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PhD IN EVOLUTIONARY BIOLOGY AND ECOLOGY

XXX CYCLE

Macroinvertebrates in riverine systems with different degree of intermittence: influence of community dynamics and environmental variables at different spatial scales

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CONTENTS

Thesis abstract

Macroinvertebrate communities exhibit high variability in diversity, abundance and structure at different spatial scales. Space is currently used as an explicit predictor to discriminate between environmental forcing and biotic processes at large and medium sized scales, but it is generally neglected at smaller scales, to which macroinvertebrate community organization is generally studied only within the environmental filtering framework, disregarding processes other than those based on abiotic factors. To fill this gap, in the first section of the present thesis, we considered environmental as well as spatial variables with the aim of explaining diversity, abundance and community patterns of macroinvertebrate community at small scale, by using geostatistical and multivariate spatial analysis. At first, we performed a pilot sampling campaign in May 2015 using a specific *in situ* sampling design along a reach of an intermittent system (Baganza stream, Northern Italy). Overall, 5493 organisms belonging to 25 taxa were collected and identified being Chironomidae, Baetidae and Naididae the most abundant taxa. Space explained a consistent fraction of abundance and taxa richness variability both singularly and jointly with depth. Moreover, the latter was a good predictor of abundance, but not for taxa richness and community structure. Our results suggest that while organisms seem to be able to occupy almost any position in the watercourse, their abundance is modulated by habitat preference. This study represents a starting point for understanding how niche-based and dispersal processes act on macroinvertebrates communities at very small scale. Findings improve the knowledge about the fine scale organization of macroinvertebrate communities in intermittent streams. Restoration ecology, habitat suitability modelling and biomonitoring sampling methods could benefit from our approach.

Based on the results of the pilot study we did a second and more in-depth study, working in three rhithral sections of perennial streams located in the Po River Basin (Northern Italy) by means of specific *in situ* 50-points random sampling grids. Benthic Organic Matter (BOM), velocity, depth and substrate were collected, as environmental factors, together with spatial coordinates for each sample. The relationship among metrics (taxa richness, abundance and biomass) and environmental and spatial variables was checked by means of Generalized Additive Models (GAMs). Regarding the analysis of communities, coordinates were used to produce Principal Coordinates of Neighbour Matrices (PCNM) in order to detect additional spatial structures. Data were then analysed by means of variance partitioning methods, considering spatial coordinates, PCNM and environmental factors as groups of explanatory variables. Environmental factors (primarily BOM), both with and without

spatial structure resulted the main drivers affecting taxa richness, abundance and community composition. On the other hand, coordinates and PCNM accounted for a minor fraction of explained variance. Nevertheless, we found that in systems with a greater riverbed structuration, PCNMs variables had higher explanatory power, highlighting the importance of space, as a proxy of small-scale community processes. Our results suggest that trophic factors are useful predictors of macroinvertebrate community organisation in rhithral sections of perennial streams.

The second section of this PhD thesis concerns the organization and variability of macroinvertebrate community in braided rivers. These systems are among the most variable and dynamic riverine systems. Changes in braided rivers are sudden and frequent, driven by the high hydrological variability. They host high levels of local heterogeneity, with many different habitats in close proximity establishing a mosaic of patches. This provides the conditions for high levels of biodiversity, with strong community variability in particular among the different habitats at the stream-reach level. Nevertheless, these systems are still poorly studied and their complexity is often not taken into account in biomonitoring protocols. We applied mixed effects modelling, spatial ordination techniques and beta-diversity partitioning (into nestedness and turnover components) with the aim of improving the knowledge of braided rivers, investigating: i) the organization of macroinvertebrate communities among the different habitats of a river reach, and ii) the temporal variability of this organization (both among seasons and during summer). We predicted a differentiation of macroinvertebrate communities between distinct habitats within rivers, with this differentiation increasing during the low-flow period. We carried out our study in four braided rivers and streams of the Po River basin (Northern Italy) sampling three different kinds of mesohabitats (main channel, secondary channel and pool) in eight stations during seven campaigns from June 2015 to April 2016. We found a high variability of taxa richness, abundance and community structure among mesohabitats, with marginal ones accounting for the greater part of macroinvertebrate diversity. Secondary channels resulted as being the habitat hosting greater taxa diversity, with 10 exclusive taxa. Surprisingly the mesohabitat communities differed greatly during the seasonal phase, whereas their dissimilarity decreased during summer. This could be explained considering the summer flow reduction as a homogenizing force, leading to a general loss of the most sensitive taxa. However, the summer taxa turnover value resulted higher than nestedness, suggesting a strong environmental control on community organization, with taxa well adapted to the different conditions of mesohabitats and able to manage the effects of flow reduction. Our work represents a remarkable issue for biomonitoring protocols, highlighting the importance of taking into account the whole complexity of braided rivers for a more realistic evaluation of macroinvertebrate communities.

In the third section of this thesis, we focussed the attention on the study of large-scale distribution of macroinvertebrates and on differences in community organization in watercourses with different hydrology. Flow regime and its alterations deeply affect macroinvertebrate communities, especially considering the shift in conditions which is occurring in several mediterranean and temperate rivers. This topic has been deeply explored in mediterranean systems, but the effect of regime shift is less known in temperate areas and seldom considering it in the framework of metacommunity ecology, incorporating also space in explanatory variables. With this in mind, we did our work aiming to understand the effect of flow intermittence on the large-scale distribution of benthic invertebrates and the differential importance of explanatory variables related to different spatial scales in permanent (P) versus intermittent (I) watercourses. We carried our work in 24 watercourses (11 intermittent and 13 permanent) of the Po River Basin (N Italy) before the summer dry phase. We applied mixed effect modelling and spatial ordination techniques in order to evaluate the variation of metrics and community structure between I and P streams and variance partitioning for assessing the relevance of the different spatial scales in I versus P. Communities of I streams resulted characterized by a greater randomness than in P streams, with, in general, greater levels of diversity in P ones. Moreover, we found out that both in I and P streams, local environmental variables are the most powerful predictors of community structuration. Our findings represent valuable insight in the effects of flow alterations in the perspective of best-strategy planning to face the regime-shift phenomenon.

General Introduction

THESIS FRAMEWORK

Lotic ecosystems occupy a minor fraction of the Earth surface (0.8%) and represent a small fraction of freshwaters (0.01%). Nevertheless, they host a disproportional number of species (> 6%, Vörösmarty et al. 2010). Given their historical central role in human activities, riverine systems are among the most vulnerable and threatened environments (Allan & Castillo 2007), with plenty of human-induced alterations that modify their natural equilibrium. At the global scale, currently nearly the 65% of all riverine systems is subject to severe threats (Vörösmarty et al. 2010) due to the combined effect of several factors such as global warming, increased extreme events, fragmentation of habitats and multiple man-made impacts (Domisch et al. 2013). The decline of biodiversity in freshwater is greater than in any other ecosystem (Allan et al. 2005; Dudgeon et al. 2006; Stendera et al. 2012): for example, about 2251 animal species (41%) of the 5435 included in the IUCN 2000 Red List live in aquatic environments (Stendera et al. 2012). In particular, in the Mediterranean basin, the human species is present since ages; it is in this area that most of the ancient civilizations developed and so the human is acting on the environment for a very long time.

Besides their astonishing diversity, lotic systems are a very peculiar environment and possess several features that make them unique. For instance, they present a hierarchical organization (e.g. Frissell et al. 1986), which implies that local patches can be affected by processes operating at larger (or smaller) spatial scales (Manfrin et al. 2016). Another feature that characterize riverine systems is their dendritic/fractal structure (e.g. Brown et al. 2011; Altermatt 2013), which generate an anisotropy in space and a preferential direction of dispersion. All these factors make watercourses unique environments, but at the same time get more difficult the understanding of their functioning, which is essential in order to protect them in a changing world. A better understanding of lotic systems and factors affecting their biotic communities is therefore of crucial importance for their conservation. This PhD thesis focusses on the role played by different drivers in structuring macroinvertebrate communities in lotic systems at three spatial scales (Fig. 1). Macroinvertebrates are of paramount importance in lotic ecosystems, since they occupy key positions in food webs and can play a crucial role in nutrient cycles, primary productivity, decomposition of allochthonous organic matter and translocation of materials (Wallace & Webster 1996).

According to the hierarchical organization proposed by Frissell et al. (1986) we considered, from the smallest to the largest:

- *MICROSCALE* (Fig. 1A, Section I) – The microhabitat level (10^{-1} m)
  Studying the microscale organization, we worked at a very fine sampling unit (surber level), considering the variability of communities among microhabitats (like cobbles, boulders, gravel and roots) within a watercourse segment. We considered both spatial and environmental variables, comparing their influence in different streams and in different seasons.
- *MESOSCALE* (Fig. 1B, Section II) – The mesohabitat level (10^{1} m)
  The second level considered in this study is the "mesohabitat" level, concerning the variability among the different habitats that can be found in a river reach: we have choosen to focus our work on three categories of habitats, namely main channels, secondary channels and pools. For this section, we worked on braided systems, i.e. those rivers and streams composed by multiple channels.
- *MACROSCALE* (Fig. 1C, Section III) – The stream level (10^{3} – 10^{4} m)
  The last part of this project regards the broad scale organization of communities, considering their variability among watercourses (both within and among catchments) with different hydrological regimes.

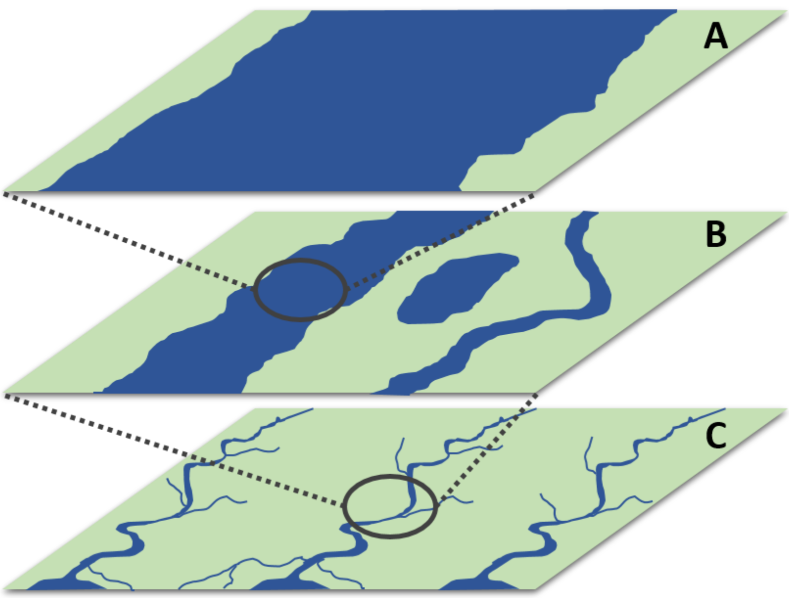

Figure 1: Spatial extents considered in the three sections of the present PhD thesis. Following a top-down direction: microscale (A), mesoscale (B) and macroscale (C).

This PhD thesis falls within the sphere of community ecology, which is the study of taxa interactions, distributions and drivers at various spatial scales. Spatial dynamics and the distribution of organisms are getting growing attention in the last years, since local communities are dynamic objects, which exhibit high levels of spatial and temporal variability. As riverine systems, they can be seen as open systems, interacting between them and with the environment (e.g. Heino et al. 2015b; Datry et al. 2016). Moreover, factors affecting community structure are numerous and time- and scale-dependent (Lamouroux et al. 2004; Cottenie 2005; Mykrä et al. 2007; Brown et al. 2011; Straka et al. 2012; Heino & Grönroos 2013). In this context, the metacommunity framework represents a very suitable tool for understanding the distribution of organisms. Metacommunities are defined as "networks of local biotic communities in which inter-community dispersal and intra-community interactions affect species persistence and turnover" (Leibold et al. 2004, Larned et al. 2010). Four different perspectives have been proposed to explain metacommunity dynamics, differing for the heterogeneity of the environment and for species features (McLaughlin et al. 2013).

1) *Patch Dynamic* (PD) – habitat patches are homogeneous, and suitable for being colonized by organisms that differ for dispersal ability. There is a colonization-competition trade-off, with best colonizers occupying habitats at the beginning of the ecological succession and best competitors occupying more stable environments (Logue et al. 2011; McLaughlin et al. 2013, Heino et al. 2015b).

2) *Species Sorting* (SS) – habitat patches are environmentally heterogeneous and filter the species assemblages, provided that the rate of dispersion is high enough to allow species to track environmental differences (Logue et al. 2011).

3) *Mass Effect* (ME) – habitat patches are environmentally heterogeneous, but higher dispersion rates generate a sink-source dynamic that masks the influence of environmental gradients and allows organisms to live in suboptimal conditions (Heino et al. 2015b)

4) *Neutral Model* (NM) – habitat patches are homogeneous and species are assumed as being ecologically equivalent. Similarity in communities will be negatively related with distance between sites and their composition will be driven only by stochastic processes in a colonization-extinction framework (Cottenie 2005; Thompson & Townsend 2006; Altermatt 2013).

Since the introduction of the metacommunity framework by Leibold et al. (2004), several studies focussed on seize the natural communities from the point of view of these four perspectives (Heino et al. 2015b). However, one of the most common conclusions is that natural communities cannot be described univocally with one of these models, rather they are the result of a combination of more than one perspective (e.g. Cottenie 2005; Gravel et al. 2006; Logue et al. 2011; Altermatt 2013; Heino et al. 2015b) and therefore the new focus is to quantify their relative importance (Moritz et al. 2013). This is accomplished by quantifying the importance of community drivers.

Main drivers commonly proposed to explain metacommunity organization are environmental or abiotic factor, biotic interaction and dispersal driven dynamics (often linked to geographic location), with dispersal ability mediating the importance of factors related to different spatial scales. These drivers can also be seen in the spatial scale perspective and therefore grouped into two general categories: local drivers, which include organism interactions and local environmental conditions, and regional drivers that are mainly related to the dispersal of organisms among local parches (Brown et al. 2011). One of the key issue in metacommunity ecology is the study of the spatial scale at which communities exhibit higher variability and factors of which scale exert stronger influence in shaping such communities (e.g. Van Looy et al. 2017). The influence of environmental factors is related to this issue. Indeed the strength of environmental control is mediated by the dispersion of organisms: with intermediate levels of dispersion, the organisms are able to track environmental changes, choosing the more suitable conditions, and therefore there is a strong environmental control of assemblages (this is the case of species sorting in the metacommunity framework). On the other hand, if dispersion rate is too high or too low, the environmental control is low since it is masked by the mass effect or organisms are not able to reach the suitable habitats (Smith and Lundholm 2010, Heino et al. 2015a). The intensity of dispersion depends on two main factors: the dispersal ability of taxa and the distance and spatial arrangement among sites. The spatial component has been for a long time neglected or treated as “nuisance” (Cottenie 2005), and only in recent years several authors have proven its importance, especially considering larger spatial extents (e.g. Cottenie 2005; Mykrä et al. 2007; Altermatt 2013; Soininen 2016). The influence of space, as stated before, can exert strong influence on freshwater assemblages, with space commonly considered as a proxy for dispersion (Heino et al. 2015a). As a consequence, including space as explicit covariate in metacommunity studies could really improve our understanding of freshwater ecosystems (e.g. De Cáceres et al. 2012; Dray et al. 2012).

Besides to the organization of macroinvertebrate communities and the factors driving them, the present PhD thesis also deals with other topics, presented in the three sections. The first one of these side topics is discussed in Section II and regards the environmental heterogeneity in braided rivers. These systems are characterized by the presence of multiple channel with variable hydrological conditions and with variable level of connectivity among them. Despite these systems are widespread, their complexity is often not taken into account into monitoring programs, with possible consequences on the validity of biotic indices and conservation programmes. Another issue considered in this thesis is the phenomenon of regime-shift/increasing intermittence (discussed in Section III), reported by several authors both in the Mediterranean and Temperate regions (e.g. Bonada et al. 2007; Larned et al. 2010; Gallart et al. 2012; Datry et al. 2014; Prat et al. 2014). Such situation is deeply altering the natural trends of aquatic communities, with responses that are highly variable and depending on the harshness of droughts. This topic should be regarded also considering the problem of ecological flow that aims to evaluate which is the threshold below which there is a heavy alteration of lotic communities.

In Sections I and III we decided to consider different life stages of organisms (larval, pupa and adult) as separated taxonomic units. We made this choice because the same taxa considered as larva or as adult can have different needs, behaviour and limiting factors and therefore keeping them separated can improve the resolution of community studies.

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SECTION I: Microscale

ASSESSING SMALL SCALE DISTRIBUTION OF BENTHIC MACROINVERTEBRATES: A PILOT STUDY IN AN INTERMITTENT STREAM

INTRODUCTION

Macroinvertebrate communities exhibit high levels of variability in diversity, abundance and structure at different spatial scales (e.g. Parson et al. 2003; Mykrä et al. 2007). Numerous works have been carried out at regional or catchment scale, where habitat filtering, biotic interaction and dispersal driven dynamics concepts explain this variability (e.g. Gutiérrez-Cánovas et al. 2015; Siqueira et al., 2012; Astorga et al. 2014). However, the knowledge about the role played by these processes in structuring macroinvertebrate community at small-scale is still limited and often contradictory.

Studies focused on different spatial scales report high unexplained variation at small scale (e.g. Boyero & Bailey 2001; Boyero 2003b; Heino et al. 2004; Lamouroux et al. 2004; Bruno et al. 2014). Water velocity and depth (Brooks et al. 2005; Karaouzas & Płóciennik 2016), algal cover and litter characteristics (Downes et al. 2000; Kobayashi & Kagaya, 2002), substrate composition and sediment grain size (Barnes et al. 2013; Bo et al. 2007; Boyero 2003a) are usually considered key factors related to macroinvertebrate abundance and richness. Depth has been stressed to be crucial not only in shaping macroinvertebrate communities (e.g. Bournaud et al. 1998; Gayraud & Philippe 2001; Fenoglio et al. 2004) but also directly affecting lotic ecosystems proprieties as leaf-litter decomposition (Martinez et al. 2016). Space has historically received little attention, but it can play a determinant role in explaining the structuring of biological communities and it should be gathered as a covariate and explicitly introduced into statistical models (Tolonen et al. 2017). Surprisingly, the spatial location of samples is explicitly considered at large scale (e.g. Grönross et al. 2013), but generally neglected at small-scale.

The study of spatial autocorrelation, namely the tendency of closer objects to be more similar than things further apart in space (Bonada et al. 2012), could help to discriminate among factors affecting macroinvertebrate community structure and to identify specific patterns. This phenomenon has been linked to the dynamics internal to the community itself (especially to the dispersal ability, which largely depends on the distance) or to missing environmental covariates (Diggins & Newman 2009). Furthermore, the combined focus on small-scale and intermittent streams on macroinvertebrate communities has not been widely considered thus far, despite temporary river ecology represents a main worldwide challenge in aquatic science (Datry et al. 2014) and intermittent systems are the most common aquatic systems in South Europe (Tockner et al. 2009).

To fill this gap, our research focuses on an intermittent system aiming to investigate the contribution of environmental parameters and space in structuring macroinvertebrate community. We hypothesize that at the reach level, macroinvertebrates present a spatially structured community driven by depth features of the watercourse. Accordingly, dynamics internal to the community are supposed to be a minor driver since organisms show no dispersal limitations at small-scale.

METHODS

Study area

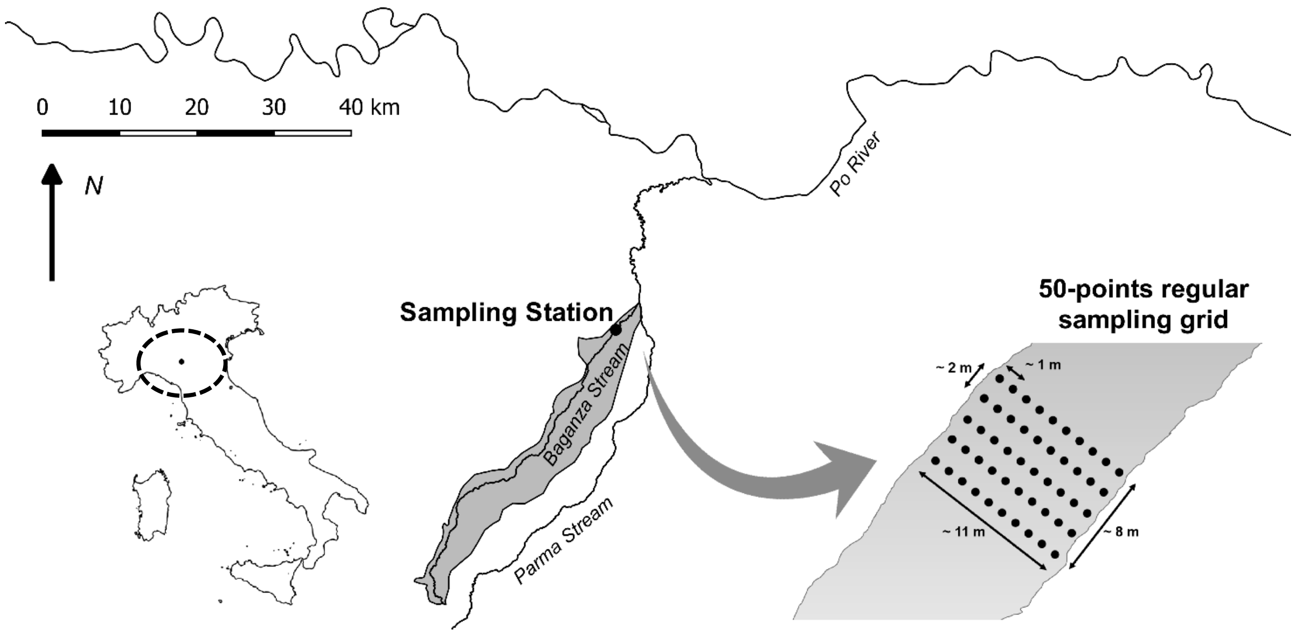

Figure 2: Study area and the sampling grid on Baganza stream, with indications of dimensions of the grid. Black dots represent the 50 surber points (marked stones).

