committee for Standardization 2003), chosen in correspondence of three of the five microhabitats and merged together in a unique sample for subsequent analyses (see Falasco et al. 2018). Diatoms were identified mainly according to reference floras Krammer and Lange-Bertalot (1986, 1988, 1991a, b) as well as on recent bibliography including series of Diatoms of Europe Iconographia Diatomologica and relevant scientific papers (Krammer and Lange-Bertalot 1986, 1988, 1991a, b; Krammer 1997a, b, 2002, 2003; Reichardt 1999; Lange-Bertalot 2001; Werum and Lange-Bertalot 2004; Blanco et al. 2010; Hofmann et al. 2011; Bey and Ector 2013; Falasco et al. 2013; Ector et al. 2015). Diatom species were then assigned to one category of life-form based on classification provided by Rimet and Bouchez (2012) and to one eco-morphotype, which combines the ecological guilds with size classes, based on the classification provided by B-Béres et al. (2017).

Data analysis

To test whether aquatic communities completely recovered after desiccation, we compared the composition and diversity of aquatic communities between DIS and CON sections. We assumed that aquatic communities in intermittent sections would have reached a complete recovery if their composition and diversity was comparable to that of communities sampled in perennial sections. All statistical analyses were performed using R software (R Core Team 2017).

Environmental variables at CON and DIS stretches

To explore potential differences between CON and DIS stretches with respect to environmental conditions, we performed a Principal Component Analysis (PCA) using the 13 habitat variables. A Permutational Multivariate Analysis of Variance PERMANOVA (Anderson 2001) was then applied to the environmental dissimilarity matrix based on Euclidean distances to test for differences between CON and DIS stretches with the function "adonis" in the *vegan* package (Oksanen et al. 2015). Statistical significance was tested via 9999 random permutations with the ID of each stream as stratum.

Community composition

We first visually inspected whether taxa dissimilarity differs among CON and DIS sections by means of a Principal Coordinate Axes (PCoA) using the Bray-Curtis dissimilarity index. We then tested whether dissimilarity in taxa composition between CON and DIS was significantly different from a random distribution with a PERMANOVA. Statistical significance was tested via 9999 random permutations with the stream identity as stratum. Both analyses were conducted with the *vegan* package (Oksanen et al. 2015). We then performed the Indicator Species Analysis (Dufrêne and Legendre 1997) with the function "multipatt" in the *indicspecies* package (De Caceres and Legendre 2009) to identify which taxa are more specifically linked to CON and DIS stretches for both diatoms and benthic invertebrates.

α-, β- and γ-Diversity

To test whether differences between CON and DIS sections were due to taxa loss (α- and γ-diversity) or species replacement (β-diversity), we followed the approach proposed by Xiao et al. (2018). We decomposed taxa richness of benthic invertebrates and diatoms into local (α) and total (γ) diversity, wherein local diversity is obtained as the average value of taxa richness at each sampling section and total diversity is given by the total number of encountered taxa in the entire sampling area. Beta diversity was calculated with the Whittaker's multiplicative formula as the ratio between gamma and alpha diversity. Statistical significance was calculated through a non-parametric, randomization test, where the null expectation of each metric is defined by permuting sampling sections between treatments and recalculating the taxa richness for each permutation. Significant differences between treatments can thus be obtained by comparing the observed value of the taxa richness to the null expectation when treatments are randomly permutated across the sampling sites (Legendre and Legendre 1998). At the α scale where there are replicate plots to summarize over, we calculated the ANOVA F-statistic as our test statistic for the observed values and we compared it with the distribution of F-statistics obtained for each permutation. At the γ scale, for which we only have a single value for each treatment (and therefore cannot use the F-statistic), we compared the observed absolute difference between the treatments with the distribution of those obtained from permutations (see McGlinn et al. 2018 for further details). These statistical analyses were performed with the R package *mobr* (Xiao et al. 2018).

Functional profile of benthic communities in CON and DIS sections

To check whether differences between CON and DIS sections were due to selective environmental filtering of some functional traits, we considered functional feeding groups (FFGs), ecological and biological groups (Usseglio-Polatera et al. 2000) for benthic invertebrates, whereas for diatoms we evaluated life-forms (Rimet and Bouchez 2012) and eco-morphotypes (B-Béres et al. 2017). We calculated this set of functional metrics as the community-level weighted means of trait values, with the function “functcomp” of the *FD* package (Laliberté and Legendre 2010; Laliberté et al. 2014). This function builds a site-by-trait matrix by multiplying the site-by-species matrix with a species-by trait matrix. For continuous and ordinal traits, like diatom body size, this function calculates the mean trait value of all species present in the community weighted by their relative abundances. For nominal and binary traits, like FFGs, ecological and biological groups for benthic invertebrates and life-forms and ecological guilds for diatoms, the relative abundance of taxa belonging to each individual class is returned. In addition, such comparison was applied also to those taxa potentially most threatened by desiccation. For benthic invertebrates, we focused on the EPT (Ephemeroptera, Plecoptera, Trichoptera) taxa richness because they are a key component of benthic assemblages in Alpine streams and sensitive to flow variability (Laini et al. 2018). For benthic diatoms, we considered the Red List (RL) taxa richness because it showed to be negatively affected by desiccation in Mediterranean streams (Falasco et al. 2016a). Significant effects of hydrological conditions on functional metrics were tested by means of a one-way ANOVA with the stream identity as error stratum. Data were log-transformed to achieve normal distribution and homoschedasticity of residuals.

Results

Environmental parameters (Table S1) were comparable across the investigated streams and between the two sampling sections. The first two axes of the PCA explained 40.5% of the total variance in the environmental variables across all sites (Fig. 2). The first axis (22.8%) represents a gradient of rheophily, being positively correlated with flow velocity, % cobbles, oxygen saturation and dissolved oxygen, and negatively with % pebbles and temperature. The second axis (17.7%) represented a streambed sediment gradient, being negatively correlated with % pebbles, % sand and positively correlated with % cobbles and pH. PERMANOVA showed that environmental variables did not differ between CON and DIS sections (PERMANOVA, $P = 0.111$, Table 1). The PCoA depicted differences in species composition between CON and DIS sections for benthic invertebrates (Fig. 3a), whereas this pattern was not evident for diatoms (Fig. 3b). These patterns revealed significant differences between CON and DIS sections for benthic invertebrates (PERMANOVA, $F_{1,128} = 5.84$; $P < 0.001$) but not for diatoms (PERMANOVA, $F_{1,24} = 0.692$; $P = 0.072$).

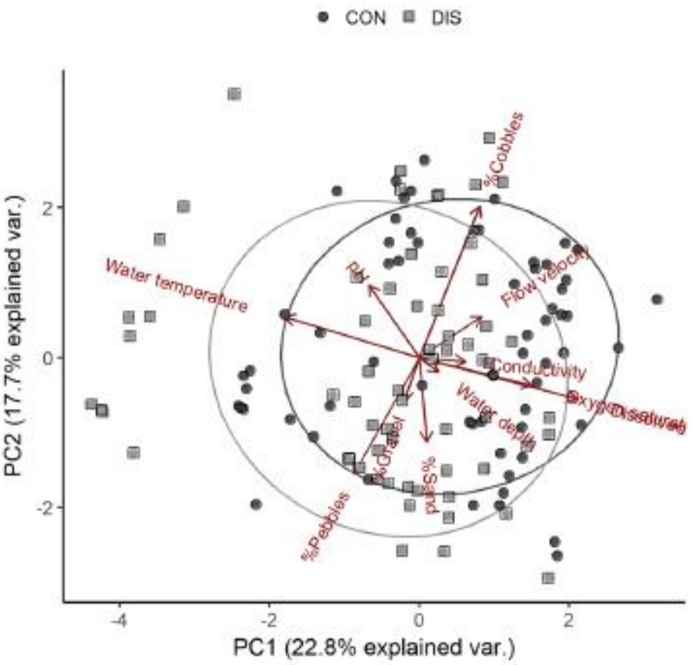
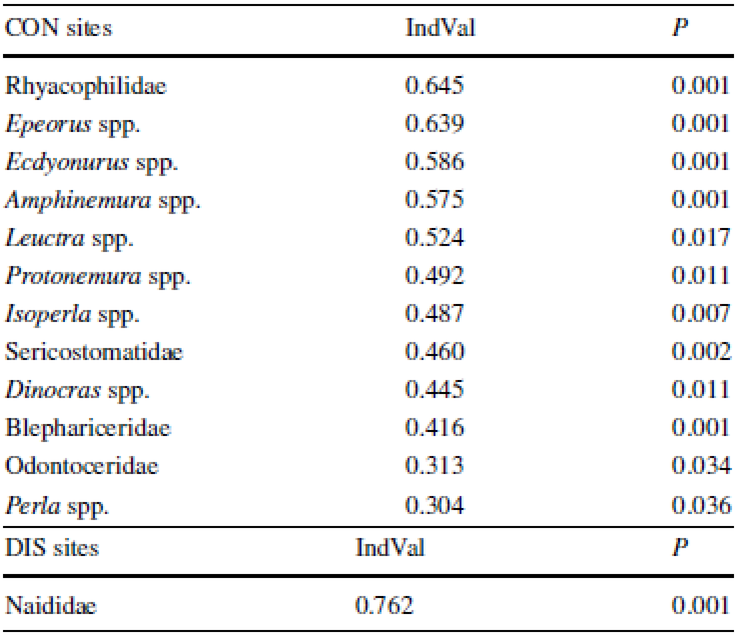


Fig. 2 Result of the PCA. Points represent each single sample while arrows represent the loadings of each environmental variable included in the analysis. Ellipses represent standard deviations around the centroids of the two groups



CON sites	IndVal	P
Rhyacophilidae	0.645	0.001
Epeorus spp.	0.639	0.001
Ecdyonurus spp.	0.586	0.001
Amphinemura spp.	0.575	0.001
Leuctra spp.	0.524	0.017
Protonemura spp.	0.492	0.011
Isoperla spp.	0.487	0.007
Sericostomatidae	0.460	0.002
Dinocras spp.	0.445	0.011
Blephariceridae	0.416	0.001
Odontoceridae	0.313	0.034
Perla spp.	0.304	0.036
DIS sites	IndVal	P
Naididae	0.762	0.001

Table 1 Results of the Indicator Species Analysis for benthic invertebrates

The Indicator Species Analysis identified 12 indicator taxa in CON sections and 1 indicator taxon of DIS sections for benthic invertebrates (Table 1), whereas no indicator species were identified for diatoms. The results of the diversity partitioning showed significant effects of the hydrological conditions on macroinvertebrate taxa richness at both alpha and gamma scale (Fig. 4a), which drops from an average value of 11.6 in CON stretches to 8.58 in DIS stretches. Conversely, we did not find statistical differences in any of the diversity metric considered between CON and DIS sites for diatoms (Fig. 4b). Considering the community functional profile of benthic invertebrates, we found significant effects of the hydrological conditions on EPT richness (Table 2; Fig. 5a) and FFG composition (Table 2; Fig. 6), with particularly evident impacts on predators, scrapers and shredders. In CON sites, the abundances of predators, scrapers and shredders are on average 4%, 8% and 9%, respectively, but drop to 3%, 6% and 3% in DIS sites. Conversely, results showed a significant effect of hydrological conditions on collector-gatherers (Table 2; Fig. 6), with higher values in DIS (70%) compared to CON sites (59%), whereas no significant effects (Table 2; Fig. 6) were recorded for filter-feeders (20% in CON sites and 18% in DIS sites). Significant negative effects of nonflow hydrological conditions were observed for organisms preferring medium to fast flowing, oligotrophic waters and living on coarse mineral substrata and vegetation (ecological groups A and C; Table 2; Fig. 6). Percentages of individuals belonging to ecological groups A and C showed significant lower values in DIS than in CON stretches, dropping from 12% and 21–6% and 13% respectively. For taxa preferring lentic habitats (ecological group F), we observed a significant increase from CON (36%) to DIS (48%) sites (Table 2; Fig. 6). Significant effects of hydrologic conditions were also recorded for several biological groups (Table 2; Fig. 6). Percentages of semivoltine organisms, large-sized predators and crawlers (biological group d), monovoltine organisms, medium-sized shredders and crawlers with aquatic respiration (biological group f), and small to medium-sized swimmers or crawlers, with aerial respiration (biological group g), showed a significant decreasing pattern (Table 2; Fig. 6) from CON (2%, 15% and 9% respectively) to DIS (1%, 8% and 4% respectively) stretches. Conversely, plurivoltine, small-sized organisms (biological groups h and

e) showed significant higher values in the DIS (11% and 76% respectively) than in CON (5% and 64% respectively) sites (Table 2; Fig. 6). Considering the community functional profile of benthic diatoms, we could not detect any significant effect of hydrological conditions on red list species abundance (Table 2; Fig. 5b) and on functional groups (Table 2), except for high profile species of size class 4, which resulted more abundant in DIS than in CON sites.

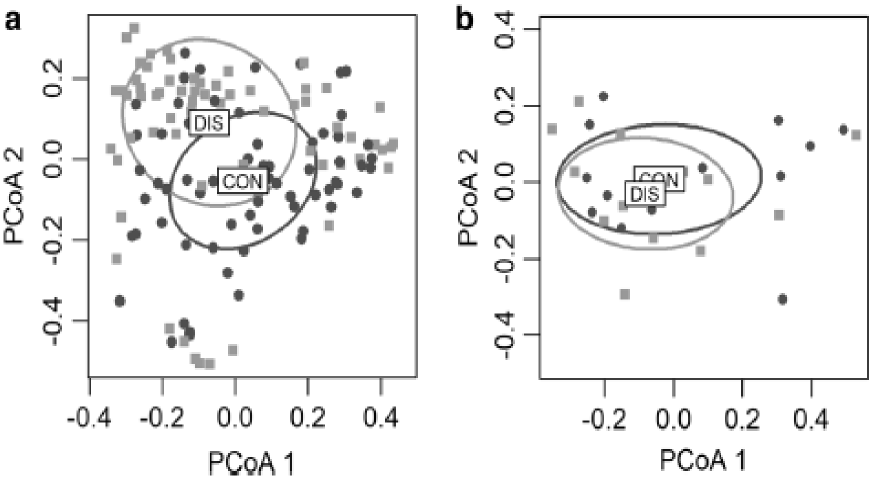


Fig. 3 Ordination of the sampled benthic invertebrate (**a**) and diatom (**b**) communities according to the first two PCoA axes (Dark grey circles = CON sites; light grey squares = DIS sites). Ellipses represent standard deviations around the centroids of the two groups.

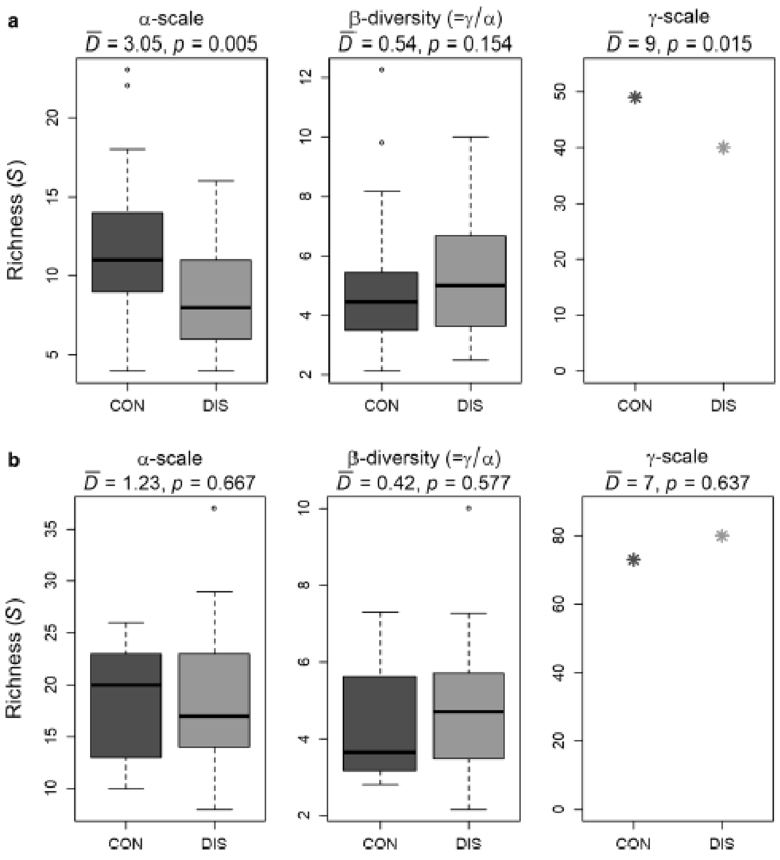
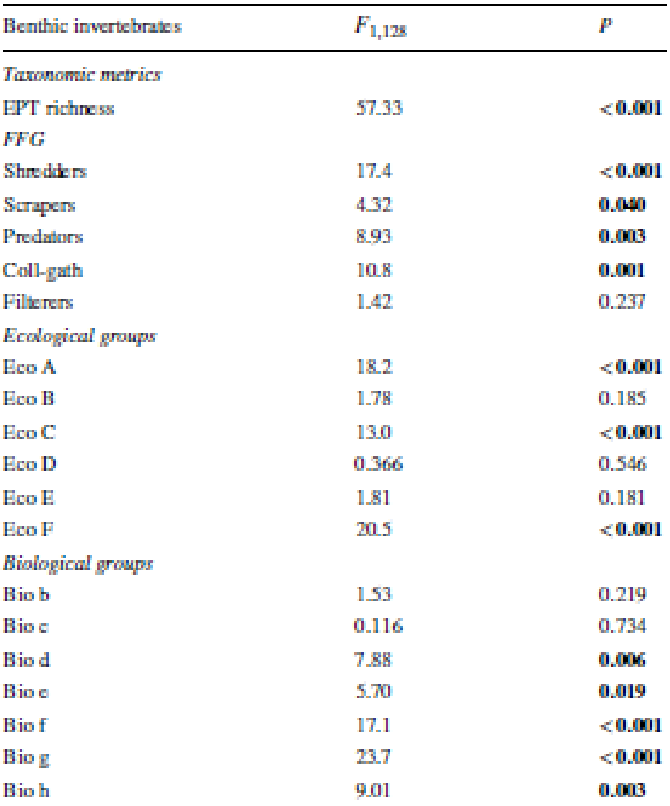


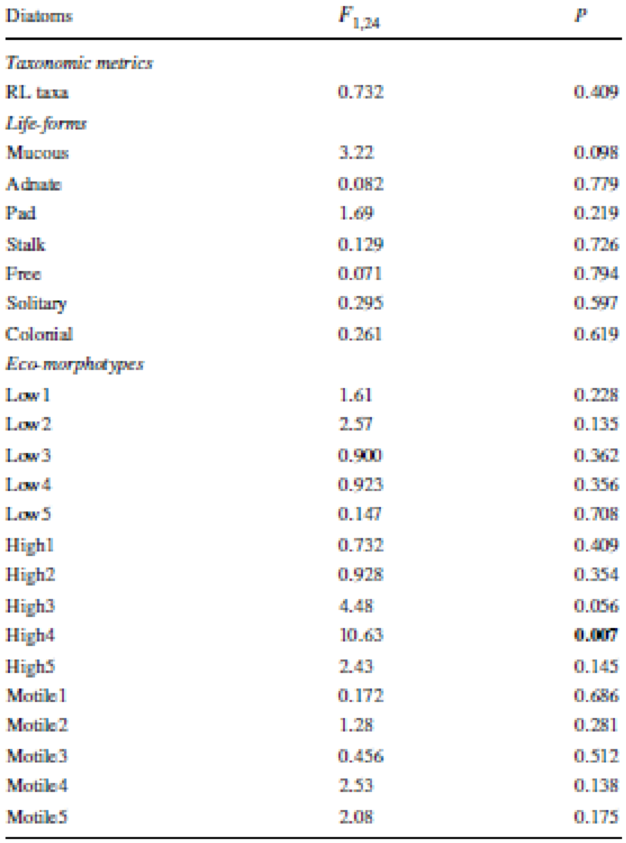
Fig. 4 Diversity metrics for benthic invertebrates (a) and diatoms (b) at the α (i.e., single stretch), beta (i.e., between stretches) and γ (i.e., all stretches) scales. The *p*-values are based on 999 permutations of the treatment labels

Discussion

Alpine and mountainous streams are affected by multiple pressures that often cause the alteration and sometimes even the almost complete destruction of these ecosystems (Wohl 2006). In particular, increasing water abstraction (e.g. for hydroelectric power generation, drinkable waters, irrigation and snow generation) combined with the global climate change effects have severe repercussions on Alpine streams, with increasing frequency of non-flow events in previously perennial systems (Fenoglio et al. 2010). Such events are expected to affect biotic communities, whose composition changes substantially during non-flow periods, but recovers quite rapidly after water returns (Lake 2003; Fowler 2004). However, this might not be true in recently temporary streams, where biotic communities still lack adaptations to such extreme events. Although environmental variables in permanent and intermittent sites were similar in our study, we recorded differences in benthic community diversity and composition, which could be ascribed to the long-term effects of desiccation. This result clearly strengthens the role of biological communities in assessing ecological conditions as their response integrates temporal aspects. Our results revealed that in recently temporary Alpine streams benthic macroinvertebrates, but not diatoms, reflect a negative effect of water intermittency, confirming earlier findings in Mediterranean (Boix et al. 2010) and other temperate regions (Calapez et al. 2014; Elias et al. 2015).



Benthic invertebrates	$F_{1,128}$	P
Taxonomic metrics		
EPT richness	57.33	**<0.001**
FFG		
Shredders	17.4	**<0.001**
Scrapers	4.32	**0.040**
Predators	8.93	**0.003**
Coll-gath	10.8	**0.001**
Filterers	1.42	0.237
Ecological groups		
Eco A	18.2	**<0.001**
Eco B	1.78	0.185
Eco C	13.0	**<0.001**
Eco D	0.366	0.546
Eco E	1.81	0.181
Eco F	20.5	**<0.001**
Biological groups		
Bio b	1.53	0.219
Bio c	0.116	0.734
Bio d	7.88	**0.006**
Bio e	5.70	**0.019**
Bio f	17.1	**<0.001**
Bio g	23.7	**<0.001**
Bio h	9.01	**0.003**



Diatoms	$F_{1,24}$	P
Taxonomic metrics		
RL taxa	0.732	0.409
Life-forms		
Mucous	3.22	0.098
Adnate	0.082	0.779
Pad	1.69	0.219
Stalk	0.129	0.726
Free	0.071	0.794
Solitary	0.295	0.597
Colonial	0.261	0.619
Eco-morphotypes		
Low1	1.61	0.228
Low2	2.57	0.135
Low3	0.900	0.362
Low4	0.923	0.356
Low5	0.147	0.708
High1	0.732	0.409
High2	0.928	0.354
High3	4.48	0.056
High4	10.63	**0.007**
High5	2.43	0.145
Motile1	0.172	0.686
Motile2	1.28	0.281
Motile3	0.456	0.512
Motile4	2.53	0.138
Motile5	2.08	0.175

Table 2 Results of the one-way ANOVA performed on functional traits. Significant results are reported in bold.

Benthic invertebrate response

Our results depicted how river reaches experiencing non-flow periods supported different and less species-rich communities compared to perennial reaches. These results suggest that the shift in macroinvertebrate community composition was partly determined by a species filtering process, such that only a limited set of species—with particular trait combinations—can survive in the intermittent sections, similarly to Giam et al. (2017). To further investigate whether desiccation favours tolerant- disturbance species or species that share particular traits, we tested whether communities in perennial and intermittent river reaches differed in their functional trait composition. In accordance with literature (Dewson et al. 2007; Datry et al. 2014), we observed that taxa depletion in intermittent sites was mainly driven by the loss of the EPT (Ephemeroptera, Plecoptera and Trichoptera) taxa, which represent the dominant groups in the Alpine streams and the most sensitive taxa as well. Likewise, several EPT taxa can be found almost exclusively in perennial sections, as demonstrated by the Indicator Species Analysis. Consistent results were obtained by Doretto et al. (2018) who conducted a manipulative experiment in artificial flumes located in Eastern Italian Alps. In the cited work, taxonomic richness of benthic invertebrates and EPT richness decreased after two consecutive non-flow events. Considering the response in terms of biological groups of benthic invertebrates, large semivoltine taxa with long larval life, such as *Dinocras* sp., *Perla* sp. and Athericidae, which are unable to survive in periodically dry environments, showed lower values in intermittent than in perennial sections. Conversely, small, fast growing, plurivoltine organisms, such as Chironomidae, Simuliidae and *Baetis* sp., showed an opposite trend. Voltinism has been recognized as one of the most sensitive traits (Bonada and Dolédec 2018) and a shift from multivoltinism to semivoltinism has been observed from perennial to intermittent hydrological regimes in Mediterranean streams (Corbet et al. 2006; López-Rodríguez et al. 2009a, b): in particular, these authors demonstrated that species inhabiting temporary habitats complete their life cycle in less than 1 year, emerging just before the loss of surface water. However, in Alpine streams, where non-flow events are human-induced, the match between life-history adaptation and non-flow periods might be lost with severe consequences for species survival. Similarly, small body size is also a common attribute of macroinvertebrate taxa living in temporary Mediterranean streams, since reduced dimensions allow fast development and population growth (Bonada et al. 2007b). These attributes assure higher survival rates in temporary watercourses, where life-cycle has to be completed before water disappears.

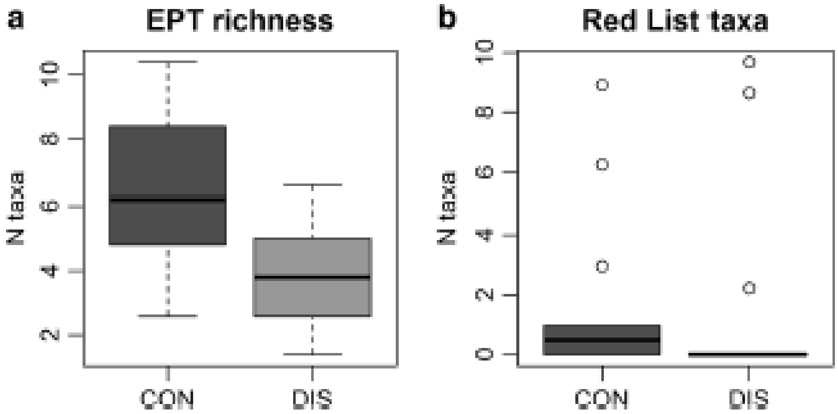


Fig. 5 Boxplots representing differences in EPT richness (a) of benthic invertebrates and Red List species abundance (b) of diatoms between CON and DIS sites

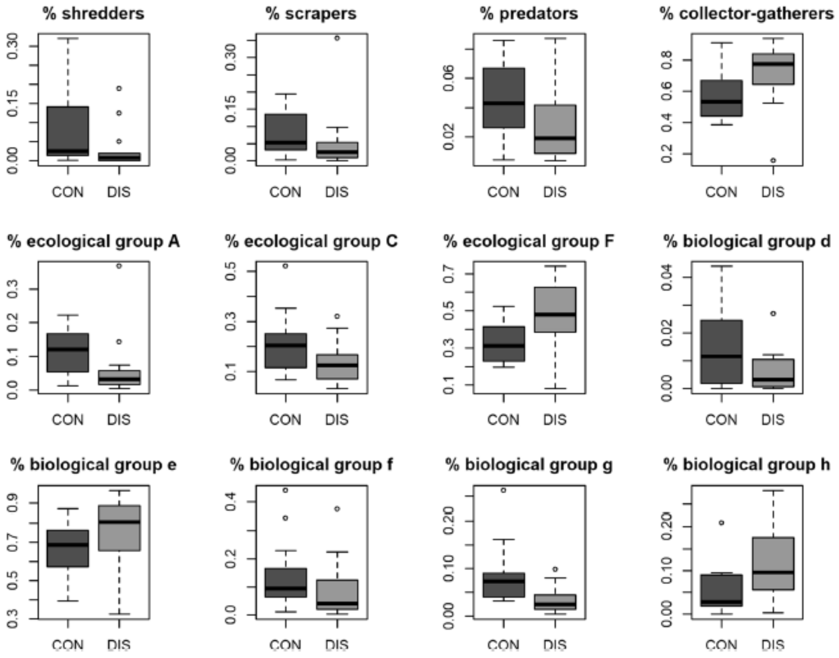


Fig. 6 Boxplots representing differences in functional groups between CON and DIS sites for benthic invertebrates. Only significant results are reported. Ecological group A: rheophilous organisms preferring coarse mineral substrates in oligotrophic and cold waters; ecological group C: organisms living on mineral substrates or vegetation at slow or medium current velocities; ecological group F: organisms living in lentic and stagnant habitats. Biological group d: semivoltine, large-sized predators with aerial dispersal; biological group e: small to medium-sized plurivoltine crawlers with aquatic respiration; biological group f: monovoltine, medium-sized shredders and crawlers with aquatic respiration; biological group g: small to medium-sized swimmers or crawlers, with aerial respiration; biological group h: monovoltine burrowers, deposit-feeders.

When looking at ecological niches, we could observe that rheophilous taxa preferring coarse substrata, such as *Epeorus* sp. and *Rithrogena* sp., decreased from perennial to intermittent sites, while there was an increase of lentic taxa, mainly Chironomidae, able to survive in environments with fluctuating water levels. This is in accordance with Acuña et al. (2005), Pace et al. (2013) and Chessmann (2015), who depicted negative effects of desiccation on rheophilous taxa in Mediterranean streams. Our results on macroinvertebrates confirm early findings of Fenoglio et al. (2007) who observed similar responses along a gradient of desiccation in a study on the Po river. However, as they developed their work during the lentification phase, it remained less clear to what extent such functional alteration of benthic invertebrate community was transient or persistent in time. Since we here sampled intermittent sections six month after water returned, our data strongly suggest the long-term effect of non-flow events on macroinvertebrate communities in Alpine streams. Moreover, with the analysis of functional groups, we could reveal that desiccation does not only filter non-adapted taxa, but this taxonomic loss is at least partly compensated by positive selection of tolerant species. This desiccation-driven replacement of species mirrors the effects of climatic change, whose impacts have been reported for many taxonomic groups (Parmesan and Yohe 2003; Hickling et al. 2006). Our results also demonstrate that non-flow events in Alpine streams alter macroinvertebrate functional feeding groups, with possible repercussions on the ecosystem functionality. We here highlighted how specialised functional feeding groups, such as shredders and scrapers, mainly represented by Limnephilidae and Heptageniidae respectively, decreased from perennial to intermittent sections. Conversely, the generalist group of collector-gatherers, including *Baetis* sp. and Chironomidae, increased. This suggests that drying events can persistently alter the composition of macroinvertebrate assemblages in streams and this might be particularly true for non-adapted Alpine communities. The absence of water disrupts some ecological processes, such as the microbial breakdown of allochthonous inputs (Boulton and Lake 1992). In this context, Wenisch et al. (2017) demonstrated how the loss of shredder benthic taxa may significantly alter the decomposition rate of leaf litter in streams with consequent alteration of ecosystem functionality. Such results are critical also because flow reduction and desiccation are strictly associated with heavy fine sediment accumulation (i.e., clogging), since lower water velocity enables more sediment to settle out of suspension (Dewson et al. 2007; Rolls et al. 2012). Such phenomenon is also responsible for altering the organic material decomposition (Doretto et al. 2016, 2017), exponentially increasing the negative effect of non-flow events on stream nutrient cycling. Although intermittent flow might prevent the establishment of permanent periphytic biofilms (Wood and Petts 1999), this explanation is not plausible to justify the strong reduction of scrapers in intermittent sections observed in our study, since we could not record any clear response of benthic diatoms. However, in our sites, scrapers are mainly represented by the family Heptageniidae, which includes extremely rheophilous organisms (Usseglio-Polatera et al. 2000). Therefore, they are probably negatively affected more by flow reduction than by trophic processes. In addition, desiccation promotes heterotrophy (Acuña et al. 2015) and alter fatty acid composition (Sanpera-Calbet et al. 2017) in the biofilm, likely affecting scrapers (Guo et al. 2016). Further investigations in this sense could unravel mechanisms behind this pattern.