Sampling was carried out in the Baganza Stream (Emilia-Romagna, Northern Italy), an intermittent 58 km long watercourse located in the Po River Basin, with a basin surface of 225 km^2 and a mean discharge of 5.2 m^3 s^{-1}. Intermittent streams are widespread in the studied area (Skoulikidis et al. 2017) where they present an extended dry phase (minimum 3 months) during the entire summer and large part of the autumn with relevant discharges usually from winter to spring (following rain distribution). The sampling site (44°45'35.0"N 10°16'41.0"E, 150 m a.s.l.) is part of a near-natural lowland stretch, with a mixture of riffles and runs, no deep pools and a limited slope. Here active channel width is approximately 155 m, but just a limited portion is occupied by a 5-10 m wide main channel. Agricultural land use and small-scattered urban areas cover the adjacent zones while dense riparian vegetation follows alongside the entire stretch.

Experimental design

In order to achieve the aims, a specific *in situ* sampling design was created by placing marked stones in the riverbed (Fig. 2) for a total of 50 points. Depth, relative coordinates and grain size were recorded for each point. Grain size was classified according to the Italian biomonitoring system (Buffagni & Erba 2007, Tab. 1).

***Table 1**: Substrate classification by Buffagni & Erba (2007).*

Microhabitat	Code	Description
Silt/Clay < 6 μm	ARG	Silt substrates, even with important organic constituents, and/or clay substrates made up of very fine granulometry
Sand 6 μm - 2 mm	SAB	Fine and coarse sand
Gravel 0,2 - 2 cm	GHI	Gravel and very coarse sand
Microlithal 2-6 cm	MIC	Small stones
Mesolithal 6-20 cm	MES	Medium size stones
Macrolithal 20-40 cm	MAC	Coarse stones
Megalithal > 40 cm	MGL	Large size stones, boulders, rocky substrates of which only the surface is sampled
Artificial	ART	Concrete and all non-granular solid substrates artificially placed in the river
Hygropetric	IGR	Thin water layer on solid substrate, often covered with mosses

Macroinvertebrates were collected just upstream each marked stone, using a Surber net of 500 μm mesh size and 0.05 m2 frame area. Samples were kept separated from each other in 1 litre PET bottles and then fixed with ethanol 90º for laboratory sorting. Identification was made at family or genus level (Ephemeroptera and Plecoptera) according to the taxonomic guide proposed by Tachet et al. (2010). To avoid bias due to temporal heterogeneity, sampling was carried out intensively during flow condition in spring (May 2015) before the drought period (summer). Samplings in spring enable us to obtain data during the wet phase and, at the same time, this season may represent a period with high invertebrate activities in these intermittent systems before the adult emergence period. A similar temporal approach was considered in numerous Mediterranean systems in South Europe (e.g. Bruno et al. 2014).

Data analysis

Three response variables were considered in this work: taxa richness, abundance and community composition. Two statistical methods were followed in order to highlight the spatial organization of macroinvertebrate community. At first taxa richness and abundance were modelled by using semivariograms, a geostatistical tool specifically targeted to measure the spatial autocorrelation of measured variables. A semivariogram is a graph in which semi-variance is plotted, on the ordinate, against distance classes among sites, on the abscissa (Legendre & Legendre 1998).

The organization of communities was explored with a non-Metric Multidimensional Scaling (nMDS), a spatial ordination technique that represents the set of objects along a predetermined number of axes maintaining the ordering relationships among them (Borcard et al. 2011). Modified Gower distance (Anderson et al. 2006) was used as dissimilarity measure and the goodness of ordination was assessed with the stress measure. Vectors proportional to depth and x-axis were also added to the ordination by means of the *envfit* function of vegan R package.

The relationship among the response variables (taxa richness, abundance and community structure) with explanatory variables (coordinates, depth and spatial structure) was then assessed by means of variance partitioning. This method enables us to assess the contribution of explanatory variables by the decomposition of R-squared as described in Peres-Neto et al (2006). Briefly, total variance is partitioned between explained and unexplained (or residual) variance, with the explained variance split into single and joint contribution. Explained and unexplained variance sum up to 100%. Variance partitioning for community structure was performed with raw and Hellinger transformed data.

Spatial structure was modelled by using principal coordinates of neighbour matrices (PCNM, Borcard & Legendre 2002; Dray et al. 2006). A similar approach was recently used by Tolonen et al. (2017) studying littoral macroinvertebrate community in a single aquatic system (Kitkajärvi lake system, Finland). PCNM method produces orthogonal spatial variables from broad to fine scale that allow taking into account spatial patterns among the replicates. In order to construct these spatial variables the procedure proposed by Borcard et al. (2011) was followed. A forward stepwise selection procedure was performed to detect significant PCNM variables for community structure, taxa richness and abundance. The last were transformed by natural logarithm prior to analysis in order to meet the assumptions of linear regression.

All analyses and graphs were performed with the base, packfor (Dray et al. 2013), geoR (Ribeiro & Diggle 2015), akima (Akima & Gebhardt 2015), fields (Nychka et al. 2015), plot3D (Soetaert 2016) and vegan packages (Oksanen et al. 2016) of the statistical software R (R Core Team 2016).

RESULTS

Overall, 5493 organisms belonging to 25 taxa were collected and identified. The most abundant taxon was Chironomidae with a total of 3726 individuals (nearly 68% of the total abundance), followed by *Baetis* with 893 individuals (16%) and Naididae with 493 individuals (9%). Abundance and detection frequencies of the collected families and genera are reported in Tab. 2. Most of the taxa (nearly 70%) were observed with detection frequencies below the 20% and 10 out of 25 could be considered as low abundance taxa (e.g., Guareschi et al. 2016).

Table 2: List of taxa found in Baganza stream, with total abundance, percentage abundance and frequency (expressed as percentage of samples with the presence of that taxa). The most abundant taxa are marked in bold.

Order	Taxa	Abundance (total)	Abundance (%)	Frequency (%)
Anthoathecatae	*Hydra*	5	0.09%	10%
Coleptera	Dytiscidae	3	0.05%	4%
	Elmidae	1	0.02%	2%
Diptera	Ceratopogonidae	4	0.07%	4%
	Chironomidae	**3726**	**67.83%**	**100%**
	Empididae	13	0.24%	22%
	Limoniidae	1	0.02%	2%
	Simuliidae	24	0.44%	38%
	Tipulidae	1	0.02%	2%
Ephemeroptera	***Baetis***	**893**	**16.26%**	**100%**
	Caenis	9	0.16%	18%
	Ecdyonurus	11	0.20%	14%
	Electrogena	12	0.22%	20%
	Ephemerella	153	2.79%	84%
	Rhitrogena	52	0.95%	46%
Gastropoda	*Bithynia*	1	0.02%	2%
	Valvata	1	0.02%	2%
Oligochaeta	Lumbricidae	3	0.05%	6%
	Naididae	**493**	**8.98%**	**72%**
Plecoptera	*Leuctra*	6	0.11%	12%
Prostigmata	Hydrachnidae	3	0.05%	6%
Trichoptera	Beraeidae	2	0.04%	4%
	Leptoceridae	1	0.02%	2%
	Hydropsychidae	7	0.13%	14%

Mean depth for each transect ranged from 26±13 to 29±14 cm; while maximum depth was 45 cm and minimum depth was 5 cm. The complete depth profile is shown in Fig. 3A while the spatial patterns of richness and abundance, according to the sampling grid and overlapped to the depth profile, are reported in Fig. 3B and 3C respectively. Substrate was dominated by microlithal (82%; diameter 2-6 cm) with minor percentages of gravel (10%; 0.2-2 cm) and mesolithal (8%; 6-20 cm).

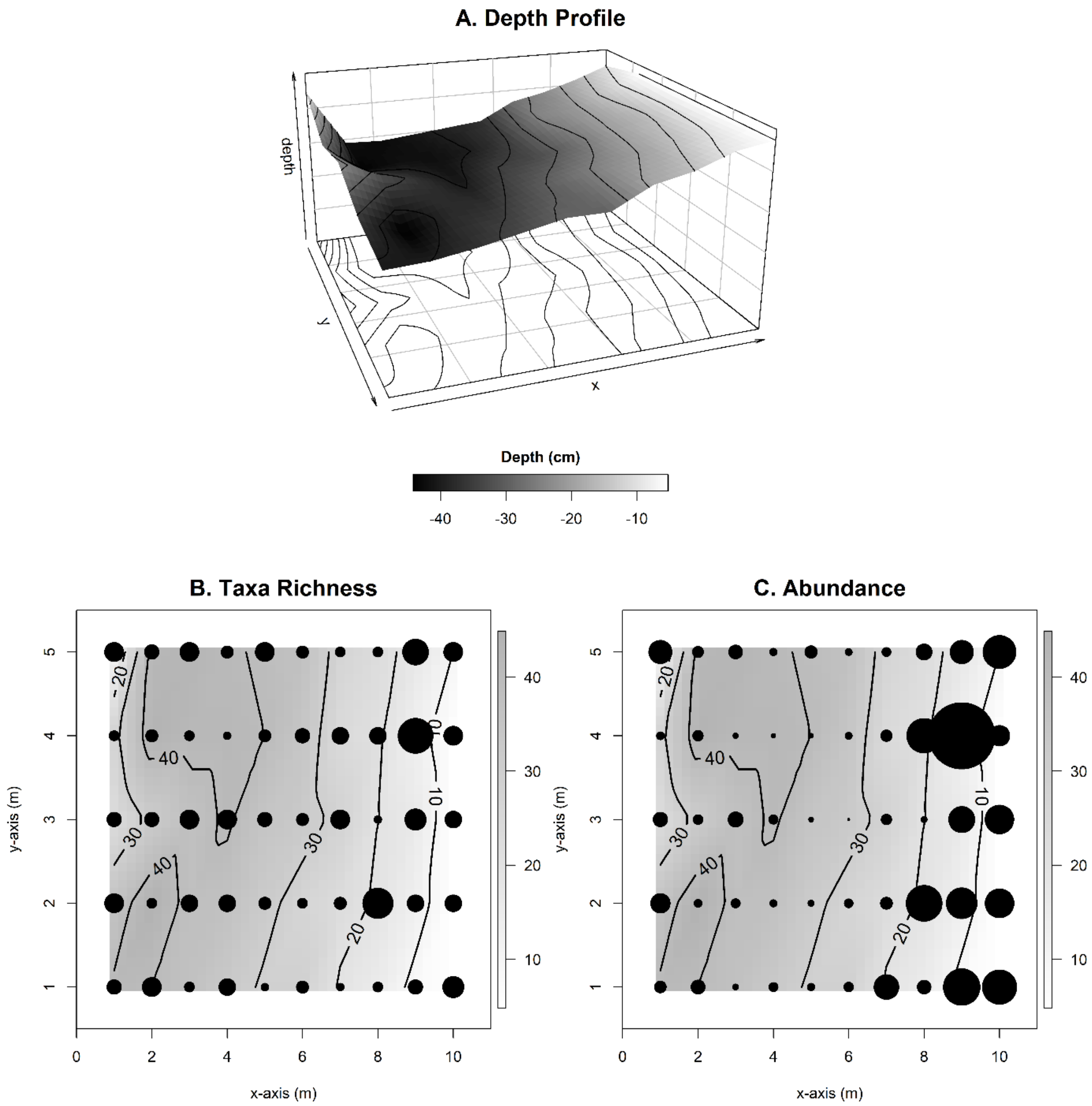

Figure 3: Depth profile (cm) of the sampling stretch in Baganza stream (A). Spatial distributions of Taxa Richness (B) and Abundance of organisms (C), represented with spatially located grey circles overlapped with the depth profile expressed in cm. The size of the circles is proportional to the metric values (taxa richness range 3-15; abundance range 11-479). X and Y values represents the coordinates of sampling points inside the grid, with transect on the y-axis and the 10 points of each transect on x-axis.

Semivariograms of log-transformed taxa richness and abundance are reported in Fig. 4 (4A and 4B respectively). Taxa richness lacks of spatial structure, while abundance showed a clear spatial autocorrelation with replicates located within a distance of 4.45 m correlated among each other.

Community ordination output is reported in Fig. 5. Points with different depth cluster in two different areas of the nMDS plot. This segregation seems to be driven by depth and x-axis.

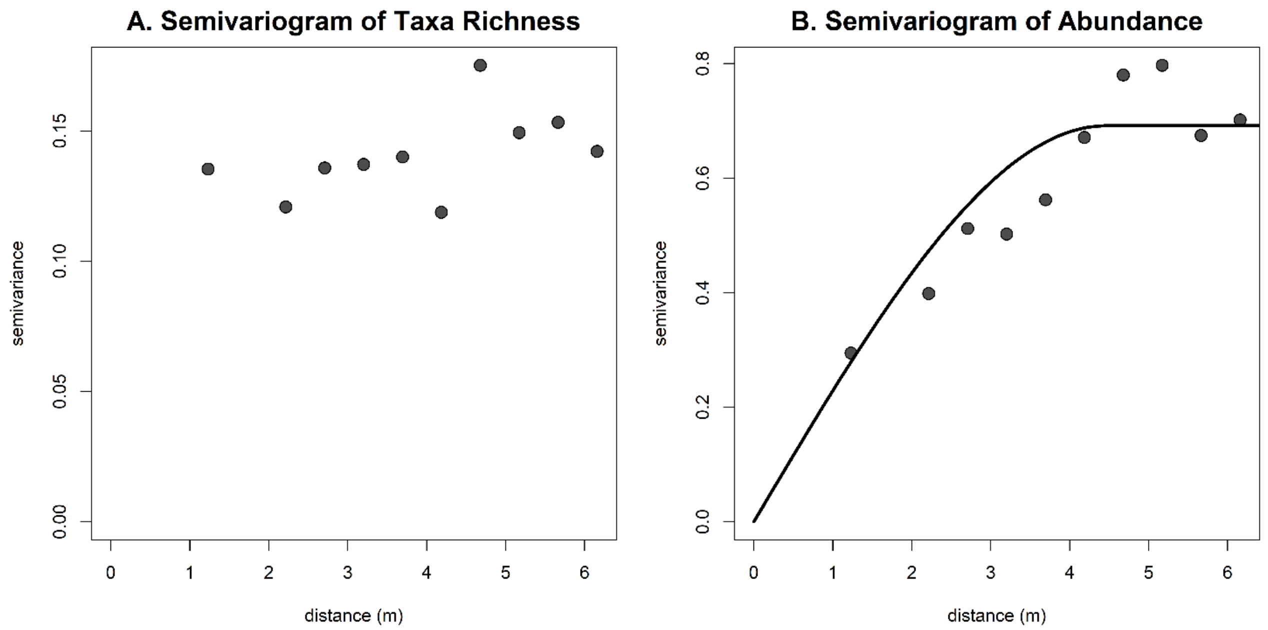

Figure 4: Semivariograms of log-transformed Taxa Richness (A) and Abundance (B). For the taxa richness there are no trends, while for abundance the trend is marked with the black line.

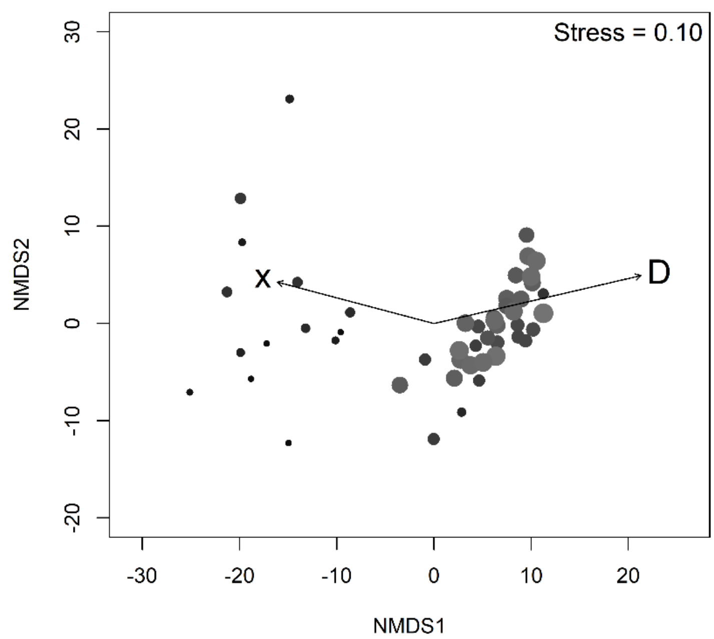

Figure 5: nMDS ordination output. Diameter of dots is proportional to the depth value. Ordination stress=0.10. Vectors representing depth and x-axis are also overlapped to the ordination.

Variance partitioning results for taxa richness and abundance are reported in Fig. 6. After forward selection only x coordinate, depth and a set of PCNM variables were retained. For taxa richness the explanatory variables account for the 24% of variance, with PCNM explaining the 20%. On the other hand, explanatory variables account for the 71% of variance for abundance, with greater contributions given by depth and PCNM variables joined (26%), PCNM variables (24%) and the three explanatory variables joined (22%). Results of variance partitioning of the whole macroinvertebrate community, both with and without Hellinger transformation, are reported in Fig. 7. For Hellinger-transformed data there is a minor explained variance (24%) with the larger contribution given by the three components joined (11%). For untransformed data there is a greater amount of explained variance (64%), with the joined contribution of the whole set of explanatory variables accounting for the 28% of total variance, followed by PCNM variables (19%) and PCNM and depth joined (17%).

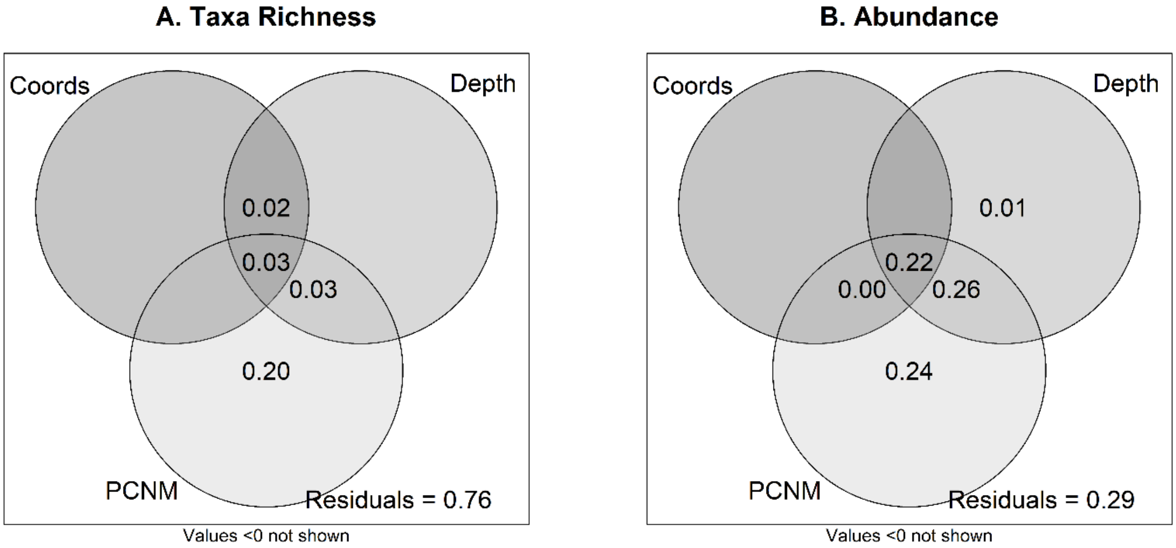

Figure 6: Variance partitioning results for log-transformed Taxa Richness (A) and Abundance (B) among the components of coordinates, depth and significant PCNM variables (see details in Methods). Residuals values are also displayed.

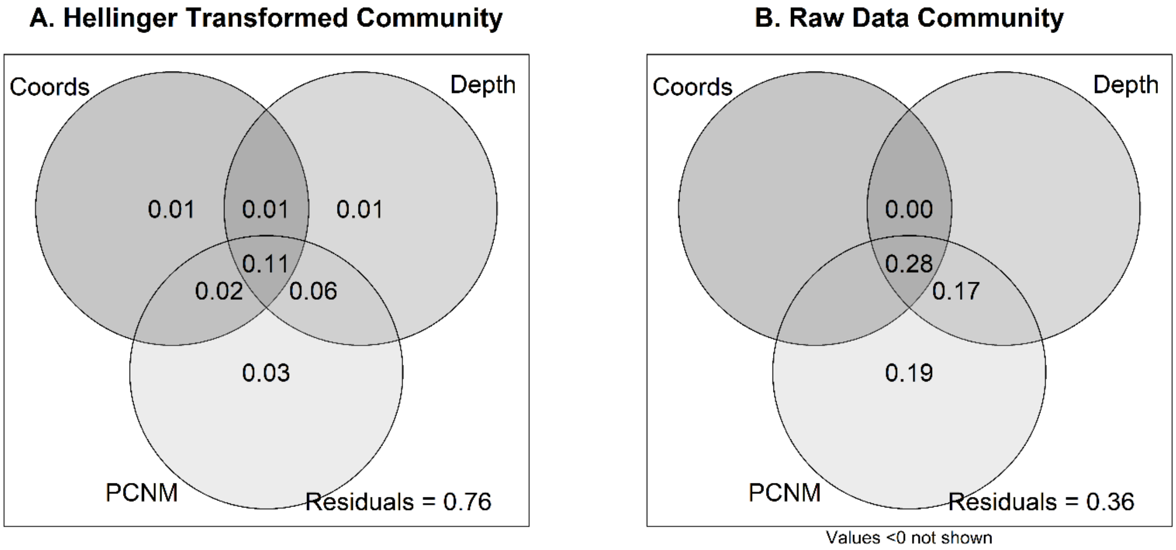

Figure 7: Variance partitioning results for community structure among the components of coordinates, depth and significant PCNM variables, operated on Hellinger transformed data (A) and on raw data (B). Residuals values are also displayed.

DISCUSSION

A spatially structured macroinvertebrate community was highlighted in the intermittent stream studied, with abundance being the variable more depending on the position inside the riverbed. Taxa richness showed a relationship only with PCNM variables, which represent a spatial structure uncovered by coordinates and depth. With the variance partitioning approach, a consistent fraction of taxa richness variance (20%) was explained by PCNM variables alone, indicating processes driven by factors not considered in this study. Community structure results, with Hellinger transformation, resemble those of taxa richness, while the results of community structure without transformation resemble those of abundance. This is due to the fact that Hellinger transformation (the square root of sample standardized to unit variance) operates a downweight of the most abundant taxa (Borcard et al. 2011), making the community matrix more similar to a presence/absence matrix.

Abundance and community structure (without transformation) exhibited a clear trend oriented transversally to the watercourse, highlighted by semivariogram, nMDS ordination and variance partitioning methods. From our results, it seems clear that for the studied reach of Baganza stream, depth is spatially structured along the x-axis (see Fig. 3A). This is mirrored by the organization of macroinvertebrates both in terms of structure and abundance (see discussion below). In fact, depth resulted a good predictor of macroinvertebrate abundance, while this was not true for taxa richness.

A negative relationship of water depth with abundance was already found in other watercourses (e.g. Brooks et al. 2005; Collier et al. 2010), while opposite results were found by Fenoglio et al. (2004) in tropical systems. The lack of a relationship between taxa richness and depth could be attributed to the small gradient measured in the Baganza stream. Moreover, community structure (with Hellinger transformed data) did not present spatial structure at all. The negative relationship between abundance and depth could be linked to greater food availability near the banks (Bournaud et al. 1998; Ferreiro et al. 2010), high water velocity in the riverbed centre that may dislodge organisms (Rempel et al. 2000) or to an interaction of these factors. In the studied stretch, macroinvertebrate community was mainly composed by collector gatherers and grazers such Chironomidae (mainly Orthocladiinae and Chironominae), *Baetis* and Naididae that commonly fed on fine particulate organic matter or algae and associated material. Furthermore, the behaviour of some aquatic insects that during emergence periods go towards shallow water could also explain this pattern (e.g. Sagnes et al. 2008). These results suggest that while different taxa can choose to occupy almost any position inside the riverbed, their abundance is strictly dependent on depth and, in general, on the spatial structure of the system: at fine scale the habitat filtering acts predominantly on abundance of organisms.

Focusing on the taxonomic list (Tab. 1) it is interesting to underline that most of the detected taxa presents low detection frequencies and/or low abundance. In general, these results seem consistent with recent data from invertebrate seedbank composition obtained in other temporal systems (e.g. Stubbington et al. 2016 in UK).

Biomonitoring in these lotic ecosystems represents an open research challenge for bioassessment science (e.g. Prat et al. 2014; Cid et al. 2015) and small scale variations may have relevant importance considering that the confidence and precision of a biological index can be closely linked to the small scale patchiness of aquatic taxa distribution (Laini et al. 2014). In this context, our findings provide useful informations on this topic, reflecting also the potential relevance of low abundance taxa in stream biomonitoring indices (e.g. Guareschi et al. 2016). Our case study represents one of the first studies trying to highlight the importance of both niche processes and community dynamics at small scale, especially in globally widespread and threatened ecosystems such as intermittent streams. Supplementary research (e.g. in different basins, conditions and with other explanatory variables) is needed and would be of scientific global interest in order to validate our results in a larger geographical context and to complement our knowledge on these systems.

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BY BOM BE DRIVEN: SPATIAL AND ENVIRONMENTAL FACTORS AFFECTING SMALL-SCALE ORGANIZATION OF MACROINVERTEBRATE COMMUNITIES

INTRODUCTION

The comprehension of factors affecting the organization and the distribution of natural communities has been a crucial issue in past decades (e.g. Johnson et al. 2007; Kuemmerlen et al., 2014), not only in community ecology but also considering the biomonitoring and restoration perspectives (Laini et al. 2014; Tolonen et al. 2017). Most of natural communities shows a high degree of patchiness and variability in distribution of organisms (McAuliffe 1984; Azovsky et al. 2000). Metacommunity theories point out environmental/habitat filtering and biotic/spatial processes as main groups of drivers for such variability. The differential relevance of these processes has been found to be highly variable and depending on the taxa features (e.g. dispersal ability) and on the different systems studied (Grönross et al. 2013; Soininen 2016) as well as on the spatial scale considered. In riverine systems, several authors report species sorting (sensu Leibold et al. 2004) as more suitable model for metacommunity dynamic (Cottenie 2005; Heino et al. 2015; Soininen 2016) though depending on dispersal limitations. Nevertheless, there is still a lack of concordance on this topic, with results depending on the variable considered.

Considering the distribution of aquatic macroinvertebrate at small-scale (meaningly the within mesohabitat scale), several authors highlight high levels of variability (Downes et al. 1993; Heino et al. 2004; Lamouroux et al. 2004; Lancaster & Belyea 2006; Costa & Melo 2008; LeCraw & Mackereth 2010), mainly ascribable to an environmental control for microhabitat differentiation (Fenoglio et al. 2004; Braccia & Voshell 2006). Depth and flow velocity ((Bournaud et al. 1998; Brooks et al. 2005), substrate composition and sediment grain size (Boyero 2003; Bo et al. 2007; Barnes et al. 2013), algal cover, litter characteristics (Downes et al. 2000; Fenoglio et al. 2005) and macrophytes (Heino & Korsu 2008) are generally identified as main environmental drivers of aquatic invertebrates. However, despite the various environmental factors considered, not always the fit between species composition and environment is strong and the unexplained fraction of variation often remains high (e.g. Lamouroux et al. 2004) with taxa distribution probably depending on other drivers than environmental ones. Indeed, as stated before, also biotic interactions can exert a strong influence on community dynamic, especially considering a spatial extent so limited (e.g. Patrick &

Swan 2011). Intra- and inter-specific interactions, including both negative (like competition, predation and parasitism) and positive interactions (like mutualism and commensalism), can play a crucial role in shaping communities (Holomuzki et al. 2010).

Assuming space as explicit covariate (and therefore integrating it into statistical models) has been proven to be a useful tool in unravelling macroinvertebrate community organization at the regional and catchment scale (Johnson et al. 2007; Mykrä et al. 2007; Soininen 2016). Space is often seen as a proxy for dispersal and biotic dynamics: its relative importance in community organization increases with increasing spatial distance due to dispersal limitation (Cottenie 2005; Mykrä et al. 2007). As a consequence, community located at a greater distance are supposed to be progressively more dissimilar between them (Mykrä et al. 2007; Heino et al. 2015). Hence, community located close to each other should be highly similar, since there are no dispersal limitations and communities can be homogenized (Soininen 2016; Tolonen et al. 2017) according to the mass effect model (sensu Leibold et al. 2004). As a consequence, the majority of studies regarding the small scale distribution of macroinvertebrate addresses only to environmental features, disregarding processes other than those based on abiotic factors. Few studies focus on discriminate between environmental and spatial drivers at this scale, using coordinates as explicit covariate (e.g. Tolonen et al. 2017). However, space (as proxy of other processes) can exert a strong influence also in structuring local communities and its omission could led to oversimplifications and misunderstandings of macroinvertebrate distributions (e.g. McLaughlin et al. 2013). Moreover, considering other organismal groups, several authors report high level of variability at small scale, largely dependent on the position inside the riverbed, for benthic algae (Bolpagni and Laini 2016), diatoms (Soininen et al. 2007), bacteria (Augspurger et al. 2010), and phytoplankton (Moresco et al. 2017).

In this contest, we conducted the present study on some very small areas, aiming to: i) identify the main variables that dictate the within mesohabitat variability of aquatic invertebrates; ii) discriminate between spatial and environmental drivers in structuring small-scale communities; and iii) quantify the role of biotic processes. With these purposes, we included space as explicit covariate in our study and we used it as a proxy for biotic interactions. Based on previous findings (e.g. Tolonen et al. 2017), we hypothesize a prevalence of environmental control in macroinvertebrate community structuration, with a dominance of niche-filtering mechanisms. Indeed, as stated before, considering the within stream scale, dispersal limitations are supposed to be very low, therefore organisms can track the environmental variability, occupying most suitable positions based on their necessity (according to the species sorting model by Leibold et al. 2004).

METHODS

Study area

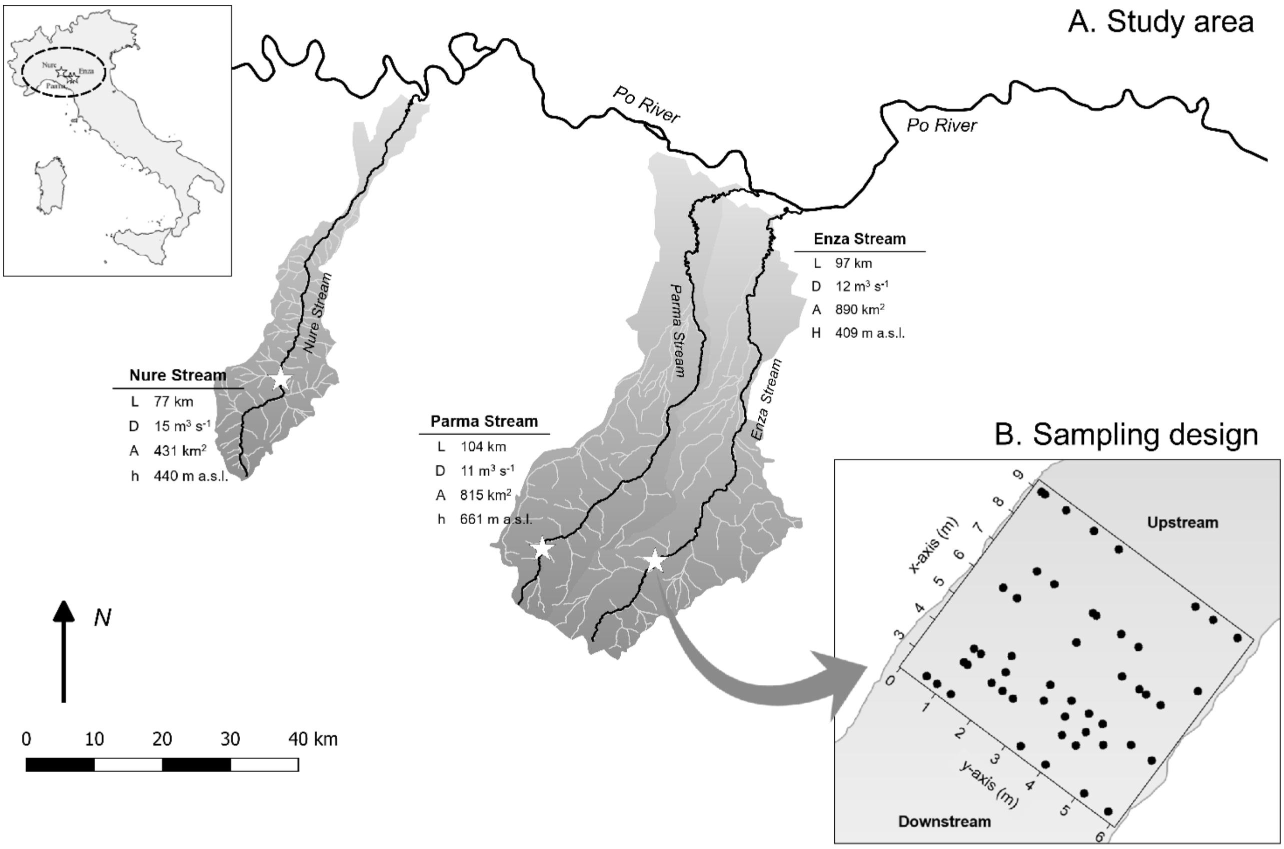

Figure 8: In A basins of Nure, Parma and Enza Streams. Sampling stations are marked with a star. Features of the streams are also reported in tables. L=total length, D=mean annual discharge, A=basin area, h=station altitude. Top-left, in the small square, the position of the sampling stations relative to Italy. In B a scheme of the sampling design is reported. Black dots represent the 50 points inside the grid, with positions varying depending on sampling campaign (for each sampling campaign coordinates were generated randomly). A Cartesian system of axes is overlapped to the scheme, representative for the axes position, with the x-axis parallel to the stream reach and the y-axis transversal to the stream reach.

Sampling was carried out in the geographical context of the Po River Basin, in Northern Italy (Fig. 8A). The Po River is the longest in Italy and it is fed by both Alpine and Apennine rivers and streams. For the present study, we selected three Apennine watercourses, right tributaries of the Po River: from W to E Nure Stream, Parma Stream and Enza Stream (Fig. 8A). These systems are included in the Cfb (warm temperate – fully humid – warm summer, for Nure Stream) and Csb (warm temperate – summer dry – warm summer, for Parma and Enza Streams) of the Köppen-Geiger Climate Classification. Because of the absence of Apennine glaciers, these watercourses are fed only by wet depositions and present two high discharge periods (in autumn and spring) and a main low water period in summer (with a secondary additional one in winter). Supplementary informations about watercourses are reported in Fig. 8A. We selected one sampling station for system, similar to each other for environmental features (e.g. mesohabitat type, substrate, width of the wetted riverbed), altitude and riparian vegetation and belonging to the same river category according to Italian legislation. Each station was sampled twice, once during summer 2016 and once during winter 2017.

Field and laboratory activities

Sampling was carried out according to a specific *in-situ* sampling design. With the aim of include small-scale spatial coordinates, we created some 50-points random sampling grids (one for each sampling campaign) at first by generating random numbers according to the size of the stream sections and then by placing marked stones as position markers for sampling inside the water (Fig. 8B). For each point, we collected a surber sample (just upstream of each marked stone) for the macroinvertebrate community using a surber net with frame area of 0.05 m^2 and mesh size of 500 μm. Samples were kept separated in PET bottles and fixed with 90° ethanol for laboratory sorting. At the same time we also recorded the relative coordinates inside the grids, the dominant substrate (according to Buffagni & Erba 2007) and flow velocity and water depth by means of a current meter (FP101-FP102 Global Flow Probe). Macroinvertebrates were counted and identified at genus level (except for Diptera, for which the family or subfamily level has been reached) according to Tachet et al. (2010). After processing, from the residual of every sample, the Benthic Organic Matter (BOM) was separated from inorganic material by elutriation (Boulton & Lake 1992), sieved with 1 mm mesh-size sieve to retain only the coarse fraction and then dried in hoven at 105° C until constant weight. Then the BOM was weighed by a precision balance (METTLER TOLEDO AB104). Macroinvertebrates were also dried and weighed for the biomass estimation, according to the same procedure followed for BOM.

Data analysis

To analyse the effect of spatial and environmental variables on macroinvertebrates two different approaches were followed, the first for community metrics and the second for community matrices.

The relation of relative coordinates (x, y) and environmental variables (BOM, velocity, depth and substrate) with community metrics (taxa richness, abundance and biomass) was modelled by means of generalized additive models (GAMs, Hastie & Tibshirani 1986), applying the smoothing function to coordinates in order to model also the spatial autocorrelation together with environmental variables. The advantage in using GAMs is their ability to handle highly non-linear and non-monotonic relationships between the response and the set of explanatory variables (Guisan et al. 2002;

Zuur et al. 2009). A Gaussian distribution family was used for biomass, while a quasi-Poisson distribution family was applied to taxa richness and abundance in order to deal with overdispersion. BOM, taxa richness, abundance and biomass were log-transformed and collinearity among environmental variables was tested (with Pearson's correlation coefficient) prior the analysis. When Pearson correlation was found to be higher than 0.7, variables were omitted from GAMs. With the same GAMs approach, we tested also the relationship of some significative taxa with environmental and spatial variables. The choice of taxon was operated according to their frequency, abundance and functional feeding group (according to Merrit & Cummins 1996), considering their diffusion not only within stream but also among different streams and, when possible, between different seasons. Lastly, we used GAMs also to explore the variability of taxa richness, abundance and biomass among rivers and seasons, again applying the smoothing function to coordinates.

The second approach was used in order to test the relative contribution of spatial and environmental variables in structuring communities. At first the sets of relative coordinates were transformed in distance matrices, later used to model the spatial structure by means of principal coordinates of neighbour matrices (PCNM, Borcard & Legendre 2002; Dray et al. 2006) according to the procedure proposed by Borcard et al. (2011). Then a forward selection procedure was performed to detect significant variables for coordinates, PCNMs and environmental variables. At last, variance partitioning was used to assess the explanatory power of these three sets of explanatory variables (X1=coordinates, X2=environmental variables, X3=PCNMs). This method enables us to assess the contribution of explanatory variables by the decomposition of R-squared as described in Peres-Neto et al. (2006).

All analyses and graphs were performed with the base, packfor (Dray et al. 2013), geoR (Ribeiro & Diggle 2015), plot3D (Soetaert 2016), mgcv (Wood 2011) and vegan packages (Oksanen et al. 2016) of the statistical software R (R Core Team 2016).

RESULTS

The bathymetric profiles of the sampled section of Parma, Enza and Nure during summer and winter are reported in Fig. 9. Profiles resulted to be variables in terms of riverbed structuration, with two general kind of situations: a spatially structured riverbed, with a well-defined depth gradient (Fig. 9 B and F) and a more chaotic riverbed configuration, with high variability of depth (Fig. 9 A, C, D, E). Mean values of depth, flow velocity, BOM and the dominant substrate are reported in Tab. 3, splitted for sampling campaign.

A total of 29364 organisms, belonging to 103 taxa was found globally, with Ephemeroptera and Diptera being the most abundant (32.3% and 33.3% respectively) and frequent (27.1% and 25.3% respectively) groups. Mean and total values of taxa richness, abundance and biomass are reported in Tab. 3, splitted for sampling campaign. Taxa richness and abundance differed significantly between seasons, with higher values during summer (F-value=36.58, p-value<0.001; F-value=21.64, p-value<0.001, for richness and abundance respectively) but not among streams, while biomass differed neither between streams nor between seasons (Fig. 10).

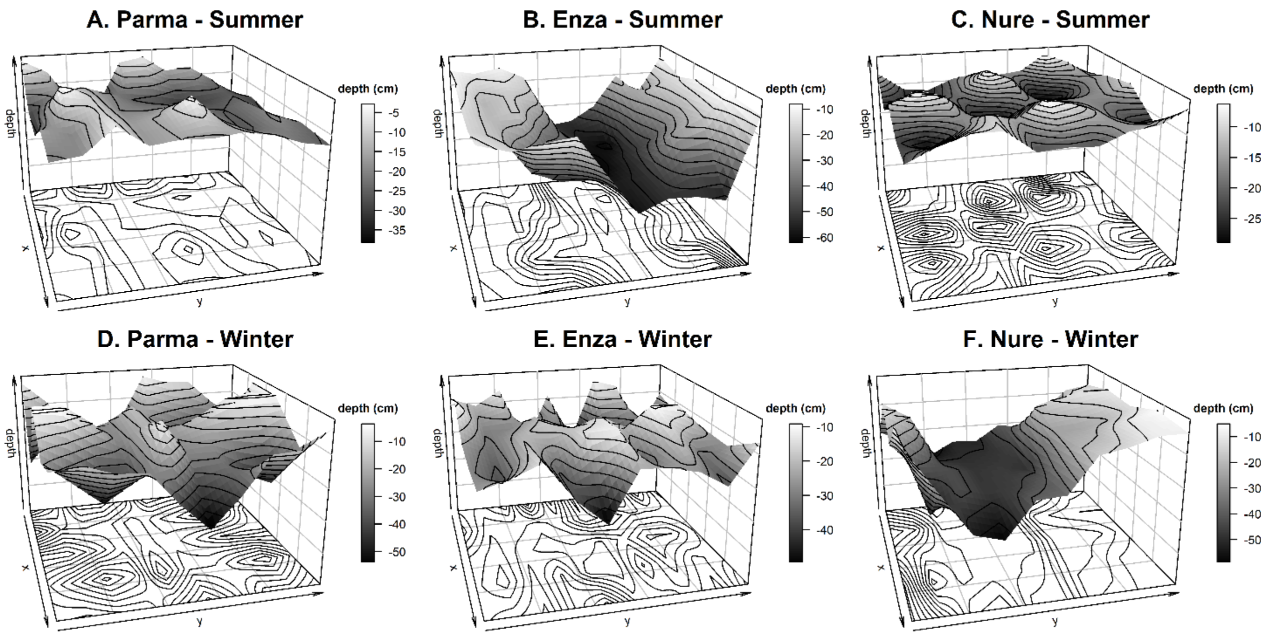

Figure 9: Bathymetric profiles of the sampled sections of Parma, Enza and Nure Streams during summer (A, B and C) and winter (D, E and F). Darker grey correspond to higher values of depth.