Benthic diatom response

Diatoms show a rapid recovery after non-flow regime in this study, given that after 6 months since the water returned diatom community in intermittent stretches is comparable to that found in perennial sites. This result was also confirmed by the analysis of the most sensitive taxa, i.e. Red List taxa, since we could not find any significant pattern. Within biofilm, diatoms are probably the most sensitive component since other groups, such as cyanobacteria and green algae, better cope with desiccation (Piano et al. 2017a). In this context, in Mediterranean streams a clear response of diatoms to stream lentification and fragmentation processes occurring during non-flow periods has been highlighted: for instance, river shrinkage causes the loss of Red List diatom taxa (Falasco et al. 2016a), with increasing proportions of motile (Falasco et al. 2016b) and tolerant (Piano et al. 2017b) species, and similar results were also reported by Falasco et al. (2018a) in Alpine streams. Considering colonization processes during rewetting phases, Artigas et al. (2012) observed a faster recolonization on bare substrates in a Mediterranean temporary stream, in comparison to a slow gradual recovery in a Central European river. Similarly, Souffreau et al. (2010) demonstrated that acclimatization increases the tolerance of diatom strains to desiccation, underlying how non-flow events can have a stronger negative impact on diatom communities in recently temporary rivers than in Mediterranean regions. Despite recovery might take more than 90 days in pristine streams (Falasco et al. 2018b), diatom communities recovered faster after a non-flow event in comparison with macroinvertebrates, probably due to their shorter life cycles and higher physiological adaptation to dryness (Calapez et al. 2014). Moreover, the high dispersal capacity of diatoms (Robson et al. 2008) combined with their fast reproduction rates guarantee a rapid recovery after desiccation (i.e. mass effect, Mouquet and Loreau 2003). The analysis of their functional groups confirmed results obtained from diversity and community composition, since communities in intermittent sites showed no significant variations in ecological guilds, size and lifeforms compared with the perennial stretches. Although changes in species diversity and composition as well as in functional groups were more clearly detected for benthic invertebrates than for diatoms, translating these observations as stronger invertebrate versus diatom response to long-term desiccation effects should be interpreted with caution. Although diatoms and invertebrates were sampled in an equal number of sites, the higher number of replicates within each site collected for macroinvertebrates could potentially lead to higher statistical power to detect invertebrate than diatom responses. Further investigations are required to shed light on this point.

Conclusions

Our results emphasize how the functional-based approach can help in unravelling the ecological responses of stream benthic communities to non-flow events. Our predictions of a persistent effect of desiccation on benthic communities was rejected for diatoms, but was confirmed for macroinvertebrate assemblages. Moreover, we disentangled the additive effect of two mechanisms on benthic invertebrates, which may act with varying intensities depending on the considered functional aspects. Species loss represents the dominant mechanism, since non-adapted species are filtered out, as confirmed by a reduction in species richness. On the other hand, species replacement also plays a role since small, plurivoltine taxa, preferring lentic habitats and feeding on streambed sediment are favoured in intermittent sections. Such results underlay how benthic communities in intermittent sections of Alpine streams are currently facing alterations caused by flow intermittency and further investigations in mountainous areas are required in the next future to better unravel these processes. In particular, even if we did not record significant differences between permanent and intermittent sites in terms of environmental parameters, results of the PCA showed how intermittent sites are slightly warmer than permanent ones. Temperature increasing values likely associated with low flows might represent a disrupting mechanism, especially in cold arctic streams, as highlighted by Heino et al. (2009). The effect of water warming on benthic communities should be investigated in Alpine streams, which are in some respect environmentally similar to arctic streams. Moreover, since alterations in benthic invertebrate functional groups could possibly have repercussions on periphyton composition (e.g. scraper decrease), despite at present no alterations were recorded for diatom community, monitoring both benthic communities will be essential to disentangle functional changes in the lotic food web.

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THE ROLE OF RECURRENT DEWATERING EVENTS IN SHAPING ECOLOGICAL NICHES OF SCRAPERS IN INTERMITTENT ALPINE STREAMS

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Abstract

Several Alpine streams are currently changing from perennial to intermittent systems with expected detrimental consequences on benthic invertebrates, through the alteration of hydrological conditions, substrate composition, and trophic resources. We examined the ecological niche of three phylogenetically- related scraper macroinvertebrates, namely Ecdyonurus, Epeorus, and Rhithrogena (Ephemeroptera, Heptageniidae) experiencing recurrent summer dewatering events in perennial and intermittent stretches in 13 Alpine streams. We evaluated the overlap among the ecological niches of the three examined taxa in terms of hydraulic stress, substrate coarseness, and total primary production and we investigated their ecological preferences in perennial and intermittent sites. Our results showed a broad overlap of their ecological niches, suggesting exploitative competition among the examined taxa. Their ecological niches were smaller in intermittent than perennial sites, pointing out a reduction of available microhabitats.

All groups were favored by substrate coarseness and total primary production in reach types, while the hydraulic stress showed an opposite effect in perennial (negative) and intermittent (positive) sites. Overall, our results emphasize how stream physical parameters play a key role in determining the ecological niche of Heptageniidae and underline how benthic communities in intermittent sections of previously perennial Alpine streams are currently under pressure.

Keywords Heptageniidae; Chlorophyll a; Froude number; Substrate Index; Physical disturbance

Introduction

Streams are dynamic ecosystems, often facing natural hydrological and geomorphological changes that might rearrange near-bed conditions, potentially reducing the availability of suitable microhabitat for some species, while increasing it for others (Gore et al., 2001). In mountainous low-order lotic systems these phenomena are usually highly predictable, with an increase in water flow and fine sediments in summer, during snow melting, and a minimum discharge in winter (McGregor et al., 1995). However, the increasing human pressure has been intensifying physical disturbance in these streams (Wohl, 2006) with detrimental consequences on benthic biota (Bona et al., 2008; Bruno et al., 2013; Doretto et al., 2016, 2017, 2019). For instance, clogging caused by the huge increase of fine sediments caused by human activities currently represents one of the most important and pervasive concern for mountainous lotic environments, especially because they are naturally characterized by coarse substrates (Bo et al., 2007; Bona et al., 2016; Doretto et al., 2016, 2017). Another current threat to mountainous lotic systems is the enhanced frequency of extreme flow events (i.e., droughts and floods) due to Climate Change and exacerbated by human activities (Brighenti et al., 2019). In particular, summer flow cessation periods are increasing in mountainous regions at the midlatitudes, because they are warming at a rate two to three times faster than the global average (Hansen et al., 2005; Pederson et al., 2011), causing rapid shrinkage of glaciers and snowpack (Rauscher et al., 2008). These changes anticipate the hydrological peak in spring and induce modifications in thermal and hydrological regimes as well as sediment transport (Brighenti et al., 2019), thereby modifying hydrogeomorphological, physical, and chemical conditions downstream (Hannah et al., 2007) causing cascading effects on the structure and function of aquatic communities (Milner et al., 2017). Moreover, these lotic ecosystems are suffering the heavy flow regulation, hydropower, and increased water abstraction aimed to fulfill local human needs (e.g., agricultural use, drinking water, etc). As a consequence, mountain streams are changing from perennial to temporary systems, characterized by recurrent non-flow events followed by rewetting phases (Fenoglio et al., 2010) as a consequence of interactive effects of both climate change and anthropogenic disturbance (Belmar et al., 2019; Bruno et al., 2019). Periodic flow intermittency is expected to alter the distribution of benthic invertebrates by influencing physical conditions and distribution of trophic resources (e.g., Datry et al., 2011; Acuña et al., 2015; Piano et al., 2017; Chessman, 2018).

From an evolutionary standpoint, near-bed hydraulic conditions and substratum composition represent strong selective pressures for benthic invertebrates (Rempel et al., 2000; Jowett, 2003; Mérigoux & Dolédec, 2004). Near-bed hydraulic conditions are of critical importance for benthic invertebrates (Statzner et al., 1988), which need to find a compromise between the energy costs of maintaining position in turbulent environments, the benefits of acquiring food and oxygen and dispersal (Chessman, 2018). The stream benthos is also dependent on substratum characteristics, whereby stable substrates provide a good supply of shelters and food resources increasing richness and abundance of benthic invertebrates (e.g., Minshall, 1984; Boulton et al., 1998; Bona et al., 2016; Doretto et al., 2017). Conversely, huge amounts of fine sediments, which are often a byproduct of human activities, impact benthic macroinvertebrates due to abrasion, clogging and burial (Fenoglio et al., 2007; Jones et al., 2012; Bona et al., 2016) and reducing refuge availability and the amount of food resources (Doretto et al., 2017). Hydraulic and substratum conditions therefore represent major physical gradients along which the benthic community is organized. However, coexistence of multiple species with the same ecological requirements translates into strong competitive interactions, especially when species are phylogenetically-related and thus share the same trophic niche and competitive abilities (Leibold & Chase, 2017). Consequently, interspecific competition for food may also influence the abundance and distribution of aquatic taxa. Likely, physical conditions and food availability are key factors in outlining the ecological niche of macroinvertebrates in streams (Resh et al., 1988). The ecological niche of a species can be defined as the range of ecological conditions in which this species can survive, grow, reproduce, and maintain a viable population (Hutchinson, 1957). In streams, it is mainly influenced by physical factors acting at multiple spatial scales (Statzner et al., 1988; Quinn & Hickey, 1994; Heino et al., 2003). At regional and global scale, the investigation of the ecological niche considers distribution patterns of species along large geographical and environmental gradients, such as variation in precipitations and temperature, to elucidate their bioclimatic ranges and biogeographic history (e.g., Boyero et al., 2011). At smaller scale, local environmental conditions become the most important factors explaining species coexistence and their competition for space and resources (Chesson, 2000; Chase & Leibold, 2003). In particular, water flow and particle size have been recognized to play a major role in the spatial distribution of macroinvertebrate organisms at within-site scale (e.g., Statzner et al., 1988; Wellnitz, 2015; Bo et al., 2016). Evidence in literature (Fenoglio et al., 2007; Datry et al., 2011; Doretto et al., 2018; Piano et al., 2019) suggests that specialized feeders, namely shredders, i.e., invertebrates feeding on coarse allochthonous organic fragments, and scrapers, i.e., invertebrates feeding on autochthonous biofilm, were often more negatively affected by dewatering events than generalist collectors and predators, in accordance with the assumptions of the River Continuum Concept (RCC, Vannote et al., 1980). In particular, dewatering events likely affect scrapers by preventing the establishment of mature periphytic biofilms (Wood & Petts, 1999), by promoting heterotrophy (Acuña et al., 2015) and by altering fatty acid composition in the biofilm (Guo et al., 2016). 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., 2019), little is known about the role of dewatering events in shaping ecological niches of scrapers. We here investigated the ecological niche of three phylogenetically-related genera of scrapers, namely Ecdyonurus, Epeorus and Rhithrogena (Ephemeroptera, Heptageniidae) in 13 streams in Italian SW Alps, where summer non-flow events, lasting on average two months, have been recorded since 2011. In particular, we aimed at: (i) shedding light on ecological niche requirements at within-site scale of the three most common genera of Heptageniidae in Italian Alpine streams; (ii) investigating the potential interspecific competition among the three examined taxa; and (iii) exploring potential effects of recurrent non-flow events on their niche partitioning.

Materials and methods

Sampling design

This study was conducted in 13 low-order streams located in the hydroecoregion of SW Alps (HER 4, Piemonte, NW Italy; Wasson et al., 2007), showing similar characteristics in terms of geology, climate, and altitude. In this area, streams flow on siliceous substrates and are characterized by a nivo-pluvial regime (Fig. S1). Several stretches are already facing seasonal hydrological alterations in summer due to the interactive effect of reduction in precipitations and the consequent increase in water abstraction for human needs. In particular, the stream selection was made based on our expertise and historical data (ARPA— Environmental Protection Agency of Piemonte) regarding their hydrology and confirmed by recognized fishermen (i.e., ATAAI—Associazione Tutela Ambienti Acquatici e Ittiofauna). According to these data, selected streams have been already facing seasonal summer droughts lasting two months since 2011, with the riverbed almost completely dry in some stretches at least 30 m long, as a combination of climatic factors and anthropogenic pressures, namely increasing water abstraction, e.g., for hydroelectric power generation, drinkable waters, irrigation, and snow generation (Borroni, 2011; ARPA, 2013). In each of the selected stream, we sampled and compared an intermittent and a perennial section in subalpine reaches during baseflow conditions to investigate differences as a result of possible cumulative effects of non-flow events. In each stream, we then selected two sampling sections differing in their hydrological regime: (i) a control section, with permanent water during the whole year (hereinafter CON section); and (ii) a disturbed section, which experiences drought during summer (hereinafter DIS section). CON sections were identified within 10 km upstream of the DIS section to reduce environmental variation between the two stretches (mean distance = 4.7 km). The selected sections were distributed over a 510 m elevational range, with the lowest and the highest sections located at 308 and 847 m.a.s.l. respectively (see Table S1 for an environmental characterization of sampling sections). We performed our sampling campaign in April 2017 (6 months after the last drying period), under moderate flow (Qmean = 3.98 ± 4.56 m3/s) in both sections. All samples were collected within 15 days to minimize temporal variation of organism distribution related to seasonality and temperature. Water flow in DIS sections had resumed in November 2016 after a heavy rain event, interrupting the dewatering period of the streams

(Hydrological bulletins, www.arpa.piemonte.it). In each stretch we collected three Surber samples, selected within the streambed in order to cover different conditions of flow velocity, water depth, and substrate composition (3 samples 9 2 stretches x 13 streams = 78 samples). Each Surber sampled a patch in which we measured flow velocity (0.06 m from the bottom) and water depth with a current meter (Hydro-bios Kiel). Percentages of different substratum sizes (rocks, boulders, cobbles, gravel, sand) were recorded at each point by using a gravelometer and their coverage was visually estimated. Collected samples were conserved into plastic jars with 75% ethanol. 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 Heptageniidae were considered for further analysis. To obtain synthetic measures of hydraulic stress and substrate composition, we calculated the Froude number (Gordon et al., 1992) and the Substrate Index (SI, modified by Quinn & Hickey, 1994 after Jowett et al., 1991). The Froude number is a measure of hydraulic turbulence, hence high values correspond to rheophilous microhabitats. It is calculated as: v/H(d 9 g), where v is the flow velocity, measured as m/s, d is the 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 9 %Rocks + 0.7 9 %Boulders + 0.6 9 %Cobbles + 0.5 9 %Gravel + 0.4 9 %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). In addition, we expected that these parameters would affect the distribution of the three examined genera, which are medium-sized, monovoltine crawlers, preferring coarse rheophilous substrates, on which they feed on the periphytic biofilm (Tachet et al., 2010). In order to quantify the scraper food availability we measured the amount of periphyton biomass. Within each Surber sample, we selected one cobble where we took three measures of epilithic chlorophyll a with the BenthoTorch®. This instrument is a Pulse Amplitude Modulated (PAM) fluorimeter emitting light pulses at three different wavelengths (470, 525, and 610 nm), recording the response of cyanobacteria, diatoms, and green algae at 690 nm wavelength (Kahlert & McKie, 2014). We then selected the median value of chlorophyll a concentration for each autotrophic group, and we calculated the total chlorophyll a concentration as the sum of their values as an explanatory variable and a proxy for resource availability for subsequent analyses.

Statistical analysis

All statistical analyses were performed with R software (R Core Team, 2017). To investigate whether the three examined taxa displayed similar ecological niches, we first built their multidimensional niche hypervolumes (sensu Hutchinson, 1957) using a set of variables referring to hydrological conditions, substrate composition, and trophic resources. We then assessed their potential competition by calculating the overlap between the resulting geometrical solids. Finally, we compared their niches in permanent and intermittent sites. Among the three genera of Heptageniidae recorded in our samples, Rhithrogena resulted the most abundant and widely distributed taxon, followed by Epeorus and Ecdyonurus (Table 2).

Before proceeding with the analysis of the ecological niches of examined taxa, we investigated whether hydrological stress (Froude number), substrate coarseness (Substrate Index) and periphyton biomass (total chlorophyll a concentration) differed between CON and DIS sites by means of the non-parametric Wilcoxon test for paired data.

Niche hypervolume

In a first step, we compared the overall ecological niches of the examined taxa based on presence data in both CON and DIS sites to investigate whether their ecological requirements overlap, thus suggesting possible exploitative competition. To perform this, we calculated their ecological niche sensu Hutchinson (1957) via the hypervolume R package (Blonder, 2015) based on the Froude number, SI, and total chlorophyll a measured at each Surber sample. This approach relies on a kernel density estimation (KDE) procedure, which permits to quantify the multidimensional Hutchinsonian hypervolume and to assess the intersection between the hypervolumes of two taxa (mathematical details in Blonder et al., 2014). Before the analysis, we standardized all variables in order to achieve the same dimensionality for all axes following the assumptions of the algorithm. 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 (Silverman, 1992) as recommended by Blonder et al. (2014), and we set a threshold that included 100% of the total probability density. The intersection between the hypervolumes and their overlap statistics were obtained via the hypervolume_set and hypervolume_ overlap_statistics R commands respectively (Blonder, 2015) for each pair of taxa, for a total of three pairs. 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)

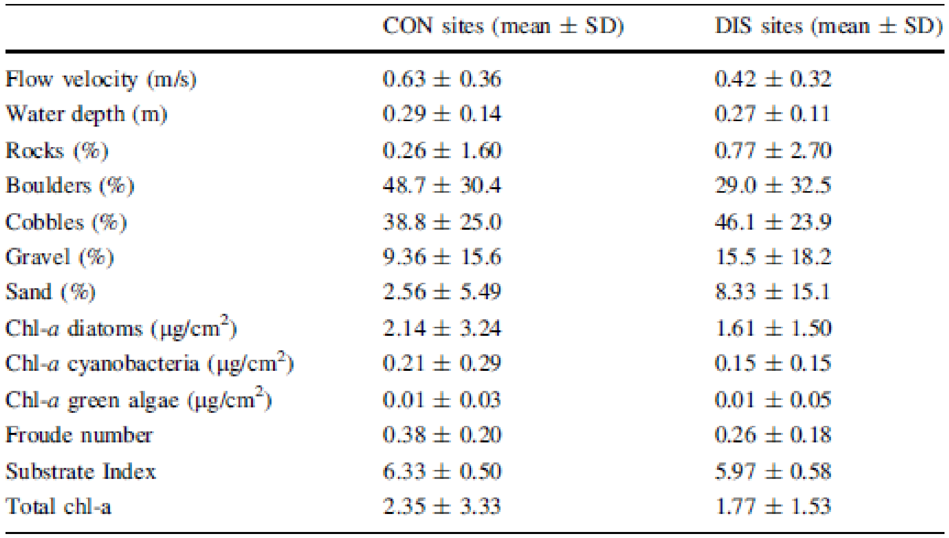
In a second step, we examined whether recurrent dewatering events alter the ecological niche of the three examined taxa by means of the Outlier Mean Index (OMI) analysis on CON and DIS sites separately. The OMI is a two-table ordination technique that positions the sampling units in a multidimensional space as a function of environmental parameters (Dolédec et al., 2000). The distribution of species in this space represents their realized niches and considers two aspects: marginality and tolerance. The marginality measures the distance between the mean habitat conditions used by a taxon and the mean habitat conditions across the study area. Taxa with high values of OMI have marginal niches (occur in atypical habitats within the study area), whereas those that get low values have non-marginal niches (occur in typical habitats within the study area). The tolerance measures the niche breadth, which means the amplitude in the distribution of each species along the sampled environmental gradients. Low values mean that a species is distributed across a limited range of conditions, while high values imply that a species is distributed across habitats with widely varying environmental conditions. 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, 2017).

Results

The observed values of environmental parameters observed in this study in both CON and DIS sites are reported in Table 1. The Wilcoxon test highlighted significant differences among CON and DIS sites in terms of Substrate Index (P = 0.008) and Froude number (P = 0.015), while no differences were observed for total chlorophyll a (P = 0.873). Among the three genera of Heptageniidae recorded in our samples, Rhithrogena resulted the most abundant and widely distributed taxon, followed by Epeorus and Ecdyonurus (Table 2).

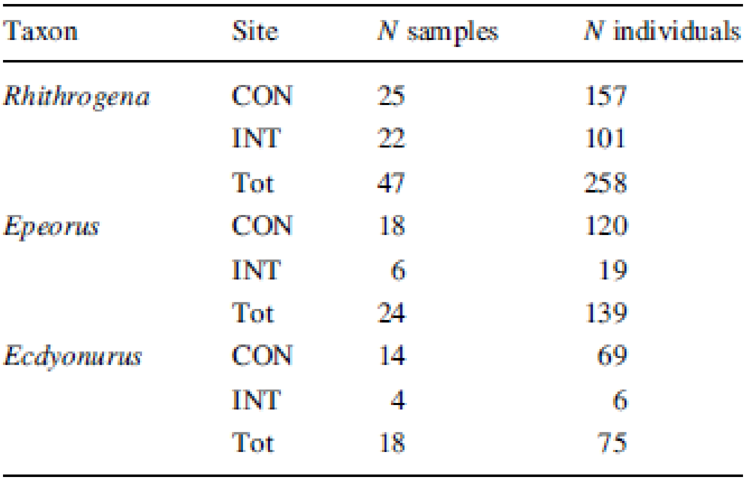
Niche hypervolume

Among the three examined taxa, *Epeorus* showed the highest dimension of the three-dimensional hypervolume (184.7), whereas *Ecdyonurus* and *Rhithrogena* displayed lower and similar values (*Ecdyonurus* = 128.2; *Rhithrogena* = 131.5). The three hypervolumes remarkably overlap (Fig. 1) as demonstrated by the similarity indices (*Ecdyonurus* vs *Epeorus*: Jaccard = 0.64, Sorensen = 0.78; *Ecdyonurus* vs *Rhithrogena*: Jaccard = 0.66, Sorensen = 0.79; *Epeorus* vs *Rhithrogena*: Jaccard = 0.65; Sorensen = 0.79).



	CON sites (mean ± SD)	DIS sites (mean ± SD)
Flow velocity (m/s)	0.63 ± 0.36	0.42 ± 0.32
Water depth (m)	0.29 ± 0.14	0.27 ± 0.11
Rocks (%)	0.26 ± 1.60	0.77 ± 2.70
Boulders (%)	48.7 ± 30.4	29.0 ± 32.5
Cobbles (%)	38.8 ± 25.0	46.1 ± 23.9
Gravel (%)	9.36 ± 15.6	15.5 ± 18.2
Sand (%)	2.56 ± 5.49	8.33 ± 15.1
Chl-*a* diatoms (μg/cm^2)	2.14 ± 3.24	1.61 ± 1.50
Chl-*a* cyanobacteria (μg/cm^2)	0.21 ± 0.29	0.15 ± 0.15
Chl-*a* green algae (μg/cm^2)	0.01 ± 0.03	0.01 ± 0.05
Froude number	0.38 ± 0.20	0.26 ± 0.18
Substrate Index	6.33 ± 0.50	5.97 ± 0.58
Total chl-a	2.35 ± 3.33	1.77 ± 1.53

Table 1 Average values (mean) and standard deviations (SD) for environmental parameters in CON and DIS sites



Taxon	Site	*N* samples	*N* individuals
Rhithrogena	CON	25	157
	INT	22	101
	Tot	47	258
Epeorus	CON	18	120
	INT	6	19
	Tot	24	139
Ecdyonurus	CON	14	69
	INT	4	6
	Tot	18	75

Table 2 Number of samples (N samples) and abundance of individuals (N individuals) recorded in perennial (CON) and intermittent (INT) sites, as well as their total values (Tot) in the study area, for the three examined genera

Outlying Mean Index (OMI)

The first two axes of the OMI analysis were selected and they accounted for the 99.4% and 100% of total explained variance in CON and DIS sites, respectively. In CON sites, the first and second axes contributed for the 79.2% and 20.2% to the total explained variance, respectively (Table 3). *Ecdyonurus* and *Epeorus* showed the highest and lowest marginality values (%OMI) respectively, while they have similar values of tolerance (%Tol). *Rhithrogena* had the narrowest niche (%Tol) (Table 4). *Ecdyonurus* is negatively affected by Froude number (- 0.72), but positively by SI (0.46), while total chlorophyll a has a positive low influence (0.02). The main environmental variables favoring *Epeorus* are total chlorophyll a (0.24) and SI (0.23), while Froude number has a low but negative effect (- 0.08) (Fig. 2). *Rhithrogena* is favored especially by total chlorophyll a (0.54) followed by SI (0.30), but it is negatively affected by Froude number (- 0.62) (Fig. 2). In DIS sites, the first axis alone contributed for the 87.5%, while the second axis contributed for the 12.5% to the total explained variance (Table 3). *Ecdyonurus* resulted as the most tolerant taxon (%Tol), showing also the lowest marginality value (%OMI) (Table 3). *Epeorus* is characterized with the highest marginality value (%OMI), while *Rhithrogena* showed the lowest tolerance value (%Tol) (Table 4). The three taxa all positively correlate with Froude number, with *Rhithrogena* (0.56) showing the highest correlation, followed by *Epeorus* (0.35) and *Ecdyonurus* (0.14). The SI has a highly positive effect on *Epeorus* (0.72) and a weak positive correlation with *Ecdyonurus* (0.14) and *Rhithrogena* (0.11) (Fig. 2). Total chlorophyll a positively affects the three taxa, even if its effect is more evident on *Rhithrogena* (0.33) and *Epeorus* (0.17) than on *Ecdyonurus* (0.06) (Fig. 2).

Discussion

We here revisited the role of interspecific competition in shaping the general geometry of the niche of three coexisting phylogenetically-related scrapers in mountainous streams recently facing seasonal dewatering events. In agreement with our predictions, our model organisms were found to co-occur in similar environmental conditions and were negatively affected by recurrent dewatering events, since they are less represented in DIS than in CON sites. Correlation among taxa and OMI axes indicated that all three environmental variables (Froude number, Substrate Index, and total chlorophyll a concentration) were important in explaining the spatial distribution of macroinvertebrates within microhabitats. Several studies underlined that the examined taxa prefer rheophilous microhabitats with coarse mineral substrates (Rempel et al., 2000; Usseglio-Polatera et al., 2000; Mérigoux & Dolédec, 2004; Beauger et al., 2006; Wellnitz, 2015) and our results partially confirmed previous findings. On one hand, we observed a positive consistent effect of the Substrate Index on the three examined taxa. In particular, this environmental feature was highly positively correlated with occurrence of *Ecdyonurus* and *Epeorus* in perennial and intermittent sections respectively. It should be noted that, although the flow had recovered since 6 months at the sampling moment, significant differences were observed in terms of Substrate Index among CON and DIS sites, with the former displaying more coarse substrates than the latter, possibly as a consequence of fine sediment deposition due to the dewatering event (average ± SD %Sand: CON sites = 2.56% ± 5.49; DIS sites = 8.33% ± 15.1). In fact, fine sediment accumulation is strictly associated with flow reduction and droughts, because lower water velocity enables more sediments to settle out of suspension (Dewson et al., 2007; Rolls et al., 2012).

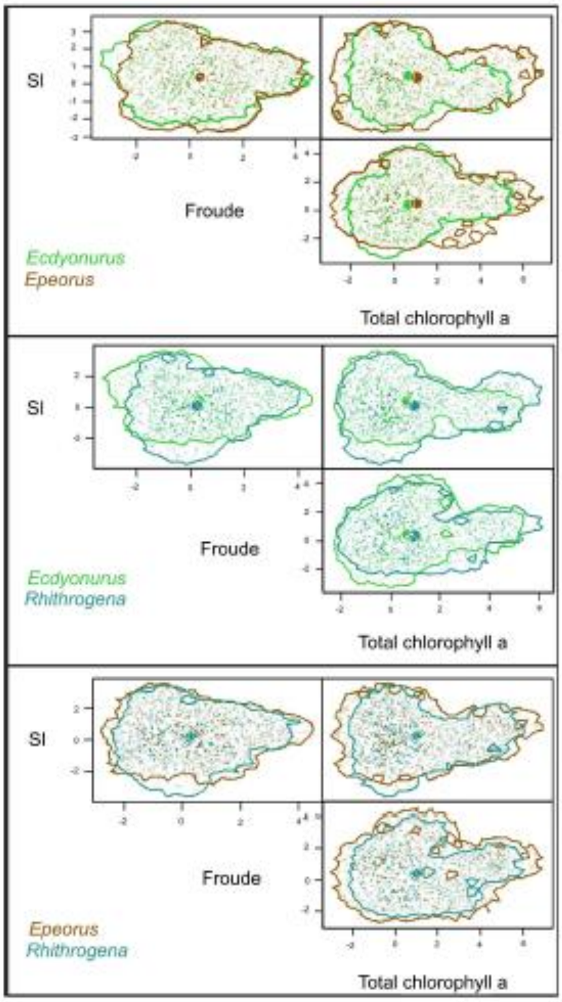


Fig. 1 Pair plots showing the estimated three-dimensional Hypervolumes for the three examined taxa. The colored points for each taxon reflect the centroids (large points), original observations (intermediate points) and the stochastic points sampled from the inferred hypervolume (small points). All variables are standardized

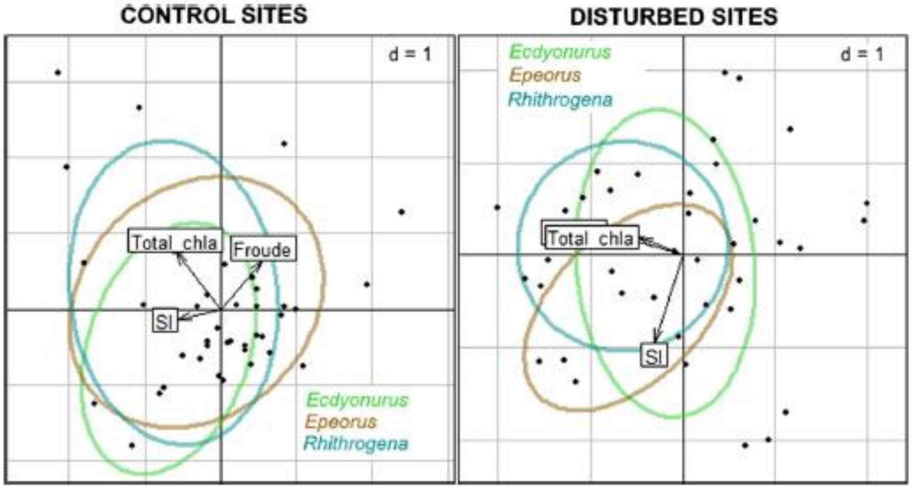
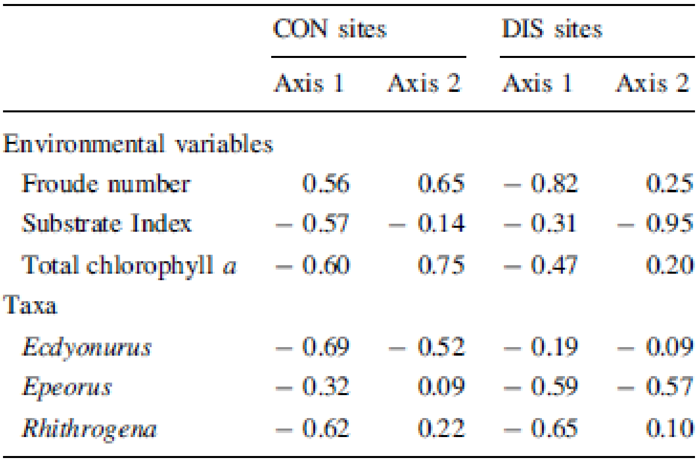
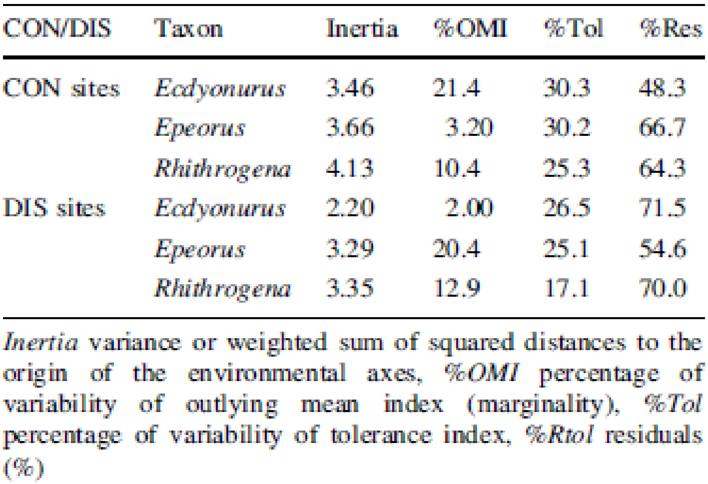


Fig. 2 Projection of environmental variables on the axis of OMI analysis in CON (left panel) and DIS (right panel) sites and representation of ecological niches of the three examined taxa. Values of distances among one square and the other along the two axes are determined by the d value reported in the top right corner of the pictures



	CON sites		DIS sites	
	Axis 1	Axis 2	Axis 1	Axis 2
Environmental variables				
Froude number	0.56	0.65	− 0.82	0.25
Substrate Index	− 0.57	− 0.14	− 0.31	− 0.95
Total chlorophyll *a*	− 0.60	0.75	− 0.47	0.20
Taxa				
Ecdyonurus	− 0.69	− 0.52	− 0.19	− 0.09
Epeorus	− 0.32	0.09	− 0.59	− 0.57
Rhithrogena	− 0.62	0.22	− 0.65	0.10

Table 3 Correlation values between OMI axes and both environmental variables and examined taxa in CON and DIS sites



CON/DIS	Taxon	Inertia	%OMI	%Tol	%Res
CON sites	*Ecdyonurus*	3.46	21.4	30.3	48.3
	Epeorus	3.66	3.20	30.2	66.7
	Rhithrogena	4.13	10.4	25.3	64.3
DIS sites	*Ecdyonurus*	2.20	2.00	26.5	71.5
	Epeorus	3.29	20.4	25.1	54.6
	Rhithrogena	3.35	12.9	17.1	70.0

Inertia variance or weighted sum of squared distances to the origin of the environmental axes, *%OMI* percentage of variability of outlying mean index (marginality), *%Tol* percentage of variability of tolerance index, *%Rtol* residuals (%)

Table 4 Niche parameters of Heptageniidae taxa

On the other hand, the role of the hydrological stress is controversial. Heptageniidae display morphological adaptations to fast currents, i.e., flat head and femora (Weissenberger et al., 1991) and abdominal gills that overlap to form friction pads (Ditsche-Kuru & Koop, 2009). In addition, their abdominal gills are largely immobile, making Heptageniidae nymphs unable of generating effective ventilatory movements. Consequently, they are likely physiologically restricted to fast-flow waters with high oxygen exchange rates (Baumer et al., 2000). Surprisingly, examined taxa were favored by high hydraulic stress in intermittent sites, but this relationship was opposite in perennial sites. In particular, *Rhithrogena* was strongly disturbed by high hydraulic stress in perennial sites, despite being known as an extremely rheophilous taxon (Rempel et al., 2000; Mérigoux & Dolédec, 2004; Beauger et al., 2006). Evidence in literature suggests that this inverse relationship might be due to different grazing rates at different flows, which may also vary within the same family. For instance, Poff et al. (2003) showed an increasing grazing rate with increasing flow velocity for the caddisfly *Glossosoma verdona* Ross, 1938, but Wellnitz et al. (2001) observed an opposite trend for the related species *Agapetus boulderensis* Milne, 1936. We might hypothesize that differences in flow velocity among perennial and intermittent sites are responsible of altered grazing efficiency with consequent inconsistent relationship among the examined taxa and hydraulic stress. In addition, the architecture and taxonomic composition of periphyton is shaped by stream current. Microhabitats differing in their flow velocity may host periphytic mats on which some grazers can feed and forage more effectively than others. For instance, thin biofilms are more readily consumed by some species (Wellnitz & Poff, 2012), while thicker mats may hinder grazer movements across substrates (Hoffman et al., 2006). Also, current-mediated periphytic structure also has the potential to foster positive interactions among grazers if one grazer's foraging facilitates another's access to algal food (Wellnitz & Poff, 2012). A comprehensive investigation of these aspects in recently temporary streams may, therefore, improve our ability to predict the effect of flow changes on macroinvertebrates. Availability of trophic resources positively affected the distribution of the three examined taxa. In particular, *Rhithrogena* and *Epeorus* were strictly linked to the total chlorophyll a concentration, whereas this relationship was less evident for *Ecdyonurus*. This pattern might result from different ecological preferences of the three examined taxa. For instance, low water velocity and turbulence allow the accrual of algae, with consequent high abundance of scrapers (Manfrin et al., 2016). Conversely, light attenuation in deeper areas may reduce periphyton growth and thus food availability for scrapers (Quinn & Hickey, 1994; Mérigoux & Dolédec, 2004). As their ecological niches broadly overlapped, we may expect that the interaction among the three examined taxa results in exploitative competition. This is particularly true in DIS sites, where their niches are smaller compared to CON sites, suggesting how dewatering events likely reduce the availability of suitable niches and, consequently, enhance competition among coexisting taxa. In our streams, *Epeorus* resulted to be the most competitive taxon among Heptagenidae, as demonstrated by the widest hypervolume, and this is particularly true in perennial sites, where it showed the lowest marginality value. The lower hypervolumes suggest that in perennial stretches *Rhithrogena* and

Ecdyonurus are less competitive. Among them, *Rhithrogena* seems the most specialized taxon since it had the narrowest niche both in permanent and intermittent sections. On the contrary, *Ecdyonurus* had its optimal niche in intermittent sites, where it had the lowest marginality. In fact, interestingly, while showing similar niche width, *Epeorus* and *Ecdyonurus* were characterized by opposite marginality values in perennial and intermittent sites, with the former being favored in perennial sites and viceversa. We might hypothesize that, in intermittent sites, the less competitive taxon, namely *Ecdyonurus*, replaces *Epeorus*, which outcompetes *Ecdyonurus* in hydrologically stable sites. Another explanation could be found in the different flow conditions between perennial and intermittent sites. In fact, we observed higher values of Froude number in perennial than in intermittent sites, which are due to higher values of flow velocity. Several studies indicated *Epeorus* and *Rhithrogena* among the most rheophilic organisms (Rempel et al., 2000; Mérigoux & Dolédec, 2004; Beauger et al., 2006; Wellnitz, 2015), whereas *Ecdyonurus* showed preference for intermediate or low flow velocity (Mérigoux & Dolédec, 2004; Beauger et al., 2006). However, flow velocity and water depth, here summarized into Froude number, showed opposite effects on examined taxa in perennial and intermittent sections, whereas the positive relationship between their occurrence and total chlorophyll a concentration is consistent across sampling sites. This pattern seems therefore to corroborate the hypothesis of lower competition ability of *Ecdyonurus* compared to *Rhithrogena* and *Epeorus*, which emerged from the hypervolume and marginality values. This hypothesis is furtherly supported by Peralta-Maraver et al. (2017), who recently analyzed the gut content of several macroinvertebrate taxa, among which the three Heptageniidae examined in this study. Their results showed how *Ecdyonurus* is less dependent on periphytic biofilm, which compose on average the 50% of its diet, than *Epeorus* and *Rhithrogena*, whose 68% of their average diet is composed by periphytic biofilm. Interestingly, *Ecdyonurus* can alternatively feed on allochthonous organic detritus, which represents on average 22% of its diet, and this happens especially when the three taxa temporally coexist. We can thus suggest that *Ecdyonurus* is more generalist and less competitive than *Epeorus* and *Rhithrogena* in the access to trophic resources. However, translating these observations into interspecific competition among Heptageniidae for trophic resources should be interpreted with caution. Although our data suggest that *Epeorus* and *Rhithrogena* outcompeted *Ecdyonurus* in the access to trophic resources in perennial flow conditions, their high mobility could allow them to partition their trophic niche. In fact, mayflies are extremely agile crawlers on surfaces exposed to fast current and they can move from one area to another, eating only small amounts of algae at each location (Hill & Knight, 1987). Moreover, spatial segregation within the periphyton mat may also occur, with some taxa feeding mainly on tightly attached forms and others preferring superficial layers (Tall et al., 2006). Further investigations should be therefore performed to shed light on this point. Overall, our results emphasize how stream physical parameters play a key role in determining the ecological niche of Heptageniidae. Although we did not highlight any difference between perennial and intermittent sections in terms of substrate and trophic resource preferences, recurrent dewatering events negatively affect the examined taxa, which are less represented in DIS than in CON sites and the controversial role of hydraulic stress corroborates this hypothesis. In addition, flow reduction is strictly associated with fine sediment accumulation, because lower water velocity enables more sediments to settle out of suspension (Dewson et al., 2007; Rolls et al., 2012). Both phenomena may in turn alter primary productivity and algae composition (Bona et al., 2012, 2016; Piano et al., 2017) with consequent detrimental effects on scrapers. These phenomena will reduce the availability of suitable conditions for the three examined taxa, as demonstrated by the narrower niches in DIS than in CON sites. Since taxa living in previously perennial streams do not have evolved adaptations to dewatering events, they are likely exposed to higher risk of extinction compared to organisms living in Mediterranean streams, which naturally include extreme variations of flow discharge in their hydrological cycle (Fenoglio et al., 2010). In addition, Alpine and mountainous streams are affected by a variety of different human pressures that may alter and sometimes almost destroy these ecosystems (Wohl, 2006). In particular, the increasing water abstraction, e.g., for hydroelectric power generation, drinking, irrigation, and snow generation, add up to the global climate change effects, is exacerbating the effects of flow reduction on mountainous streams (Maiolini and Bruno, 2007). Water flow reduction and dewatering events are expected to have dramatic consequences on stream ecosystem functionality by altering the availability of energetic inputs (e.g., Ledger et al., 2008; Datry et al., 2011; Piano et al., 2017; Falasco et al. 2018). The expected decrease in autochthonous trophic resources will likely cause a bottom-up effect in the food web, directly influencing the survival, growth, and reproduction of invertebrate scrapers. The reduced grazing pressure may also favor the bloom of toxic cyanobacteria, whose growth is enhanced by water flow reduction and temperature increase (Piano et al., 2017). Since low-order mountainous streams are currently facing alterations caused by flow intermittency, further investigations in mountain areas are required in the next future to better unravel these processes.

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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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Abstract

Several Alpine streams are currently facing recurrent summer drying events with detrimental consequences on stream detritivores, i.e., shredders, due to negative effects via changes the organic matter (CPOM) availability. We examined the ecological requirements 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 events. We evaluated the overlap among their ecological niches measured in terms of hydraulic stress, substrate composition, changes in CPOM availability and competition with other shredder taxa (i.e., presence of individuals of other shredders) and we examined potential changes in their ecological niches between permanent and intermittent sites. The ecological niches of *Protonemura* and *Amphinemura* overlap broadly, but not with *Nemoura*, suggesting only partial potential competition. The reduced CPOM availability decreased the individual abundance of the three genera in intermittent sites, where they consistently preferred microhabitats with high CPOM availability and low competition with other shredder taxa, 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 bottom up effects on stream ecosystem functionality.

Keywords Organic matter; Ecological niche; Hypervolume; Nemouridae; Stream detritivores

Introduction

Mountain 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 mountain 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; Piano et al., 2019a; Doretto et al., 2020a). 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 mountain 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; Webster & Meyer, 1997; 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 late 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 before the expected increase in flow due to snow melt (González & Graça, 2003; Fenoglio et al., 2005; Tierno de Figueroa et al., 2009; Bo et al., 2013). Therefore, as flow intermittency events occur in summer, they are expected to impact shredders during the oviposition and early instars, greatly reducing their survival and abundance, 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 usually reduces 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). 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 resources, in terms of both reduced instream production, due to the abrasion of autotrophic biofilms (Henley et al., 2000; Bona et al., 2016), and allochthonous coarse organic matter availability through burial (Doretto et al., 2016). On top of that, such dramatic consequences on organic matter availability and decomposition may persist even after several months following flow resuming, because of the so-called ‘‘drying memory’’ (Datry et al., 2011; Pinna et al., 2016), thus affecting also the late instars of shredders (Piano et al., 2019a). Physical alterations and consequent changes in food resources induced by flow intermittency thus represent strong environmental filters that may influence the ecological niche of shredders. Although some studies investigated the effect of flow intermittency on the diversity of this trophic group in Alpine streams (e.g., Fenoglio et al., 2007; Doretto et al., 2018; Piano et al., 2019a), little is known about how these events may alter the ecological parameters that determine the niches of shredder taxa. Here, we 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 Nemouridae (Plecoptera) family. 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 mountain Italian streams, which has already published data on other macroinvertebrate groups (i.e., scrapers, see Piano et al., 2019b). We hypothesized that: (i) the ecological niches of the three examined genera overlap, thus suggesting possible exploitative competition; (ii) flow intermittency negatively affects their abundances; 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). We selected PSs within 10 km upstream of the ISs to reduce environmental variation between the two reaches. At the same time, due to the low drift propensity of Nemouridae (e.g., Bruno et al., 2013), permanent and intermittent sites within the same stream may be considered independent sampling units. 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 (Qmean = 3.98 ± 4.56 m3/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 long stream section of longitudinal distribution, overall spaced at least 5 m apart, which were randomly selected to cover different habitat 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 m2, 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 lm mesh size; 0.062 m2 area) and we collected both the retained CPOM and macroinvertebrates (Doretto et al., 2020b). Collected samples were preserved in plastic jars with 75% ethanol. In the laboratory, CPOM was washed through a 250 lm 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, expressed as mg/m2, was then considered a proxy of CPOM 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 Table S1 and Fig. S1 for a complete checklist of shredders collected in this study). The numbers of individuals of the three genera *Nemoura*, *Protonemura* and *Amphinemura* were used as dependent variables in our analyses, while we used the number of individuals of the other shredder taxa as a proxy for inter-specific 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) 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/H(d 9 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 9 %Rocks + 0.7 9 %Boulders + 0.6 9 %Cobbles + 0.5 9 %Gravel + 0.4 9 %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).

Ecological niches of Nemouridae

In order to characterize the ecological niches of Nemouridae, we compared the overall (i.e., independent of streams and sections) 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 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).

Effects of flow intermittency on the ecological niches of Nemouridae

To test the effect of flow intermittency on the ecological niches of Nemouridae, we first checked whether environmental and biotic parameters, namely Froude number, SI, CPOM mass and the abundance of individuals belonging to shredders (hereinafter Competition), and abundances of the three examined genera differed between PSs and ISs with the nonparametric Wilcoxon test repeated for each variable. In a second 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

All the three genera of Nemouridae typical of the Alpine area, namely *Nemoura*, *Protonemura* and *Amphinemura*, were present in the samples and were generally more abundant in PS than in IS sites. We collected 492 Nemouridae, 450 individuals in PS sites and 72 individuals in IS sites, recorded in 11 and 6 reaches, respectively. Among the three genera, *Protonemura* resulted the most abundant and widely distributed genus, with 264 individuals recorded in 13 out of 28 reaches, with higher occurrence and abundance in PS (Occurrence = 8 reaches; Abundance = 225 individuals) than IS sites (Occurrence = 5 reaches; Abundance = 39 individuals). *Amphinemura* was slightly less abundant than *Protonemura*, with a total of 236 individuals of *Amphinemura*, 205 in PS sites and 31 in IS sites, recorded in 10 reaches and 4 reaches, respectively, for a total of 14 reaches. *Nemoura* showed the lowest occurrence and abundance, with only 22 individuals collected, 20 in PS sites and 2 in IS sites, recorded in 8 and 2 reaches, respectively, for a total of 10 reaches. Of the 28 investigated reaches, 11 had no Nemouridae, 2 had only one genus, 10 hosted 2 genera and 5 reaches accounted for the three examined genera.

Ecological niches of Nemouridae

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 (Table 1). 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 Table 1). The highest overlap value is observed between the ecological niches of *Amphinemura* and *Protonemura* (Table 1). The overlap between ecological niches (Fig. 1) indicates that the unique portion of *Nemoura* niche is mainly determined by the fact that it is not limited by the presence of other shredder taxa (Competition), but it seems the be the most 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 hydromorphological conditions (Fig. 1).

Effects of flow intermittency on the ecological niches of Nemouridae

The Wilcoxon test highlighted significant differences among PS and IS sites for all the three genera, as PS sites had higher abundances and more favorable habitat conditions than in IS sites (Table 2), and among PS and IS sites in terms of Substrate Index, CPOM mass, and Competition, while no differences were observed for the Froude number (Table 2). In particular, our results highlight how PS sites have significant higher values of SI, CPOM and Competition than IS sites. To evaluate how flow intermittency affects the ecological preferences of the three examined genera, we performed the OMI analysis, which showed how responses to hydrological intermittency seems to be genera-specific. In PS sites, *Amphinemura* showed the highest marginality and tolerance values, thus being the genus that occupies the most marginal microhabitats, whereas *Nemoura* showed the lowest marginality, which means that it occupies the most typical microhabitats in permanent sites (Table 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

(Table 3). In IS sites, *Nemoura* has the highest marginality, thus occupying the most marginal microhabitats in intermittent sites, whereas *Protonemura* has the lowest marginality value, thus occurring in the most typical microhabitats of intermittent sites (Table 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 (Table 3). When considering the role of environmental variables, *Nemoura* was 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 (Table 4 and 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 (Table 4 and Fig. 2b). *Protonemura* was 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) (Table 4 and Fig. 2a) in PS sites. 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) (Table 4 and Fig. 2b).

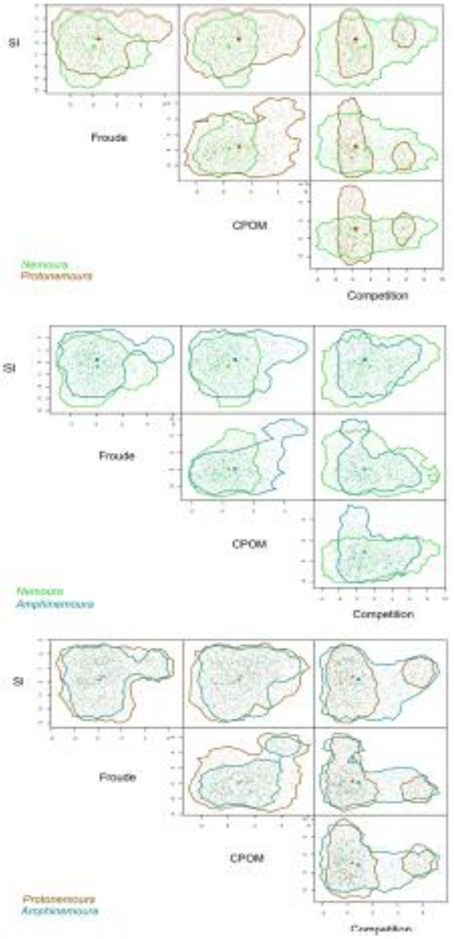
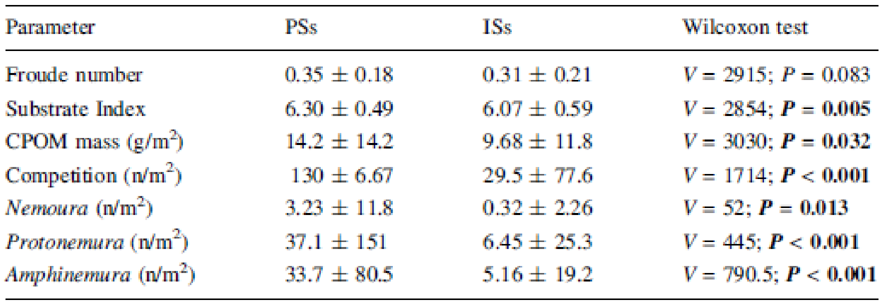
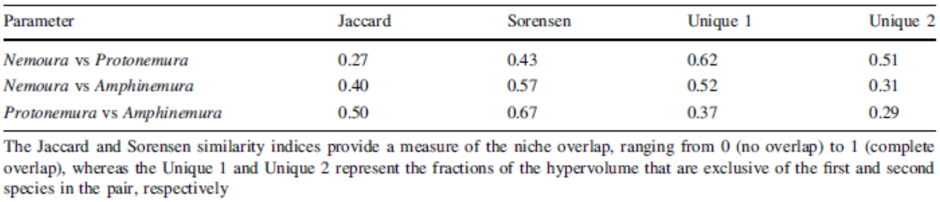


Fig. 1 Bi-plots showing the two-dimensional aspects of the estimated four dimensional hypervolumes in a pair-wise comparison among the three genera. The colored points for each genus reflect the centroids (large points), original observations (intermediate points) and the stochastic points sampled from the inferred hypervolume (small points). All variables are standardized



Parameter	PSs	ISs	Wilcoxon test
Froude number	0.35 ± 0.18	0.31 ± 0.21	$V = 2915$; $P = 0.083$
Substrate Index	6.30 ± 0.49	6.07 ± 0.59	$V = 2854$; **$P = 0.005$**
CPOM mass (g/m^2)	14.2 ± 14.2	9.68 ± 11.8	$V = 3030$; **$P = 0.032$**
Competition (n/m^2)	130 ± 6.67	29.5 ± 77.6	$V = 1714$; **$P < 0.001$**
Nemoura (n/m^2)	3.23 ± 11.8	0.32 ± 2.26	$V = 52$; **$P = 0.013$**
Protonemura (n/m^2)	37.1 ± 151	6.45 ± 25.3	$V = 445$; **$P < 0.001$**
Amphinemura (n/m^2)	33.7 ± 80.5	5.16 ± 19.2	$V = 790.5$; **$P < 0.001$**

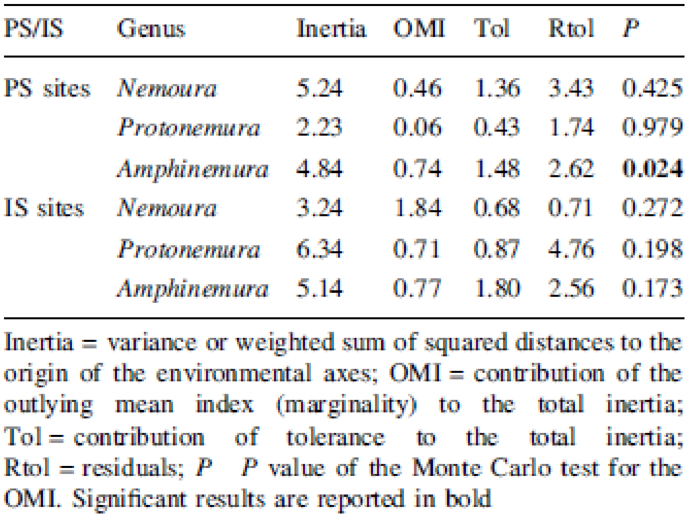
Table 1 Average values and standard deviations for environmental parameters and abundance of the three examined genera in PS and IS sections. Significant results are reported in bold



Parameter	Jaccard	Sorensen	Unique 1	Unique 2
Nemoura vs *Protonemura*	0.27	0.43	0.62	0.51
Nemoura vs *Amphinemura*	0.40	0.57	0.52	0.31
Protonemura vs *Amphinemura*	0.50	0.67	0.37	0.29

The Jaccard and Sorensen similarity indices provide a measure of the niche overlap, ranging from 0 (no overlap) to 1 (complete overlap), whereas the Unique 1 and Unique 2 represent the fractions of the hypervolume that are exclusive of the first and second species in the pair, respectively

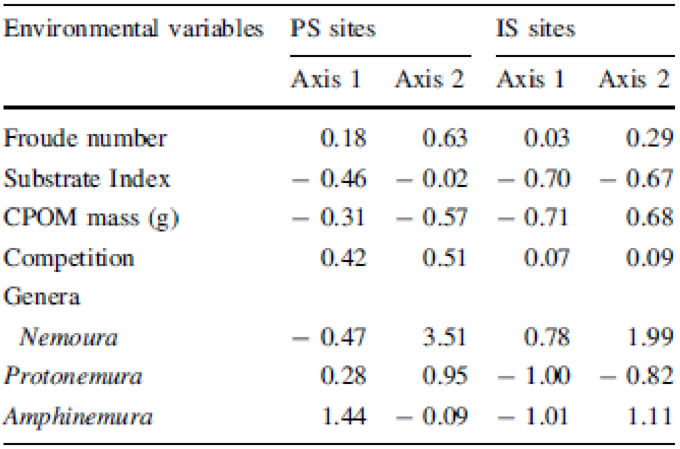
Table 2 Results of the hypervolume overlap statistics for the three pairs of genera examined here



PS/IS	Genus	Inertia	OMI	Tol	Rtol	*P*
PS sites	*Nemoura*	5.24	0.46	1.36	3.43	0.425
	Protonemura	2.23	0.06	0.43	1.74	0.979
	Amphinemura	4.84	0.74	1.48	2.62	**0.024**
IS sites	*Nemoura*	3.24	1.84	0.68	0.71	0.272
	Protonemura	6.34	0.71	0.87	4.76	0.198
	Amphinemura	5.14	0.77	1.80	2.56	0.173

Inertia = variance or weighted sum of squared distances to the origin of the environmental axes; OMI = contribution of the outlying mean index (marginality) to the total inertia; Tol = contribution of tolerance to the total inertia; Rtol = residuals; *P* *P* value of the Monte Carlo test for the OMI. Significant results are reported in bold

Table 3 Niche parameters of Nemouridae genera



Environmental variables	PS sites		IS sites	
	Axis 1	Axis 2	Axis 1	Axis 2
Froude number	0.18	0.63	0.03	0.29
Substrate Index	− 0.46	− 0.02	− 0.70	− 0.67
CPOM mass (g)	− 0.31	− 0.57	− 0.71	0.68
Competition	0.42	0.51	0.07	0.09
Genera				
Nemoura	− 0.47	3.51	0.78	1.99
Protonemura	0.28	0.95	− 1.00	− 0.82
Amphinemura	1.44	− 0.09	− 1.01	1.11

Table 4 Eigenvalues of projected environmental variables and examined genera on OMI axes in PS and IS sites

Amphinemura 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) (Table 4 and Fig. 2a) in PS sites. 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) (Table 4 and 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* 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* selects microhabitats with coarser substrates and high competition and CPOM availability in permanent sites, while it is not affected by competition in intermittent sites.

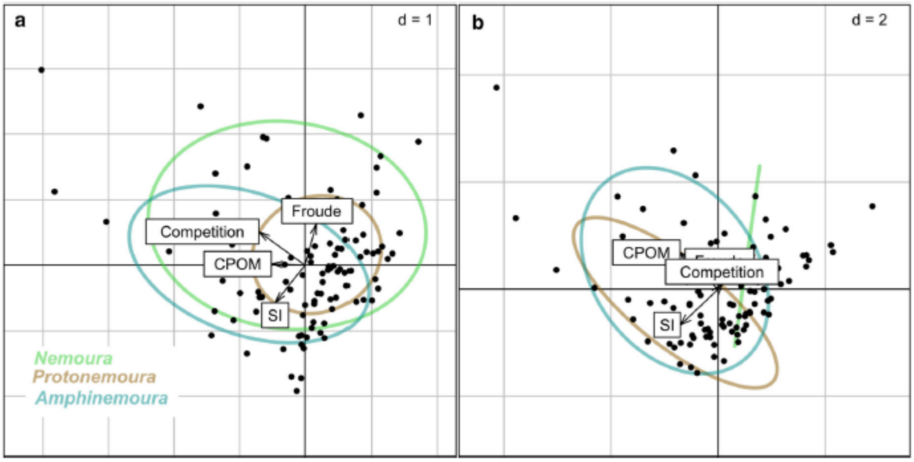


Fig. 2 Projection of environmental variables on the axis of OMI analysis and representation of ecological niches of the three examined genera in: a PS sites (axis 1 = 92.0% of total explained variance, and it represents a gradient of increasing competition and decreasing coarseness of the substrate; axis 2 = 7.82% of the total explained variance, and it is a gradient of decreasing CPOM mass and increasing hydraulic stress); and b IS sites (axis 1 = 82.7% of total explained variance, and it represents a gradient of decreasing CPOM mass and SI; axis 2 = 12.5% of total explained variance, and it represents a gradient of decreasing SI and increasing CPOM mass). The d value reported in the top right corner of the pictures represents the measure of one square along the two axes

Discussion

We here evaluated the role of flow intermittency in shaping the niche of three coexisting phylogenetically related shredders in Alpine 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, as high fine sediment deposition buries CPOM, in the subsequent months, we here tested whether and how flow intermittency affects the three shredder genera belonging to the Nemouridae family, namely *Nemoura, Protonemura* and *Amphinemura*. By first highlighting how these three genera share similar ecological requirements, we then demonstrated a clear negative effect of flow intermittency on both the abundance and ecological preferences of *Nemoura, Protonemura* and *Amphinemura.*

Ecological niches of Nemouridae

As we expected differences in the ecological requirements of Nemouridae, we first examined whether the examined genera differed in the dimension of their realized ecological niches and whether they overlap in their ecological requirements. Our results displayed only a partial overlap of the niche hypervolumes of the three genera, partially confirming our first hypothesis. Although our model organisms were found to co-occur in similar hydromorphological conditions, they differ in their niche dimension especially in terms of CPOM availability and potential competitive pressure. *Nemoura* 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 support this second hypothesis as *Nemoura* occupied 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).

Voltinism has been recognized as one of the most sensitive traits (Bonada & Dolédec, 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). In fact, plurivoltine species can overcome the negative effects of flow intermittency events on individuals inhabiting the stream during summer by producing multiple generations per year. Conversely, individuals semivoltine species, i.e., completing their life cycle in more than 1 year, have to face the drying up of the riverbed, thus being strongly affected by these events. Although being highly sensitive to flow intermittency, *Protonemura* and *Amphinemura* resulted to be less influenced by flow intermittency than *Nemoura*, possibly because species belonging to these genera are mainly monovoltine (Fochetti et al., 2009), and their life cycle allows them to escape flow intermittency events by emerging before their occurrence. This hypothesis of a similar response of these two genera to flow intermittency is further supported by the fact that their ecological niches broadly overlap. 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 compared to permanent sites, *Amphinemura* was the most tolerant genus to flow intermittency among the three Nemouridae genus studied. 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).

Effects of flow intermittency on the ecological niches of Nemouridae

When considering the effect of flow intermittency on the three examined genera, our results showed that abundances of the three genera were lower in intermittent than in permanent reaches, in agreement with our second hypothesis. 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 due to 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 prevents 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 quantity of energy inputs, as it affects the amount of autotrophic production (Henley et al., 2000; Bona et al., 2016) and allochthonous coarse organic matter availability in Alpine streams (Doretto et al., 2016, 2017). In particular, the burial of leaf litter by sediments reduces availability of this resource and alters the composition of the microbial community involved in its degradation (Receveur et al., 2020), with consequent negative effects on shredder invertebrates (Danger et al., 2012). When focusing on the differences in ecological niches of the three genera between permanent and intermittent sites, the relationship with CPOM is weak in permanent sites, except for *Amphinemura*, but it becomes consistently and highly positive in intermittent sites, suggesting food limitation under flow intermittency conditions. Competition with other shredders positively influences the examined genera in permanent sites, except for *Protonemura*, suggesting that in permanent sites, where CPOM availability is high, different shredder taxa can coexist. Conversely, in intermittent sites, competition with other shredders has a consistent weak negative effect, possibly because the access to trophic resources is dominated by exploitative competition among shredder taxa due to food limitation, with detrimental effects on Nemouridae. Our suggestion is supported by 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). While the role of CPOM availability and competition in determining the ecological niches in permanent and intermittent sites is consistent among the three examined genera, the role of the hydromorphological parameters is more controversial. When considering the Froude number, *Nemoura* and *Protonemura* were favored in reophilous microhabitats in permanent but not in intermittent sites, whereas *Amphinemura* was weakly affected by this factor. Our sampling sites are located in mountain 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, hydraulic conditions have an indirect effect on the examined genera, with high Froude numbers likely negatively affecting 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 (Quinn et al., 2007). Given that microhabitats with low flow velocity are deposition areas not only of CPOM but also of fine sediments (Quinn et al., 2007), the substrate preference can also indirectly affect the examined genera by conditioning the availability of CPOM. In fact, we observed again contrasting results among the three examined genera, and even between permanent and intermittent sites for *Protonemura*, which could be ascribed to the relationship between CPOM availability and substrate composition. In fact, *Protonemura* is mainly found on finer substrates in permanent sites whereas it shifts on coarser substrates in intermittent sites. This change can 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.