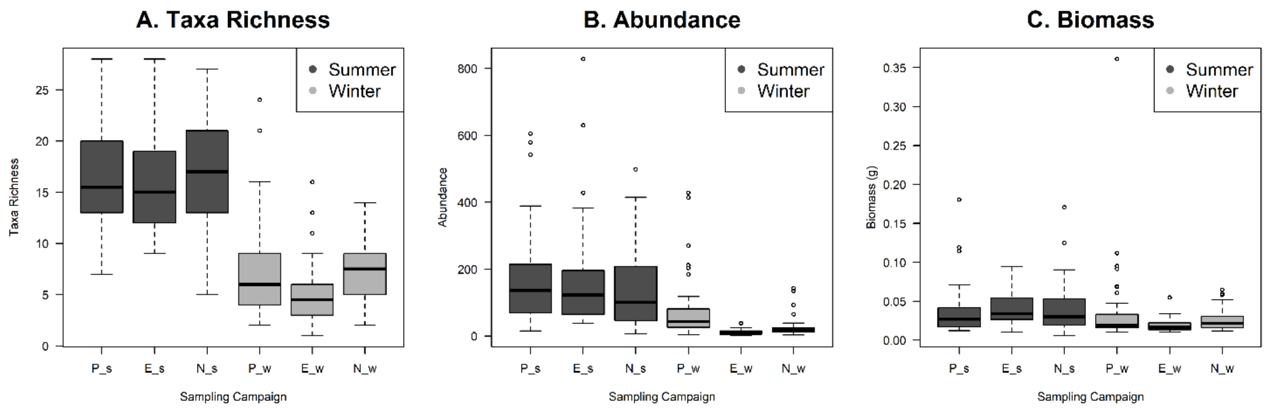

Figure 10: Variation of taxa richness, abundance and biomass of the studied watercourses between summer and winter. Metrics resulted to be very similar in the three watercourses but varied between seasons (except biomass, which differed neither between streams nor between seasons).

Results of GAMs are reported in Tab. 4 (and Tab. 5 for single taxa). The amount of BOM resulted to be the strongest driver of taxa richness, abundance and biomass, being almost always highly significant with F-values ranging from 9.068 with p-value < 0.01 to 71.547, with p-value < 0.001 (see also Fig. 11 for the relation among BOM and metrics). The effect of other variables resulted to change with watercourse, season and metric considered. E.g. flow velocity becomes more significant during winter especially for abundance and biomass, while the influence of coordinates is more related to the watercourse or metric considered (with biomass more related to spatial coordinates than other metrics).

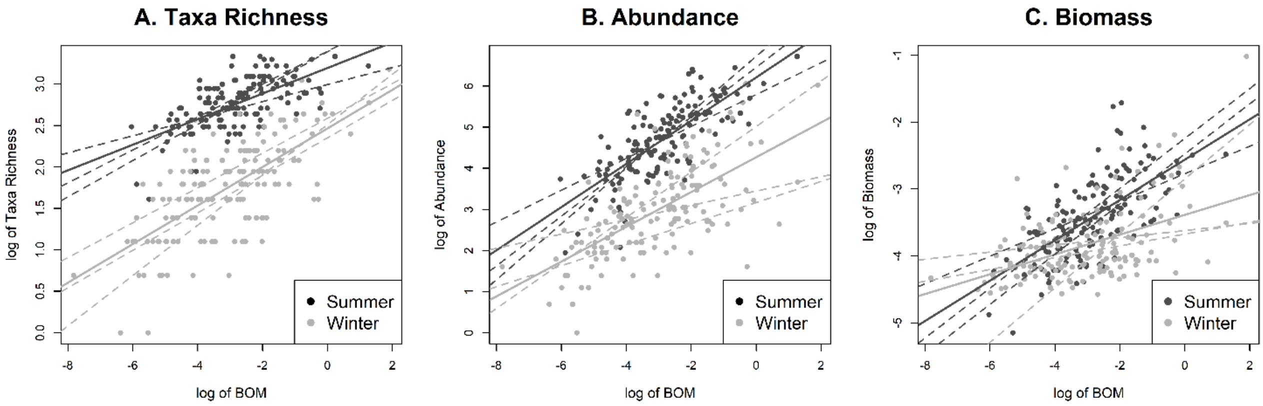

Figure 11: Trends between community metrics and BOM. In dark grey summer data, in light grey winter data. Solid lines represent the general seasonal trend, dashed lines the ones in the single watercourses.

The relationship between the ratio of biomass and abundance versus the amount of BOM is reported in Fig. 12. We found that with the increase of BOM there is a decrease of the ratio, according to a hyperbolic trend. In other words, with high amount of BOM, there is a high number of small organisms, while with low quantities of BOM there are fewer and bigger macroinvertebrates. Considering the taxa individually (Tab. 5), we found higher values of significance for space compared to metrics results. In addition, the analysis showed that the same taxa may have different drivers, depending on the watercourse and the season. E.g. the genus *Esolus* (Coleoptera, Elmidae) resulted to be mainly related to the amount of BOM in Nure Stream (summer), to the depth in Parma Stream (summer) and to coordinates in Enza Stream (summer). A similar trend has been found also for the genus *Leuctra* (Plecoptera, Leuctridae) for which the main drivers resulted to be BOM in Parma and Nure Streams during summer and coordinates in Enza Stream during summer.

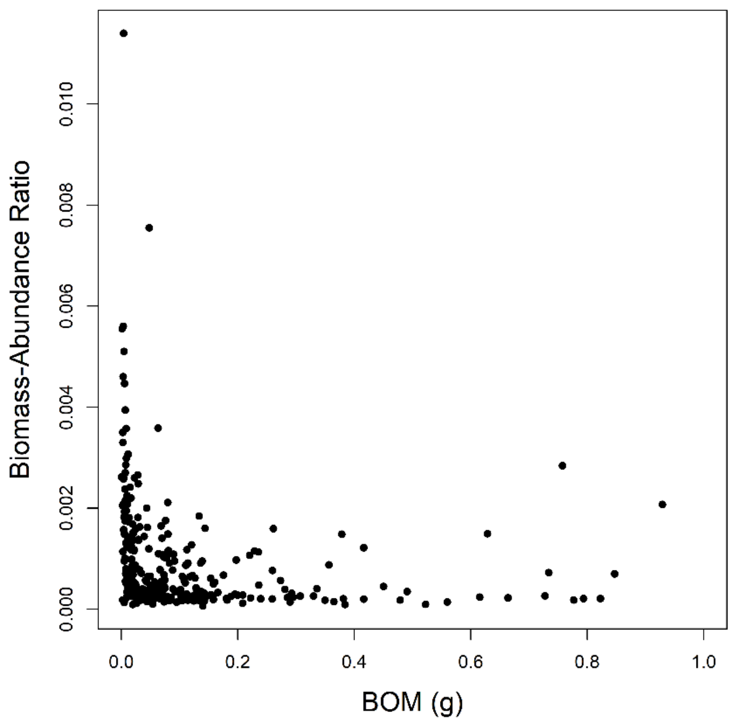

Figure 12: Relationship between the biomass-abundance ratio and the amount of BOM (g).

Regarding community structure, whose results are reported in Fig. 13 and Tab. 6, we highlighted two kind of situations. In the first case, encountered for Parma summer, Nure summer, Parma winter and Enza winter (Fig. 13 A, C, D, E), we found that the environmental set of variables has the greatest explanatory power, with BOM and flow velocity being the more frequently selected variable during the forward selection procedure. The percentages of variance explained by the pure environmental fraction resulted to be 26%, 25%, 18% and 47% for Parma summer, Nure summer, Parma winter and Enza winter respectively. In the second case, encountered for Enza summer and Nure winter (Fig. 13 B and F) we found that the pure PCNMs fraction has the greatest explanatory power, with 26% and 13% of total variance explained respectively. Coordinates resulted to be less important in explaining variance of communities, with values ranging from 0% to 5% of explained variance.

Table 3: Mean values of environmental parameters and community metrics recorded for each sampling campaign. P_s=Parma Stream summer campaign, E_s=Enza Stream summer campaign, N_s=Nure Stream summer campaign, P_w=Parma Stream winter campaign, E_w=Enza Stream winter campaign, N_w=Nure Stream winter campaign.

Sampling campaign	**Flow Velocity**	**Depth**	**Dominant substrate**	**BOM**	**Taxa richness (mean)**	**Taxa richness (tot)**	**Abundance (mean)**	**Abundance (tot)**	**Biomass (mean)**	**Biomass (total)**
	$m\ s^{-1}$	cm	%	g per 0.05 m^2	#taxa per 0.05 m^2	#taxa	#organisms per 0.05 m^2	#organisms	g per 0.05 m^2	g
P_s	0.48±0.46	19.9±9.0	MES (62%)	0.10±0.16	17±5	51	143±124	7171	0.04±0.03	2.03
E_s	1.49±1.14	31.7±18.4	MES (46%)	0.09±0.09	17±5	51	168±139	8410	0.04±0.03	1.80
N_s	1.12±0.81	17.1±7.0	MES (46%)	0.23±0.54	16±5	60	165±151	8266	0.04±0.02	2.08
P_w	0.74±0.67	25.0±15.6	MES (44%)	0.14±0.32	8±3	43	25±28	1246	0.02±0.01	1.25
E_w	1.26±0.98	25.7±12.1	MES (34%)	0.32±0.95	7±5	39	75±90	3734	0.04±0.05	1.75
N_w	1.26±0.77	31.5±17.5	MES (58%)	0.14±0.52	8±3	29	11±8	536	0.02±0.01	0.95

Table 4: GAM results for metrics in each sampling campaign. Significant results are marked in bold. Taxa richness, abundance, biomass and BOM are log transformed. P_s=Parma Stream summer campaign, E_s=Enza Stream summer campaign, N_s=Nure Stream summer campaign, P_w=Parma Stream winter campaign, E_w=Enza Stream winter campaign, N_w=Nure Stream winter campaign. BOM=Benthic Organic Matter, V=flow velocity, D=depth, Sub=substrate, s(x,y)=coordinates with smoothing function.

	BOM		**V**		**D**		**Sub**		**s(x,y)**	
	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value
Taxa richness										
P_s	**51.996**	***	0.548		0.009		0.104		3.136	.
E_s	**27.684**	***	0.016		N.D		0.113		**5.981**	**
N_s	**9.068**	**	0.127		N.D.		0.097		2.644	.
P_w	**25.151**	***	1.211		N.D.		0.551		0.817	
E_w	**25.412**	***	0.068		0.011		**6.658**	*	1.302	
N_w	**25.962**	***	0.795		N.D.		**14.402**	***	0.931	
Abundance										
P_s	**71.547**	***	0.132		0.055		0.227		2.134	
E_s	**37.064**	***	0.101		N.D		0.508		**2.754**	*
N_s	**34.366**	***	1.643		N.D.		0.009		0.300	
P_w	**30.198**	***	**6.398**	*	N.D.		**5.198**	*	**1.946**	*
E_w	**33.954**	***	3.061	.	1.367		**7.025**	*	2.445	.
N_w	**25.683**	***	**4.163**	*	N.D.		**15.620**	***	0.547	
Biomass										
P_s	**51.652**	***	0.004		**5.087**	*	0.539		**5.235**	***
E_s	**14.636**	***	0.002		N.D		1.682		**6.144**	**
N_s	**14.244**	***	**9.892**	**	N.D.		2.299		0.443	
P_w	**16.968**	***	**12.760**	**	N.D.		0.004		**2.534**	**
E_w	**26.182**	***	**6.354**	*	3.869	.	0.217		0.322	
N_w	**15.258**	***	**4.475**	*	N.D.		0.668		1.383	

Table 5: Results of GAMs for single taxa for each sampling campaign. Significant results are marked in bold. In brackets the FFG (Functional Feeding Group, according to Merritt & Cummins 1996) to which they belong: CG=collector-gatherers, CF=collector-filter, BOM=Benthic Organic Matter, V=flow velocity, D=depth, Sub=substrate, s(x,y)=coordinates with smoothing function.

	BOM		**V**		**D**		**Sub**		**s(x,y)**	
	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value
Parma summer										
Baetis (CG)	**23.512**	***	**28.827**	***	1.804		**14.710**	***	**3.035**	***
Chironominae (CG)	**63.258**	***	2.310		**5.739**	*	3.308	.	**3.513**	***
Esolus A (Sc)	1.584		0.039		**9.284**	**	**5.972**	*	**2.937**	*
Leuctra (Sh)	**32.508**	***	0.092		0.166		1.061		1.441	
Orthocladiinae (Sc)	**88.130**	***	0.372		0.592		**4.280**	*	**3.864**	***
Hydracnidia (P)	**9.643**	**	0.489		0.191		1.012		0.884	
Enza summer										
Baetis (CG)	**17.118**	***	0.016		N.D.		0.007		**6.489**	**
Chironominae (CG)	**19.778**	***	3.481	.	N.D.		0.923		**10.42**	***
Esolus A (Sc)	0.756		0.037		N.D.		3.298	.	**12.74**	***
Leuctra (Sh)	**8.011**	**	0.001		N.D.		2.352		**9.845**	***
Orthocladiinae (Sc)	**20.845**	***	0.002		N.D.		0.846		**3.864**	***
Hydracnidia (P)	**17.118**	***	0.016		N.D.		0.007		**6.489**	**
Simuliidae (CF)	2.197		2.519		N.D.		0.951		**5.343**	**
Nure summer										
Baetis (CG)	**36.181**	***	3.073	.	N.D.		0.232		1.282	
Chironominae (CG)	**16.751**	***	0.173		N.D.		**5.104**	*	**3.451**	**
Esolus A (Sc)	**5.201**	*	1.719		N.D.		0.220		0.480	
Leuctra (Sh)	2.885	.	0.244		N.D.		0.932		1.186	
Orthocladiinae (Sc)	**52.163**	***	0.111		N.D.		**5.630**	*	**7.008**	***
Hydracnidia (P)	**23.546**	***	0.733		N.D.		1.775		0.293	
Simuliidae (CF)	**14.649**	***	**8.245**	**	N.D.		**32.531**	***	**4.566**	*
Parma winter										
Brachyptera (Sh)	**41.325**	***	**15.008**	**	N.D.		0.069		**21.07**	***

Ecdyonurus (Sc)	0.127		1.169		N.D.		0.918		**2.326**	*
Leuctra (Sh)	**18.16**	***	0.810		N.D.		0.70		**5.750**	***
Amphinemura (Sh)	**6.841**	*	0.973		N.D.		0.013		1.704	
Orthocladiinae (Sc)	**7.359**	*	0.018		N.D.		**66.557**	***	**5.152**	***
Hydracnidia (P)	3.996	.	3.201	.	N.D.		1.924		0.948	
Enza winter										
Brachyptera (Sh)	**10.709**	**	0.180		2.421		0.105		0.981	
Orthocladiinae (Sc)	**28.187**	***	1.454		2.340		**20.385**	***	0.215	
Hydropsyche (CF)	**16.714**	***	0.832		2.302		**7.253**	*	1.712	.
Simuliidae (CF)	0.204		**36.870**	***	2.369		0.034		**3.34**	**
Hydracnidia (P)	**5.781**	*	0.362		0.849		0.236		0.796	
Nure winter										
Brachyptera (Sh)	**11.329**	**	**23.804**	***	N.D.		3.855		0.021	
Ecdyonurus (Sc)	**8.256**	**	1.676		N.D.		0.925		1.814	.
Hydropsyche (CF)	**9.702**	**	0.446		N.D.		0.394		**2.77**	*
Hydracnidia (P)	0.745		1.722		N.D.		0.014		0.732	

Table 6: Variables selected with the forward selection procedure. P_s=Parma Stream summer campaign, E_s=Enza Stream summer campaign, N_s=Nure Stream summer campaign, P_w=Parma Stream winter campaign, E_w=Enza Stream winter campaign, N_w=Nure Stream winter campaign. BOM=Benthic Organic Matter, V=flow velocity, D=depth, Sub=substrate, s(x,y)=coordinates with smoothing function.

	Coordinates	Environment	PCNMs
P_s	y	BOM, V	V14
E_s	x, y	BOM, V, D	V8, V1, V16, V7, V9, V2, V14
N_s	No variables selected	BOM, V	V3
P_w	y	Sub, V	V8, V1
E_w	No variables selected	BOM, Sub	No variables selected
N_w	y	BOM, V	V5, V3, V4, V8

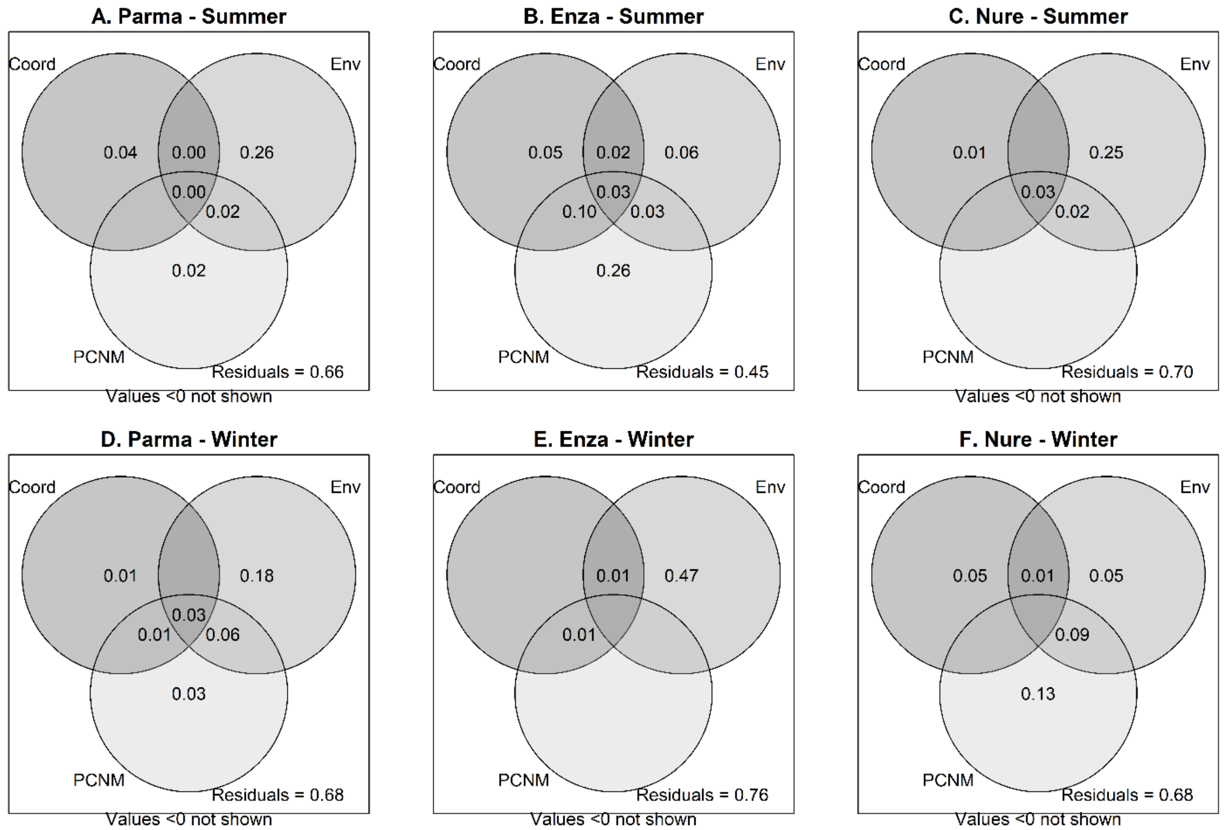

Figure 13: Variance partitioning results for communities among three sets of selected explanatory variables (Coord=relative coordinates, Env=environmental variables and PCNM). Fractions showed are adjusted R2 values. Residuals values are also displayed.

DISCUSSION

Aquatic invertebrates in the studied streams showed a very strong relationship with the amount of BOM found locally, highlighted both with GAMs and variance partitioning methods. The number of taxa, abundance and biomass resulted to be positively related to the amount of organic matter. These results are consistent with those reported e.g. by Graça et al. (2004), Fenoglio et al. (2005), Ortiz at al. (2006, but only for density and biomass) and Straka et al. (2012). Conversely, Braccia & Voshell (2006) found that the relationship between assemblages and organic matter was weaker than those with physical habitat features. Furthermore, we found that this relationship persist both in summer and winter. This is surprising, especially if considering that the availability of BOM changes during seasons; in fact we found higher values during summer, probably due to the growth of algal mats. The positive relation detected between aquatic invertebrates and BOM can be addressed to two main functions of BOM: food resource and in-stream refugia.

The function of BOM mainly as food resource has been greatly supported (e.g. Richardson 1992; Dudgeon and Wu 1999; Graça et al. 2004) especially in the framework of the FFGs. Macroinvertebrates play a key role in the allochthonous organic matter decomposition (Fenoglio et al. 2005) and therefore they are strictly related to its distribution, as supported by our data. Moreover we considered not only allochthonous organic matter but also algal mats, so the relationship resulted stronger and not limited to shredder organisms. The presence of BOM can promote also the retention of fine particulate organic matter (e.g. by trapping, Eggert et al. 2012) therefore affecting also macroinvertebrates feeding on it. Unfortunately, we were not able to assess the amount of fine particulate organic matter since we sampled by means of a surber net with mesh size of 500 μm, which do not hold the finest particles of organic matter. BOM can act also as in-stream refugia for benthic organisms, in addiction to the trophic function (Bo et al. 2010). The presence of algal mats and allochthonous detritus can promote benthic communities, acting as a shelter from the current, living surface and refugia from predators and droughts (e.g. Lancaster & Hildrew 1993; Haapala et al. 2003; Merten et al. 2014). In addition, several authors suggest that the BOM can act simultaneously as food resource and refugia/microhabitat (e.g. Straka et al. 2012). This could be the case of our study: we found a strong relationship with BOM for organisms belonging to different functional groups (though stronger for those who directly feed on BOM), supporting the food-resource hypothesis, while the relation observed between the biomass-abundance ratio and the amount of BOM (Fig. 12) could support the refugia hypothesis. However, this should be thoroughly investigated, with more targeted studies, since the presence of a large number of small individuals in correspondence of big amounts of BOM could be also due to the trapping effect of BOM for fine particulate organic matter. Besides the organic matter, also other variables resulted to be related to macroinvertebrates, however these relationships were weaker and stream and season dependant. E.g. flow velocity resulted to be a good predictor for biomass during winter, while during summer this relationship disappears. This probably due to the fact that with the low flow summer period the velocity of water is generally low, and does not represent a limiting factor for aquatic insects.

In our study, also space (both as coordinates and PCNMs) resulted to be an important driver of macroinvertebrates. Similar results were found also by Vilmi et al. (2016), which detected a relation between spatial variables and community structure at small scale. Conversely, Rezende et al. (2014) reported that space has little influence in shaping community at local scale. Among metrics, the one more affected by the relative coordinates resulted to be the biomass, while the effect on taxa richness and abundance resulted weaker. The effect of coordinates resulted stronger also considering the taxa individually. Moreover, the influence of space has been found to be very stream-dependent. For community organization, we highlighted that PCNMs have the greatest explanatory power in Enza Stream during summer and Nure stream during winter (Fig. 13B and 13F). In these two situations we also observed riverbeds more spatially structured, with the "U" shape channel more pronounced than in the other studied sections, which presented a higher randomness in riverbed configuration (Fig. 9). Such correspondence between riverbed and community spatial structuration may be the result of small-scale biotic processes, since that the dominant fraction is represented by the pure PCNMs (not shared with environment nor coordinates). The greater riverbed structuration may enhance the dispersion of macroinvertebrates, resulting in a more emphasized mass effect or in the establishment of stronger biotic interactions (e.g. Mouquet & Loreau 2003, Smith & Lundholm 2010). We highlighted also a surprising difference of drivers' effect considering taxa individually (Tab. 5): we found that the same taxon can have different drivers depending on stream and season. These findings support the idea that the local configuration of the systems affects the prevalence of spatial or environmental filtering. However, we have to underline that both in the case of community and single taxa results, the range of variability of environmental factors can affect the presence or absence of a relationship between such environmental factors and single taxa or community structure. Indeed, if an environmental variable presents a strong gradient in the studied system, this gradient could reach values that are out of the tolerance range of some taxa and therefore the variable represents a limiting factor. On the other hand, if the gradient of the variable is not very wide, this variable is not tracked as environmental driver as it does not represent a limiting factor. In this context, the evaluation of what is the level beyond which a factor becomes limiting could be of remarkable interest.

The differential importance of spatial versus environmental factors should be also regarded from the point of view of environmental heterogeneity. As pointed out by Heino et al. (2015), under mass effect there is a trade-off between excessive dispersion, that masks the influence of local environmental conditions and sufficient dispersion, which is needed for taxa to track the local environmental conditions. Given the absence of dispersal limitations at small scale, organisms can occupy any position inside the riverbed, only depending on their microhabitat preferences and a strong environmental (and spatially structured environmental) signal can therefore be expected at small scale. Moreover, the strength of this environmental signal could be strictly related to the degree of environmental heterogeneity: a greater local environmental heterogeneity could lead to a stronger environmental signal, while a smaller environmental heterogeneity involves a stronger spatial signal in community. This is the case of our systems: in random-configured riverbed the high heterogeneity may promote an environmental control on assemblages, while in structured riverbed the small-scale heterogeneity is lower, enhancing the movement of organisms.

Another interesting point is the finding that community metrics and structure respond in different ways to the spatial and environmental drivers, with community being more dependent than single metrics from space. This can be explained thinking to the taxa replacement: the same position inside the riverbed can be occupied by different taxa, having the same functional role (e.g. Smith & Lundholm 2010). This would have an effect on community matrix but not on richness nor abundance.

Based on these findings, we suggest a strong environmental control on macroinvertebrate distribution at small-scale, mainly ascribable to the local amount of BOM. Nevertheless, the environmental filtering is not always dominant and it depends on the spatial configuration of the systems, which mediate the selection of microhabitats and the possible prevalence of biotic interactions in shaping macroinvertebrate communities.

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SECTION II: Mesoscale – Braided Rivers

MESOHABITAT MOSAIC IN LOWLAND BRAIDED RIVERS: SHORT-TERM VARIABILITY OF MACROINVERTEBRATE METACOMMUNITIES

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INTRODUCTION

Braided rivers (BRs) are defined as systems composed by multiple channels, with bars and islands, often with coarse textured unstable banks (Tockner et al. 2006). This kind of systems is spread worldwide and can be found in delta areas, where rivers enter lakes and oceans, or in floodplains in presence of low slope and sandy or gravel-filled substrates (Dodds 2002). The main feature that shapes BRs is the extreme flow variability. The alternation of sudden and frequent flow changes, spanning flash floods and dry conditions, generates a mosaic of patches, which undergoes rapid evolution (Gray & Harding 2009). Events like the displacement of channels or disconnection of habitats can happen within short periods, spanning from a few weeks to a few hours. Van der Nat et al. (2003) estimated the turnover time of the different habitats in a BR system (Tagliamento, NE Italy) and they found a high level of variation, with a total replacement of all the aquatic habitats of 82% during the period of study (2.5 years). Nevertheless, they reported that the relative proportion of the various habitats remained quite consistent. Based on these results, BRs can be conformed to the "shifting mosaic steady model" that identify systems where the habitat turnover is high but the proportions of habitats are constant (Tockner et al. 2006; Gray & Harding 2011).

Based on these attributes, BRs can be considered as very suitable systems for studying metacommunity dynamics. According to the metacommunity theory (Leibold et al. 2004) environmental heterogeneity and taxa features (e.g. dispersal ability and competition) determine the structure and evolution of metacommunities at different spatial scales (McLaughlin et al. 2013; Siqueira et al. 2012). The paradigm of the heterogeneity-diversity relationship (Heino 2009) is a widely accepted concept. In general, habitat heterogeneity has a positive effect on taxa richness (Poff & Ward 1990; Garcia et al. 2012; Astorga et al. 2014), enhancing the niche availability and allowing the co-occurrence of taxa with different requirements. The high dynamism of BRs generates a great heterogeneity, especially at the scale of river reach, with a wide range of different habitats, spanning from lotic to lentic conditions and with a time-variable level of connection. The degree of influence of dispersal dynamics and environmental forcing is strictly related to the connectivity of habitats, besides the dispersal ability of taxa (Padial et al. 2014). In riverine systems, the level of connectivity can change widely in time and among them BRs are one of the most dynamic and complex (Ward et al. 2002). All these conditions are the basis for the presence of biodiversity hot-spots, with high levels of diversity variation in particular among the different habitats at the stream-reach level. In fact, several authors pointed out high levels of lateral variation in taxa diversity and community structure for braided systems. (e.g. Arscott et al. 2005; Gray & Harding 2007 respectively in north-eastern Italy and New Zealand). The variation among habitats can be considered as a beta-diversity variation and therefore it can be ascribed to two different phenomena: nestedness and spatial turnover. Nestedness occurs when there is a non-random taxa loss, with the result that the poorer communities are a subset of the richer ones, while turnover is the result of taxa replacement (Baselga 2010). Datry et al. (2016) highlighted that turnover is more related to environmental filtering, while nestedness is given by dispersal limitation. These two processes can assume differential importance in shaping local communities, in particular during low-flow periods, when connectivity among habitats is more variable.

Although these systems are widespread and considered as diversity hot-spots, for years they have been poorly studied (Gray & Harding 2007), with a lack of knowledge, especially in how the different habitats in the river segment contribute to the total diversity and how these patterns change in time. This topic is particularly relevant considering that BRs are often located in areas with a heavy human presence, with all the possible consequences, like considerable water withdrawals, canalization and reduction or loss of lateral areas (e.g. Tockner et al. 2006; Gray & Harding 2011; Karaus et al. 2013). These phenomena lead to a trivialization of BRs, with the consequent reduction of habitat variability. Therefore, a good understanding of habitat contribution to the local diversity becomes a key point for biodiversity conservation.

The aims of this study are therefore i) to evaluate the seasonal structure and variation of benthic macroinvertebrate communities within the highly patchy environments of BRs and ii) to evaluate the short-term variability of these communities, assessing their variability between the different kinds of habitats during the low-flow period. For this work, we focused on the mesohabitat sampling unit, demarcated according to the hydrodynamic characteristics. Tickner et al. (2000) defined mesohabitats as “medium-scale habitats which arise through the interactions of hydrological and geomorphological forces”. We hypothesize that: i) in general there is a differentiation of macroinvertebrate communities between different habitats within rivers, and between considered seasons ii) during low-flow periods, with the increasing disconnection of habitats there is an increase in community dissimilarity, with higher turnover in the less disconnected habitats and higher nestedness in the more disconnected ones.

METHODS

Study area

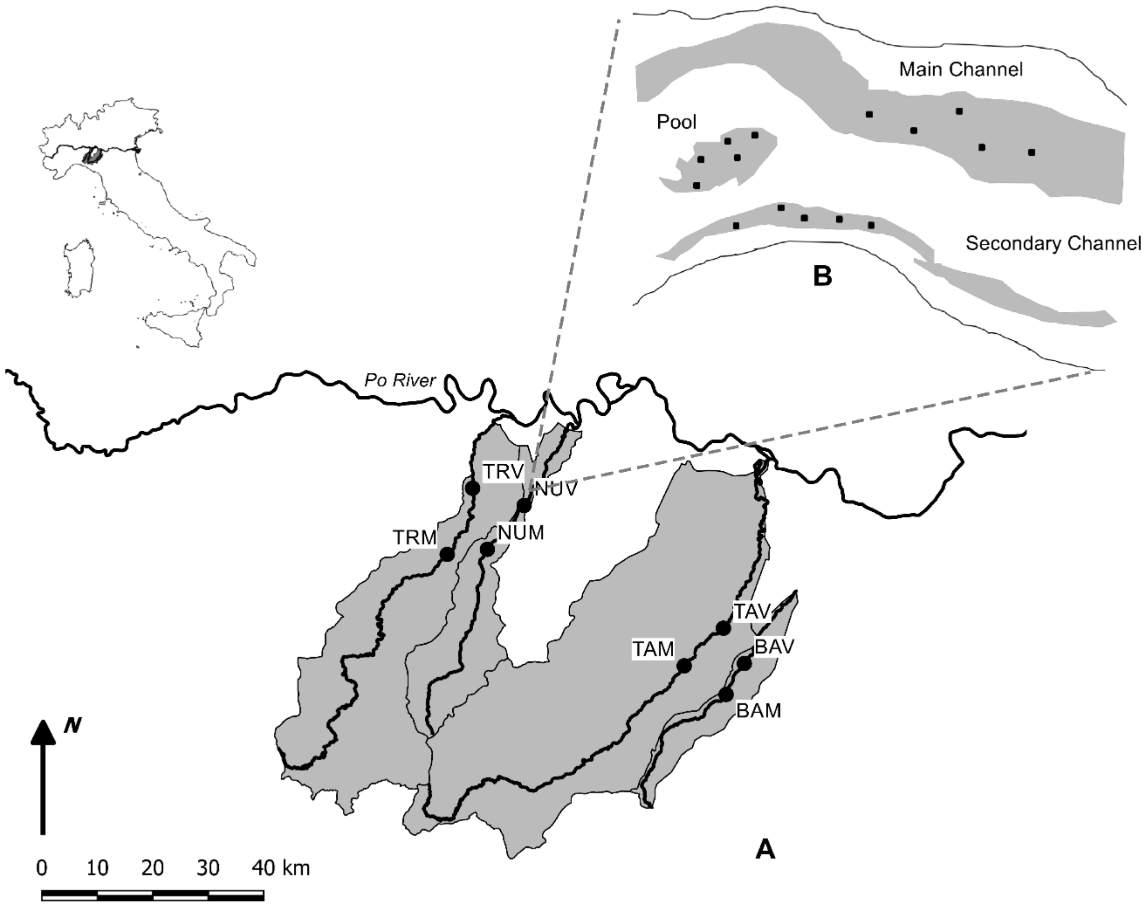

Figure 14: Map of study, with the four studied basins coloured in grey (A) and the sampling design (B) repeated in each of the eight sampling stations. Black squares represent the random sampling plots.

The study was performed in four braided watercourses of the Po River basin (Northern Italy, Fig. 14A). From west to east: Trebbia River, with annual mean flow Qm = 22.0 m^3 s^{-1}, total length of 120 km and basin area of 1083 km^2, Nure Stream Qm =15.0 m^3 s^{-1} , total length of 77 km and basin area of 458 km^2, Taro River with Qm = 40.5 m^3 s^{-1}, total length of 138 km and basin area of 2051 km^2 and Baganza Stream Qm =5.2 m^3 s^{-1}, total length of 59 km and basin area of 228 km^2. These systems are fed only by wet depositions and they present two high discharge periods (in autumn and spring) and a main low water period in summer (with a secondary additional one in winter). They are included in the Cfa and Csa climatic regions (according to http://koeppen-geiger.vu-wien.ac.at). For each watercourse we selected two sampling stations in order to take into account the within river variability. Within each station, three model mesohabitats were further selected: main channel, secondary channel, and pool (Fig. 14B). For the first part of the study (seasonal phase) sampling was carried out in November 2015 and in April 2016 in the whole set of systems. For the second part (summer phase) the set of investigated systems was reduced to two (Trebbia and Taro Rivers) and the sampling was carried out in five occasions in the period of low flow, from June to September 2015. The downsizing of sampled area was operated because Nure and Baganza streams completely dried up during the summer season and to contain the sampling and processing effort.

Physical and chemical variables

In order to check the difference between mesohabitats, for each sampling environmental data were collected with five random replicates (Fig. 14B). Flow velocity, water depth, temperature, conductivity and dissolved oxygen were recorded in situ by means of a current meter (FP101-FP102 Global Flow Probe) and a multi-parametric probe (HI 9828; Hanna Instruments). Water samples were collected for a descriptive analysis of ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), soluble reactive phosphorous (SRP), Dissolved Silica (SiO_2) and Total Dissolved Inorganic Carbon (TCO_2). Detection limits were 0.01 mg l^{-1} for NH_4^+, NO_3^-, SRP and SiO_2, 0.005 mg l^{-1} for NO_2^- and 0.02 mM for TCO_2. Precision ranged between ±3% and ±5%. Chemical analyses were performed by means of spectrophotometric techniques, according to A.P.H.A (2005), Valderrama (1977) e Golterman et al. (1978).

Macroinvertebrates

In each mesohabitat, a ~ 50 m long stretch was sampled, choosing five random sampling points (Fig. 14B). Samples were collected using a surber net with frame area of 0.1 m^2 and mesh size of 500 μm. The five replicates were cumulated for each mesohabitat. Samples were filtered and preserved in 70% ethanol for laboratory sorting, where the organisms were counted and identified to family or genus level, according to Tachet et al. (2010).

Data analysis

The difference between mesohabitats, in terms of physical variables, was assessed by means of mixed effects modelling, considering mesohabitat and time (sampling date) as fixed effects, and station and site (namely the specific sampling location) as hierarchically organized random effects. A similar approach was followed also for testing the influence of mesohabitat and time on richness and abundance. The use of these models allows us to work with correlated and non-normally distributed data (McCulloch & Neuhaus 2005), typical of nested and hierarchical designs. The significance was checked by means of a likelihood-ratio test. The effect of covariate was tested both for seasonal and summer data. Then the distribution of taxa between mesohabitats at station level was checked, by estimating the mesohabitat contribution to the total number of taxa. We did this by computing the percentage ratio for each station between the richness of each mesohabitat and the total richness of the station.

The organization of community structure in mesohabitats was explored with a non-Metric Multidimensional Scaling (nMDS), a spatial ordination technique that represents the set of objects along a predetermined number of axes maintaining the ordering relationships among them (Borcard et al., 2011). As dissimilarity measure Bray-Curtis distance was used and the goodness of ordination was assessed with the stress measure.

To assess the nature of diversity variation between mesohabitats during the summer phase we performed a partition of beta-diversity, following Baselga (2010). This method produces three metrics: the total beta-diversity (the Sørensen Dissimilarity index), for all the possible pairwise comparisons, and its two additive components: nestedness, expressing the taxa loss between mesohabitats, and the turnover, expressing the taxa substitution. The values of nestedness and turnover were normalized by dividing them by the Sørensen dissimilarity value. We checked the effect of time for the Sørensen Dissimilarity index by means of linear mixed effect models and then we adjusted the p-values using the Bonferroni correction for multiple comparisons. We also applied mixed effects modelling to check the difference between beta-diversity components and their variation in time.

All analyses and graphs were performed with the statistical software R (R Core Team 2016), with base version and with ggplot2 (Wickham 2009), lme4 (Bates et al. 2015), vegan (Oksanen et al. 2016) and betapart (Baselga et al. 2013) packages.

RESULTS

Physical and chemical variables

Mean values of measured physical and chemical variables are reported in Tab. 7, according to season and mesohabitat. The distinction between mesohabitats was tested for physical variables considering the whole dataset (seasonal and summer data) and we found that they differ greatly for flow velocity ($p\text{-value}<0.001$) and water depth ($p\text{-value}<0.001$), while the others variables (temperature, conductivity and percentage of dissolved oxygen) varied significantly only in time (p-value<0.001) but not between mesohabitats.

Table 7: Physical and chemical variables (mean values and standard deviations) for mesohabitats in each season. NH4+, NO2- and SRP values are not shown because always lower than detection values.

		Autumn		**Spring**		**Summer**	
		mean	*sd*	*mean*	*sd*	*mean*	*sd*
Flow Velocity (m s^{-1})							
	main	0.47	0.24	0.46	0.07	0.42	0.16
	sec	0.31	0.36	0.14	0.07	0.22	0.19
	pool	0.08	0.15	0.00	0.01	0.07	0.13
Water Depth (cm)							
	main	22.56	7.73	28.26	6.45	21.29	6.85
	sec	13.49	9.97	10.69	8.67	14.29	8.00
	pool	24.31	9.33	13.08	7.55	19.05	6.83
Temperature (°C)							
	main	12.98	1.15	14.13	1.25	23.47	1.97
	sec	13.81	1.47	14.88	2.17	23.69	3.83
	pool	13.32	1.33	15.39	1.59	24.61	1.91
Conductivity (μS cm^{-1})							
	main	249.50	28.86	253.13	23.88	328.40	44.98
	sec	286.88	81.29	272.00	35.31	382.60	142.54
	pool	268.00	38.36	274.00	27.11	374.95	74.43
Dissolved Oxygen (%)							
	main	104.09	5.81	103.78	12.15	107.47	8.06
	sec	100.46	12.11	101.95	11.15	96.27	18.77
	pool	93.95	11.29	88.28	12.56	105.10	18.42
NO_3^- (mg l^{-1})							
	main	0.29	0.08	0.16	0.08	0.23	0.14
	sec	0.36	0.22	0.17	0.09	0.49	0.64
	pool	0.31	0.07	0.21	0.06	0.44	0.67
SiO_2 (mg l^{-1})							
	main	1.36	0.27	1.24	0.36	2.62	0.62
	sec	1.37	0.32	1.32	0.38	3.00	0.80
	pool	1.38	0.34	1.39	0.23	3.10	0.38
TCO_2 (mM)							
	main	2.14	0.21	2.32	0.23	1.88	0.40
	sec	2.34	0.58	2.20	0.29	1.92	0.50
	pool	2.21	0.32	2.51	0.43	1.96	0.53

Macroinvertebrates

A total of 74122 organisms, belonging to 94 taxa was found globally. The sample with the highest taxa richness (34 taxa) was collected at the beginning of the summer period in the upstream pool of Trebbia River, while the one with the lowest taxa richness (7 taxa) in the downstream pool of Nure Stream, during the November sampling campaign. The mean values of taxa richness and abundance were 18 and 837 for main channels, 17 and 426 for secondary channels and 22 and 796 for pools. The list of most abundant (A) and frequent (B) taxa is reported in Fig. 15. Chironomidae was both the most abundant and frequent taxon (abundance=29.3%, frequency=99.1%). Detection probabilities for the other most common taxa were unrelated to their abundance. Some taxa were found to be exclusive of one kind of mesohabitat: we found 5 exclusive taxa in the main channels (*Heptagenia*, *Notonecta*, Gordiidae, *Besdolus* and *Brachyptera*), 6 in the pools (*Pseudocentroptilum*, *Pisidium*, Dixidae, *Hydrometra*, Haplotaxidae and *Protonemura*) and 10 in the secondary channels (Hydridae, Blephariceridae, Dolichopodidae, Ephydridae, Rhagionidae, *Valvata*, *Gerris*, *Helobdella*, *Nemoura* and Lepidostomatidae).