Conclusions

Overall, our results emphasize how stream physical parameters, resource availability and their interaction 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 reduce shredder biodiversity by altering the availability of their energetic inputs, with potential dramatic effects on stream ecosystem functionality (e.g., Ledger et al., 2008; Datry et al., 2011; Piano et al., 2019b). In fact, 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. Further investigations in mountain areas are thus required in the next future to better unravel how the interaction between flow intermittency and CPOM availability may affect shredders in lotic ecosystems.

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EFFECTS OF FLOW INTERMITTENCE ON ECOSYSTEM PROCESSES IN MOUNTAIN STREAMS: ARE ARTIFICIAL AND FIELD EXPERIMENTS COMPARABLE?

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Abstract

Flow intermittence, and specifically riverbed drying, is becoming increasingly frequent due to the effects of climate change and human pressures, including in mid-elevation streams and rivers in the Alpine area. Due to the highly dynamic flow regimes of intermittent streams and interactions among multiple environmental factors, experiments in laboratory and field-based mesocosms can be used to simplify the set of abiotic factors that influence a biotic response. Results from such mesocosms require evaluation and, if necessary, calibration with data from field experiments. To assess the applicability of manipulative experiments to natural conditions, we compared the results of a field-based flume experiment simulating leaf litter processing with those obtained from natural field data. We compared an intermittent treatment (in which the surface sediments dried) with a control treatment with perennial flow in (a) an artificial flume system fed by a mountain stream and (b) two piedmont mountain streams. In both experiments, we sampled the macroinvertebrate community and measured leaf decomposition rates at comparable time intervals. We observed consistent patterns in both spatial and temporal total β-diversity and leaf mass loss in the two experiments, despite differences in shredder abundance. Where flowing water was present during the whole experiment, leaf litter decomposition was faster and macroinvertebrate communities were more stable and taxonomically richer. These results highlight drying events as a key influence on ecological communities, enabling their characterization in both controlled and natural conditions. Our results suggest that flume experiments can provide an effective proxy for naturally occurring processes in stream ecosystems.

Keywords: leaf bags; dry river; flow intermittency; climate change; leaf litter decomposition; benthic invertebrate; temporary river.

Introduction

River drying events are increasing in both frequency and intensity due to global change (Datry et al. 2014a; Vicente-Serrano et al. 2014; Afzal & Ragab 2020), causing previously perennial streams to shift to intermittent flow regimes. This disruption of hydrological regimes has marked effects on lotic habitats and communities, with consequences for instream processes and thus ecosystem functioning (Abril et al. 2016; Leigh & Datry 2017; Falasco et al. 2020). The effects of flow intermittence are mediated by changes in aquatic habitat availability:

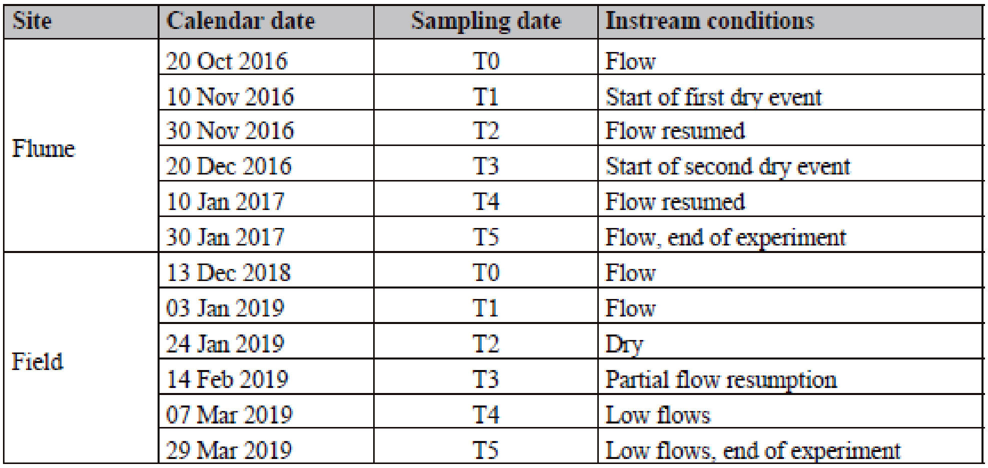
rheophilic taxa become less abundant as flow regimes shift from perennial to intermittent, while lentic taxa increase, due to the greater availability of habitats matching their preferences (Pace et al. 2013; Chessman 2015; Buffagni 2021). In the last few decades, shifts to intermittent flow caused by climate-driven decreases in winter rain/snowfall and increases in temperatures coupled with increasing water abstraction have become widespread in watercourses that drain the Italian Alps (Zolezzi et al. 2009; Ravazzani et al. 2015; Vezzoli et al. 2015), but our understanding of the effects on stream biota remains limited (Fenoglio et al. 2010; Rogora et al. 2018; Lencioni 2018). However, some studies suggest that many macroinvertebrate taxa within these streams lack adaptations to persist in reaches that dry (Lytle & Poff 2004; Domisch et al. 2011). For example, in intermittent reaches in the Upper Po River catchment, Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa decrease in abundance in response to dry conditions, alongside taxa with a long, desiccation-sensitive aquatic juvenile life stage (Doretto et al. 2018; Piano et al. 2019a). Drying events also negatively affect macroinvertebrate functional feeding groups such as shredders, decreasing their abundance and thus disturbing trophic interactions (Corti et al. 2011; Datry et al. 2011) and ecosystem processes such as leaf litter breakdown (Ledger et al. 2011; Wenish et al. 2017). In addition, microbial activity can be reduced by water loss, decreasing microbial conditioning of coarse particulate organic matter (CPOM) and thus its palatability to macroinvertebrates (Boulton & Lake 1992; Arias-Real et al. 2020). Flow intermittence is thus expected to impact the structure of lotic food webs, causing nutrient processing to become spatially patchy and food chain length to shorten, with dramatic consequences for biodiversity, river functionality and self-purification capacity (McIntosh et al. 2017; Stubbington et al. 2017). Decomposition rates may thus represent river ecosystem functioning and health, because drying disturbances simplify food webs, making CPOM processing less efficient (Ledger et al. 2013). The occurrence of aseasonal dry events (i.e. in late autumn/winter) may further impact Alpine stream ecosystems, because low water levels exacerbate the effects of low temperatures, increasing the extent and rate of freezing (Tolonen et al. 2019). The drying of previously perennial streams is also likely to alter ecological processes from primary instream production to allochthonous CPOM degradation (Fenoglio et al. 2015; Pinna et al. 2016). Leaf litter is an important energy source in low-order streams and its decomposition plays a key role in nutrient cycling (Petersen & Cummins 1974; Graça & Canhoto 2006), including leaching of soluble compounds, physical abrasion, microbial conditioning and invertebrate fragmentation activity. Intrinsic factors such as the C:N ratio, tannin content, and leaf texture can affect decomposition, reducing breakdown rates in low-quality litters (Simon et al. 2018; Zhang et al. 2019). Extrinsic factors also influence leaf litter decomposition, especially during dry periods: variation in parameters such as temperature and dissolved nutrient concentrations (Tank et al. 2010; Chessman 2018) can affect bacterial and fungal activity in wet refuges (e.g. pools, saturated interstitial spaces), with indirect impacts on macroinvertebrate communities (Boyero et al. 2011; Follstad Shah et al. 2017). The impacts of winter dry events on aquatic communities and ecosystem processes have been little researched through field studies in mountain and piedmont areas. Here, allochthonous CPOM input is highest in autumn and early winter, decomposition continues under very low temperatures, and flow reduction and winter dry events could subject leaf litter to emersion–immersion cycles, affecting CPOM processing. However, field studies are hampered by the unpredictable timing and duration of dry events, as well as variability introduced by the distribution of refuges and co-occurrence of anthropogenic impacts. Experiments in artificial flumes enable discrimination among such covariables (Rumbos et al. 2010; Santschi et al. 2018; Truchy et al. 2020), but represent a simplified version of natural conditions. CPOM decomposition has been widely investigated in manipulative field experiments (Corti et al. 2011; Calapez et al. 2014; Elias et al. 2015), mainly by placing standardized leaf bags in natural streams (Yoshimura et al. 2010) but, to our knowledge, no studies have directly compared CPOM decomposition data from natural field and artificial flume experiments (but see Bækkelie et al. 2017). By comparing data from a field study and a flume experiment, our aim was to estimate if CPOM processing rates and trends in macroinvertebrate community composition in artificial flume experiments can act as an effective proxy for natural conditions. We hypothesized that, in both types of experimental conditions: a) decomposition rates of comparable leaf litter types are higher at perennial than intermittent sites; b) intermittence reduces macroinvertebrate community taxa richness; c) shredder abundance is positively related to the presence of flowing water; and d) data generated by flume experiments represent a valid proxy to assess CPOM processing.

Materials and methods

Two analogous experiments were carried out under different conditions: a simulation in flumes connected to the Fersina Stream (Trentino Alto Adige, eastern Italian Alps), and a field experiment in the Pellice and Varaita Streams (Piemonte, western Italian Alps). The two experiments were comparable in: a) duration (100 and 105 days, respectively); b) leaf litter quality, i.e. European beech (*Fagus sylvatica*) and oak (*Quercus robur*), respectively, which are both low-quality litters and were selected as the dominant riparian species in the study areas (McKie & Malmqvist 2009; Sanpera-Calbet et al. 2009; Martínez et al. 2016); c) river type (gravel-bed streams *sensu* Rinaldi et al. 2016, draining from the Southern Alps) and the dominant sediment type (see below). The study sites were in slightly different geographic settings and the experiments were conducted in different seasons: the flume experiment from autumn to winter boreal seasons, and the field experiment from winter to early spring. Despite these differences, both experiments were carried out in cold seasons during which natural leaf litter input peaks due to abscission, and benthic communities comprise taxa adapted to exploit these seasonal resources (Richardson 1991; Johnson et al. 2012). Leaf litter inputs from riparian vegetation represent an important energy source, especially in low- and mid-order streams (Doretto et al. 2017). Moreover, the absence of the chrysophyte algae *Hydrurus foetidus* in the selected reaches (which is generally preferred over allochthonous material by aquatic decomposers in mountain streams; Zah et al. 2001; Niedrist & Füreder, 2017) supports our expectation that allochthonous leaf material is the primary food resource in both experiments. We thus expected the effects of drying on macroinvertebrate communities and CPOM processing rates to override seasonal effects.

Artificial flumes

The artificial experiment was conducted in a set of open-air metal flumes situated in the riparian zone of the Fersina Stream (46° 04' 33" N; 11° 16' 27" E), a 2nd order perennial stream at an elevation of 580 m asl. The Fersina is a 14-km snowmelt-fed gravel-bed stream with a 171-km^2 catchment area, and with numerous small tributaries inflowing from lateral valleys. The experimental set-up—which has been used in similar ecological experiments e.g. by Bruno et al. (2013), Grubisic et al. (2017) and Doretto et al. (2018)—was located on the right bank of a wandering reach (*sensu* Rinaldi et al. 2016), and comprised five 20-m long, 30-cm wide, 30-cm high metal flumes. The flumes were filled with two layers of ca. 10-cm diameter cobbles and a layer of silt, sand and gravel, which naturally collected on the flume base. Each flume has sluice gates to control discharge and supply from a 1-m^3 loading tank fed directly by diverted stream water. Sluice gates were closed to block incoming flow and simulate dry periods. Flumes were run undisturbed from early April 2016 at a baseflow of 5 l s^{-1}, velocity 0.4 m s^{-1}, to allow natural colonization by benthic macroinvertebrates and periphyton, e.g. by drift from upstream sites and/or egg deposition. The experiment started on 20 October 2016 and lasted 100 days, ending on 30 January 2017. We used two flumes: a control flume, in which continuous flow was maintained throughout the experiment; and an intermittent treatment flume, in which we alternated 20 days of flow with 20 dry days two times, ending the experiment with 20 days of flow after the second dry period. Due to low air temperatures limiting evaporation, 3–5 cm of water remained around some cobbles, forming irregular 20–30-cm long, flume-width pools, similar to those that occur in natural conditions. In each flume, we installed 50 leaf bags (weight 3.0 ± 0.1 g) with a 1-cm mesh (to allow macroinvertebrate colonization) and filled with *F. sylvatica* leaves, which were collected in September 2016, air-dried for 15 days and stored in dark, dry conditions until the experiment began. We tied 25 leaf bags to each of two ropes, which were in turn fixed to the flume base. After leaf bags were deployed on 20 October (i.e., T0), sampling was conducted every 20 days on five subsequent dates (i.e., T1–T5), regardless of the duration of flow/dry period (Table 1). Before the experiment, conductivity, pH and dissolved oxygen concentrations were measured in each flume using WTW© meters (WTW GmBH, Weilheim, Germany). A HOBO® TidbiT v2 water temperature data logger (Onset Computer Corporation, Bourne, MA, USA) was placed in each flume to measure water temperature at 6-h intervals throughout the experiment, and data used to identify dry periods, with lower, more variable temperatures indicating that loggers were exposed to the air. On each sampling date, seven leaf bags were randomly removed from each flume, placed into a container, refrigerated and transported to the laboratory, where the leaf material from each bag was rinsed with distilled water to remove macroinvertebrates and inorganic particles.



Site	Calendar date	Sampling date	Instream conditions
Flume	20 Oct 2016	T0	Flow
	10 Nov 2016	T1	Start of first dry event
	30 Nov 2016	T2	Flow resumed
	20 Dec 2016	T3	Start of second dry event
	10 Jan 2017	T4	Flow resumed
	30 Jan 2017	T5	Flow, end of experiment
Field	13 Dec 2018	T0	Flow
	03 Jan 2019	T1	Flow
	24 Jan 2019	T2	Dry
	14 Feb 2019	T3	Partial flow resumption
	07 Mar 2019	T4	Low flows
	29 Mar 2019	T5	Low flows, end of experiment

Table 1. Sampling dates for flume and field experiments, indicating the instream conditions in the intermittent treatment in each experiment. T0, leaf bag deployment; T1–T5, sample collection.

The remaining leaf bag material was rinsed with water, air dried for 24 h in dark, dry and cool (approx. 13°C) conditions to prevent microbial activity, and subsequently oven-dried (24 h, 80°C) and weighed to the nearest 0.1 g, to determine dry mass and leaf litter mass loss. All macroinvertebrates were preserved in 80% ethanol, and then later identified to family or genus following Campaioli et al. (1994, 1999) and Waringer & Graf (2011). Shredder macroinvertebrates (mainly Nemouridae, Sericostomatidae, Limnephilidae, Tipulidae and Gammaridae) were classified as such following Tachet at al. (1984).

Field experiment

The Pellice Stream is approximately 53 km long, has a 975-km^2 catchment, and is the largest left tributary of the Po River in the Cottian Alps area. The sampling site (hereafter, *perennial*) was a reach in the upstream, unconfined, cobble/gravel-dominated wandering section (44° 49' 2.41" N; 7° 18' 47.77" E, 372 m asl) in which flowing surface water was present throughout the study. The Varaita Stream is the first right tributary of the Po River, is approx. 92.4 km long with a 605-km^2 catchment area. The Varaita sampling site (hereafter, *intermittent*) was a reach in the upstream, unconfined, cobble/gravel-dominated wandering section (44° 35' 1.06" N; 7° 31' 28.99" E, 398 m asl) in which drying events occurred during the study. In recent years, this section has experienced rapid-onset drying events that culminate in complete surface water loss (see Falasco et al. 2018). Our knowledge of these drying events is supported by our recent research in the study area (e.g. see Piano et al. 2019a). More information about environmental characteristics of two study sites are reported in Falasco et al. (2018). At each site, we deployed 60 1-cm-mesh leaf bags, each containing 5.0 ± 1.0 g dry oak (*Q. robur)* leaves, which were collected in October 2018, air-dried for 15 days and stored in dark, dry conditions until the start of the experiment. The field campaign began on 13 December 2018 (i.e, T0), lasted 105 days (ending on 29 March 2019), and included five sampling dates at 20–21-day intervals (i.e., T1–T5, Table 1). Conductivity, pH and dissolved oxygen concentrations were measured on each sampling date using a Hydrolab Quanta probe, and water temperatures were logged and used to assess dry periods as in the flumes (Fig. 1). Seven randomly selected leaf bags were removed every 21 days and stored in 80% ethanol prior to processing. In the laboratory, leaves were rinsed with water to remove ethanol, oven-dried at 105°C for 24 h, then dried material weighed to the nearest 0.1 g to determine dry mass and leaf litter mass loss. All macroinvertebrates were treated as in flume experiments, with Campaioli et al. (1994, 1999), Sansoni (1988) and Tachet at al. (1984) used to classify shredders.

Data analysis

We used Cleveland dot plots to identify outliers and removed four shredder abundance values to enable pattern detection, two each from the flume and field datasets. For both datasets, variation in CPOM decomposition rates over time in the perennial and intermittent treatments was analyzed following Benfield et al. (2017).

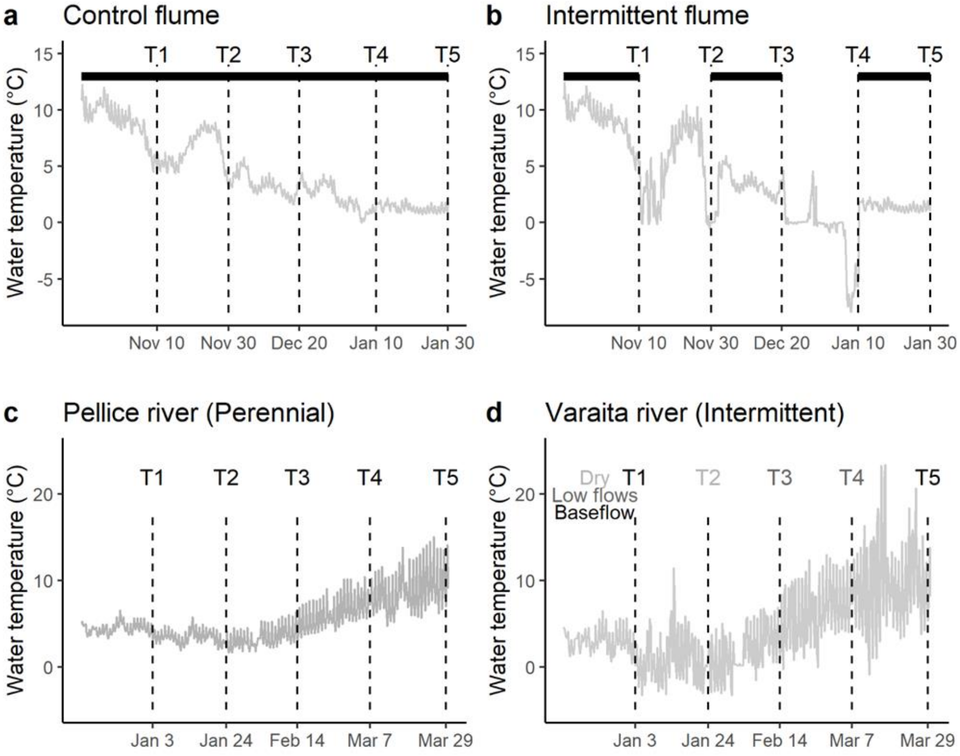
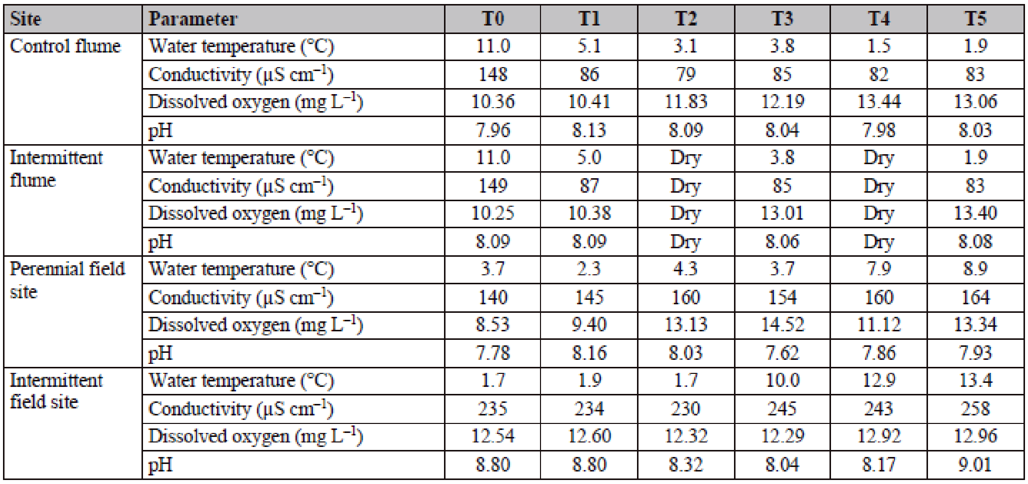


Fig. 1. Water temperature recorded at 6-h intervals during the experiment in flumes (a–b; black solid lines indicate water flow) and at field sites (c–d).



Site	Parameter	T0	T1	T2	T3	T4	T5
Control flume	Water temperature (°C)	11.0	5.1	3.1	3.8	1.5	1.9
	Conductivity (µS cm^{-1})	148	86	79	85	82	83
	Dissolved oxygen (mg L^{-1})	10.36	10.41	11.83	12.19	13.44	13.06
	pH	7.96	8.13	8.09	8.04	7.98	8.03
Intermittent flume	Water temperature (°C)	11.0	5.0	Dry	3.8	Dry	1.9
	Conductivity (µS cm^{-1})	149	87	Dry	85	Dry	83
	Dissolved oxygen (mg L^{-1})	10.25	10.38	Dry	13.01	Dry	13.40
	pH	8.09	8.09	Dry	8.06	Dry	8.08
Perennial field site	Water temperature (°C)	3.7	2.3	4.3	3.7	7.9	8.9
	Conductivity (µS cm^{-1})	140	145	160	154	160	164
	Dissolved oxygen (mg L^{-1})	8.53	9.40	13.13	14.52	11.12	13.34
	pH	7.78	8.16	8.03	7.62	7.86	7.93
Intermittent field site	Water temperature (°C)	1.7	1.9	1.7	10.0	12.9	13.4
	Conductivity (µS cm^{-1})	235	234	230	245	243	258
	Dissolved oxygen (mg L^{-1})	12.54	12.60	12.32	12.29	12.92	12.96
	pH	8.80	8.80	8.32	8.04	8.17	9.01

Table 2. Physicochemical parameters recorded on each the sampling dates (T0–T5) in flume and field experiments.

The leaf mass remaining, as a % of the initial weight of each leaf bag, was log-transformed and linearly regressed against time (number of days since the experiment onset), with the negative slope of the regression line corresponding to the processing coefficient (i.e. -*k*; Benfield et al. 2017). Due to the temporal dependency of our data, we calculated this slope using linear mixed models (LMM) as per Doretto et al. (2020), with *% leaf mass remaining* as the dependent variable, *day*, *treatment* and their interaction as explanatory variables, and *sampling date* as a random factor. For each flume/stream, differences among sampling dates (i.e. T1–T5) in taxa richness and shredder abundance were tested with Kruskal-Wallis tests (H) and, when significant, pairwise comparisons were performed with Mann-Whitney U-tests. To account for differences in colonization by shredders in relation to CPOM quantity, shredder abundance was standardized by dividing the number of shredders by the quantity (g) of mass remaining for each leaf bag. We visualized variability in the taxonomic composition of macroinvertebrate assemblages in flume and field leaf bags using two-dimensional non-metric multidimensional scaling (NMDS) ordinations. A two-way permutational analysis of variance (PERMANOVA; Anderson 2001) was used to test the effects of *treatment* (intermittent, control/perennial), *sampling date* (T1–T5) and their interaction on community composition. NMDS and PERMANOVA were based on a dissimilarity matrix of Bray-Curtis distances calculated using log(x+1) abundance data. We analyzed the multivariate homogeneity of group dispersions (PERMDISP) as a measure of β-diversity, for both the control and intermittent communities in each experiment (Anderson 2006; Piano et al. 2020). Welch two-sample t-tests were used to statistically compare distances from the PERMDISP group centroid for the two communities. Indicator Species Analysis (i.e. IndVal; Dufrêne & Legendre 1997) was used to identify taxa indicative of perennial and intermittent conditions in both experiments and thus responsible for compositional differences. To investigate the mechanisms underlying variation in community composition, we calculated the Sørensen dissimilarity index as a measure of total β-diversity and partitioned it into its nestedness and turnover components following Cardoso et al. (2015, 2020). To evaluate the temporal changes in each component associated with leaf litter breakdown, taxa recorded in leaf bags on the first removal date (T1) were pooled and considered as baseline communities for each flume/site. We compared total β-diversity, nestedness and turnover between the baseline communities and those present on each subsequent sampling date (i.e. T1 vs. T2, T1 vs. T3 etc.) for each flume/stream. Analysis of variance (ANOVA) was used to evaluate differences in total β-diversity between the two experiments. All analyses were performed in R (R Core Team 2019), using basic functions and the packages lme4 (Bates et al. 2011) for LMM, vegan (Oksanen et al. 2015) for NMDS, PERMANOVA and PERMDISP, indicspecies (Cáceres & Legendre 2009) for Indicator Species Analysis and BAT (Cardoso et al. 2015, 2020) to partition β-diversity. Plots were drawn using the packages ggplot2 (Wickham 2016) and ggpubr (Kassambara 2017).

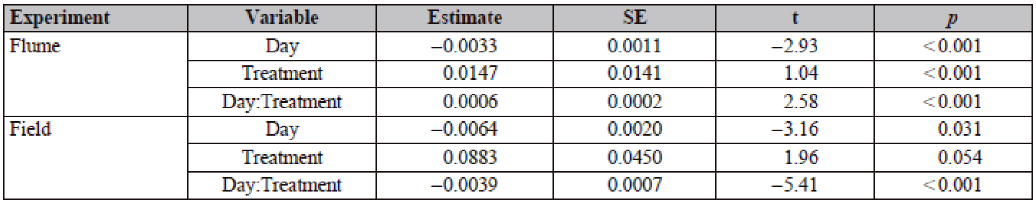
Results

Environmental conditions

In the field experiment, lower, more variable temperatures indicated that a dry phase started in the intermittent stream between T1 and T2 (i.e. after 21–42 days), completely exposing most leaf bags to air (Fig. 1d). Water flow then partially returned by T3 (after 63 days) due to inflows from a small tributary, and low flows continued until the end of the experiment (Table 2). In the flumes, water temperatures decreased from approx. 13°C at the beginning of the experiment to 3°C at the end (mean 4.06 ± 3.60°C, range: -8.06 – 12.44°C), whereas in the field, temperatures increased over time from approx. 5 to 20°C (mean 4.88 ± 3.85°C, range: -3.32 – 23.38°C; Fig. 1). Despite these contrasting thermal patterns, water temperatures always declined during dry events, in both experiments.

Leaf litter decomposition

In the flume experiment, day, treatment and their interaction had significant effects on the % leaf mass remaining (LMM; Table 3). Mass decreased over time in both flumes, but the decrease was steeper in the control than the intermittent flume, and the % leaf mass remaining was thus lower in the control at the end of the experiment (Fig. 2a; Table 3). The significant treatment effect reflected a higher % leaf mass remaining in the intermittent treatment (mean ± SD; 74.24 ± 3.54%) compared to the control (70.22 ± 4.50%). Taxa richness was comparable on all dates in the control flume (11.6 ± 3.8 taxa per leaf bag; Kruskal-Wallis test, H = 2.82; df = 4; p = 0.589; Fig. 2b). Richness was also stable over time in the intermittent flume (8.1 ± 3.8 taxa per leaf bag; H = 6.82; df = 4; p = 0.146) although we observed a non-significant decline in richness between T1 and T4 (p = 0.05), when taxa richness was particularly and consistently low (Fig. 2c).



Experiment	**Variable**	**Estimate**	**SE**	**t**	***p***
Flume	Day	–0.0033	0.0011	–2.93	<0.001
	Treatment	0.0147	0.0141	1.04	<0.001
	Day:Treatment	0.0006	0.0002	2.58	<0.001
Field	Day	–0.0064	0.0020	–3.16	0.031
	Treatment	0.0883	0.0450	1.96	0.054
	Day:Treatment	–0.0039	0.0007	–5.41	<0.001

Table 3. Results of linear mixed models testing the effects of treatment (control/perennial, intermittent) and day on % leaf mass remaining in the flume and field experiments.

Temporal variability in shredder abundance differed between the two flumes. In the control flume, shredder abundance was comparable on all dates (3.5 ± 4.8

individuals g^{-1} CPOM; H = 4.07; df = 4; p = 0.397; Fig. 2d). In the intermittent flume, shredder abundance was highest at T1 (4.4 ± 2.5 individuals g^{-1} CPOM), decreased at T2 (1.5 ± 1.6) and then remained low (H = 14.97; df = 4; p = 0.005; Fig. 2e). In the field experiment, day and its interaction with treatment, but not treatment alone, had significant effects on the % leaf mass remaining (LMM, Table 3). As in the flume experiment, the % leaf mass remaining decreased over time in both streams (Fig. 3a), this decrease was greater in the perennial than the intermittent stream, and thus the % mass remaining was higher in the perennial stream at the start of the study but lower in this stream by the end. Taxa richness was moderately higher in the intermittent stream (4.57 ± 2.37 taxa per leaf bag) compared to the perennial stream (2.14 ± 1.35) at the beginning of the experiment, and the two streams then experienced contrasting temporal changes in taxa richness. In the perennial stream, mean richness increased between T1 (2.1 ± 1.4 taxa per leaf bag) and T4 (6.7 ± 2.3; Kruskal-Wallis test, H = 16.97; df = 4; p = 0.002; Fig. 3b) whereas in the intermittent stream, richness was comparable on all dates (3.7 ± 2.1; Kruskal-Wallis test = 7.07; df = 4; p = 0.132; Fig. 3c). In the perennial stream, mean shredder abundance (1.6 ± 4.5 individuals g^{-1} CPOM) increased from T2 to T4 then fell sharply at T5 (Mann-Whitney test, U = 21.99; df = 4; $p < 0.001$; Fig. 3d), whereas abundances were comparable on all dates in the intermittent stream (U= 2.4507; df = 4; p = 0.654; Fig. 3e).

Macroinvertebrate community composition

The taxa recorded in each experiment and each treatment are listed in Tables S2–S5. Community composition was characterized by EPT taxa in the control/perennial reaches, mainly *Isoperla* and Rhyacophilidae (mean ± SD community composition differed between dates (PERMANOVA: $F_{4,69} = 3.25$; $p < 0.001$); 6.0 ± 9.0 and 5.0 ± 4.8 individuals per leaf bag, respectively) in the control flume and Nemouridae and Limnephilidae (5.0 ± 5.7 and 3.6 ± 2.7, respectively) in the perennial stream. Intermittent reaches were dominated by Diptera, mainly Chironomidae (1442 ± 568 individuals per leaf bag in the flume; 199 ± 203 in the field) and the Ephemeroptera genus *Baetis* in the intermittent flume (651 ± 237). In the flumes, macroinvertebrate community composition differed between dates (PERMANOVA: $F_{4,69} = 3.25$; $p < 0.001$), treatments ($F_{1,69} = 15.34$; $p < 0.001$) and their interaction ($F_{4,69} = 3.72$; $p < 0.001$). The NMDS ordination (2D-stress = 0.138) showed compositional overlap in the control and intermittent flumes at T1, but by T2 the intermittent flume communities were more dispersed than those in control flumes (PERMDISP; Welch two-sample t-test: $t = 5.07$; $df = 65$; $p < 0.001$; Fig. 4a), and this dispersion increased over time. Seven taxa (*Rhithrogena*, *Isoperla*, Rhyacophilidae, Nemouridae, Leptophlebiidae, *Ecdyonurus*, *Perlodes*) were significantly associated with control flumes, whereas no taxa were indicative of the intermittent treatment (IndVal; Table S1). Mean β-diversity was lower and relatively stable over time in the control flumes (Sørensen dissimilarity index; 0.35 ± 0.05, range 0.26–0.39; Fig. 4b), and was higher and more variable in the intermittent flumes (0.51 ± 0.12, range

0.64–0.91; Fig. 4c). In the control flume, differences in the total β-diversity between baseline (T1) communities and those sampled at T2 and T5 were due mainly to nestedness (98%), whereas the contribution of turnover was 63% at T3 and 47% at T4 (Fig. 4b). In the intermittent flume, nestedness contributed ≥ 97% to β-diversity on all sampling dates (Fig. 4c).

The NMDS ordination (2D-stress = 0.037) showed similar patterns to the flume experiment: at T1, composition overlapped in the perennial and intermittent streams, then from T2 onwards, the intermittent stream communities were more dispersed than perennial communities (PERMDISP; Welch two-sample t-test: $t = 4.32$; $df = 54$; $p < 0.001$; Fig. 5a) and, over time, the intermittent stream communities became increasingly variable.

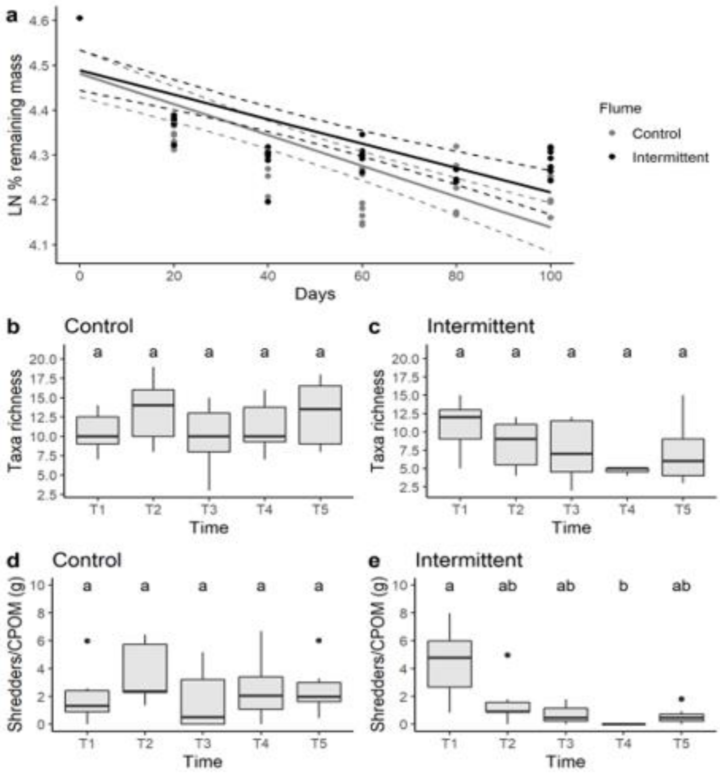


Fig. 2. Results from the flume experiments: (a) log-transformed % leaf mass remaining on each sampling date (days 20–100, equivalent to T1–5 on b–e) following deployment (day 0). Metrics measured on each sampling date: taxa richness in (b) the control flume and (c) the intermittent flume; shredder abundance in (d) the control flume and (e) the intermittent flume. Black line: median value; lower and upper box edge: first and third quartiles, respectively; vertical lines: whiskers (± 1.5 interquartile distance). Letters above boxes indicate comparable results (b–d) and significant differences (e) identified by pairwise Mann-Whitney U-tests. In the field experiment, community composition differed between dates (PERMANOVA; $F_{4,67} = 5$; $p < 0.001$), streams ($F_{1,67} = 30$; $p < 0.001$) and in response to their interaction ($F_{4,67} = 3$; $p < 0.001$).

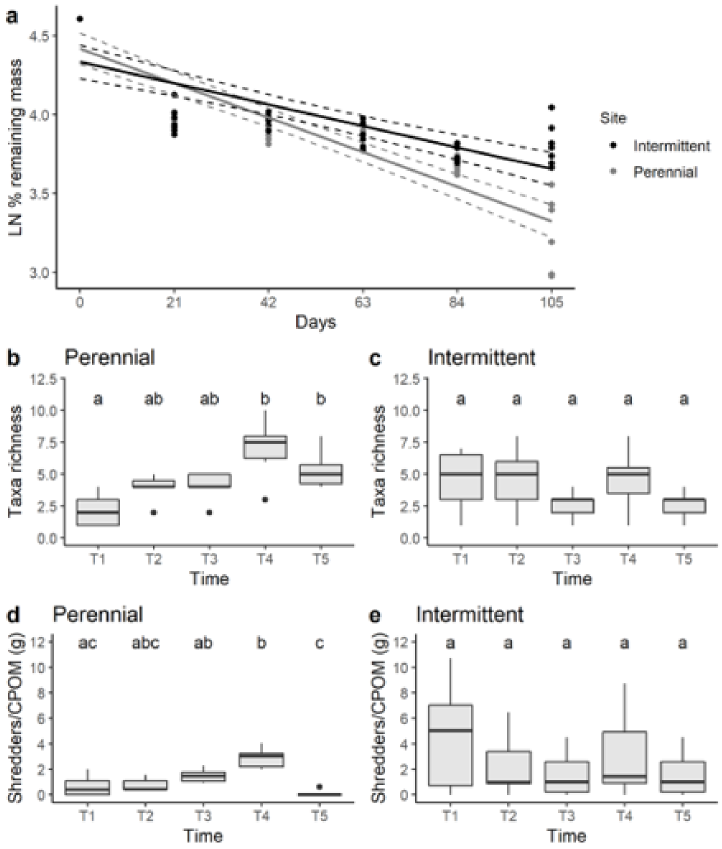


Fig. 3. Results from the field experiments: (a) log-transformed % leaf mass remaining; taxa richness in (b) the perennial stream and (c) in the intermittent stream; shredder abundance in (d) the perennial stream and (e) the intermittent stream. Further detail is provided in the Fig. 2 legend.

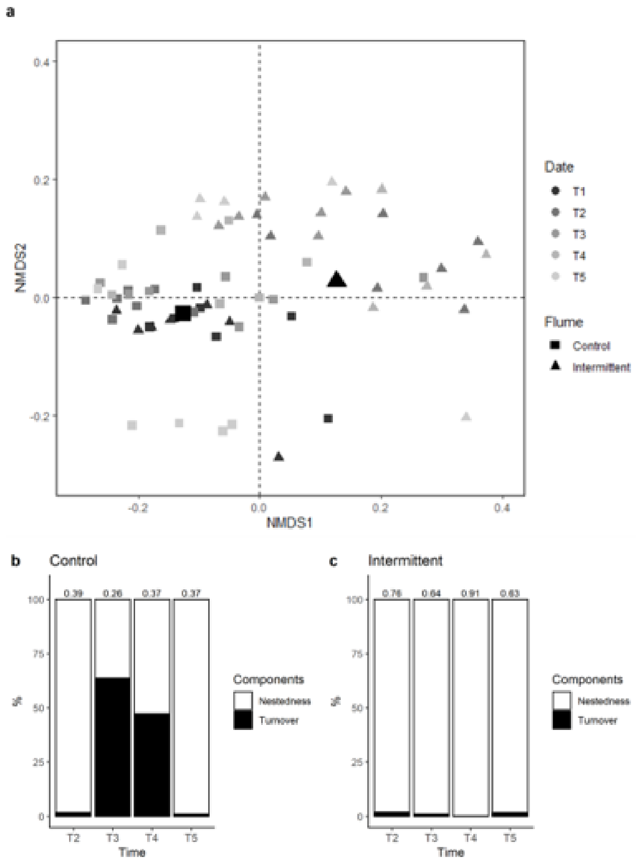


Fig. 4. (a) Non-metric multidimensional scaling (NMDS) ordination of macroinvertebrate community composition in the artificial flumes; missing symbols reflect the removal of the outliers (*n* = 2), and larger black symbols represent centroids for each flume. The % of total β-diversity explained by nestedness and turnover for (b) control and (c) intermittent flumes. Numbers above the bars indicate total β-diversity: 0 and 1 indicate that communities have no taxa or all taxa in common, respectively. T1 was used as a baseline against which to compare β-diversity, nestedness and turnover.

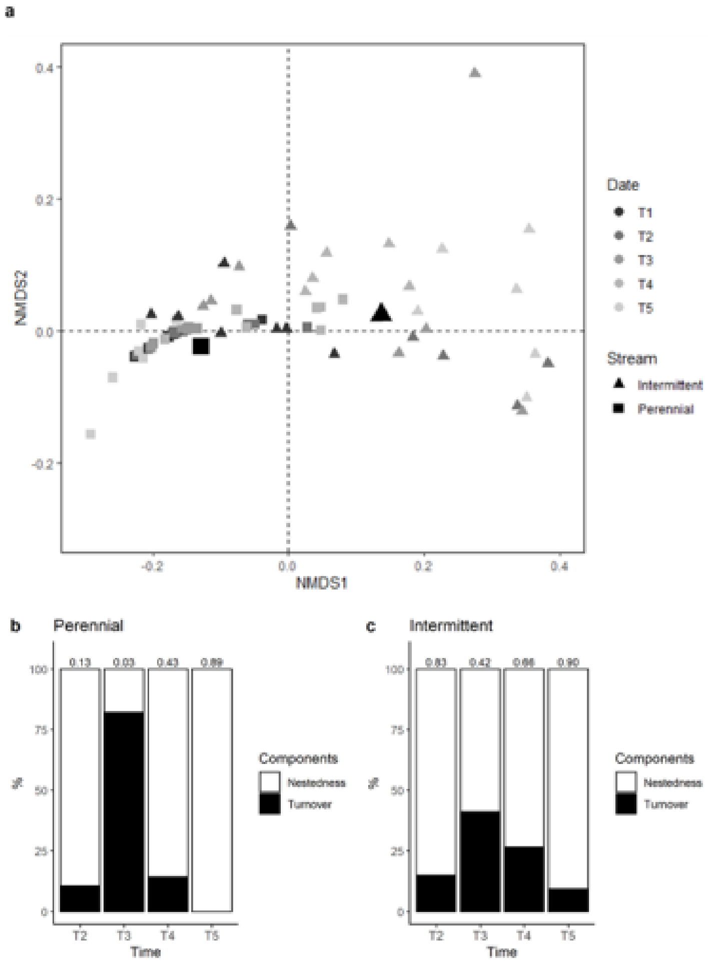


Fig. 5. (a) Non-metric multidimensional scaling (NMDS) ordination of macroinvertebrate community composition in the natural streams; missing symbols reflect the removal of the outliers (*n* = 2), and larger black symbols represent centroids for each stream. The % of total β-diversity explained by nestedness and turnover for (b) perennial and (c) intermittent streams. Further detail is provided in the Fig. 4 legend.

Three families (Nemouridae, Limnephilidae, Ceratopogonidae) were significantly associated with the perennial stream, and three other families (Elmidae, Simuliidae, Athericidae) were indicative of intermittent conditions (IndVal; Table S1).

In the perennial stream, β-diversity was very variable (Sørensen dissimilarity index; 0.37 ± 0.36, range 0.03–0.89), being particularly low at T3 (0.03) and very high at T5 (0.89). In the intermittent stream, the trend was similar but less pronounced: mean β-diversity (0.70 ± 0.19) was lowest at T3 (0.42) and increased to a peak at T5 (0.90). In the perennial stream, community changes were mainly (≥86%) due to nestedness on all dates except T3, when minimal β-diversity was explained largely (82%) by turnover (Fig. 5b). Nestedness was also the primary contributor to total β-diversity in the intermittent stream (85%), with the contribution of turnover peaking at 41% at T3 (Fig. 5c).

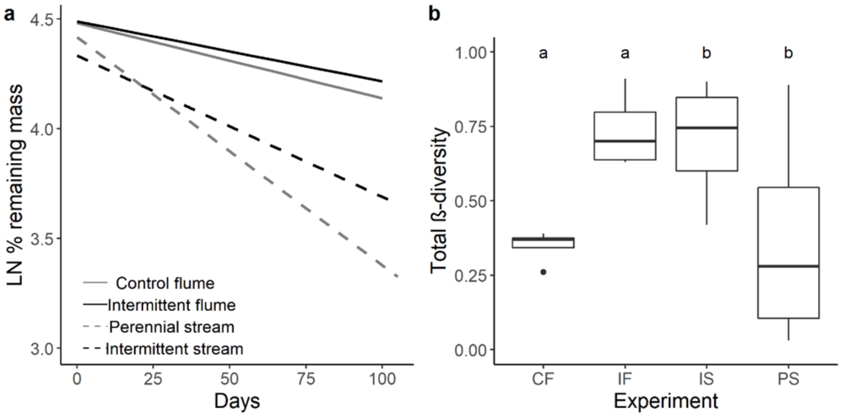


Fig. 6. Comparison of flume and field experiments: (a) log-transformed % leaf mass remaining on deployment and on each sampling date. (b) Total β-diversity in intermittent and perennial conditions in flume and field experiments. CF = Control flume; IF = Intermittent flume; IS = Intermittent stream; PS = Perennial stream.

Comparison between flume and field experiments

We observed some differences between our flume and field experiments, especially in relation to leaf litter decomposition. Differences in temporal trends in CPOM processing between flume and field experiments were greater than differences between control and intermittent treatments: decomposition rates (-*k*, which corresponds to the LMM estimates in Table 3) were two-fold higher in streams (-0.0064) than flumes (-0.0033; Fig. 6a). However, similarities were also observed: first, in both the flume and field experiments, leaf litter processing rates were lower in intermittent compared to control/perennial conditions, with a lower % leaf mass remaining in the latter at the end of both experiments (Fig. 6a). Second, total community β-diversity was higher in the intermittent compared to control/perennial conditions in both experiments (ANOVA: $F_{3,28} = 8$; $p < 0.001$; Fig. 6b).

Discussion

Our primary aim was to assess the validity of artificial flume systems as a proxy for natural streams in ecological experiments. Macroinvertebrate community composition and leaf litter decomposition responses differed in flume and field experiments, reflecting natural environmental variability in field experiments, which was controlled in flumes. Despite this variability, we detected some consistent patterns, suggesting that ecosystem processes in controlled flume experiments can approximate the general trajectories of those occurring in natural conditions. Our study also provided an opportunity to examine the effects of flow intermittence on leaf litter breakdown and macroinvertebrate community composition, including β-diversity. Despite the differences between the field and flume experiments and despite flumes representing simplified systems (Stewart et al. 2013; Menczelesz et al. 2020), we observed strong effects of dry events on CPOM decomposition, with consistent differences in the relative magnitude of leaf litter mass loss indicating streambed drying as a primary determinant of the structure and function of intermittent stream communities (Schlief & Mutz 2011; Leigh & Datry 2017). The differences in final absolute values of % leaf mass remaining probably reflect differences in water temperature trends between experiments: temperatures gradually decreased and increased during the flume and stream experiments, respectively, but became lower and more variable during dry phases in both experiments.

Leaf litter decomposition

In our experiments, dry phases occurred in cold seasons and results showed both contrasting and comparable patterns between artificial flumes and natural streams. First, direct comparison of temporal variation in the % mass remaining indicated that–regardless of treatment–CPOM decomposition rates were two times higher in streams than flumes and comparable to previously recorded rates (Petersen & Cummins 1974; Schlief & Mutz 2009), suggesting that the experiment type influenced CPOM decomposition rates. Compared to the field experiment, flume water temperatures were lower and decreased over time as the season shifted from autumn to winter. Low temperatures could have affected microbial conditioning of leaf litter, with occasional freezing potentially constraining fungal diversity, activity and biomass (Fernandes et al. 2009) and thus delaying leaf decomposition (Nikolcheva & Bärlocher 2005). Despite this variability, when the experiments were considered separately—and perennial and intermittent conditions thus were analyzed under comparable thermal conditions—we detected similar trends. First, the decrease in % leaf mass remaining was consistently greater in the control/perennial treatment compared to intermittent treatments, so that at the end of both experiments, the % remaining was lower in perennial conditions. As a generalizable pattern rather than a context-dependent result, this finding may be more ecologically meaningful than the absolute magnitude of the CPOM decomposition. As such, our findings suggest that, as in other regions (e.g. Datry et al. 2011), CPOM decomposition in Alpine streams is faster and more efficient in perennial than intermittent streams, because dry conditions desiccate leaves, reduce microbial activity and affect macroinvertebrate communities, including taxa which feed primarily on CPOM (i.e. shredders).

Macroinvertebrate community composition

Macroinvertebrate community composition overlapped in the control/perennial and intermittent conditions after 20–21 days in both experiments, indicating similar colonization processes in both flow regimes. As the dry period progressed, and despite the later onset of drying in the stream than the flume, community composition in both intermittent streams and flumes became increasingly variable—more so than in the corresponding perennial stream/flume. Similarly, in a study conducted in the same flumes, macroinvertebrate communities exposed to alternating flowing and dry phases had a more heterogeneous taxonomic composition than those experiencing continuous flow, due to their temporal instability (Doretto et al. 2018). Such compositional heterogeneity probably reflects the greater influence of priority effects during the initial stages of colonization after a drying disturbance ends (Little & Altermatt 2018). Indicator Species Analysis identified no indicator taxa for the intermittent flume communities, which were dominated by Diptera, especially Chironomidae, and Ephemeroptera of the genus *Baetis*—both small-sized, plurivoltine taxa with rapid larval development (Tachet et al. 1984). In contrast, seven taxa were indicative of the control flumes, all EPT, highlighting the sensitivity of these orders to flow cessation and drying (Dewson et al. 2007; Datry et al. 2014b). Usseglio-Polatera et al. (2000) classify these taxa into groups “c” (*Isoperla, Perlodes* and Rhyacophilidae) and “f” (*Ecdyonurus,* Leptophlebiidae, Nemouridae and *Rhithrogena*), which include medium to large-sized, univoltine macroinvertebrates, mostly predators or shredders. Similarly, in the field experiment, the medium to large-sized families Nemouridae, Limnephilidae (group “f”) and Ceratopogonidae (group “c”) were identified as indicative of perennial flow. In contrast, Elmidae, Simuliidae (biological group “e”) and Athericidae (biological group “d”) were associated with intermittent flow, of which Elmidae and Simuliidae are small-sized and plurivoltine (*sensu* Usseglio-Polatera et al. 2000). The contrasting results of flume and the field experiments may also reflect differences in the availability of instream refuges such as pools and/or saturated hyporheic sediments, which can support macroinvertebrate persistence during dry phases (Chester & Robson 2011; Stubbington 2012). However, in the intermittent Varaita Stream, the water table rapidly drops deep beneath the bed after dry phase onset and pools are lost (Bruno et al. 2020; Doretto et al. 2020), and similarly, pools and saturated subsurface sediments were not well-developed during dry periods in the flumes. Equally, the minimal contribution of aerial colonists to macroinvertebrate communities is likely to have been comparable in the two experiments, which were both conducted during winter (Doretto et al. 2018). Drift from upstream reaches was thus probably the main mechanism facilitating community recovery after dry periods (Doretto et al. 2020), with the densities and taxonomic identities of colonists differing in the flumes and stream. Despite any variability introduced by such differences, we observed consistent patterns in field and flume experiments, with macroinvertebrate communities in control/perennial conditions comprising medium to large-sized, univoltine shredders and predators, whereas these traits were selected against by flow intermittence (Acuña et al. 2015; Piano et al. 2019b).

Beta diversity: the mechanisms behind variation in community composition

Communities that colonized both control flumes and perennial field sites were taxonomically richer and more stable than those experiencing intermittent flow, with nestedness indicating that dry events acted as an environmental filter on community composition. Turnover explained most of the variation within total β-diversity only on T3 (i.e. 60 days after the experiment began) and only in control/perennial conditions, when competitive macroinvertebrates probably began to colonize CPOM after its conditioning in water and to replace early colonists (Rossi 1985; Hieber & Gessner 2002). Specifically, taxa including *Epeorus* and Sericostomatidae replaced *Leuctra*, Polycentropodidae and Ceratopogonidae in the control flume, while *Isoperla*, Rhyacophilidae, Limnephilidae and Ceratopogonidae replaced Baetidae and *Serratella* in the perennial stream. The dominance of nestedness on all other dates mainly reflected the recruitment of new taxa in perennial conditions, and taxa loss in intermittent conditions. Moreover, total β-diversity was particularly stable over time in the control flumes, whereas wider temporal variation in intermittent flumes indicated that drying reduced the temporal stability of their leaf-bag assemblages. Total β-diversity could also have been limited in flumes because their communities comprise a subset of the taxa present in natural streams, mainly due to the simplified morphology of the flumes. Other studies (Ledger et al. 2009; Bækkelie et al. 2017) have reported a lower diversity in experimental flumes than in natural stream ecosystems, and simplified communities may limit biotic and trophic responses to drying, including leaf litter processing.

Conclusions

Experimental flumes have become a common tool to study biological community responses to changes in instream conditions, enhancing the study of ecological processes in freshwater ecosystems (Bruno et al. 2013; Menczelesz et al. 2020 and references therein). The controlled experimental conditions in artificial flumes allow investigation of the independent and interactive effects of specific factors, thus characterizing relationships which are difficult to disentangle in field conditions. Research conducted in artificial streams can thus enable generation of testable hypotheses (Lamberti & Steinman 1993), which can be validated in natural stream ecosystems. We are not aware of previous research comparing field and mesocosm experiments in mountain and/or mid-elevation running waters. In particular, the effects of drying events and shifts from perennial to intermittent flow remain poorly investigated in these streams, especially during winter (Fenoglio et al. 2007; Bruno et al. 2020). Our comparison provided both contrasting and comparable results: major differences between experiment types included CPOM mass loss rates, which was markedly lower in flumes than streams regardless of flow treatment. Subtler differences were observed in temporal variation in shredder abundance and in the contribution of nestedness and turnover to total β-diversity. Equally, consistent patterns were observed across experiments. First, leaf litter decomposition was faster in perennial conditions; second, macroinvertebrate communities at intermittent sites were compositionally different, less temporally stable and taxonomically poorer than those at perennial sites. These compositional differences reflected community nestedness, with the loss of medium to large-sized, univoltine and trophic specialist (shredder and predator) taxa driven by drying (also see Aspin et al. 2019). The comparable patterns observed in the flume and field experiments could enable identification of generalizable patterns and/or predictions. Although our study encompassed few sites in different geographical and seasonal settings, and the flume experiment lacked replicates, our results provide a positive initial evaluation of the use of open-air artificial flumes to assess macroinvertebrate roles and dynamics in organic matter decomposition. Our study indicates that flumes can represent a useful tool to investigate litter breakdown (as also evidenced e.g. by Gessner & Chauvet 2002; Boyero et al. 2011), by solving field-related issues (e.g. loss or burial of leaf bags) and by generating data which can be statistically analyzed with less complex factorial designs than in natural systems. However, our flume study underestimated natural rates of CPOM mass loss, suggesting that mesocosm designs require refinement to enhance their capacity to replicate natural ecosystem processes, especially their magnitude. Improvements may be achieved by mesocosm designs that support macroinvertebrate assemblages comparable to those in natural communities (Ledger et al. 2009), and by increasing the number of replicates (e.g. Ledger et al. 2013). Such refinements could enhance future studies of intermittence which simulate disturbance events and/or manipulate of food webs (e.g. Ledger et al. 2013; Aspin et al. 2019). Indeed, stream-fed open flume systems similar to those used herein are still few compared to closed, often indoor flumes/mesocosms, which are not connected to a natural source of potential colonizers (e.g. see http://mesocosm.org). Open systems represent a useful means of assessing riverine processes, as they enable control of potential confounding factors and can therefore indicate the proportion of variation in field experiments explained by flow intermittence. In any case, our results highlight the need for calibration experiments that quantify the capacity of flume systems to represent natural processes. Such advances could support understanding of how drying events are altering biodiversity and ecosystem functions in mountain streams and more widely, as intermittence increases in extent due to ongoing global climate change.

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Authors' contributions

Conceptualization: LG, AD; writing—original draft preparation: LG; formal analysis: LG, AD; laboratory work: LG, AD, MCB; all authors set the experimental designs, performed the field work, contributed critically to the drafts and gave final approval for publication.

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FLOW INTERMITTENCY AFFECTS LEAF DECOMPOSITION AND BENTHIC CONSUMERS COMMUNITIES OF ALPINE STREAMS: A CASE STUDY ALONG THE PO RIVER

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Abstract

Streams and rivers are becoming increasingly intermittent in Alpine regions due to the global climate change and related increases of local water abstractions, making it fundamental to investigate the occurrence of supraseasonal drying events and their correlated effects. We aimed to investigate leaf litter decomposition, the C:N ratio of the litter, and changes in associated macroinvertebrate communities in three reaches of the Po River: One upstream, consistently perennial, a perennial mid-reach with high hydrological variability, and an intermittent downstream reach. We placed leaf litter bags of two leaf types—chestnut and oak; both showed comparable decomposition rates, but the remaining litter mass was different and was attributed to the C:N ratio and palatability. Furthermore, (1) in perennial reaches, leaf litter decomposed faster than in the intermittent ones; (2) in intermittent reaches, the C:N ratio showed a decreasing trend in both leaf types, indicating that drying affected the nitrogen consumption, therefore the conditioning phase; (3) associated macroinvertebrate communities were richer and more stable in perennial reaches, where a higher richness and abundance of EPT taxa and shredders was observed. Our results suggest that the variations in the hydrology of mountain streams caused by global climate change could significantly impact on functional processes and biodiversity of benthic communities.

Keywords: leaf bags; global climate change; dry rivers; benthic community; CPOM decomposition

Introduction

Over the last few decades, the phenomenon of riverbed drying in mountain streams has gained increasing attention from researchers [1–3]. In the Italian Alps, effects of climate change such as increasing temperatures and decreasing winter precipitation are also coupled with anthropogenic pressures (i.e., water abstraction) [4,5]. This phenomenon is quite recent in those streams that were previously considered perennial, and its effects must be investigated to fully understand the potential short- and long-term ecological consequences to river and watershed ecosystems. Riverbed drying events can negatively affect ecological processes from primary instream production to allochthonous coarse particulate organic matter (CPOM) decomposition [6]. Since leaf litter decomposition plays a key role in low-order stream nutrient cycling, studying this process has been proposed as a tool to assess river ecosystem quality and functional stream integrity [7,8]. Indeed, river drying disturbances simplify food webs, making litter processing less efficient [9].

CPOM degradation process is facilitated by both physicochemical (i.e., leaching of soluble compounds and physical abrasion) and biological (i.e., microbial conditioning and invertebrates' fragmentation activity) mechanisms [10–12]. Intrinsic factors, such as litter quality, are also important [13], such as leaf C:N ratio, tannin content, and texture [14,15]. Extrinsic factors also contribute to leaf litter decomposition, including temperature and dissolved nutrient concentrations known to affect bacterial and fungal conditioning [16], with impacts on macroinvertebrate communities [17]. When the flow regime shifts from perennial to intermittent, rheophilic macroinvertebrates, among others, are negatively affected because of reduced lotic habitat availability while lentic taxa increase [18,19]. In the Upper Po River catchment, for example, Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa and those with long, desiccation-sensitive aquatic juvenile life stages decrease in abundance in response to dry conditions [20,21]. These events negatively affect shredders as well [22], and their decrease in abundance can further affect trophic interactions and in-stream metabolism [23,24]. Furthermore, the microbial richness can be reduced by water loss, and their activity modified, decreasing leaf litter conditioning important to certain functional feeding groups [25–28]. In mountain piedmont areas, the impacts of supraseasonal drying events (during winter, for example) on benthic communities have been poorly investigated; in such streams, allochthonous CPOM input peaks in autumn and early winter [29], and litter decomposition may be altered by emersion–immersion cycles due to the occurrence of dry periods during this season. Some studies suggest that many macroinvertebrate taxa within these streams lack adaptations to face drought periods [30,31]. To investigate this phenomenon along a significant Alpine river, we analyzed the decomposition process for two different quality leaf types and hypothesized that (1) decomposition rate would differ between leaf types; (2) CPOM breakdown would be faster and with a lower percentage of leaf mass remaining where water was present during throughout the study period; (3) macroinvertebrate communities from the intermittent reach would be less stable and diverse; (4) shredders abundance would be positively related with flowing water presence.

Materials and Methods

Study Site

The study was carried out along the Po River (Piemonte, western Italian Alps), a major economically and ecologically important watershed in Europe; the headwaters are located in the Cottian Alps, and it is approximately 652 km long. Three sampling sites were selected along the mountain section of the river (Figure 1): (1) one upstream site, (hereafter Crissolo; 44°42’3.20’’ N, 7°7’58.98’’ E), that is perennial and stable; (2) one downstream site (hereafter Sanfront; 44°39’16.65’’ N, 7°19’26.62’’ E) considered perennial but with high flow variability; (3) an intermittent reach site (hereafter Revello; 44°37’50.35’’ N, 7°24’25.84’’ E) where periodic drying events occur. Our knowledge of the occurrence of drying events is supported by our recent research in the study area [21,32,33] and by historical data of the Environmental Regional Agency [34]. The three reaches show different sediment types, according to the River Continuum Concept: Crissolo sampling site is characterized by rocks and boulders, Sanfront by boulders and cobbles, and Revello by cobbles and gravel.

Experimental Design

In each sampling site, we deployed a total of 120 coarse mesh (1 cm) leaf bags: 60 containing oak leaves (*Quercus robur*) and 60 chestnut leaves (*Castanea sativa*). The leaf species were selected because they are the two most abundant in the stream catchment and are characterized by different qualities. Oak is classified as low-quality leaf litter, with coriaceous leaves, while chestnut has high quality, with less tough and more easily decomposable leaves. Each leaf bag contained 5.0 ± 1.0 g of dry leaves, collected in October 2018 after the abscission, air dried for 15 days, and stored in dark and dry conditions until the onset of the study. The sampling campaign began on 13 December 2018, with sampling occurring every 21 days and ending on 19 April 2019, for a total of 6 sampling dates (Table S1). The seasonal choice was led by the fact that during this season, allochthonous energetic input is particularly significant and, consequently, so is the degradation process. To assess dry periods, water temperature during sampling was logged at 6 h intervals using HOBO® TidbiT v2 data loggers (Onset Computer Corporation, Bourne, MA, USA). Conductivity, pH, and dissolved oxygen concentrations were measured on each sampling date using a Hydrolab Quanta probe. At each 21-day sampling date, 10 randomly selected leaf bags of each type were removed with 7 stored in 80% ethanol before processing and 3 frozen for carbon and nitrogen content analysis. In the laboratory, leaf material was rinsed with water to remove ethanol, oven dried at

105 °C for 24 h, and then weighed to the nearest 0.1 g to determine dry mass (i.e., % residual mass remaining) loss over the sampling dates. Data were expressed as leaf mass remaining.