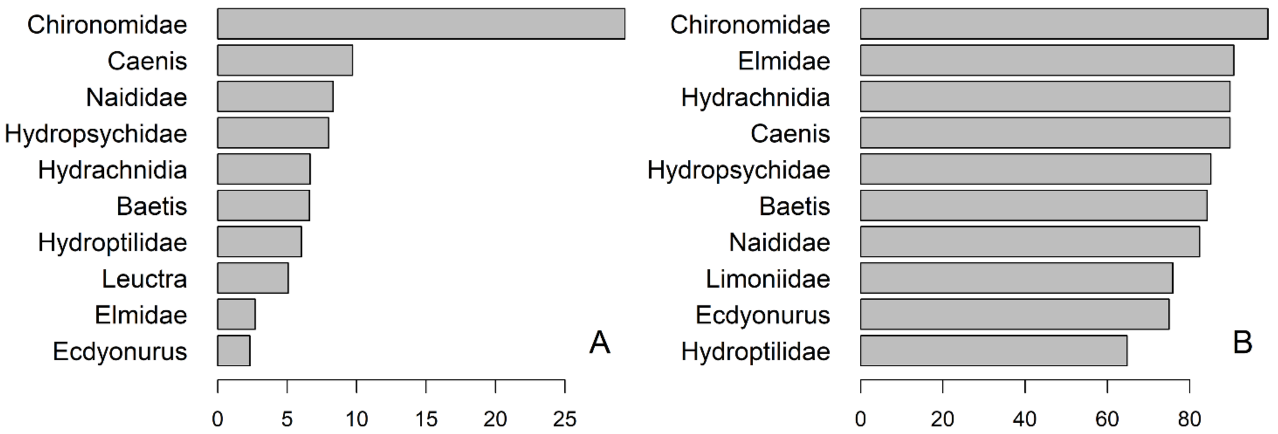

Figure 15: Barplots of the first 10 taxa in terms of abundance percentage (A) and frequency percentage (B).

The significance of mesohabitat and time for taxa richness and organism abundance was tested by means of several mixed effects models for seasonal and summer phases. Both taxa richness and abundance resulted related with mesohabitats, especially for seasonal data (p-values 0.002 and 0.003 respectively), while for summer data these relations resulted weaker (p-values 0.078 and 0.060). Time resulted significant only for the seasonal taxa richness (p-value=0.026), with a variation between November and April, while no significant variation was found during summer nor for abundance. Mesohabitats control resulted high, also considering community composition patterns (Fig. 16A, B): points corresponding to the three kinds of mesohabitats group into different areas of the nMDS plot, both for seasonal and summer communities. Moreover, comparing the two graphs, it can be seen that the segregation between mesohabitats resulted slightly greater during the seasonal phase (November and April) than for summer. We also found a variability of communities in time (Fig. 16C), with a clear segregation of autumn, spring and summer data in three different clusters.

The mesohabitats contribution to the taxa richness at station level resulted significantly different, either seasonally (p-value=0.002) or during the summer (p-value=0.045). The greater contribution was the one given by marginal habitats and by the secondary channels in particular, while the importance of main channels resulted limited (Fig. 17).

Variation during the summer phase of the Sørensen Dissimilarity index and of the beta-diversity partition for the pairwise comparisons between mesohabitats are reported in Fig. 18 (A and B, respectively). The dissimilarity values (Sørensen Dissimilarity index) showed similar trends in all comparisons, with a decrease in June and July (T1:T5 in Fig. 18A) and a new increase at the beginning of Autumn. Nevertheless this trend resulted significant only considering the comparison between main channels and pools (p-value=0.027). The two components of beta-diversity resulted significantly different for all the comparison (p-value<0.001), with higher values for taxa turnover. No significant temporal trends resulted from the analysis.

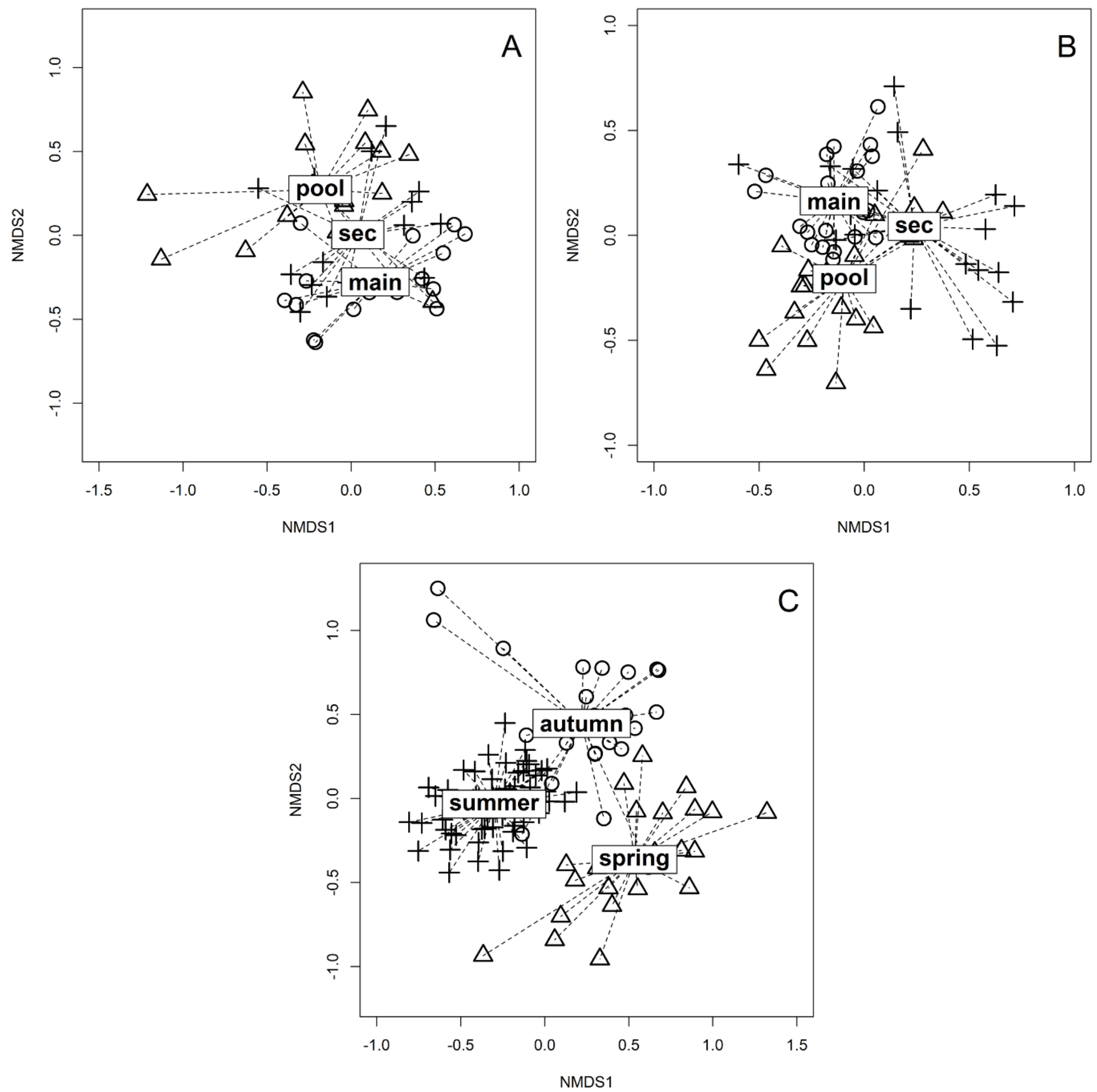

Figure 16: nMDS ordination outputs for seasonal (A), summer (B) and all (C), data. For seasonal and summer data (A and B) the mesohabitat segregation is shown, while for the all data graph (C) the temporal (between seasons) segregation of communities is shown. Seasonal nMDS stress=0.176; Summer nMDS stress=0.157; All data nMDS stress=0.175.

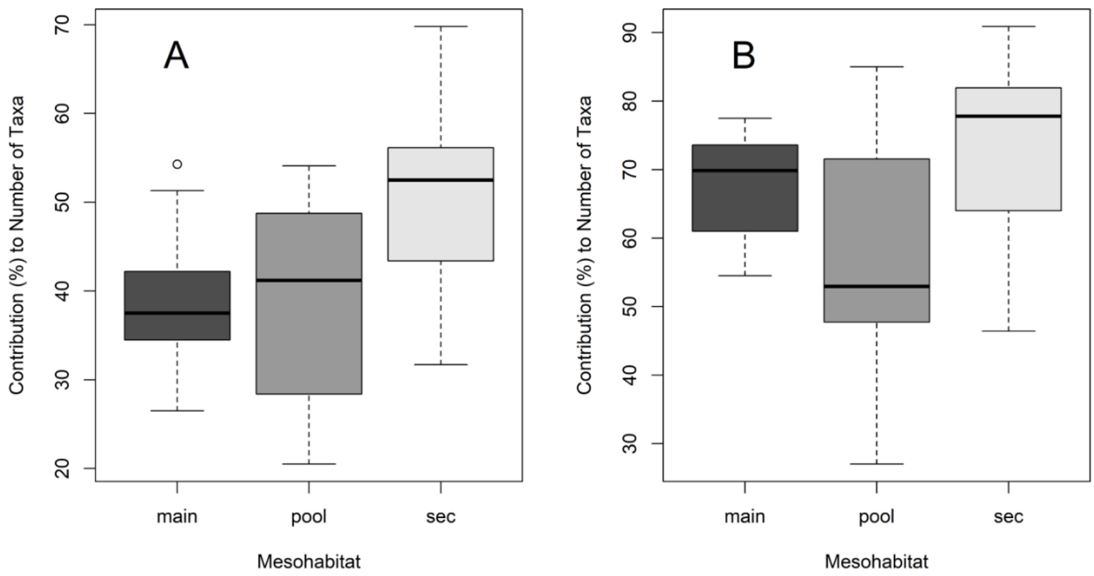

Figure 17: Mesohabitat contribution to the total number of taxa for seasonal (A) and summer data (B).

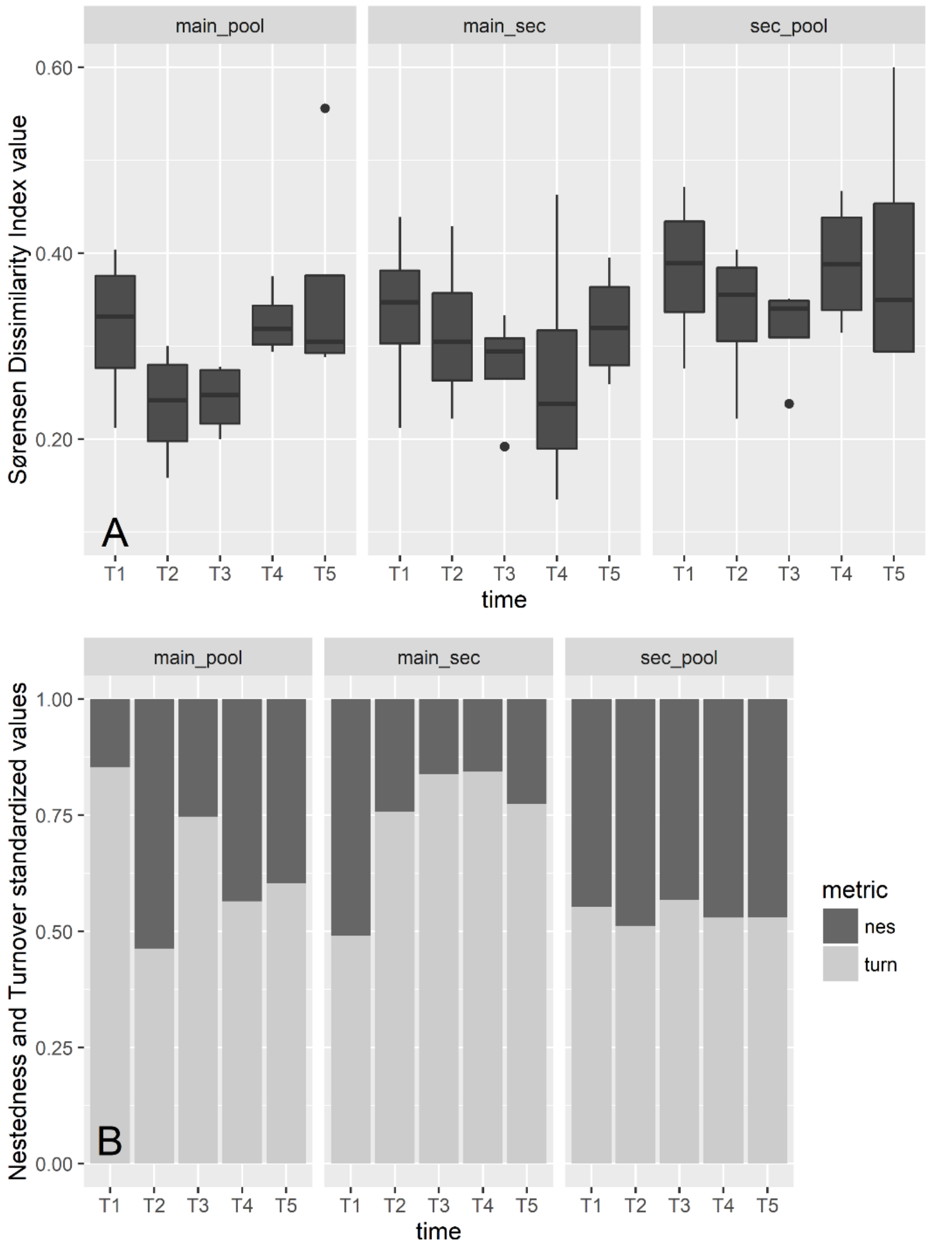

Figure 18: Short-term variation of Sørensen dissimilarity index (A) and nestedness and turnover components (B), in the three pairwise confrontations between mesohabitats (main_pool=main channel–pool, main_sec=main channel–secondary channel, sec_pool=secondary channel–pool). Nestedness and turnover values were standardized by dividing by the Sørensen index.

DISCUSSION

This study highlights a strong variability of macroinvertebrates in BRs, with different habitats hosting different communities. These findings are consistent with the findings of Gray & Harding (2009), Zilli & Marchese (2011), Karaus et al. (2013) and Starr et al. (2014), that reported significant levels of variation of taxa richness and abundance among habitats inside river reaches. Arscott et al. (2005) found greater diversity in macroinvertebrate communities of backwaters areas of Tagliamento River, while Gray & Harding (2009) pointed out spring creeks, spring sources and ponds and Zilli & Marchese (2011) isolated lakes as habitats hosting greater diversity in New Zealand rivers and in Panamá River floodplain, respectively. By contrast, in our systems secondary channels resulted as being the habitat hosting greater taxa diversity, both for seasonal and summer data, while in the other works these habitats resulted in those with low diversity compared to the other ones. This higher diversity could be explained considering that secondary channels were often located near the margin of riverbeds and presented a higher heterogeneity of microhabitats (cobbles, gravel, clay, algal mats and roots). These features have been shown to be critical in enhancing the within site richness (e.g. Downes et al. 2000).

Several factors have been proposed as main drivers for BRs macroinvertebrate community differentiation: conductivity and percentage of sand (Zilli & Marchese, 2011), flow velocity (Arscott et al. 2005), nature of substrate (Beisel et al. 1998). In our work, we considered the mesohabitat category as a proxy of physical environment differentiation: in particular we found that main channels, secondary channels and pools mainly differed in flow velocity and water depth. Given the high significance of mesohabitats for macroinvertebrate, we hypothesized a strong physical control of communities, with a selection of taxa based on their habitat necessities, also suggested by the presence of unique taxa for different mesohabitats.

Time resulted to be an important factor for community organization. We observed minor values of richness and abundance during the seasonal sampling. Similar results are reported also for Tagliamento River by Arscott et al. (2003), who found the highest values of density at the end of the summer. Several explanations have been proposed for this kind of trend: a concentration effect for the decrease of available living area (Verdonschot et al. 2015), changes in food resources (Suren et al. 2003), alterations of habitat suitability with the proliferation of more tolerant taxa (Feio et al. 2010) and the annual life cycles of macroinvertebrate, especially insects (Bêche et al. 2006).

Another temporal trend that arose from our results is a difference in the importance of mesohabitats between seasonal and summer phases, supported by both mixed effects modelling and nMDS ordination. These findings are in contrast with the ones of Starr et al. (2014) and Arscott et al. (2003), who reported an increase of compositional heterogeneity coming from the increasing isolation of sampling sites in the first study, and from flood homogenization in the second. García-Roger et al. (2011) instead found similar results, with a mesohabitat differentiation smaller during the dry season for a decrease of habitat heterogeneity. The greater differentiation of mesohabitat communities observed in the present study during seasonal sampling (November and April) could be the result of a major connectivity that allows organisms to actively choose the best living place, according to their necessities. This generates a high environmental control on the community from the moment that there are no dispersal limitations and the choice of the most suitable environmental features drives the community. On the other hand, during the summer the disconnection increases, hampering the dispersion of organisms. Unlike our initial hypothesis, in this phase we observe a temporal trend of dissimilarity reduction between mesohabitats, coupled with a prevalence of taxa turnover on taxa loss. These phenomena could be due to a general loss of the more sensitive and specialized taxa, which leads to the homogenization of communities. Nevertheless, the turnover remains greater than nestedness, suggesting the presence of taxa well adapted to the different conditions of mesohabitats and able to manage the effects of flow reduction.

This work provides significant insights, also into the biomonitoring procedures. The Italian legislation provides only the main channel sampling, but this, based on our data, could lead to samples unrepresentative of the real communities. In BRs the distribution of benthic macroinvertebrates exhibits high levels of heterogeneity and therefore the ecological status cannot be evaluated only in the main channels, but should be assessed considering the river ecosystem as a whole, including marginal water bodies that are common in these environments.

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SECTION III: Macroscale – Intermittence

LARGE SCALE DISTRIBUTION OF AQUATIC INVERTEBRATES IN PERMANENT AND INTERMITTENT STREAMS

INTRODUCTION

Regarding the distribution of organisms across a wide range of riverine systems, the flow regime is considered as a "*master variable*", which deeply shapes the organization of macroinvertebrate communities (Poff et al. 1997; Gray & Harding 2011). Indeed, flow alterations has received growing attention in the last decades (e.g. Poff et al. 1997; Allan et al. 2005; Döll & Schmied 2012; Arnell & Gosling 2013; Acreman et al. 2014) since, in the last century, natural regimes have been deeply altered (e.g. Malmqvist & Rundle 2002) and forecasts for the future indicate a continuation, if not a worsening of this trend (Fig. 19). Alterations of the natural flow regime originate from two main categories of phenomena, namely the anthropogenic pressures and the climate changes (Fenoglio et al. 2007; Arthington et al 2014; Datry et al 2016).

The growing demographic increase of the world has led to obvious impacts on the hydrological system, with both direct effects for the increased water demand (for irrigation, energy production, industrial use, civil and other), as well as indirect effects, caused by the increased soil consumption and consequent increase in habitat fragmentation (e.g. Prat & Munné 2000; Cooper et al. 2013; Bonada & Resh 2013; Datry et al. 2014b). Forecasts on land use and water requirements aggravate the current situation, with a predicted further decrease in flow rates (Merelender & Matella 2013).

Besides the anthropogenic pressures, also the climate change will lead significant alterations of riverine systems (Allan et al. 2005; Arnell & Gosling 2013). Schneider et al. (2013) point out that, at European level, climate change will considerably modify the natural flow regime, through the alteration of precipitation, temperature and snow cover. Based on climate change scenarios, there will be an increase in frequency and extent of droughts, in particular at medium latitudes, with a possible progressive shift from permanent water bodies to systems characterized by an increasingly temporary/stochastic character (e.g. Fenoglio et al. 2010; Larned et al. 2010). Projections to 2050 foresee further regime shifts, involving strong changes in habitat and ecosystem conditions (Döll & Schmied 2012). Increased climatic instability manifests itself not only as changes in temperatures and precipitations, but also as an increase of extreme phenomena, in terms of frequency, intensity and unpredictability (Fenoglio et al. 2007; Calapez et al. 2014). Indeed, one of the outcomes of the global warming is an increase of atmospheric energy, which is dissipated through the occurrence of more intense atmospheric events (Houghton et al. 2001). Floods and droughts represent the two extremes of hydrological spectrum of watercourses and both of them can exert an effect on ecological patterns and processes (e.g. Clausen & Biggs 2000, Robinson & Uehlinger 2008). Given the actual spread of these phenomena, it is of essential importance to understand how aquatic communities respond and recover from these events (e.g. Domisch et al. 2013; Bogan et al. 2015).