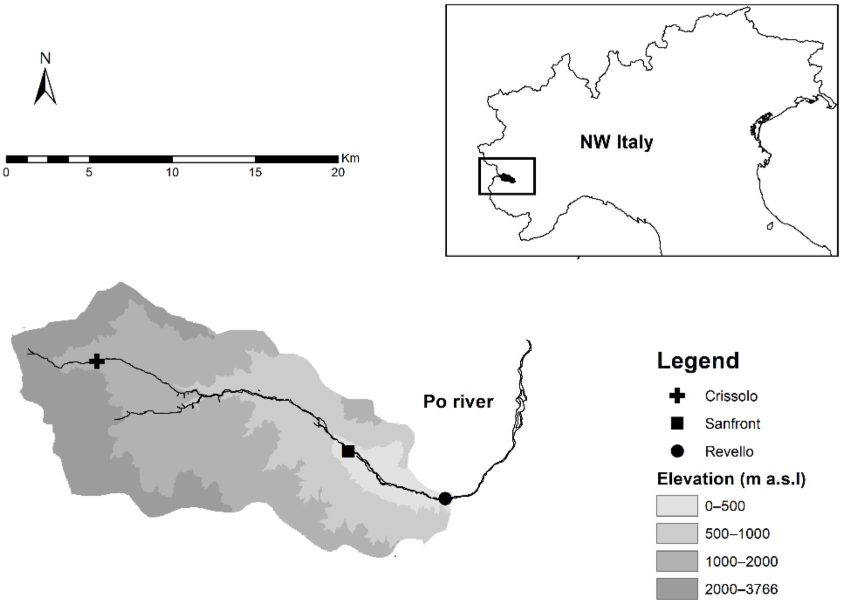

Figure 1. Map of the Po river with the three sampling reaches selected for the experiment.

All macroinvertebrates associated with leaf bags were preserved in 80% ethanol and identified to family or genus (Plecoptera and Ephemeroptera), following [35–37]. Shredder macroinvertebrate taxa were designated as such, according to [38,39].

The identified shredders were mainly part of the following families: Nemouridae, Leuctridae, Capniidae, Taeniopterygidae, Sericostomatidae, Limnephilidae, Tipulidae, and Gammaridae. After thawing, the previously frozen samples were rinsed with water, oven dried for 24 h at 70 °C, and then sent to the laboratory to perform carbon and nitrogen content analyses. Elemental analyses were performed with Elementar Unicube® (CE Instruments NA2100, Rodano, Italy), following the ISO 10694: 1995 protocol.

Data Analysis

Prior to performing the statistical analyses, data extraction was carried out according to [40], and outliers were removed because they deviated from the general trend: Two for oak (one for total abundance and one for EPT abundance) and three for chestnut (one for total abundance and two for EPT abundance). The final number of samples used for the analyses of oak leaf litter is 124 and 123 of chestnut. For both leaf types, CPOM decomposition was analyzed as described in [41]. The leaf mass remaining, as a % of residual dry mass for each leaf bag, was log transformed and regressed with time (number of days since the experiment onset), with the slope representing the processing coefficient (i.e., –k) [41]. Due to the temporal dependency of the data, we calculated this slope using linear mixed models (LMM), with % leaf mass remaining as the dependent variable, day, treatment (i.e., the three sites: perennial, perennial with higher flow variability and intermittent) and their interaction as explanatory variables, and sampling date as random factors. Generalized additive models were used to assess the nonlinear response of the C:N ratio over time, expressed in terms of days since the study onset, and sampling date was included as a random factor. For each sampling site, differences among sampling dates (i.e., T1–T6) in taxa richness, total abundance, EPT richness, EPT abundance, and shredder abundance were tested with ANOVA and log(x + 1) transformed data. When significant, pairwise comparisons were performed with the Tukey test (S3–S12). We evaluated heterogeneity in macroinvertebrate community composition using two-dimensional non-metric multidimensional scaling (NMDS) ordination and two-way permutational analysis of variance (PERMANOVA) [42], to test the effects of the sites (Crissolo, Sanfront, and Revello), sampling dates (T1–T6) and their interaction on community compositions. NMDS and PERMANOVA were based on a dissimilarity matrix of Bray–Curtis distances calculated using log(x + 1) abundance data. We analyzed the multivariate homogeneity of group dispersions (PERMDISP) as a measure of β diversity [43,44]. ANOVA was used to test distances from the PERMDISP group centroid for the communities. Indicator species analysis (i.e., IndVal; Table S13) [45] was used to identify taxa indicative of the three reaches, and thus responsible for compositional differences. All analyses were performed in R [46], by using basic functions and the packages lme4 [47] for LMM; vegan [48] for NMDS; PERMANOVA and PERMDISP for indicspecies [49] for indicator species analysis. Plots were drawn using the packages ggplot2 [50] and ggpubr [51].

Results

Environmental Conditions

Dataloggers revealed substantial variability in water temperature (from –4.15 to 22.9 °C; mean ± SD: 5.44 ± 3.16). In Crissolo and Sanfront reaches had similar temperature variability, while in Revello, there were major fluctuations (Figure S1): Between T1 and T2, for example, water was present but began to decrease to low flow (mean ± SD: –0.23 ± 2.34), while between T3 and T4, temperature drastically increased (5.80 ± 2.71), and surface water disappeared, leaving the riverbed completely dry and leaf bags exposed to air. At the end of the experiment, water temperature remained high, because of the low flow after the dry period (11.55 ± 3.94). In the three sampling reaches, water temperature increased over time during the whole experiment. However, in Revello, the sharp fluctuations and higher temperature ranges highlighted the occurrence of drying events and the slow subsequent recovery of flowing water. Physical and chemical values (i.e., conductivity, dissolved oxygen, and pH) of the three sampling sites are reported in Table S2.

Leaf Decomposition

For both leaf types, day, treatment (i.e., sites), and their interaction had significant effects on leaf litter decomposition rate (LMM; Table 1). For oak litter, the decomposition rate was highest in Crissolo, where the river is relatively pristine and with a stable, consistent flow (% of mass remaining mean ± SD: from 45.13 ± 0.86

at T1 to 4.92 ± 2.03 at T6); decomposition rate was similar in Sanfront, although the % remaining mass was higher than Crissolo (from 49.65 ± 3.69 at T1 to 17.14 ± 5.64 at T6). Overall, in absence of drying events the decomposition process was significantly faster (Figure 2a, Table 1). In Revello, dry events occurred from T3 to T5, and leaf decomposition was significantly slower (from 50.73 ± 3.02 at T1 to 37.81 ± 2.55 at T6; Figure 2a, Table 1). There were similar patterns of leaf litter decomposition for chestnut leaves but with lower absolute values of leaf mass remaining (Figure 2b, Table 1). In Crissolo, chestnut leaf decomposition was faster and more pronounced than in oak leaves (from 63.82 ± 3.73 at T1 to 2.00 ± 1.38 at T6), while in Sanfront, the observed values were similar to oak (from 66.19 ± 3.31 at T1 to 16.86 ± 7.75 at T6). In Revello, chestnut leaf decomposition was slower than in the other two reaches, compared with oak (from 66.05 ± 4.21 at T1 to 58.80 ± 9.31 at T6).

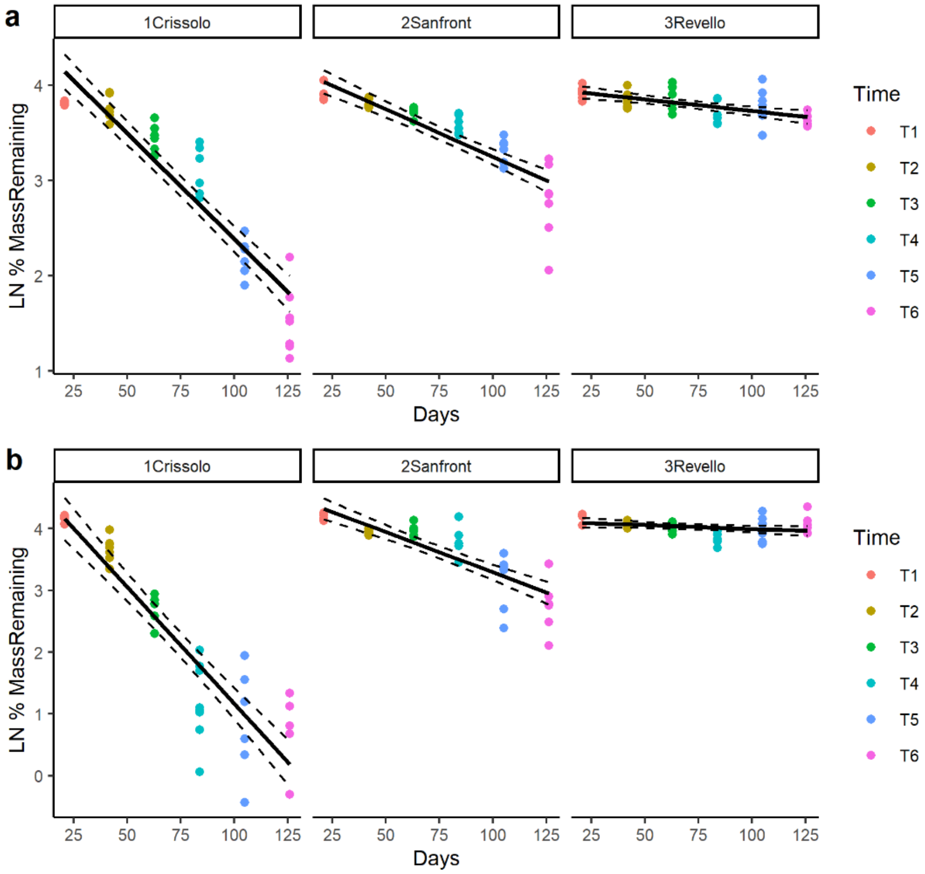

Figure 2. LN % of leaf mass remaining on each sampling date (days 21–126) following deployment (T0), in the three sampling reaches: (**a**) LMM for oak leaves, slope: –0.02; (**b**) LMM for chestnut leaves, slope: –0.04.

Significant temporal variation was observed in both leaf types for C:N ratio (Table 2). At the beginning of the experiment, C:N was 38.98 ± 0.47 for oak and 47.64 ± 0.47 for chestnut. Regarding oak leaves, the C:N ratio decreased from T1 to T4 and increased again between T4 and T5 until the last sampling campaign in Crissolo; in Sanfront, it decreased until T4 and then remained low, while in Revello, it continued to decrease slowly until the end of the experiment (Figure 3a–c). Chestnut leaves showed a different pattern: in Crissolo, the C:N ratio decreased until T2, increased again with a peak between T3 and T4, and then decreased again until the end of it; in Sanfront, it decreased until T3, then increased until T5 and decreased again, while in Revello, it decreased from the beginning to the end of the experiment (Figure 3d–f).

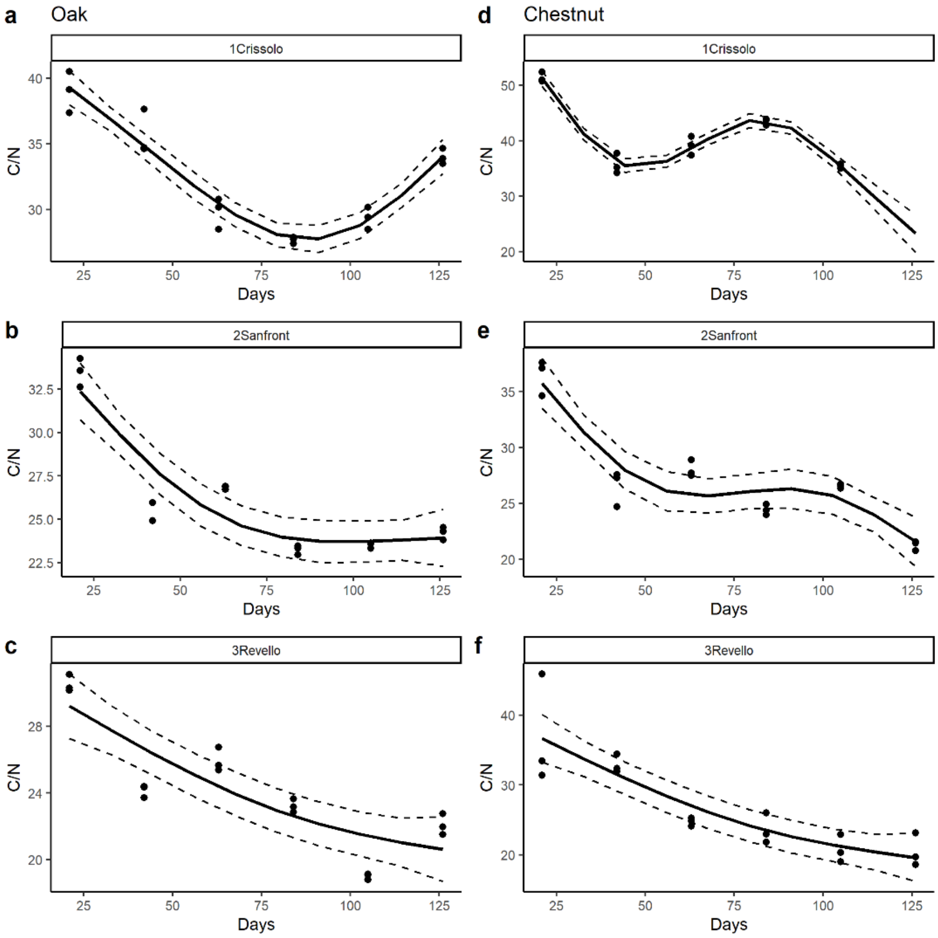

Figure 3. Generalized additive models for C:N ratio in the selected sampling reaches along the Po River: (**a**–**c**) *Quercus robur*; (**d**–**f**) *Castanea sativa*. Black lines represent the predicted values, dashed lines the 95% confidence interval. On the (**d**) plot, T6 values are missing, because chestnut leaves were almost completely degraded, and the remaining material was not enough to perform the analysis.

Leaf Type	Variable	Estimate	SE	t	*p*
Quercus robur	Days	−0.02	0.002	−11.46	<0.001
	Site2Sanfront	−0.36	0.10	−3.55	<0.001
	Site3Revello	−0.61	0.10	−6.02	<0.001
	Days:Site2Sanfront	0.01	0.00	9.85	<0.001
	Days:Site3Revello	0.02	0.00	15.33	<0.001
Castanea sativa	Days	−0.04	0.00	−18.40	<0.001
	Site2Sanfront	−0.34	0.19	−1.74	0.08
	Site3Revello	−0.81	0.19	−4.09	<0.001
	Days:Site2Sanfront	0.02	0.00	9.81	<0.001
	Days:Site3Revello	0.04	0.00	14.54	<0.001

Table 1. Results of linear mixed models testing the effects of site and day on % leaf mass remaining for both leaf types. For the categorical variable “site”, Site1Crissolo does not appear in the table because it was selected as reference level.

Metric	Leaf Type	Int	SE	t	Site	F	*p*
C:N	Quercus robur	27.6303	0.5674	48.7	Crissolo	5.933	0.003
					Sanfront	7.804	0.007
					Revello	9.003	0.004
	Castanea sativa	28.9510	0.6734	42.99	Crissolo	32.31	<0.001
					Sanfront	14.81	<0.001
					Revello	33.35	<0.001

Table 2. Statistics of the generalized additive models for C:N ratio.

Macroinvertebrate Community

For oak leaf litter, all macroinvertebrate metrics (i.e. taxa richness, total abundance, EPT richness and abundance, shredder abundance) were significantly affected by site, time and their interaction (ANOVA: $p \leq 0.001$). In general, all metrics were highest and most consistent in Crissolo and Sanfront, but significantly reduced and variable in Revello (Fig. 4). We detected smaller differences when comparing these metrics in Crissolo and Sanfront, while the differences between these two sampling reaches and Revello were more pronounced; for example, total abundance (Fig. 4b) was high in Crissolo (65 ± 47 organisms per leaf bag) and Sanfront (69 ± 56 organisms per leaf bag), while in Revello this metric was consistently lower (28 ± 34 organisms per leaf bag).

For chestnut leaf litter, we observed the same trend: all macroinvertebrates metrics were significantly affected by site, time and their interaction (ANOVA: $p \leq 0.001$). These values were highest and most consistent in Crissolo and Sanfront, and significantly reduced and variable in Revello (Fig. 5). Total abundance, for example (Fig. 5b), showed the highest values in Crissolo (129 ± 149 individuals per leaf bag), high but more variable in Sanfront (75 ± 82 individuals per leaf bag) and low throughout the whole experiment in Revello (27 ± 37 individuals per leaf bag). Tukey test comparisons of all the metrics are reported in supplementary materials (Tables S3 – S12).

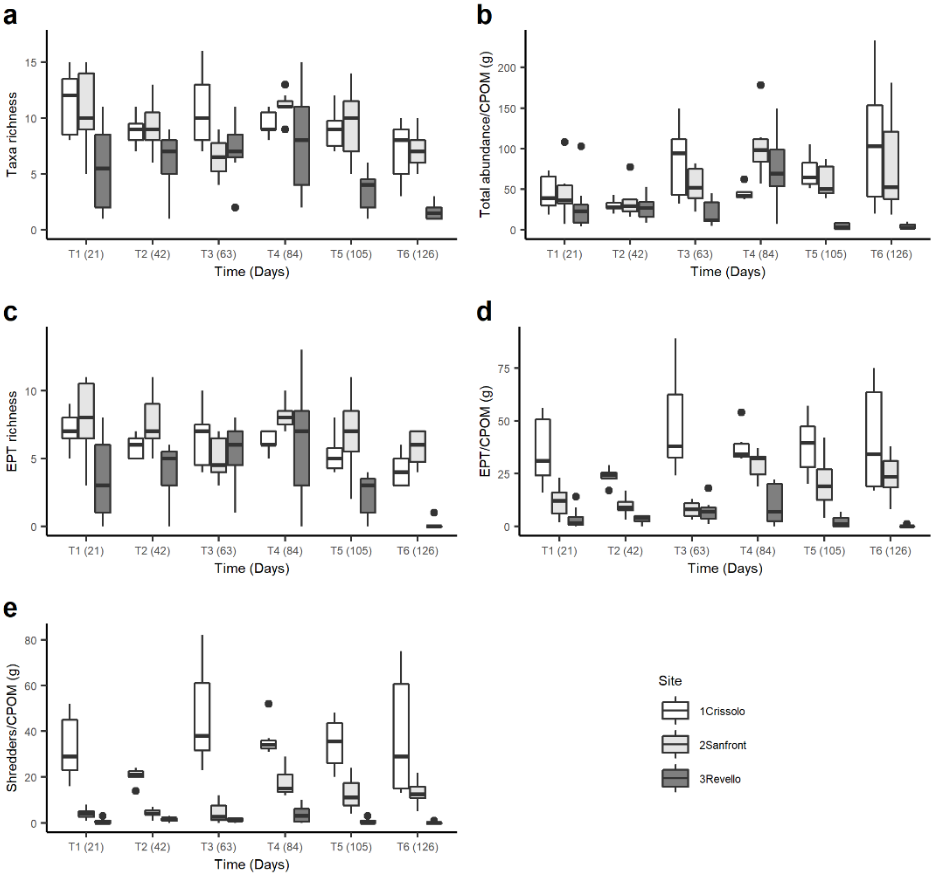

Figure 4. Metrics measured during sampling days in the three sampling reaches for oak leaves: (**a**) taxa richness; (**b**) total abundance; (**c**) EPT richness; (**d**) EPT abundance; (**e**) shredders abundance. Black line represents the median value; lower and upper box edges represent first and third quartiles, respectively; vertical lines represent whiskers (± 1.5 interquartile distance).

For both leaf types, community composition was characterized mainly by EPT taxa in Crissolo and Sanfront, and by Diptera in Revello. Regarding oak leaf litter, community composition was significantly affected by time (PERMANOVA; $F_{5,126} = 4$; $p < 0.001$), treatment (PERMANOVA; $F_{2,126} = 34$; $p < 0.001$), and their interaction (PERMANOVA; $F_{10,126} = 4$; $p < 0.001$). In Crissolo, the community was most represented by *Nemoura* (6 ± 6 individuals/ CPOM (g)), *Protonemura* (4 ± 5 individuals), and Limnephilidae (19 ± 17 individuals), In Sanfront, communities were represented by *Amphinemura* (5 ± 5 individuals), *Paraleptophlebia* (1 ± 2 individuals), Hydropsychidae (2 ± 3 individuals), and Rhyacophilidae. For Revello leaf litter, the communities were dominated by Ceratopogonidae (Diptera) (1 ± 2 individuals/ CPOM(g)). For chestnut leaf litter, a similar trend was observed, in which community composition was significantly affected by time (PERMANOVA; $F_{5,124} = 4$; $p < 0.001$), site (PERMANOVA; $F_{2,124} = 29$; $p < 0.001$), and their interaction (PERMANOVA; $F_{10,124} = 4$; $p < 0.001$). The relative proportion of shredder taxa with respect to the other functional feeding groups are 1.06 in Crissolo, 1.025 in Revello, and 1.02 in Sanfront. In Crissolo the community was mainly defined by *Isoperla* (1 ± 1 individuals/CPOM(g)), *Protonemura* (6 ± 11 individuals), and Limnephilidae (46 ± 93 individuals), while in Sanfront, by *Habroleptoides* (1 ± 2 individuals), *Paraleptophlebia* (1 ± 2 individuals), and Amphinemura (5 ± 8 individuals), and in Revello only by Ceratopogonidae (1 ± 2 individuals/CPOM (g)). The results of the indicator species analysis are presented in Table S13, where IndVal and p values are reported. This analysis helps to understand which taxa best characterize each of the three sampling sites. Further information about the macroinvertebrate communities is reported in Tables S14–S16. The NMDS ordination showed similar results for both oak (2D-stress = 0.133;

Figure 6a) and chestnut (2D-stress = 0.123; Figure 6b)—namely, community composition was unique for Crissolo but comparable for Sanfront and Revello at the beginning of the experiment and then, from T3 (after 63 days) onward, Revello showed a more dispersed community than the one of the other two sampling reaches (ANOVA; $p < 0.001$ for both leaf types).

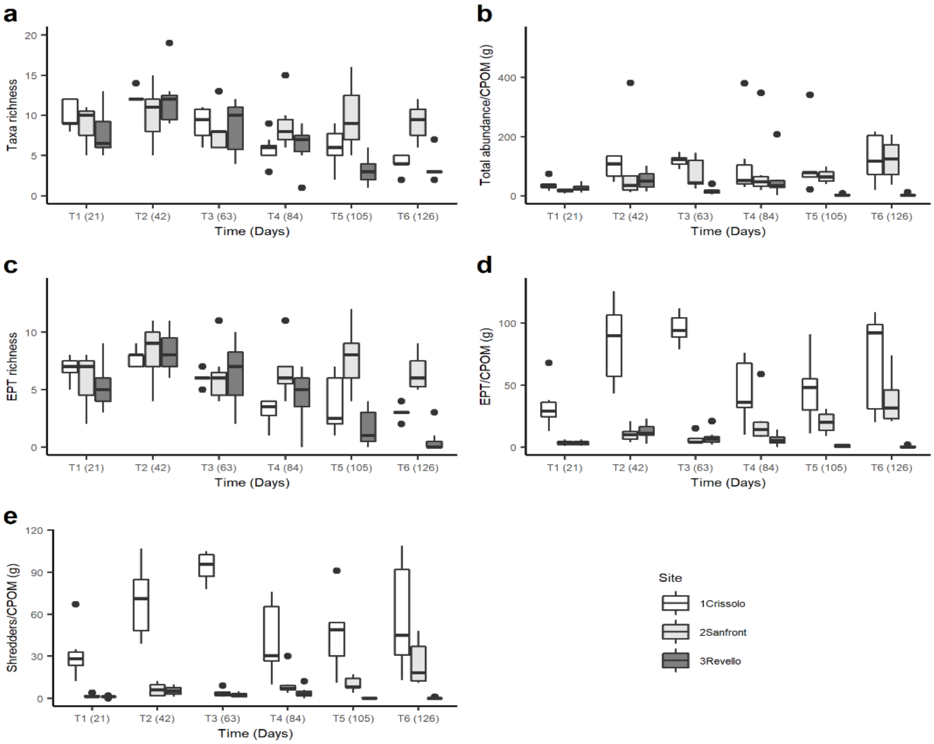

Figure 5. Metrics measured during sampling days in the three sampling reaches for chestnut leaves: (**a**) taxa richness; (**b**) total abundance; (**c**) EPT richness; (**d**) EPT abundance; (**e**) shredders abundance. Black line represents the median value; lower and upper box edges represent first and third quartiles, respectively; vertical lines represent whiskers (± 1.5 interquartile distance).

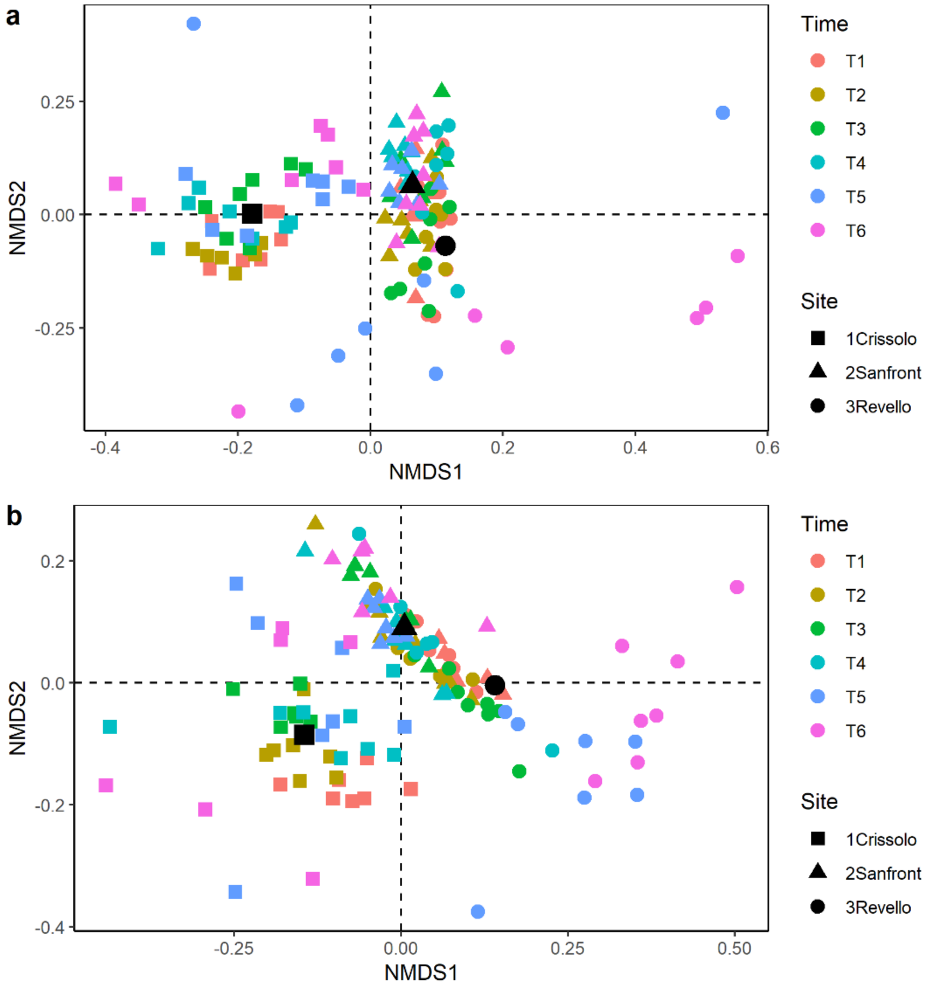

Figure 6. Non-metric multidimensional scaling (NMDS) ordination of macroinvertebrate community composition in (**a**) oak leaves and (**b**) chestnut leaves; larger black symbols represent centroids for each sampling reach.

Discussion

Our main aim was to evaluate CPOM decomposition processes and associated macroinvertebrate communities among river reaches differentially affected by variable flow conditions along an economically and ecological important watershed. The results of this study shed light on the negative effects caused by riverbed drying in mountain streams that were historically considered perennial.

Leaf Litter Decomposition

In this study, we evaluated the effect of supraseasonal dry events, which occur mainly in winter when the input of allochthonous material (mainly deciduous leaves from the autumn season) is higher, on the leaf litter decomposition process [52]. We observed very slow leaf litter decomposition rates associated with a reach that experienced substantial riverbed drying over three months, variable water quality conditions during that time, and significant changes in associated macroinvertebrate communities. Indeed, in the sampling reaches that did not experience dry phases (perennial and perennial with flow variability), leaf litter decomposition was faster, with flowing water that likely allowed natural colonization by bacteria and fungi, making leaves more palatable for macroinvertebrates [53,54]. In the sampling site where dry events occurred, desiccation may have influenced the conditioning process and, consequently, reduced biotic decomposition activity [55,56]. Leaf quality, however, played an important role in the process: Oak leaves, considered as low-quality leaf litter because of high recalcitrance and nutrient content [57], showed higher leaf mass remaining; however, chestnut leaves, which have been shown to decompose more readily [58,59], had very low leaf mass remaining at the end of the study, especially in the perennial sites. In the Revello site, where dry events occurred, leaf type did not have such a major impact, as all decomposition was very low. The C:N ratio, even with some differences, decreased in both leaf types and then increased again in Crissolo, the most pristine sampling reach in our study design. Indeed, the nitrogen content is expected to increase in early spring in both chestnut and oak due to higher temperatures [60] and also because of the high conditioning of the leaves surface, which leads to higher consumption of nitrogen [61]. The C:N decrease in chestnut leaves in Crissolo after its increase was due to the minimum amount of leaf litter remaining in the last two sampling dates when our leaf bags were almost completely decomposed. We hypothesize that the pattern observed for oak's C:N ratio in Crissolo could be the same one detected in chestnut leaf litter but slowed down over time because of the recalcitrant nature of the leaves. In Sanfront, for both leaf types, we observed a decrease in this ratio and then stabilization, even if with slight differences between chestnut and oak, mainly due to their intrinsic nutrient content. Regarding Revello, the sampling reach where dry events occurred, for both leaf types, a decrease in C:N was observed, probably due to the conditioning processes, which were affected by drying. These results suggest that, when the microconsumer conditioning is impacted, higher mineralization of the leaves may occur, due to the oxidation of the organic material exposed to the air. Moreover, some studies highlight that chemical diversification of leaf litter, due to the emersion–immersion variation during flow intermittency, can play an important role in decomposition processes and therefore in better understanding ecosystem functioning [62].

Macroinvertebrate Community

At the beginning of the experiment, macroinvertebrate community composition was similar in Sanfront and Revello sampling reaches, making it possible to directly compare these two sites—one perennial but variable and one intermittent. After 21 colonization days, community composition was very similar; then, as the low flow and dry phase occurred in Revello, the macroinvertebrate community became less diverse and with lower abundances of most taxa. For both leaf types, this was associated with a loss of EPT taxa and medium-to-large-sized univoltine trophic specialists that led to a community dominated by Diptera, mainly Chironomidae and Ceratopogonidae; such results suggest that intermittent flow periods have a large effect on aquatic macroinvertebrates associated with leaf litter that is independent of—and supersedes—the importance of leaf litter quality [63,64]. Drift from upstream reaches was probably the main process that facilitated community recovery after drying [65]. Moreover, as demonstrated in previous studies on intermittent rivers and ephemeral streams [66], predators typically increase as flow ceases, and in our experiment, the relative proportion of predators is highest (1.79) in Revello, when compared with Crissolo and Sanfront (1.6 and 1.5, respectively). Along with this increase, shredder macroinvertebrates decrease, as demonstrated by our study. These changes in the trophic chain can affect leaf litter decomposition and therefore river functionality, making previously perennial Alpine streams at severe risk, both in terms of exploring their biodiversity and their ability to efficiently process nutrients, which can influence the general health of these ecosystems. The Crissolo sampling site showed a different community composition at the beginning of the experiment, likely explained by its higher elevation and more pristine environmental conditions, where human activities have less impact on the ecosystem. The leaf litter macroinvertebrate community was highly diverse and remained stable through the study. In a study conducted using open-air mesocosms, macroinvertebrate communities showed the same heterogeneity in taxonomic composition when facing flowing and drying phases, compared with the ones in which the continuous flow was maintained [20]; the same results were obtained in another experiment, comparing mesocosms and field data [67], highlighting that this pattern can also be observed in natural conditions, such as in this study. It is important to note that for both leaf types, EPT richness and abundance and shredder abundance were always higher in the perennial sampling sites. EPT taxa are sensitive to flow cessation and drying [68,69], and shredder macroinvertebrates likely colonized leaf litter that was made more palatable by conditioning [70]; furthermore, this process could have been disturbed by dry events, which promote bacteria and fungi desiccation.

Conclusions

Many studies have been conducted regarding the effects of drying events, mainly in Mediterranean streams [70–72]. In the last decade, research attention has been shifted to those streams that were previously considered perennial but which are now facing dry events more frequently. It has been argued that the communities of these once perennial streams evolved with continuous flow and that they are facing negative consequences with frequent drying events, especially in mountain streams. This explorative research aimed to investigate only one (but widely studied by our research group throughout the years) Alpine stream, with the purpose to increase the current knowledge regarding the possible effects of climate change and human pressures on these threatened ecosystems. Our results clearly showed that the loss of surface running water negatively affected CPOM decomposition, one of the key factors affecting the river ecosystem's functionality. The macroinvertebrate community was also negatively affected, with the loss of specialist/ sensitive taxa replaced by more generalists. The differences in decomposition rates were mainly driven by leaf species and quality, but the results are comparable, highlighting the fact that the occurrence of dry events has the same negative effects, despite leaf type. The instream refuges (such as hyporheic sediments) were not investigated in this study, but they certainly function as a support for macroinvertebrate persistence during drying [73,74]. Indeed, further research is needed to understand the long-term effects of drying on these ecosystems, and the capability of recovery if and when flow resumes.

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to the analysis and interpretation of the results, M.E.B. All authors set the experimental designs, performed the fieldwork, contributed critically to the drafts. All authors have read and agreed to the published version of the manuscript.

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CHAPTER 3: MICROBIAL COMMUNITY INVOLVED IN LEAF LITTER DECOMPOSITION PROCESSES

HIDDEN DECOMPOSERS: THE ROLE OF BACTERIA AND FUNGI IN INTERMITTENT ALPINE STREAMS FUNCTIONAL PROCESSES

This work is still to be submitted as:

Gruppuso L.*, Receveur J.*, Fenoglio S., Bona F. & Benbow M. E.

Abstract

The frequency of flow-intermittency and drying events in Alpine rivers is expected to increase due to climate change. These events can have significant consequences for stream ecological communities, though the effects of reduced flow conditions on the microbial communities of decomposing allochthonous leaf material require additional investigation. In this study, we investigated the bacterial and fungal communities associated with the decomposition of two common species of leaf litter, chestnut (Castanea sativa) and oak (Quercus robur). Sampling of experimentally placed leaf bags was spread over six collection points (up to 126 days after placement) at seven sites in the Western Italian Alps, which have historically differed in streamflow conditions. Bacterial and fungal communities from these leaf bags were identified using high-throughput amplicon sequencing. Chestnut and oak leaf bags harbored distinct bacterial and fungal communities, with a number of taxonomic groups differing in abundance, though bacterial communities began to converge at later sampling dates. Historical flow conditions (intermittent vs perennial rivers) and observed conditions (normal flow, low flow, ongoing drying event) had comparatively weak observed effects on bacterial and fungal communities to that of leaf type and collection date (i.e. length of decomposition). Our findings highlight the importance of leaf characteristics (e.g., C:N ratios, recalcitrance, etc.) to the in-stream conditioning of leaf litter and the need for additional investigations of drying events in Alpine streams. This study provides new information on the microbial role in leaf litter decomposition with expected flow changes associated with a global change scenario.

Keywords: Bacteria; Fungi; leaf litter decomposition; intermittent rivers; in-stream conditioning; Alpine streams

Introduction

In low-order streams, allochthonous leaf litter is a fundamental energy source, and its decomposition plays a key role in nutrient and energy cycling (Petersen & Cummins, 1974); therefore, coarse particulate organic matter (CPOM) decomposition has been widely used to assess river function (Gessner & Chauvet, 2002), and is the basis for the River Continuum Concept (Vannote et al. 1980). In general, leaf litter decomposition consists of three primary phases: leaching, conditioning and fragmentation (Webster & Benfield, 1986). After initial immersion, leaves begin to leach chemicals and nutrients (usually within 24-48 h); this is followed by microbial conditioning by fungi and bacteria that degrades the leaf tissues and facilitates additional decomposition over days to weeks; lastly, remaining recalcitrant leaf material experiences physical abrasion through macroinvertebrates feeding and shredding activity that leads to fragmentation with associated particles drifting downstream, usually over the course of weeks in temperate regions of the world (Cummins, 1974; Purahong et al., 2016).

The process and rate of leaf litter decomposition is driven by complex intrinsic and extrinsic factors (Moore et al., 2004; Lecerf et al., 2007). Among the intrinsic factors, the C:N ratio, leaf texture and secondary compound content (e.g., tannins and phenols) affect the rate of leaf litter decomposition. Extrinsic factors also strongly influence CPOM decomposition, including microbial communities that are known to provide a priming effect (e.g. bacteria, fungi, algae) that is considered the conditioning process (Danger et al., 2013). Leaf conditioning releases nutrients and tannins and makes the leaf material more palatable and pliable for shredder macroinvertebrates (Cummins et al., 1973). For instance, many studies demonstrated that fungi secrete enzymes that break down complex molecules, such as cellulose, hemicellulose and lignin (Purahong et al., 2014; Peršoh, 2015). Moreover, initial fungal colonization is known as a priority effect, influencing later-arriving fungal species and leaf litter decomposition rate (Cline & Zak, 2015). In addition, senesced leaves carry a unique community of phyllosphere microorganisms, which can be detected on the leaf surface and are able to persist in constraining environmental conditions; among them, bacterial genera such as *Pseudomonas*, *Sphingomonas* and *Massilia* are the most common, while for fungi the most frequent ones are *Cladosporium* and *Alternaria* (Sivakumar et al., 2020).

The effective role of bacteria in leaf litter decomposition is recognized, even if it is less clear than the role of fungi, but bacteria are known to provide essential micronutrients for fungal communities and benefit from the degradation of complex molecules (Romaní et al., 2006; Frey-Klett et al., 2011). Other studies have suggested that bacteria and fungi interact with each other during CPOM decomposition (Purahong et al., 2015). This community of microconsumers is thus important to leaf litter decomposition and has been an area of investigation even before there were high throughput sequencing approaches to characterize them (Witkamp, 1966; Xu et al., 2013; Mora-Gómez et al., 2018). Ongoing advances in genomic sequencing technologies allow for new insights into the role of microbial communities in leaf litter and other forms of necromass decomposition, including how they vary among leaf litter sources (e.g., originating taxa; Larson et al., 2020), along abiotic gradients (e.g., temperature, water quality and land use effects) and in the presence of other macroconsumers (Pauls et al., 2014; Benbow et al., 2019).

In this study, we investigated aquatic leaf litter microbial communities of decomposing leaves of two tree species with different nutrient quality: high quality and lower recalcitrant chestnut (*Castanea sativa*) and low quality and more recalcitrant oak (*Quercus robur*). Leaf litter bags were placed at seven sites to assess decomposition processes in three Alpine streams of Italy: the Po River, Pellice River and Varaita River. These rivers are impacted by recent flow intermittence due to anthropogenic pressures and climate change (Falasco et al., 2018; Piano et al., 2019a and b). Leaf litter microbial communities were characterized from leaf disks followed by high throughput sequencing of the 16S rRNA and ITS gene amplicons for bacteria and fungi, respectively. Our goal was to characterize the variability of microconsumer communities from different leaf litter sources (i.e., tree species) among three rivers affected by flow intermittency. We hypothesized that: i) bacterial and fungal communities would be characterized by some genera typical of the leaf phyllosphere such as *Pseudomonas, Sphingomonas, Massilia, Cladosporium* and *Alternaria*, especially at the beginning of the experiment; ii) as leaf litter decomposition progresses, we expect to observe a shift in microbial families from leaf specific taxa, such as Oxalobacteraceae and Pleosporaceae, to litter breakdown specific, such as Hyaloscyphaceae and Helotiaceae; iii) leaf litter microbial community composition would be significantly different in river sites that underwent drying events compared to those where flow was not interrupted.

Materials and methods

The field experiment was conducted from 13 December 2018 to 19 April 2019, with sampling occuring every 21 days (six sampling dates) in three mountain rivers in the Western Italian Alps: the Po River, Pellice River and Varaita River. There were four sites in the Po river (Crissolo, Ostana, Sanfront and Revello, upstream to downstream respectively), two in the Pellice River (Pellice M, i.e. upstream and Pellice V, downstream) and one in the Varaita River. Sites were selected based on previous experiences of known prolonged drying events (see Falasco et al., 2018 and Piano et al., 2019a and b) and historical discharge data of the Environmental Regional Agency (ARPA, 2013). To measure leaf litter decomposition rates and associated microbial and invertebrate communities, leaf litter bags were employed using two leaf species abundant in the watersheds and with distinct differences in quality/recalcitrance: chestnut (*Castanea sativa*) with 47.64 ± 0.47 C:N content at the beginning of the experiment, and oak (*Quercus robur*), 38.98 ± 0.47 initial C:N content, both collected in autumn after abscission. For more details regarding experimental setting please see (Gruppuso et al., 2021 and Gruppuso et al., 2022). Three leaf bags per leaf species were collected on each sampling date, placed into individual plastic bags and stored at -20°C until DNA extraction and microbial community sequencing.

Frozen leaf litter samples were thawed and sampled using a cork-borer under sterile conditions. For each sample, 1 g of leaf tissue was stored in cryovials with Sigma-Aldrich RNAlater® and kept frozen at -20°C. DNA extractions were performed using Qiagen MagAttract® DNA Extraction Kit, following manufacturer's instructions except for the addition of lysozyme (15 mg/ml) during the lysis step. For bacterial communities, the V4 hypervariable region of the 16S rRNA gene (Claesson et al., 2010; Caporaso et al., 2011; Haas et al., 2011) was amplified using dual indexed Illumina compatible primers 515f/806r as described by Kozich, J. J. (2013). For fungal communities we used ITS4 + ITS7 primers complementary to the internal transcribed spacer (ITS) region (Nilsson et al., 2009; Schoch et al., 2012; Lindahl et al., 2013). PCR products were batch normalized using Invitrogen SequalPrep DNA Normalization plates and the products recovered from the plates pooled. The pool was cleaned using an Amicon Spin Filter and AmpureXP magnetic beads. Samples were quantified with a combination of Qubit dsDNA HS, Agilent 4200 TapeStation HS DNA 1000 and Kapa Illumina Library Quantification qPCR assays.

Library preparation and sequencing (2 x 250 bp reads) was performed at the Michigan State Research Technology Support Facility on the Illumina MiSeq platform. The sample pool was loaded onto an Illumina MiSeq v2 standard flow cell and sequencing was performed in a 2x250 bp paired end format using a MiSeq v2 500 cycle reagent cartridge. Custom sequencing and index primers complementary to the amplicon sequences were added to appropriate wells of the reagent cartridge as described in Kozich, J.J. (2013). Base calling was done by Illumina Real Time Analysis (RTA) v1.18.54 and output of RTA was demultiplexed and converted to FastQ format with Illumina Bcl2fastq v2.20.0.a

Data Processing and Statistical Analysis

Raw sequence files were demultiplexed and quality filtered using default settings in DADA2 and QIIME 2 (2020.11; see Callahan et al., 2016 and Bolyen et al., 2019). Taxonomy was assigned to Amplicon Sequencing Variants (ASVs) using a Naive Bayes classifier and the SILVA (13.8; see Quast et al., 2013) reference dataset. Shannon diversity was calculated using the phyloseq package (McMurdie & Holmes, 2013) and compared using ANOVA tests or paired Wilcoxon tests depending on comparison. Differences in taxonomic relative composition between groups was compared with Kruskal-Wallis and Wilcoxon tests and a False Discovery Rate (FDR) correction for multiple comparisons.

Differences in beta diversity were compared with PERMANOVA tests conducted using the vegan package (Oksanen et al., 2007), Bray-Curtis dissimilarity, and 999 permutations. Random forest models were built using genus level taxonomy and

Out Of Bag (OOB) error to predict leaf and river type using the randomForest package (v 4.6-14; see Liaw & Wiener, 2002). Only bacterial genera which comprised greater than 1% of the total relative abundance across all samples were included in random forest modeling. Figures were created using a combination of ggplot2 (3.3.5), phyloseq (1.36.0), ggpubr (v 0.4.0), and vegan (v 2.5-7) packages (see McMurdie & Holmes., 2013; Wickham, 2016; Kassambara, 2020). All analysis was conducted using R (v 4.1.3; R Core Team, 2022). R code used in this study has been deposited on GitHub (https://github.com/JPReceveur/AlpineStreamMicroGruppuso2022).

Results

Bacterial communities

Sequencing of 84 bacterial samples resulted in 6,262,055 reads with a mean reads per sample of 74,548. Based on alpha rarefaction curves, bacterial samples were rarefied to a depth of 7,000 reads per sample.

Impact of flow intermittency and leaf type on bacterial communities

To examine the impact of flow intermittency on leaf bag microbial communities, four perennial sites (Crissolo, Ostana, Pellice Upstream and Sanfront, hereafter addressed as CRI, OST, PEM, and SAN) were compared with sites that frequently experience intermittent drying events (Pellice Downstream, Revello and Varaita, hereafter addressed as PEV, RE, and VAR). This approach was chosen because those three alpine streams (Po, Pellice and Varaita) have been widely investigated by our research group within the framework of the PRIN NO ACQUA project, focused on the effects of hydrological intermittency on biodiversity, functionality and resilience mechanisms in previously perennial alpine streams. Further information about drying events in the three intermittent sites throughout the experiment are reported in **Table 1**.

Bacterial Shannon diversity differed significantly by river type (ANOVA, perennial or intermittent, $P < 0.001$) and leaf type (chestnut or oak, $P = 0.003$) with a nearly significant impact of date ($P = 0.098$, **Table S1**). Both chestnut and oak leaf bags had significantly higher bacterial diversity in intermittent streams ($P > 0.05$, **Table S2**) with higher mean diversity for all dates except oak leaves at day 126 (**Figure S1a**). While diversity in chestnut leaf bags was influenced by time (ANOVA, $F = 2.9$, $P = 0.029$), increasing at later sampling days, oak bacterial diversity remained relatively constant over the study duration ($F = 0.24$, $P = 0.94$). When paired chestnut and oak leaf communities were compared (i.e. comparing between leaf bags sampled at the same site and date), oak leaves had on average 0.24 (SEM +/- 0.04) higher values for Shannon diversity (Wilcoxon, $P = 0.001$, **Figure S1b**) and 28.8 more Amplicon Sequencing Variants (ASVs, +/- 11.0 SEM, $P = 0.083$).

Stream	Sampling site	Time	Date	Hydrology
Po River	Revello	T0	13-dec-18	Flow
		T1	03-jan-19	Flow
		T2	24-jan-19	Low Flow
		T3	14-feb-19	Dry
		T4	07-mar-19	Dry
		T5	29-mar-19	Dry
		T6	19-apr-19	Low Flow
Pellice River	Pellice V	T0	13-dec-18	Flow
		T1	03-jan-19	Flow
		T2	24-jan-19	Low Flow
		T3	14-feb-19	Low Flow
		T4	07-mar-19	Flow
		T5	29-mar-19	Flow
		T6	19-apr-19	Flow
Varaita River	Varaita	T0	13-dec-18	Flow
		T1	03-jan-19	Flow
		T2	24-jan-19	Dry
		T3	14-feb-19	Low Flow
		T4	07-mar-19	Low Flow
		T5	29-mar-19	Low Flow
		T6	19-apr-19	Low Flow

Table 1. Occurrence of drying events in the intermittent sampling sites selected for the experiment. Pellice River did not experience extended dry events during the experiment, but only very low flow; however, previously collected data reported that dry events occurred in this river at the downstream site (Pellice V).

Bacterial abundance at the phylum level was relatively stable across dates for both perennial and intermittent sites with bacteria from the phylum Proteobacteria comprising greater than 50% of the relative abundance across all sample groups (**Figure 1a**). For chestnut leaves, there were no phyla which differed significantly in relative abundance across date within either type of river (perennial or intermittent, KW, P-adj > 0.05) or between intermittent and perennial rivers (KW, P-adj > 0.05). In oak leaf bags, the relative abundance of Actinobacteriota was different among dates in perennial rivers (KW, P-adj = 0.014) with mean abundances on the first two sampling days (day 21 & 42) double that of later dates (**Figure 1b**) though no differences in relative abundance were observed between river types (KW, P > 0.05). Similarly, no differences were identified when comparing between samples collected during different flow characterizations (i.e. normal flow, low flow, dry/no flow, KW, P-adj > 0.05, **Table 1**) for either oak or chestnut leaves.

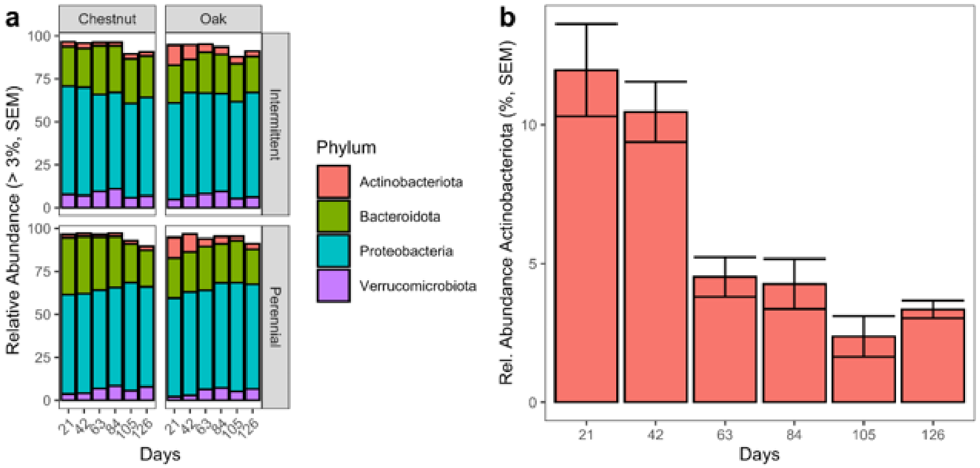


Figure 1. Leaf bag bacterial communities **a)** Relative bacterial abundance among samples from different leaf and river types. **b)** Relative abundance of Actinobacteriota in oak leaf bags from perennial rivers.

At the family level, bacterial communities were broadly similar, with few differences between river or leaf type (KW, P-adj > 0.05, **Figure 2a, 2b**) though several taxa differed significantly in abundance across sampling date (e.g. Sphingomonadaceae decreasing at dates 105 & 126, KW, P-adj < 0.05, **Table S3**).

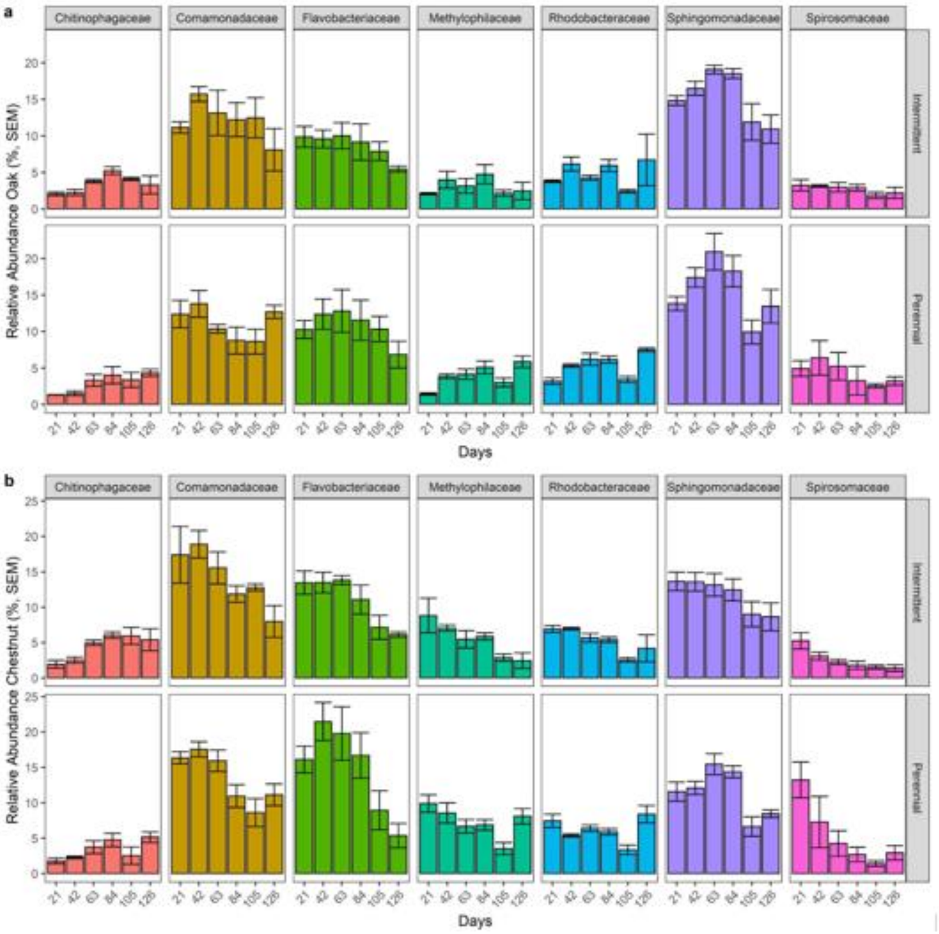


Figure 2. Family level bacterial abundance. **a)** Oak bacterial abundance by date. **b)** Chestnut bacterial abundance by date. Error bars are SEM. Only bacterial families that were greater than 3% of the total relative abundance across all samples and differed significantly for at least one comparison are shown (Kruskal-Wallis p-adj < 0.05). A list of comparisons is available in **Table S3**.

Genus level community composition analyses revealed highly distinct communities between oak and chestnut leaf bags with 94% (79/84) of samples correctly classified to leaf species (Random Forest, OOB error) based on their bacterial composition. All of the top 12 predictors (Mean Decrease Accuracy score > 5) were significantly different between leaf types, with *Caulobacter* and *Methylotenera* higher in chestnut leaves, while *Massilia* and *Sphingomonas* in the oak ones (Wilcoxon, P-adj > 0.05, **Figure 3, Table S4**).

Within individual leaf species, the differences between perennial and intermittent rivers were less distinct with only 66% (28/42) of chestnut leaves and 64% of oak leaves (27/42) correctly classified to river type. Of the top predictors within chestnut leaves, the abundance of only one group (an unassigned genus from the family Verrucomicrobiaceae) differed significantly between river type (KW, P-adj = 0.038, **Figure S2**). Within oak leaves, only the genus *Caulobacter* (Family: Caulobacteraceae) was significantly different (KW, P-adj < 0.05) between intermittent (rel. abu. = 1.14% +/- 0.1) and perennial rivers (rel. abu. = 0.77% +/- 0.1, **Figure S2**).

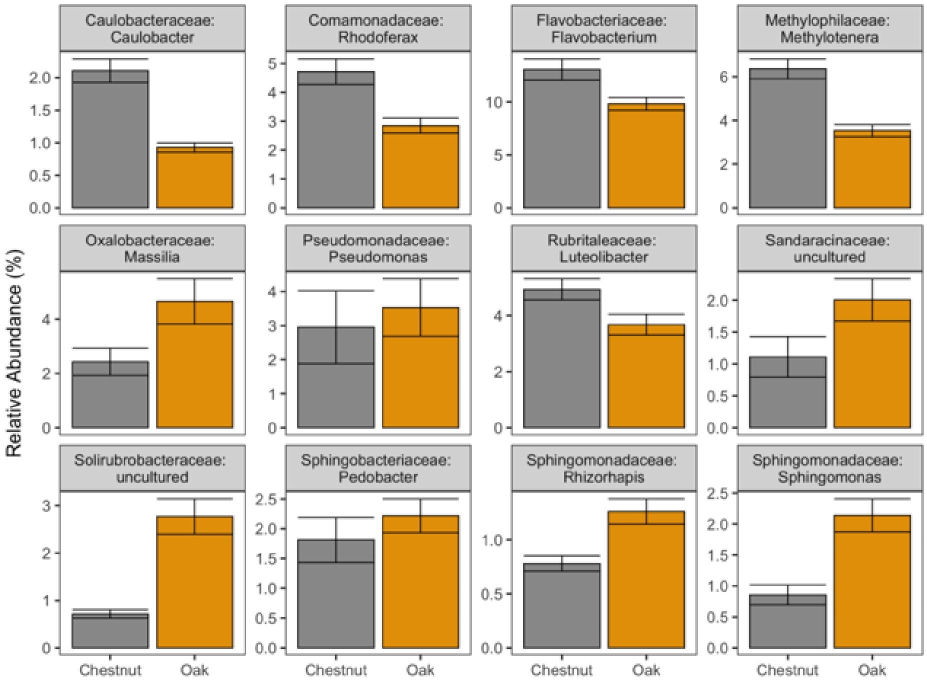


Figure 3. Top genus level random forest indicators for predicting leaf type. Error bars are SEM. Samples from different river types are combined.

Bacterial beta diversity

Bacterial beta diversity (Bray-Curtis dissimilarity) was determined by river/leaf types as well as site and date. River/Leaf type, site and sampling day all had significant effects on beta diversity (PERMANOVA, F > 6.0, P < 0.001, **Table S5**) with leaf type (chestnut vs oak) having the largest effect size (F= 11.1, P < 0.001), though the magnitude of differences between chestnut and oak communities changed over time (**Figure 4**). At early sampling timepoints (Days 21, 42 and 63), chestnut and oak communities clustered distinctly and were significantly different when tested in a pairwise fashion (P < 0.05, **Table S6**). However, at later dates (Days 84, 105, and 126) the leaf types became less distinct and were not significantly different (P > 0.05). All sampling sites showed similar levels of variability in beta diversity, with no site having significantly higher or lower variation than other sites (Vegan:Betadispr, F= 0.49, P = 0.82).

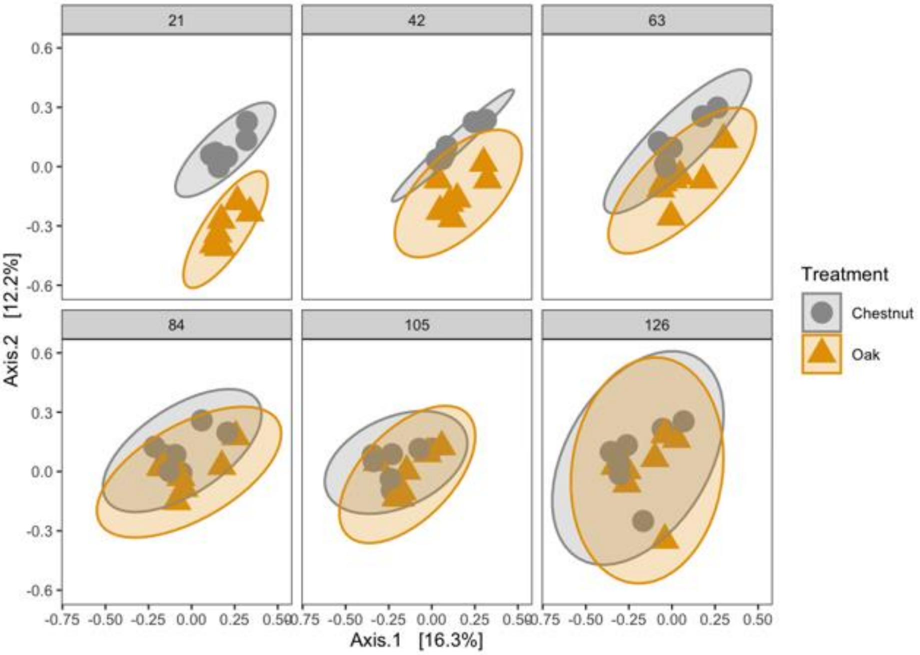


Figure 4. Difference in bacterial beta diversity between leaf types over time. PCoA is shown using Bray-Curtis Dissimilarity. Ellipses represent 95% confidence intervals for the mean of each group.

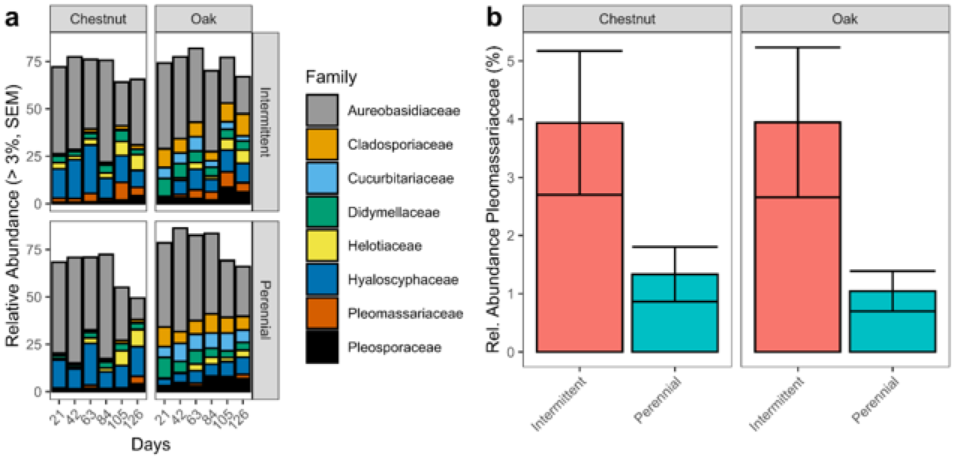
Fungal results

Across all fungal samples (N = 84), 2, 993 fungal Amplicon Sequencing Variants (ASVs) were identified with most (2,228) shared between both leaf types, though some ASVs were unique to either oak (454 unique ASVs) or chestnut (311 unique ASVs) leaves. Fugal samples were rarefied to 6,000 reads based on alpha rarefaction curves. Time had a significant effect on fungal diversity with later dates (days 105 & 126) showing higher diversity for both oak and chestnut leaves than earlier dates (**Figure S3a**, **Table S7**). River type did not strongly affect chestnut diversity (ANOVA, F = 1.98, P = 0.17) but oak leaves from intermittent rivers had higher diversity than in perennial rivers (F= 4.47, P = 0.004). Between paired samples (i.e. oak and chestnut leaves taken from the same site and date), oak leaves had Shannon diversity values higher (mean difference = 0.29 +/- 0.07 SEM) than chestnut leaves (Wilcoxon, p = 0.0003, **Figure S3b**).

Fungal taxonomic composition

Ascomycota was the dominant fungal group across all samples representing 90.45% (SEM +/- 1.08) of the relative abundance across all samples. At the phylum level, none of the four most abundant phyla (Ascomycota, Basidiomycota, Chytridiomycota, and Mortierellomycota) differed in abundance between intermittent and perennial rivers for either chestnut or oak leaf bags (**Figure S4**, KW, P-adj > 0.05). Communities described at family level were between river types (**Figure 5a**), with only a single family (Pleomassariaceae) significantly different in relative abundance between perennial (1.33% +/- 0.47) and intermittent (3.94% +/-

1.23) rivers for chestnut leaves (**Figure 5b**, KW, adj-P < 0.05). No families were significantly different between rivers for oak leaf bags (P-adj > 0.05) though Pleomassariaceae (P-adj = 0.077) displayed a similar trend as in chestnut leaves, with higher abundances in intermittent rivers (3.94 +/- 1.29 vs 1.04 +/- 0.34). There were several families which differed in abundance between chestnut and oak leaves including Cladosporiaceae, Cucurbitariaceae, Didymellaceae, Hyaloscyphaceae, and Pleosporaceae (**Table S8**, **Figure 5c**, KW, P-adj > 0.001). Of those families, Hyaloscyphaceae was in higher relative abundance in chestnut leaves (14.8 +/-1.7 vs 7.0 +/- 0.69) while the remaining families were in higher abundance in oak leaves.