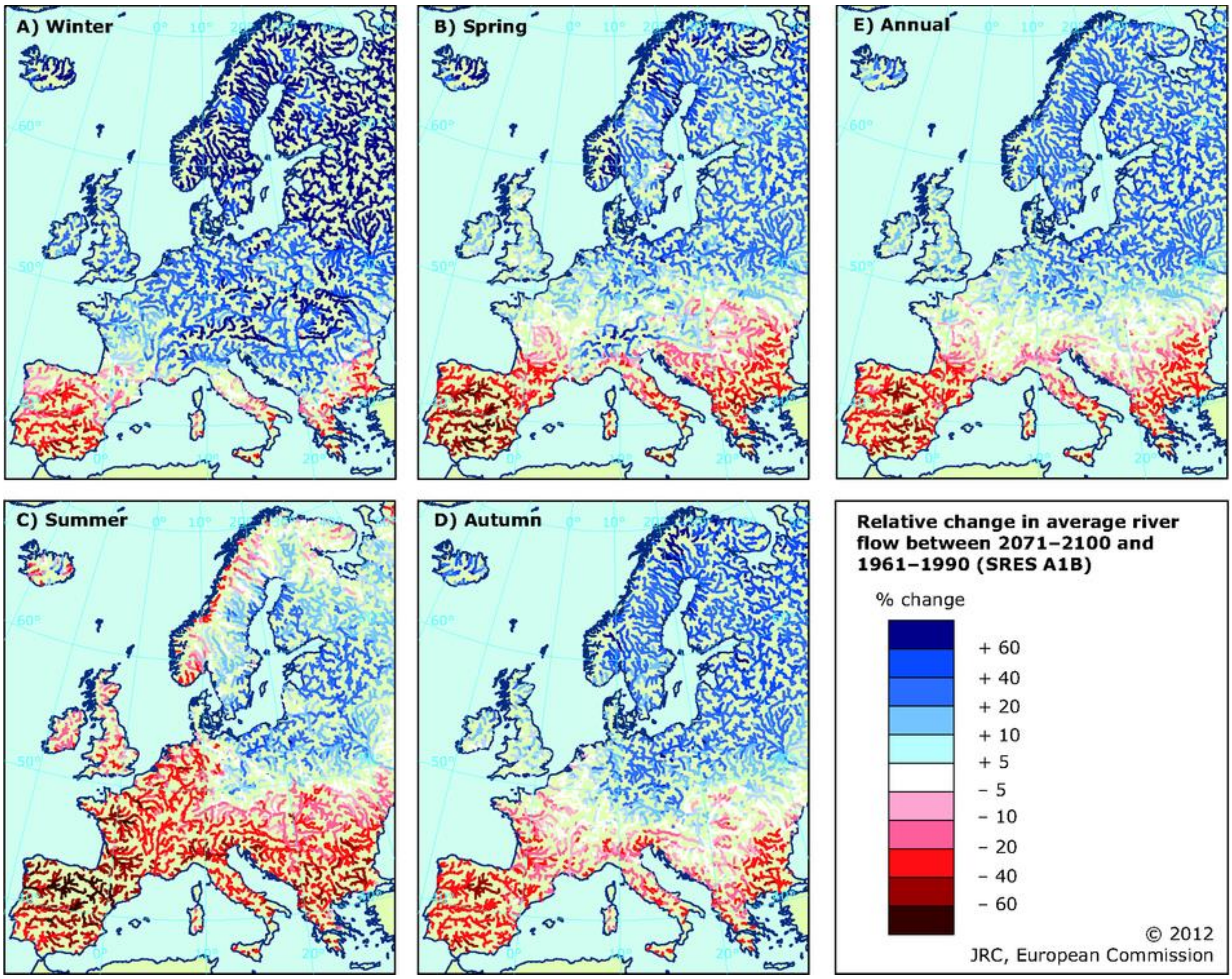

Figure 19: Prevision of flow regime alterations of European streams for the period 2071 - 2100 (Source: EEA).

Due to all these phenomena, the natural flow regime of many perennial streams and rivers is undergoing a *regime shift*, toward conditions of greater stochasticity and intermittence, and assuming features that make them increasingly similar to intermittent rivers in the Mediterranean regions (e.g. Fenoglio et al. 2007). A more in-depth knowledge of these systems can thus improve the understanding of the possible impacts of hydrological alterations and represents a useful tool for their management and recovery. This is true especially considering that while communities inhabiting arid regions are more likely to have life-history adaptations that give them resistance and/or resilience to droughts (Bogan et al. 2015), communities of perennial streams are not adapted to these new harsh conditions (e.g. Fenoglio et al. 2007; Larned et al. 2010), and therefore the impacts on communities can be severe and unpredictable. For such reasons, in the last years, the attention of scientific community focused on the study of naturally intermittent watercourses, especially in the Mediterranean region. "Intermittent streams" are defined as systems in which drought occurs at least in a reach and for at least a period of the year (Steward et al. 2012; Acuña et al. 2014; Arthington et al. 2014). Those systems are among the most dynamic freshwater environments, since they are modelled by the alternation of wet and dry periods both on annual and inter-annual cycles (e.g. Bernal et al. 2013; Prat et al. 2014; Bogan et al. 2015). Their hydrological regime reflects the trend of precipitations, so they are naturally highly variable, with a seasonally changing level of lateral and longitudinal connectivity (Prat et al. 2014). The wet season is characterized by a phase of expansion, during which there is a high level of connectivity. Conversely, the dry season is represented by a phase of contraction, during which there is a progressive decrease of the water level, with connection interruptions both in the lateral and longitudinal direction (Bonada et al. 2006). The first step is the lateral disconnection, with the loss of lateral mesohabitats. With the progress of the dry period, there is a longitudinal disconnection with a transition towards lentic conditions and the creation of several isolated pools (e.g. Fenoglio et al. 2007; Verdonschot et al. 2015). At this stage, there is a rapid deterioration of water quality, with a rise in temperature and a decrease in dissolved oxygen (Boulton 2003; Acuña et al. 2005; Lake 2011). With a further intensification of the drought, there is a full drying of the superficial water, and then also a subsurface drought, with the loss of the vertical connectivity (Bogan et al. 2015).

The occurrence of a drought period severely affects aquatic invertebrates (e.g Boulton et al. 1992) generally representing a ramp-disturbance (sensu Lake 2003), with impacts on the communities dependant on its intensity and duration and often characterised by the presence of critical thresholds of disturbance (Boulton 2003; Bonada et al. 2006; Boulton & Lake 2008). As water level decrease, taxa associated to lateral habitats are lost (Bogan et al. 2015). In addition, more rheophilic taxa decrease due to the growing stagnation of water, with a progressive substitution by more generalist ones (Poff et al. 1997; Feio et al. 2010). As reported by Datry (2012) there is a shift of community composition, with Ephemeroptera, Plecoptera and Trichoptera replaced by Coleoptera, Diptera, Microcrustacea and Oligochaeta. This shift can be the result of a real taxa replacement, with a selection of a well-adapted community (Bogan et al. 2013) or can be due to a sensitive taxa loss, with a resulting subset community (Arscott et al. 2010; Datry 2012). In the remaining pools a *concentration effect* can be observed, resulting in an apparent increase in diversity and abundance, due to a contraction in the available habitat (Fritz & Dodds 2004; Acuña et al. 2005; Storey & Quinn 2008; Verdonschot et al. 2015). However, these high densities can lead to an increase in competition and predation (e.g. Acuña et al. 2005), that together with the rapid physicochemical deterioration can generate critical conditions that can have heavy effects on communities, especially if extended over time. As the disturbance progresses, there is an increasingly intense decline in richness and abundance (Poff & Zimmerman 2010). With the complete water loss, macroinvertebrate can persist only thanks to refugia, like isolated pools (Arscott et al. 2010), leaf litter, algal mats, woody debris and damp sediment beneath large stones (Robson et al. 2011) or the hyporheic zone (Boulton et al. 1992).

Thus, the flow regime and its alterations deeply affect aquatic invertebrate assemblages. As stated by García-Roger et al. (2013) the flow regime can also alter the differential importance of community drivers and of the spatial extent at which these drivers operate. In this context, the aims of the present study are i) to investigate the responses of macroinvertebrate communities to flow intermittence, quantifying the large-scale distribution of aquatic taxa among a wide group of watercourse, in relation to the flow permanence and ii) to investigate the differential importance of spatial scale (and related environmental drivers) in permanent versus intermittent watercourses. We postulate two different conditions, based to the hydrological regime: i) in permanent systems we hypothesize the presence of a well adapted community, mainly characterized by rheophilic taxa; ii) in intermittent systems we hypothesize the presence of a reduced subset of taxa, composed only by the more generalist ones. Moreover, based on previous findings (e.g. García-Roger et al. 2013) we hypothesize a prevalence of local environmental drivers (e.g. microhabitat features) in systems characterized by an intermittent regime, while in permanent ones we hypothesize a prevalence of large-scale drivers. Indeed, in intermittent watercourses, the high hydrological variability should have a greater impact on local conditions, mirrored by macroinvertebrate communities inhabiting these environments. The present study has been carried out in the framework of the Italian National Relevant Project (PRIN2015) *NOACQUA-responses of communities and ecosystem processes in intermittent rivers*.

METHODS

Study area

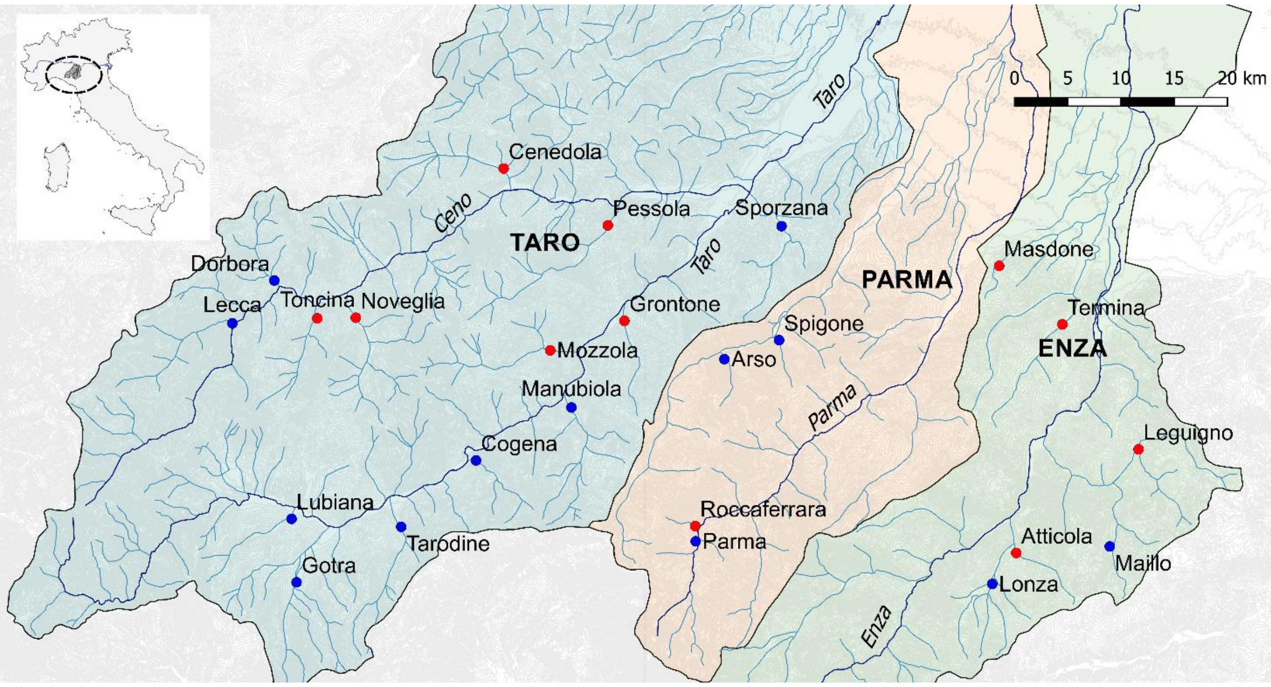

Figure 20: A map of the study area with a chromatic subdivision based on the main basin. Dots represent the sampling stations, with blue dots for stations with a permanent regime and red dots for stations with an intermittent regime. Blue thin lines represent the hydrographic network. Top-left, in the small square, the position of the sampling area compared to Italy

The present study was performed in three main basins of the Po River Basin (Northern Italy), namely Taro Basin, Parma Basin and Enza Basin (Fig. 20). These basins are all included in the Emilia-Romagna region and occupy an area of approximately 3500 km^2. They are oriented from SW to NE, perpendicular to the Po River. In the mountain sections (SW), they cover a portion of the Tuscan-Emilian Apennines, while in the lowland sections (NE) they are part of the Po Valley. All the watercourses in this area are fed only by wet depositions and present an alternation of high and low water periods, with higher values of discharge mainly in spring and autumn, and lower values during summer (and occasionally in winter). Within each basin, we identified a subset of watercourses and for each of them a sampling station having the same stream order (2nd or 3rd order, according to the Horton-Strahler classification). The selection process was carried out by means of the QGIS software, according to the fluvial order criteria, resulting in a total of 24 selected watercourses. Once the selection was done, we also extrapolated several broad scale attributes of the selected watercourses (or of the relative sub-basin), namely: primary and secondary basin, sub-basin area and perimeter, station altitude, length of the watercourse and three land use variables, i.e. the percentage of artificial, agricultural and semi-natural/forested areas inside the selected sub-basins (Tab. 8). The altitude of the sampling station ranged from a minimum of 193 m a.s.l for Sporzana Stream, to a maximum of 655 m a.s.l. for Parma Stream. The evaluation of intermittence (I) versus permanence (P) state was made prior to the field work, based on aerial photos, environmental agencies data (ARPAe) and interviews with the population. Moreover, the previous method was verified and integrated by means of field surveys during the dry phase. Unfortunately, the historical knowledge about the hydrology of the selected systems is limited and incomplete and therefore we were not able to ascertain if these systems are naturally intermittent or if they have become intermittent only in recent years, due to the effects of climate change.

Table 8: Physical and land use features of the sampled sites, grouped for basin. BI=primary basin, BII=secondary basin, A=sub-basin area (km^2), P=sub-basin perimeter (km), H=station altitude (m a.s.l.), L=watercourse length (km), LU1=artificial surfaces (%), LU2=agricultural areas (%), LU3=semi-natural and forested areas (%).

Watercourse	BI	BII	A	P	H	L	LU1	LU2	LU3
Cendedola	Po	Taro	43.2	36.7	289	26.3	2.5	31.5	64.0
Cogena	Po	Taro	17.8	20.6	360	11.3	0.4	6.2	93.3
Dorbora	Po	Taro	26.2	31.2	420	17.2	0.5	17.3	82.1
Gotra	Po	Taro	40.5	34.5	525	28.7	1.3	10.3	87.7
Grontone	Po	Taro	22.2	24.9	236	10.1	0.8	19.0	78.7
Lecca	Po	Taro	37.1	40.5	535	20.6	0.6	4.9	94.4
Lubiana	Po	Taro	153.3	73.0	451	118.7	2.7	12.6	83.7
Manubiola	Po	Taro	51.0	35.5	453	37.6	3.6	13.6	82.6
Mozzola	Po	Taro	45.2	35.9	297	24.0	1.5	21.2	76.6
Noveglia	Po	Taro	52.9	35.9	425	31.7	0.6	15.0	82.8
Pessola	Po	Taro	48.1	44.9	238	29.5	0.6	22.7	74.9
Sporzana	Po	Taro	42.4	36.5	193	30.2	3.5	48.5	46.3
Tarodine	Po	Taro	30.1	36.6	448	16.9	1.3	11.6	85.8
Toncina	Po	Taro	29.0	31.6	435	19.5	0.7	13.3	85.1
Arso	Po	Parma	10.4	18.1	587	7.7	0.6	10.9	88.5
Parma	Po	Parma	52.0	36.4	655	39.7	1.6	8.5	89.2
Roccaferrara	Po	Parma	7.6	15.4	639	3.6	0.6	4.0	95.3
Spigone	Po	Parma	10.4	16.5	427	5.9	0.5	20.4	79.0
Atticola	Po	Enza	23.4	25.4	381	14.8	3.4	51.7	44.2
Leguigno	Po	Enza	14.0	20.9	388	9.0	5.4	28.1	66.2
Lonza	Po	Enza	25.5	34.3	455	24.4	9.3	72.0	18.6
Maillo	Po	Enza	16.6	22.4	472	10.0	4.4	52.9	42.7
Masdone	Po	Enza	16.1	23.0	222	11.5	8.3	49.7	42.1
Termina	Po	Enza	78.0	61.1	210	49.9	7.2	58.3	33.4

Sampling protocol and laboratory activities

For each station, we measured the width of wetted and active channel and we recorded temperature, conductivity and pH by means of a multi-parametric probe (YSI Instruments). In a 50 m long stream section of each station, we selected 6 sampling plots, aiming to include all the microhabitats present in the stations. For each of these points we recorded flow velocity (at the bottom of water column) and depth by means of a current meter (FP101-FP102 Global Flow Probe) and dominant substrate (according to Buffagni & Erba 2007). Moreover, we collected a surber sample by means of a surber net (net with frame area of 0.05 m^2 and mesh size of 500 μm) for macroinvertebrates and benthic organic matter (BOM). Samples were kept separated in PET bottles and fixed with 90° ethanol for laboratory sorting. Macroinvertebrate were counted and identified to genus level (according to Tachet et al. 2010), except for Diptera for which the family or sub-family level was reached. BOM was separated from inorganic material by elutriation (Boulton & Lake 1992), sieved with 1 mm mesh-size sieve to retain only the coarse fraction and then dried in hoven at 105° C until constant weight. Then the BOM was weighed by a precision balance (AB104 METTLER TOLEDO) for the determination of dry weight.

Statistical analysis

Mixed effects modelling was used in order to evaluate the variability of environmental parameters recorded in each of the 6 sampling plots (flow velocity, depth and BOM) between I/P watercourses, set as fixed effect and with stream as random effect. For the other recorded environmental parameters, we used ANOVA, since no in-stream replications were not present.

We applied a procedure of best model selection in order to evaluate which of the measured environmental variables mainly affect community metrics (taxa richness and abundance). At first, we created a base model including all the measured environmental variables and the interactions among them by means of mixed effect modelling (using the sampling station as random effect). Then, we selected the best model for each metric by means of the *dredge* function of the MuMIn R package, a procedure that allow the choice of the best model based on a weight assigned to each combination of variables (with and without interaction terms) computed from the BIC criterion. Abundance was log-transformed prior the analysis to deal with overdispersion.

The effect of hydrology (I/P state) on community structure was evaluated by means of an nMDS ordination, grouping communities by means of the *ordihull* function of the vegan R package. As dissimilarity measure Bray-Curtis distance was used and the goodness of ordination was assessed with the stress measure.

The variance partitioning method (see Section I for procedure details) has been applied in order to discriminate among the differential importance of local, basin and regional scale variables for macroinvertebrate communities (e.g. Gray & Harding 2011) between P and I subsets of the whole set of sampled watercourses. As local-scale variables we selected flow velocity, depth, substrate, BOM, width of wetted and active channel, water temperature, pH and corrected conductivity. As basin-scale we selected basin area and perimeter of every sub-basin, stream length and land use categories (LU1, LU2 and LU3 in Tab.8). As regional-scale variables we selected secondary basin, elevation, latitude and longitude.

All analyses and graphs were performed with the statistical software R (R Core Team, 2016), with base version and with ggplot2 (Wickham 2009), lme4 (Bates et al. 2015), packfor (Dray et al. 2013), MuMIn (Barton 2017) and vegan (Oksanen et al. 2016) packages.

RESULTS

Environmental variables

Out of the 24 selected sites 13 resulted to have a permanent regime and 11 an intermittent regime. A full list of the watercourses with the relative regime is reported in Tab. 9, together with other environmental features recorded during the sampling campaigns. At the time of sampling three streams (Cenedola, Masdone and Termina) were disconnected, with only some remnant pools, while the other ones were connected, but with some differences in hydrological parameters. The width of the wetted riverbed resulted to be slightly variable among systems, with a maximum of 8.0 m in Gotra Stream and a minimum of 0.0 m in Masdone Stream, where we found only two remnant pools at sampling time. The width of the active bed was instead extremely variable, with a maximum of approximately 200 m for Pessola Stream and a minimum of 4.0 m for Maillo Stream. Also temperature and conductivity varied greatly among the sampling stations with maximum values of 22.9°C and 1138 μS cm^{-1} for Manubiola and Masdone Streams respectively and minimum values of 11.1°C and 192 μS cm^{-1} for Lonza and Tarodine Streams. The pH instead resulted less variable, with a maximum of 8.86 for Tarodine Stream and a minimum of 7.95 for Termina Stream. The other environmental variable, measured in each sampling point, resulted to be very variable both among and within watercourses. Flow velocity varied from zero (recorded in several watercourses, in plots near the shoreline or in pools) up to 1.37 m^3 s^{-1}, recorded in Parma Stream, while depth varied from few centimeters (found for several conditions, like near the shorelines or in thin layers of water) up to 0.38 m, found in Lonza Stream. Also the amount of BOM varied greatly, ranging from a maximum of 14.51 g for the Dorbora Stream to a minimum of 0.02 g for the Roccaferrara Stream. Most common substrate resulted to be mesolithal (6-20 cm), followed by microlithal (2-6 cm) and macrolithal (20-40 cm). Other substrate types, like gravel, sand or silt, as well as organic substrates (e.g. roots) have been detected with lower frequencies.

The variability of environmental features between I/P watercourses is reported in Fig. 21. Only conductivity varied significantly between I/P watercourses (F-value=6.48, p-value=0.02), while for the other variable we found no significant differences between the two regimes.

Table 9: Environmental variables of the sampling sites. H=hydrology (I/P state), WR=wetted riverbed (m), AR=active riverbed (m), V=flow velocity ($m^3 s^{-1}$, mean value±standard deviation), D=depth (m), Sub=dominant substrate (Buffagni & Erba 2007), BOM=dry weight of the Benthic Organic Matter (g, mean value±standard deviation), T=temperature (°C), Cond°=corrected conductivity ($\mu S\ cm^{-1}$).