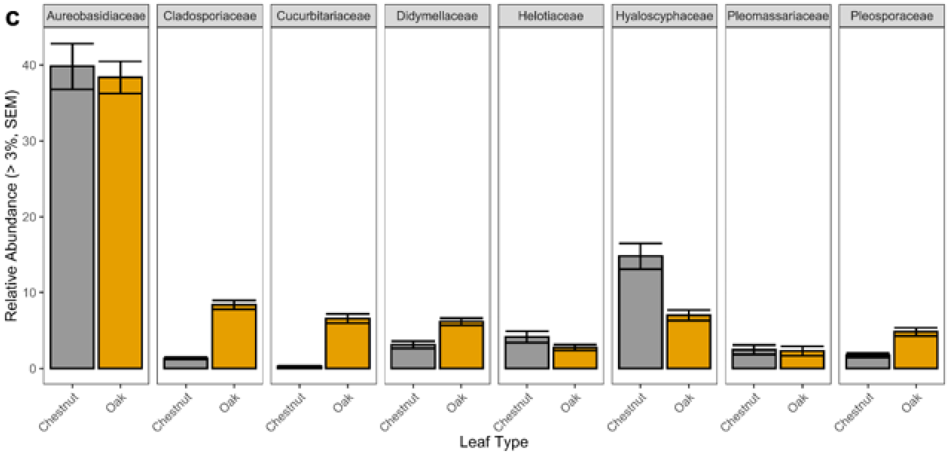


Figure 5. Family level fungal abundance. **a)** Family level abundance across sampling day by leaf and river type. Only families which comprise greater than 3% of the relative abundance across all samples are shown. **b)** Relative abundance of the family Pleomassariaceae (+/- SEM) between river types. **c)** Differences in family level relative abundance between leaf types (both river types combined). Error bars are SEM.

Genus level fungal communities were highly distinct between leaf types with only a single sample misclassified to leaf type (Random Forest, OOB error = 1.19%). All of the top genus level indicator species (N=7) in the random forest model (determined by a Mean Decrease Accuracy score > 5) were significantly different between chestnut and oak leaves (KW, P-adj < 0.01, **Figure 6**). Genus level fungal communities were less distinct between river and flow conditions with random forest models doing a poor job of classifying fungal communities to both river type (intermittent vs perennial, 30/84 samples misclassified) and flow condition (all dry samples and 87% of low flow samples misclassified).

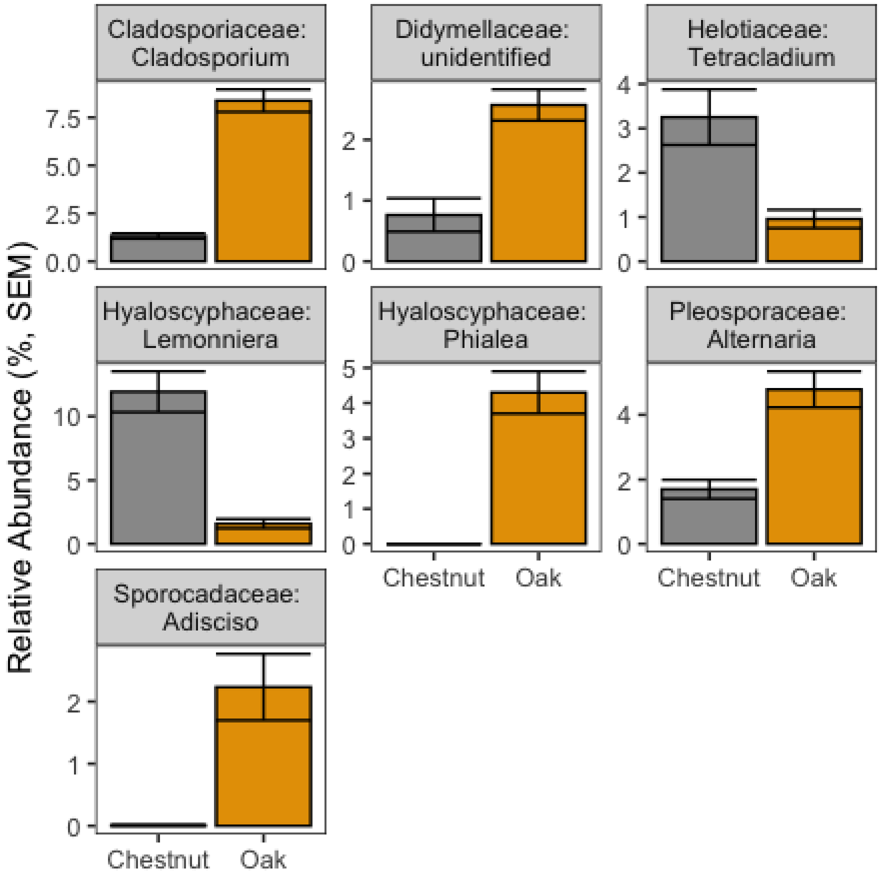


Figure 6. Top fungal genus level indicators for leaf type. Top indicators were identified by a Mean Decrease Accuracy (MDA) score greater than 5. All comparisons were significantly different (KW, P-adj < 0.01). Error bars are SEM.

Fungal beta diversity

Fungal beta diversity (Bray-Curtis dissimilarity) was significantly affected by flow conditions (dry, low flow, normal), leaf type, site, and date (PERMANOVA, $P < 0.05$, **Table S9**). Leaf type (chestnut vs oak) had the highest effect size ($F = 25.03$) and explained the greatest proportion of variation in beta diversity ($R^2= 0.19$) followed by date ($F= 4.47$, $R^2= 0.17$, **Figure 7**). River type ($F=2.48$, $R^2= 0.02$, **Table S9**) and flow conditions ($F=2.86$, $R^2= 0.04$, **Figure S5, Table S10**) only explained small proportions of the variation present in beta diversity.

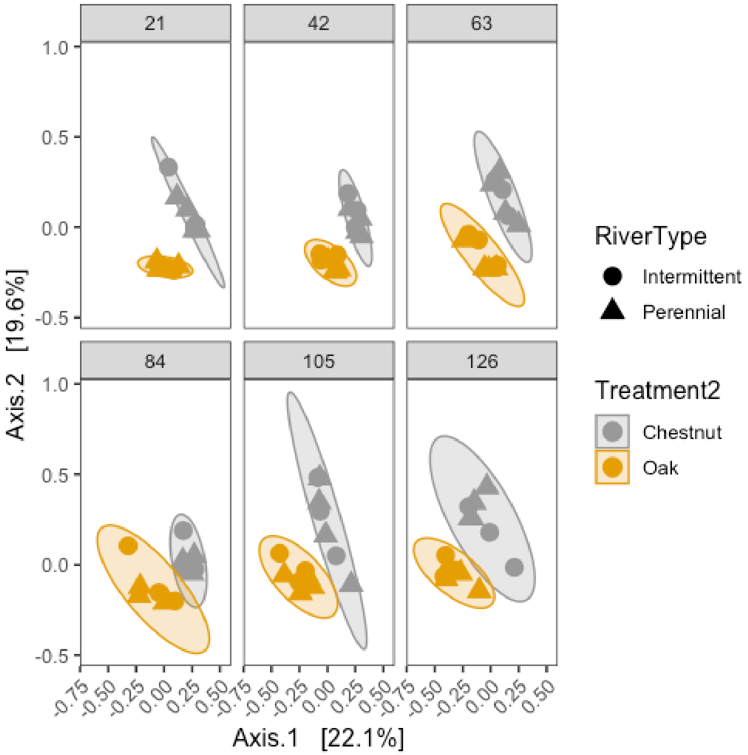


Figure 7. PCoA plot of fungal beta diversity between leaf type and river type (intermittent vs perennial). Ellipses represent 95% CI for the mean of each leaf species.

Discussion

In this study, we investigated how the bacterial and fungal community composition differed between flow conditions for two common species of aquatic leaf litter. Drying events showed limited effects on leaf bacterial communities, which were probably supported during flow disturbances by the fungal community already present since the early stages of the decomposition process, which provided an increased surface area for bacteria along with leachate and products of polymers' degradation (Das et al., 2012; Pereira & Ferreira, 2021). Similarly, leaf bag fungal diversity and taxonomic abundance were not strongly impacted by drying or low flow events. This could be due to the presence of some families which are typically present on the phyllosphere such as Cladosporaceae and Pleosporaceae (Inácio et al., 2002; Rana et al., 2019), here found in both chestnut and oak leaves and thus being more resistant to desiccation than the aquatic ones. Furthermore, the presence of desiccation-resistant taxa may have contributed to stabilizing the microbial community when flowing water disappeared. Surprisingly, oak leaves showed higher bacterial and fungal diversity between paired samples. We expected to see lower diversity in oak leaf bags due to them commonly considered as lower quality, more coriaceous (i.e., leathery), and their higher concentrations of secondary metabolites (such as phenols and tannins) which can make the substratum less palatable for both micro and macroorganisms. Potentially, the higher observed diversity of the microbial community present on the leaves is reflective of the additional complexity needed to break down more recalcitrant leaf material to process (Wolfe & Ballhorn, 2020).

Microbial community structure (as beta diversity) of oak and chestnut leaves varied over the course of this experiment. Regardless of flow conditions, chestnut and oak bacterial communities were distinct at the beginning of the experiment but converged at later sampling dates. This could be due to a higher presence of phyllosphere-specific taxa earlier in decomposition which is replaced over time by more specialized bacteria from the aquatic environment which contribute to the decomposition process. However, for fungi, we observed that chestnut and oak fungal communities remained distinct throughout decomposition. This suggests the presence of species specific fungi involved in the decomposition process, a hypothesis supported by the known differences in quality of the two leaf types (Jabiol et al., 2019): some secondary compounds such as cellulose, lignin, tannins and phenols have a role in the composition of the leaf texture and palatability, thus different fungi are necessary to break down those compounds. Moreover, it is important to remember that, at the beginning of the experiment, the two leaf species have their unique phyllosphere microbial community which can be species specific (Mustonen et al., 2016; Röhl et al., 2017). *Quercus robur* leaves were characterized by Rhodobacteraceae and Comamonadaceae, while *Castanea sativa* was mainly characterized by Rhodobacteraceae and Spingomonadaceae.

Moreover, it is important to mention that when studying a complex phenomenon such as climate changing at a global level, we cannot forget that its effects will impact riparian vegetation too, affecting the microbial phyllosphere community before leaf abscission and thus changing its composition prior the beginning of the experiment itself (Sivakumar et al., 2020). Our results demonstrated that the bacterial community present on our leaf litter bags was mainly represented by Proteobacteria and Bacteroidota, which are frequently found in environmental samples, and this was true despite the investigated flow regime. Interestingly, for intermittent sites, oak leaves had a high abundance of *Novosphingobium,* a bacteria of the family Sphingomonadaceae, which is able to degrade aromatic compounds such as phenols (Liu et al., 2005), and can play an important role in the decomposition of this coriaceus leaf type. The other genera detected as top predictors of the intermittent sites for oak leaves were *Caulobacter,* usually found in aquatic ecosystems, *Massilia*, an aerobic genus, able to use atmospheric oxygen, *Brevundimonas* and the family Verrucomicrobiaceae. Chestnut leaf communities were represented by *Brevundimonas, Novosphingobium, Pedobacter* and Verrucomicrobiaceae in intermittent sites.

Our results show that leaf bacterial communities associated with dry events were characterized by organisms able to use atmospheric oxygen to degrade aromatic compounds, along with the ones associated to the phyllosphere or to the sediment itself, able to overcome the desiccation periods (Jackrel et al., 2019). Moreover, oak leaf communities represented a high relative abundance of Actinobacteriota, a bacterial group which is able to perform mycelial growth and to decompose organic compounds with low biodegradability, such as lignin, hydrocarbon and humus (Stackebrandt et al., 1997); this result is quite interesting, because their relative abundance was higher at day 21 and 42 and then it diminishes in later sampling dates. This could be due to the need of breaking down those recalcitrant and secondary compounds into simpler molecules and nutrients, before those nutrients become available for other organisms involved in the decomposition process. Fungal communities are mainly represented by Ascomycetes (Wang et al., 2020), because Basidiomycetes usually later in leaf litter decomposition, at stages we did not investigate during our field experiment. The family Aurobasidiaceae, which is widespread in freshwater and marine ecosystems (Cannon & Kirk, 2007), was the most abundant in our samples, despite the leaf type and flow regime, likely because this family is able to adapt to different environmental conditions and thus to cope with hydrological and nutrient stressors (Cannon & Kirk, 2007). Interestingly, the fungi of the family Pleomassariaceae, widely distributed on decomposing wood and plant material (Cannon & Kirk, 2007), showed high abundance in low flow and drying conditions; this could be due to the ability to exploit the substratum's remaining moisture and an ability to degrade secondary compounds. This fungal group deserves additional investigation to understand their role in leaf litter decomposition processes during longer desiccation phases.

Oak leaf bag fungal communities had a high relative abundance of *Cladosporium,* typically found in plant material; *Alternaria,* a taxon usually present on decomposing material with high humidity (Dickinson & Bottomley, 1980); *Adisciso,* a plant pathogen (Liu et al., 2019) and *Phialea*, typical of dead leaf material (Dennis, 1963). Chestnut leaves, however, had high relative abundance of *Lemonniera,* an aquatic hyphomycetes involved in stream decomposition processes (Baschien et al., 2006); and *Tetracladium,* an important decomposer in stream food webs (Anderson & Marvanová, 2020). The presence of those genera, mainly involved in breakdown processes in aquatic environments, may explain the faster decomposition rate we found for chestnut leaves. These findings highlight the importance of bacteria and fungi in the leaf litter decomposition process, and confirms the presence of different families/genera during decomposition, and variation related in leaf type and quality and also flow regime, even if with limited effect.

Conclusions

High-throughput sequencing of microbial communities associated with in-stream leaf litter highlights the complexity of biotic interactions on aquatic decomposition processes. In Alpine river ecosystems, the occurrence of low flow phases and drying events is a recent phenomenon, caused mainly by global climate change and anthropogenic pressures (Cauvy-Fraunié, 2016). Previous studies confirm the hypothesis that the biological communities inhabiting these particular streams are not adapted yet to the phenomenon (Elias et al., 2015; Piano et al., 2019), and this can cause negative effects on the allochthonous organic matter decomposition process, altering the whole food chain. Microbial communities are fundamental in this process, their activity provides nutrients for the other organisms; however, our results showed that changing flow conditions did not strongly affect bacterial and fungal communities. This could be due to the presence of desiccation resistant forms and to the ability of these microorganisms to exploit the remaining moisture present in the leaves or contiguous sediment. Further work is needed to better understand how microbial communities change with flowing water and with the associated decomposition of leaf litter organic matter. Moreover, this work also suggests that the length of drying events can further modify the community present on leaf litter and for this reason it could be interesting to perform the same analyses using open-air mesocosms (i.e. artificial flumes). Mesocosms would allow for better control of variables such as manipulating the severity and the length of the drying events. Better understanding the impact of riverbed drying on microbial communities is fundamental within the perspective of global climate change, especially in ecosystems where this phenomenon does not occur naturally, altering their functional processes and thus their water quality and biodiversity.

Authors contributions

Conceptualization: Laura Gruppuso, M. Eric Benbow, Francesca Bona, Stefano Fenoglio; writing: Laura Gruppuso, Joe Receveur; formal analysis: Joe Receveur, Laura Gruppuso; laboratory work: Laura Gruppuso, Joe Receveur; fieldwork: Laura Gruppuso, Stefano Fenoglio, Francesca Bona; all the authors set the experimental design, contributed critically to the writing and gave final approval for publication.

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Conflicts of interests

The authors declare that they have no known competing financial interests that could have appeared to influence the work reported in this paper.

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Major results and final remarks

This thesis brings some interesting novelties in the field of stream ecology: at first, Alpine streams that are only recently facing intermittency are still poorly investigated, and trying to understand the effects of drying events on these pristine river ecosystems is a quite new approach to the study of the effects of global climate change in streams and rivers worldwide. Moreover, this project integrates the leaf litter bags method, which has been widely used in this field to assess river functionality, with modern techniques such as elemental content analyses and high-throughput sequencing. The idea of comparing the results regarding intermittency and river functionality obtained in field with the ones of artificial flumes is a novel aspect too: understanding the similarities and the differences between these two experimental settings can help the interpretation of the findings but also a better arrangement of the study design. The experiments carried out within the framework of this PhD project underlined how global climate change along with the increasing anthropogenic pressures are seriously affecting mountain river ecosystems. The effects can be seen at multiple levels: macroscopically, at a hydrological level, the loss of surface flowing water is an extremely negative data, which can cause the loss of instream tridimensional connections and the disruption of the hydrologic and nutrient cycles. When looking at benthic macroinvertebrates, our experiments highlight that they have been negatively affected by this phenomenon, leading to a poorer and less stable community, which also causes biodiversity loss. The experiments carried out during this multidisciplinary research allowed to highlight a distinction between the communities with respect to water flow: i) in perennial Alpine sites, stream macroinvertebrates were mainly represented by EPT taxa which are the most sensitive organisms, associated with good water quality and widespread in mountain stream ecosystems. Indicator Species Analysis confirmed that Nemouridae and Limnephilidae (Plecoptera and Trichoptera) were associated with permanent flowing water, along with Diptera Ceratopogonidae; ii) intermittent sites were characterized by the presence of Diptera such as Simuliidae and Athericidae, which are mainly less sensitive and more generalist organisms, along with Elmidae (Coleoptera). Community composition was almost the same at the beginning of the experiments in both perennial and intermittent sites, and then, as the dry period progressed, turned out to be more variable, highlighting the fact the riverbed drying acted as an environmental filter on the community itself, with only a limited set of species surviving thanks to particular trait combinations. For example, the nestedness and turnover characterization of total beta-diversity investigated in chapter 2, showed that the dominance of nestedness was due to new taxa recruitment in perennial sites and to taxa loss in the intermittent ones. All these observed results were true not only when referring to field experiments, but also to the ones conducted in open air mesocosms (i.e. artificial flumes) even if with different taxa involved because of the different geographical and altitude? location. Furthermore, in intermittent flumes, total beta-diversity was limited with respect to the one detected in field, but this can be due to the fact that mesocosms' community comprises a subset of the taxa naturally occurring in streams. In the intermittent streams, biotic communities' composition changed substantially during low flow and drying periods, but, as reported in literature, they could potentially be able to recover rapidly when flowing water returns. This could not be totally true for those mountain streams which are recently facing intermittency: for example, in the Alpine rivers that we investigated, benthic macroinvertebrates turned out to be negatively affected by drying events, with differences in community diversity and composition, probably due to the long-term effects of desiccation and dispersal difficulties. As previously mentioned, we thus observed that taxa depletion was mainly led by the loss of those EPT taxa, which can be found almost exclusively where water was present during the whole sampling campaign (chapters 1 and 2). Moreover, large semivoltine taxa such as *Dinocras* sp. and *Perla* sp., were less abundant in intermittent than in perennial sites, because they were not able to thrive during drying phases. Small, fast growing and plurivoltine organisms such as Chironomidae, Simuliidae and *Baetis* sp., instead, proved to be able to survive even in those conditions, showing an opposite trend. In fact, voltinism has been defined as one of the most sensitive traits, along with body size: as previously demonstrated in literature, stream macroinvertebrates can shift from multivoltinism to semivoltinism when switching from perennial to intermittent sites; besides, reduced body size dimensions allow fast development and population growth, fundamental characteristics when life cycle must be completed before the disappearance of surface flowing water. Unfortunately, in Alpine streams, where intermittency is mainly driven by anthropogenic activities, it is difficult for stream macroinvertebrates to predict when water will disappear and for how long and consequently, to adapt to this phenomenon. At an ecological niche level, the occurrence of drying events can cause shifts in niches occupation preferences, especially when looking at those key functional feeding groups in mountain streams, such as shredders and scrapers (chapter 1). We observed that scrapers co-occurring in similar environmental conditions were negatively affected by dewatering events and that the niche occupation was correlated with flow intensity, substratum type and chlorophyll *a* concentration. For example, the investigated perennial sites showed a higher coarse substratum index than the intermittent ones, where fine sediments were more abundant due to loss of flowing water and this factor influenced the presence of the studied scrapers genera (*Ecdyonurus, Epeorus* and *Rhithrogena*). The effect of flow velocity was controversial, showing that sometimes a high Froud number was not directly linked to habitat preference for the observed rheophilous genera (specifically *Rhithrogena*): we hypothesized that this was due to the different periphyton shaping caused by current velocity, which can improve or reduce grazing efficiency. Our results also showed that only *Rhitrogena* and *Epeorus* showed a strong connection with chlorophyll *a* content, suggesting that the three genera examined have different ecological preferences. Because of the observed overlapping of the niches, we found out that during flow intermittence competition among coexisting taxa can happen because of the niches' reduced volume. The effects of global warming will lead to a reduction in autochthonous trophic resources, influencing the presence and the growth of scrapers, which will lead to a bottom-up effect within the food web. Regarding shredder macroinvertebrates, the three genera examined (*Nemoura, Protonemura* and *Amphinemura*) co-occurred but had a different niche occupation based on

CPOM availability and potential competitive pressure. *Nemoura* for example, displayed the smallest niche and turned out to be the most sensitive to flow intermittency, displaying semivoltine populations, which are more affected by loss of surface water. *Protonemura* and *Ampinemura* instead, turned out to be less influenced by intermittency, mainly because species belonging to these genera are usually monovoltine. *Amphinemura* displayed the highest tolerance towards drying events when compared to the other two genera: smaller body size and monovoltinism proved to be characteristics which enabled this shredder to thrive in these difficult conditions. Thus, in perennial sites, where CPOM availability is high, the three examined shredder genera can coexist, while in the intermittent ones, competition for the resources resulted in a weak negative effect on Nemouridae presence and abundance. In addition, drying events are associated with fine sediment accumulation, which further impacts on benthic communities: organic matter burial due to fine sediments can alter microbial colonization of the litter and reduces the availability of this resource. When looking at functional groups, we noticed that desiccation positively selected tolerant species, compensating non-adapted taxa filtering. The obtained results also highlight that macroinvertebrate functional feeding groups had been altered during non-flow events, with severe repercussions on functional processes. Specifically, shredders and scrapers decreased when our sites experienced dry conditions, while collector-gatherers increased, thus producing an effect not only on community composition, but also on river ecosystem’s functionality. At a functional level, the loss of surface flowing water strongly affected leaf litter decomposition too. Instream allochthonous organic matter decomposition is fundamental in river ecosystems' nutrient cycling and the evidence that this process can be slowed down by drying events points out that the whole food chain can be seriously altered by this phenomenon. At first, changing water and air temperatures imply a different microbial community development on the leaves' surface and this effect can be further exacerbated by the complete loss of water, which causes desiccation. If microbial community changes, it will be more difficult for stream macroinvertebrates which mainly feed on leaf litter to process the detritus; the presence of particular bacteria and fungi is fundamental in order to degrade complex nutrients and macromolecules, making them available for other organisms which are involved in the decomposition process and making leaves more palatable for shredders. Analyzing the collected data, we were able to confirm our hypothesis that flow intermittency negatively affects leaf litter decomposition. As a matter of fact, in both perennial and intermittent sites the percentage of leaf mass remaining decreased over time, but the lowest values were found only in the permanent ones. Indeed, the results showed that in absence of flow disruption and riverbed drying, litter decomposition was faster, being supported by the mechanical action of current velocity and especially by the colonization of the leaf bags made by microorganisms and macroinvertebrates. In those sites where dry events occurred, decomposition was visibly slowed down due to desiccation which affected consumers' communities. Moreover, leaf litter type and quality had a strong effect on the process: good quality leaves in terms of C:N content were also less coriaceous and poorer in secondary compounds such as lignin, tannins and phenols, characteristics that contributed to a faster degradation and a more efficient shredding action made by stream macroinvertebrates. For all the above-mentioned reasons, when looking at the decomposition rate of the two different leaf types, we observed almost the same trend due to the presence/absence of flowing water, but with different absolute values of leaf mass remaining at the end of the experiment. It is interesting to notice that, regardless of leaf litter quality, in the sites which experienced major dry events, the decomposition process was equally slowed down, suggesting that the above mentioned leaves characteristics have a stronger influence on the phenomenon during normal flow conditions. Furthermore, previous studies highlighted that immersion-emersion cycles can act on the chemical diversification of litter, thus affecting leaf degradation. These results helped us to understand that loss of surface flowing water has a severe negative effect on the decomposition process, being able to affect all the nutrient cycle acting directly on its main actors, i.e. micro- and macro-consumers. At microbial community level, the results we obtained by sequencing the genetic material of both bacteria and fungi present on our leaf bags are very interesting: recalcitrant and more coriaceous oak leaves showed a high bacterial and fungal diversity, which was needed in order to support the decomposition process on this type of substratum. Moreover, for both leaf types, drying events showed limited and non-significant effects on both bacterial and fungal communities, suggesting the presence of desiccation resistant forms; these results point out that leaf type and nutrient content have a stronger effect on leaf litter decomposition than riverbed drying itself. We also noticed that, regardless of the flow regime, chestnut and oak bacterial communities resulted as distinct at the beginning of the experiment, but then converged in later sampling dates; in contrast, fungal communities showed a very different pattern, being separated by leaf type throughout the whole experiment. These observations can be explained by the fact that bacterial communities present at early decomposition stages can be species-specific and then they leave room for more generalized taxa which are required to bring forward the process, despite the leaf type. For fungi, instead, there is a higher requirement of species-specific taxa, because of the different quality and texture of the leaves, which highlight the need of different fungal families able to degrade more complex secondary compounds. Thanks to the modern sequencing techniques, we detected two bacterial families commonly associated to the leaf phyllosphere for both leaf types: Rhodobacteraceae and Comamonadaceae for *Quercus robur* and Rhodobacteraceae and Spingomonadaceae for *Castanea sativa*. The results also pointed out that the majority of the bacterial community present on our leaf bags in both perennial and intermittent sites, consisted of bacteria belonging to the Phyla Proteobacteria and Bacteroidota, frequently associated with decomposition processes. When looking and fungal community, we detected two families referring to the phyllosphere, such as Cladosporaceae and Pleosporaceae, for both leaf types. As we expected, the investigated community was dominated by Ascomycota, because usually, as previously reported in literature, Basidiomycota occur later during the decomposition process, at stages that we did not investigated in our experiment. Moreover, the family of Aureobasidiaceae, commonly widespread in freshwater and marine ecosystems, turned out to be the most abundant in our samples, despite leaf type and flow regime, likely because this family is able to adapt to different environmental conditions and thus to cope with hydrological and nutrient stressors. Another interesting result was that the fungi of the family Pleomassariaceae, widely distributed on decomposing wood and plant material, were highly abundant when dry events occurred, while being consistently lower in abundance in perennial sites. This family is able to exploit the remaining moisture of the substratum, even if flowing water is going to disappear; it will be interesting to further investigate this particular Pleomassariaceae's trait, in order to understand if it will be maintained during longer desiccation periods. As previously mentioned, leaf litter decomposition processes are difficult to investigate, because there are a huge number of biotic and abiotic factors involved. Global climate change is going to act on the majority of those factors, triggering a ripple effect which can seriously damage river ecosystems functionality and biodiversity.

This research project enabled us to understand what is happening in Alpine river ecosystems nowadays: it is difficult to predict when surface flowing water will be lost and for how long, but collecting data is essential in order to understand these complex mechanisms, working on a way to mitigate (as far as possible) the effects on benthic communities and functional processes. Unfortunately, dry events are becoming increasingly supraseasonal: usually, we expect to find lower water levels and dry events during summer, but over the past few years this phenomenon has occurred also in late autumn/winter and early spring, causing major problems not only to the anthropic activities but also (and mostly) to the river community and functionality. In some cases, post-drought recovery is fast and stream macroinvertebrates are able to thrive in these conditions, especially in Mediterranean streams and rivers, where dry events are natural and recurrent. Otherwise, in Alpine streams, community restoration is more difficult, because some taxa are strictly cold stenothermal and rheophilic and use flowing water for their dispersal, not being good crawlers able to persist in case of flow loss. These organisms, not adapted yet to this phenomenon, are going to be seriously affected by riverbed drying caused by global climate change, putting at risk the high biodiversity which characterizes these peculiar ecosystems. Due to the difficulty in predicting drying periods, during this project we compared some of our field results with the ones obtained in open-air artificial streams in the North-Eastern Italian Alps. This enabled us to highlight the importance of using mesocoms to study the effects of global climate change, in particular riverbed drying, but also suggested to interpret with care these results and to always keep in mind that what really occurs in field is a complex outcome due to multiple factors, which can be difficult to control in their totality, even using artificial flumes. However, this can be an interesting alternative, especially due to the unpredictability of changing flow conditions. The colonization of leaf litter by bacteria and fungi at first, and then by macroinvertebrates, is of utmost importance for a correct and efficient decomposition process. Global warming and anthropogenic pressures are going to directly impact these communities: desiccation due to drying events showed to be an important factor in the disruption of this complex equilibrium. The loss of those shredder taxa which mainly feed on CPOM and the variation in microbial community due to the changing litter characteristics and loss of surface water has significantly slowed down the leaf litter decomposition process. As a result, following the River Continuum Concept, nutrient cycling is affected from upstream to downstream, altering Alpine streams' functionality in a broader way. Furthermore, when a complex and broad phenomenon such as global climate change is investigated, we cannot neglect the fact that it will impact riparian vegetation too. Hence, this can have affected the microbial phyllosphere community before leaf abscission, changing its composition prior the beginning of the experiment. This is an interesting point of view, which helps us keep in mind that studying leaf litter decomposition in the perspective of global changing climate could be challenging because of the huge number of misleading factors involved.

We can thus conclude that Alpine streams are going to be among the most damaged river ecosystems in a global change scenario: their peculiar biodiversity, the pristine water quality and their fundamental role during the initial phases of nutrient cycling will be seriously affected by changing water temperatures, disruption of hydrologic cycle and riverbed drying. Since in those streams intermittency is not a natural phenomenon, biological communities showed to be not adapted yet to these environmental changes, thus are not able to thrive in those peculiar conditions. Among macroinvertebrates, the functional feeding groups of shredders and scrapers will be probably the most affected by the phenomenon: formerly, because some of the genera corresponding to these two functional feeding groups are the most sensitive and rheophilous ones and also because their trophic resources are going to be drastically modified in their nutrient content and palatability by the effects of global warming. For example, periphyton growth and quality will be influenced by current velocity and irradiation, while leaf detritus quality will be determined by the microbial community present on the fallen leaves (bacteria and fungi of the phyllosphere) and the one present on moist leaves instream during decomposition. Feeding sources of lower quality are going to affect both shredders and scrapers, causing damage at feeding groups level, at community level and at the whole food chain. Disrupted decomposition processes will alter river quality and functionality, causing even more damages downstream. Unfortunately, it is difficult to plan mitigation strategies. In general, human activities are seriously worsening the whole situation: water abstraction for agriculture and hydropower, emission of greenhouse gasses and pollution in its entire spectrum, are exacerbating the general and widespread effects of global climate change, adding more threats to the ecosystems and the biological communities inhabiting them. The river ecosystems investigated during this research project are among the most pristine in the Italian Alps, but our results showed that they are becoming more and more threatened by all these climatic changes and anthropogenic pressures, being at risk of losing their perennial flow regime and the unique biological communities present. The disruption of nutrient cycling is another key symptom: if river functionality is affected, its quality is affected and also its biological community, causing problems from upstream to downstream, following the River Continuum Concept. The only thing that can be done is beginning to act right now: every small step towards the preservation of our rivers is essential, in order to try to reduce our ecological footprint, wasting less and trying to use our water resources in a more conscious and responsible way. The results we obtained are scientifically very interesting and useful, but also highlighted the fact that our beautiful, perennial and pristine river ecosystems are seriously facing the possibility of disappearing in the next future. The world is changing and so we have to, trying to repair the damages we caused. It will be necessary to rationalize water usage, select less water demanding crops, improve the irrigation and water transport systems. Global climate change is leading not only to a decrease in water discharge, but also to a chemical and microbiological water quality worsening, because of lack of dilution. Such mountain ecosystems are important for multiple reasons: they serve as water reservoir, biodiversity hotspot, nutrient sink for the stretches located downstream, but they also are astonishing spots for sustainable tourism and sports activities, which can drive people to better appreciate those places and motivate them towards their environmental protection and preservation.

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