Watercourse	H	WR	AR	V	D	Sub	BOM	T	pH	Cond°
Cendedola	I	2.0	150.0	0.13±0.08	0.07±0.02	mes	0.23±0.13	14.3	8.25	486
Cogena	P	4.0	40.0	0.33±0.30	0.13±0.05	mes,mac	0.93±1.21	20.0	8.57	218
Dorbora	P	4.0	50.0	0.33±0.37	0.10±0.04	mes,mic	2.75±5.79	22.4	8.65	370
Gotra	P	8.0	40.0	0.23±0.23	0.25±0.08	mes	1.00±1.55	15.5	8.48	194
Grontone	I	2.5	100.0	0.33±0.31	0.07±0.02	mes	0.07±0.04	20.8	8.69	577
Lecca	P	4.0	15.0	0.15±0.12	0.10±0.03	mes	0.34±0.31	17.7	8.62	372
Lubiana	P	6.0	16.0	0.14±0.16	0.13±0.04	mes	0.56±0.47	19.0	8.15	314
Manubiola	P	7.0	60.0	0.43±0.45	0.22±0.11	mes	0.08±0.10	22.9	8.40	391
Mozzola	I	4.5	70.0	0.41±0.34	0.14±0.05	mes	0.58±0.59	22.0	8.34	476
Noveglia	I	2.0	100.0	0.22±0.30	0.09±0.07	mes	0.57±0.90	20.4	8.18	433
Pessola	I	6.0	200.0	0.26±0.26	0.08±0.03	mes	0.50±0.67	18.6	8.57	480
Sporzana	P	1.5	20.0	0.09±0.11	0.07±0.04	mic	0.35±0.26	16.1	8.36	722
Tarodine	P	7.0	50.0	0.20±0.23	0.17±0.09	mes	1.73±1.56	18.8	8.86	192
Toncina	I	4.2	40.0	0.13±0.10	0.12±0.09	mes	1.17±1.29	15.1	8.54	435
Arso	P	2.0	10.0	0.41±0.49	0.15±0.03	ghi	2.73±3.05	12.0	8.55	344
Parma	P	7.0	28.0	0.34±0.53	0.16±0.03	mes,mac	1.72±2.33	17.1	8.41	334
Roccaferrara	I	3.0	13.0	0.30±0.39	0.07±0.02	mes,mic	0.20±0.34	14.6	8.19	442
Spigone	P	2.0	7.0	0.21±0.17	0.11±0.05	mic	2.17±2.28	19.4	8.54	402
Atticola	I	6.5	34.0	0.56±0.19	0.13±0.05	mes	0.50±0.47	13.2	8.62	676
Leguigno	I	5.0	6.0	0.26±0.21	0.16±0.07	mes	1.19±0.51	11.5	8.68	759
Lonza	P	5.0	33.5	0.53±0.11	0.22±0.08	mes	0.31±0.46	11.1	8.74	404
Maillo	P	3.5	4.0	0.33±0.25	0.12±0.06	mes	0.90±0.52	11.2	8.68	885
Masdone	I	0.0	6.0	0.00±0.00	0.15±0.07	mes,mic	7.20±7.89	15.3	7.96	1138
Termina	I	1.5	6.5	0.01±0.01	0.21±0.10	mic	1.89±1.56	16.8	7.95	900

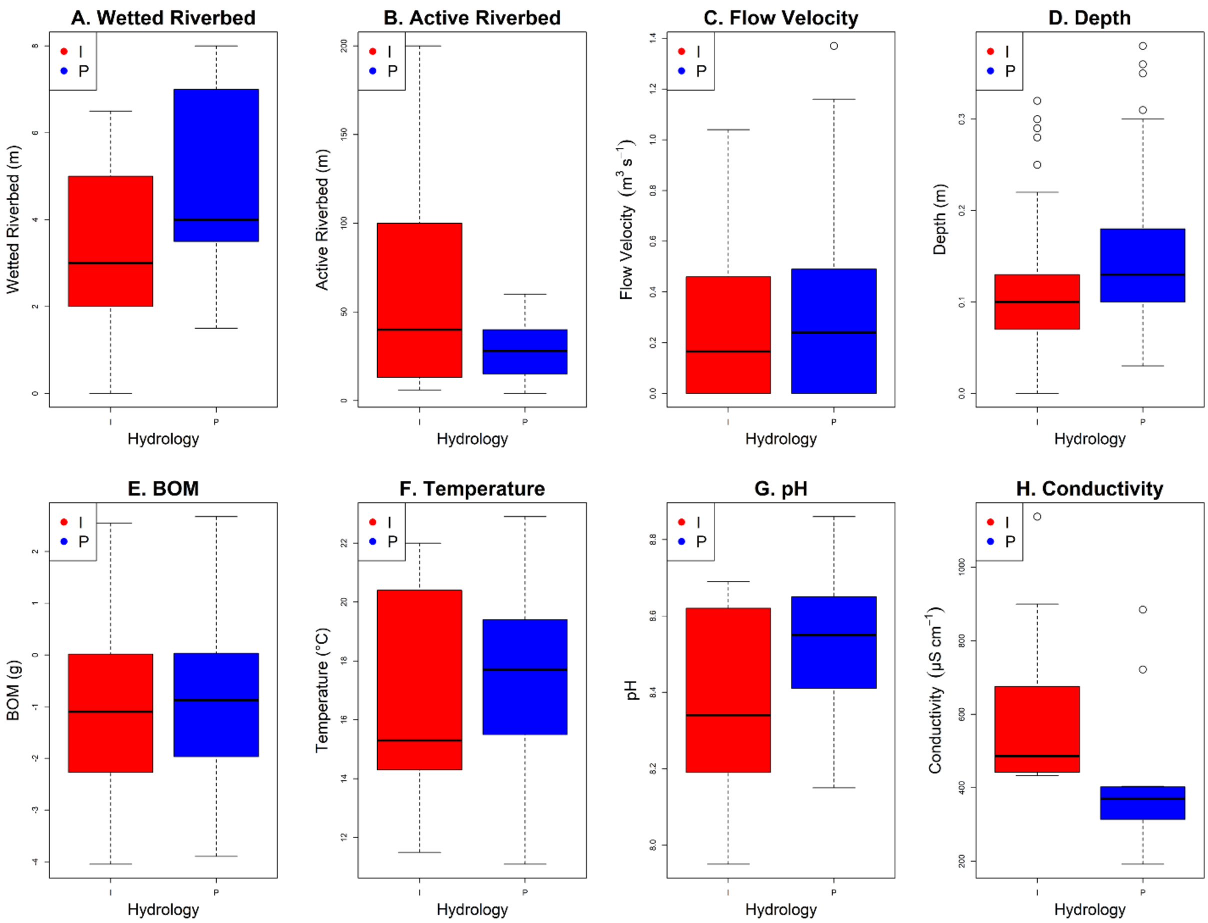

Figure 21: Variability of environmental parameters between I and P watercourses.

Metrics and macroinvertebrate community

A total of 45424 organisms belonging to 174 taxa was found globally. The highest number of taxa was found for the Cogena Stream (36 taxa) and the minimum for Grontone Stream and Roccaferrara Stream (4 taxa). For abundance the maximum was recorded for Parma Stream (5486 organisms) and the minimum again for Grontone and Roccaferrara (6 organisms). Out of the 174 taxa, the 25% has been found only in P watercourses, the 26% only in I watercourses, while the remaining 49% was found in I or P indifferently. Values of taxa richness and abundance for the studied watercourses are reported in Fig.22. Values of P watercourses (blue boxes) resulted to be higher than those of I ones (red boxes), especially for taxa richness (for which confidence intervals, dotted lines in Fig.22A, are not overlapped). Masdone and Termina Streams represent an exception to the above results: in fact these systems showed values of taxa richness and abundance higher than the mean value of I watercourses.

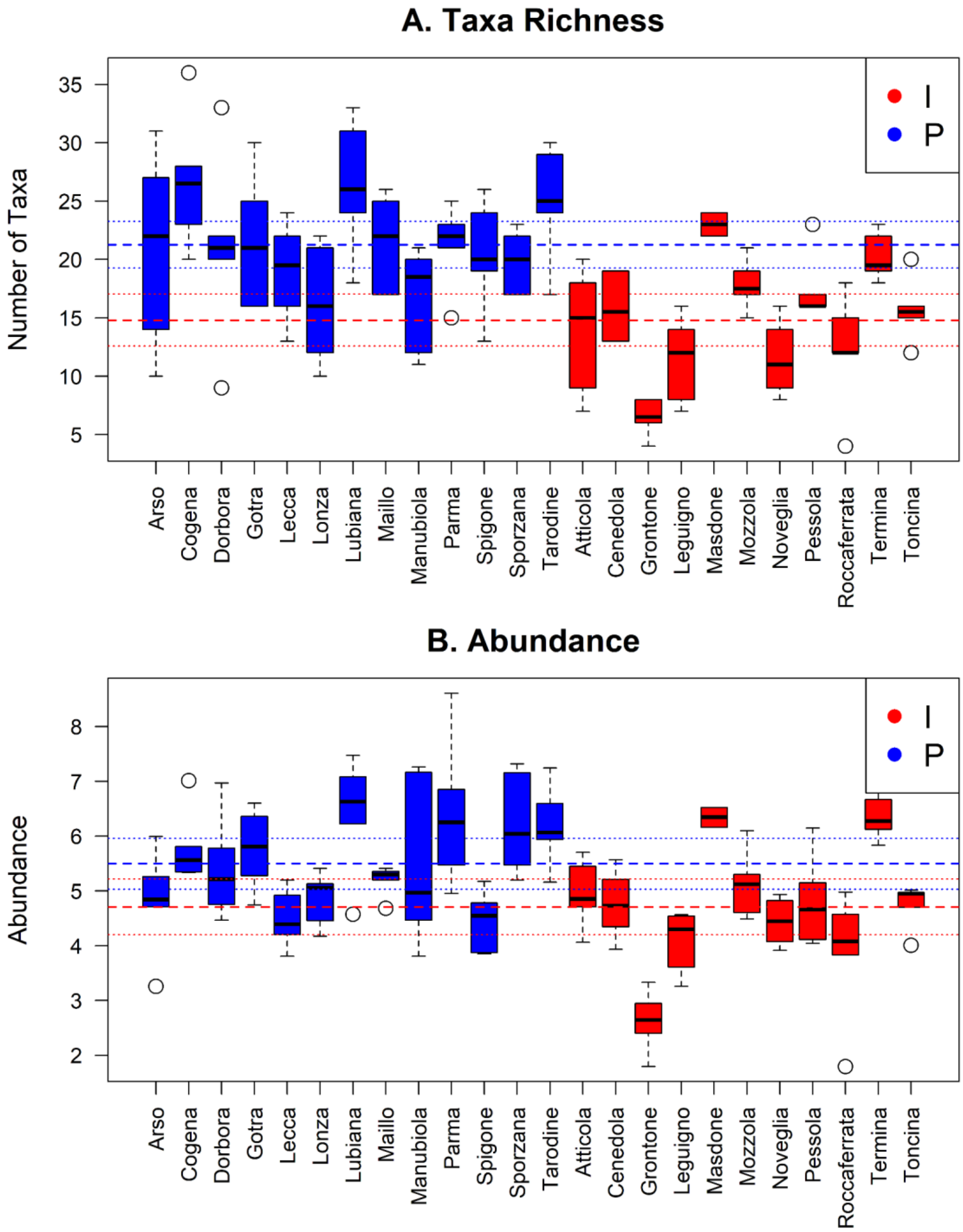

Figure 22: Taxa richness (A) and abundance (B) values for the studied watercourses. Boxes corresponding to I watercourses are filled in red, boxes corresponding to P watercourses are filled in blue. Horizontal dashed lines represent mean values of metrics for I (in red) and P (in blue) watercourses, while dotted lines represents confidence intervals for I (red) and P (blue).

The choice of environmental variables affecting metrics was operated by means of best model selection applied to mixed effect models. For taxa richness the extracted variables were BOM, hydrology, flow velocity and the interaction term between BOM and flow velocity. For the log-transformed abundance the selection procedure gave the same results obtained for taxa richness, but with the addition of the interaction term between hydrology and flow velocity.

Concerning the community structure, the nMDS ordination (Fig. 23) highlighted an overlapping of communities of I and P watercourses. Nevertheless, the spread of the point of I watercourses resulted greater than for P watercourses.

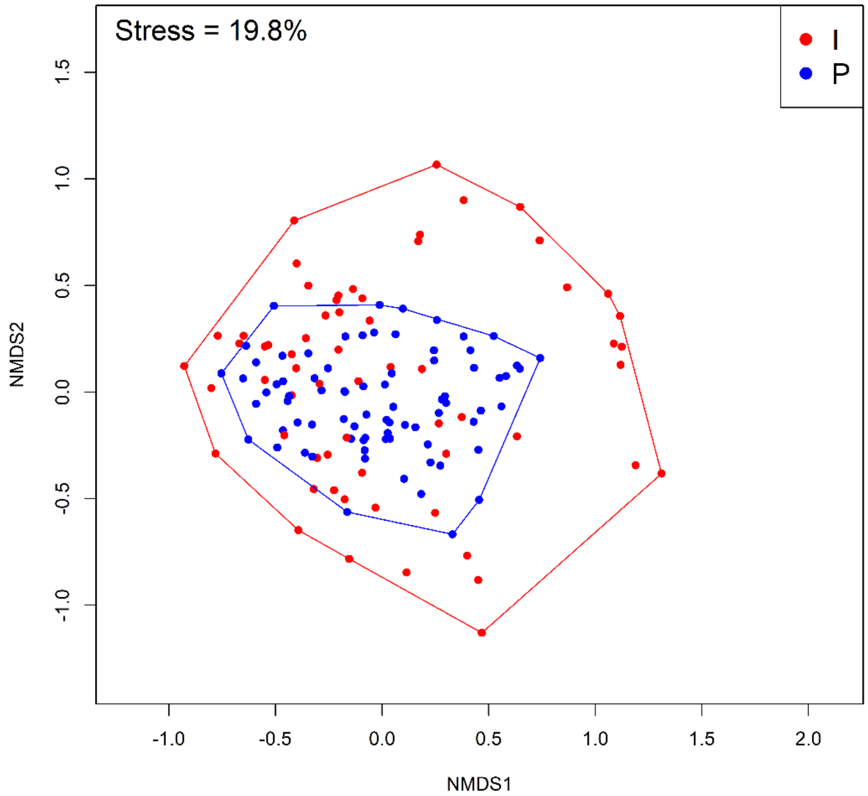

Figure 23: nMDS output for macroinvertebrate community. Red dots represents communities of I watercourses, blue dots communities of P watercourses. Stress value is also reported.

The explanatory power of local, basin and regional variables for community structure was assessed by means of variance partitioning (Fig. 24). For P watercourses the forward selection procedure gave as significant only local-scale variables (BOM, substrate and pH), while for I watercourses the procedure extracted variable of local (BOM, conductivity, pH, substrate and WR), basin (LU1, LU2, LU3 and perimeter) and regional (BII and latitude) scales. For P watercourses, we found a prevalence of local variable, with a contribution 26% to the total variance. Regional scale variable explained a lower fraction (6%) of total variance and 65% of the total variance remained unexplained. For I watercourses, only 25% of the total variance resulted unexplained, with higher contribution to explained variance given by variable of nearly all scales. Local variables resulted again the ones giving the greatest contribution (27%), together with the three sets of explanatory variables joined (28%). In addition, basin scale variables explained a large fraction (11%) of the total variance, while the contribution of regional scale variables resulted lower than for P watercourses (4%).

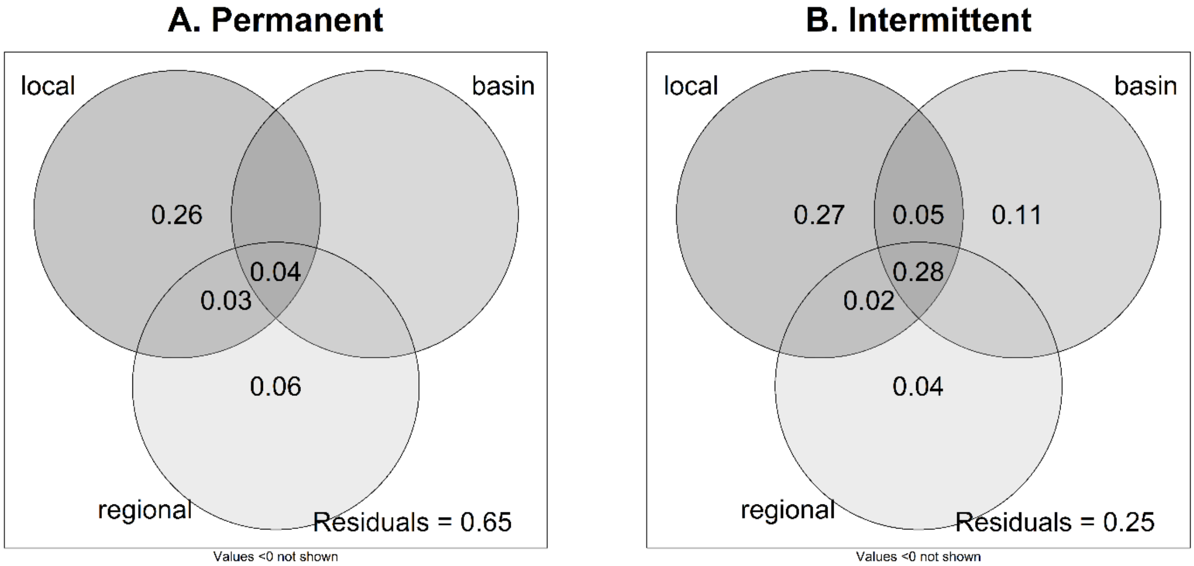

Figure 24: Variance partitioning results for communities for permanent (A) and intermittent (B) watercourses, among three sets of selected explanatory variables (relative to different spatial scales). Fractions showed are adjusted R^2 values. Residuals values are also displayed.

DISCUSSION

The main objective of this research was to investigate the large-scale distribution of macroinvertebrates, evaluating: i) their variability between I and P watercourses, and ii) the differential importance of spatial scale and related environmental variables in driving macroinvertebrate community structuration in I and P watercourses.

Concerning the first aim, we find out higher values of metrics, especially taxa richness, in P watercourses (Fig. 22), with hydrology selected as driver for all metrics during the best model selection procedure. In fact we found that, on average, in I systems there are 7 taxa less than in P ones. Our findings are in line with the ones of other authors (e.g. Boulton 2003; Fenoglio et al. 2007; Datry 2012; Bogan et al. 2013; Datry et al. 2014a), which report a decrease of taxa richness in I streams. However, the difference in richness can be ascribable to two different factors: a nestedness or a turnover of assemblages. Compositional shifts in intermittent systems have been explained in terms of one of these phenomena (e.g. Datry et al. 2014a; Leigh 2013; Bogan et al. 2013; Vidal-Abarca et al. 2013). However, in our systems, points corresponding to samples of I streams overlaps with the ones of P streams, but with a greater variability (Fig. 23). These results can be explained considering that I streams undergo to more frequent and intense disturbance events than P ones, with an alternation of floods and droughts (Heino et al. 2015). This leads to frequent changes in community composition, with higher values of heterogeneity, while the greater stability of P watercourses allows the establishment of a more selected group of taxa. Also the results of variance partitioning support this hypothesis: in fact, for I streams we found a greater fraction of explained variance that reflects a greater heterogeneity in community assemblages that hence responds greatly to the environmental variables. The effect of I/P state should be also considered in relation to the other environmental variables. E.g., in our work we found that there are no significant relationships among environmental variables and the hydrology. However, despite the absence of a direct relationship, the interaction term between hydrology and flow velocity was selected as driver for abundance, pointing out a different effect of flow velocity variation on these metrics in I versus P systems.

About the issue of Masdone and Termina Streams, for which we found a great deviation from I mean values of taxa richness and abundance, we can translate this apparent contradiction with the fact that these streams were already drying at the time of sampling, with only some remnant pools. In particular, for Masdone Stream, where we found only two pools, we found also higher values of taxa richness and abundance. Under such conditions, a concentration effect may occur in the remnant pools, with sharp increases of abundance and richness, followed by a sharp increase of predation pressure (Acuña et al. 2005; Verdonschot et al. 2015). Indeed, communities of these systems resulted to be dominated by predators, such as *Notonecta*, *Chalcolestes*, *Agabus* and Tanypodinae.

Focussing on the results of the variance partitioning, we highlighted a dominance of local-scale variables in explaining community structure, both for I and P watercourses, with similar percentage of explanatory power (26% for P and 27% for I). The dominance of local variable in explaining structure and variability of macroinvertebrate communities is widely accepted (e.g. Heino et al. 2004; Brooks et al. 2005; Costa & Melo 20008; Wilson & McTammany 2014). In our work the amount of BOM resulted one of the most important drivers for aquatic invertebrates (see Section I for more details about its role), since it was extracted both for metrics in the best model selection procedure and for communities in the forward selection procedure. Considering the basin-scale in I streams, we found that land use variables explain a good fraction of variance (11%), probably because of the greater agricultural and artificial surface and lower natural surface (Tab. 8) that can negatively affect riverine systems and their communities.

Our findings of a dominance of local-scale variables both in I and P watercourses, with a lack of differentiation in explanatory power of variables belonging to different spatial scales, are in contrast with those reported by García-Roger et al. (2013), who found a prevalence of large-scale variables in P watercourses and of small-scale variables in I ones. Such divergence could be ascribable to the fact that they sampled in different periods compared to us and, as reported by the authors themselves, the relative importance of each spatial scale changes with season. Moreover, also the difference in spatial extent of their larger scale (compared to our) could explain this discordance. Indeed they worked in six basins across Europe, while our data come from a more restricted area.

This work provides useful information about the variability of aquatic invertebrates between I and P streams, which could improve the efficiency of monitoring protocols and help the planning of more suitable strategies to face the regime-shift that is happening in temperate and mediterranean regions. Indeed, the evaluation of the environmental flows is becoming a striking objective in the European and Italian scenario, and a greater knowledge of aquatic insect communities to different hydrological conditions could benefit its assessment.

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General discussion and conclusions

MAIN FINDINGS

The general aim of this PhD thesis was to investigate the distribution of aquatic invertebrates and to discriminate the main drivers structuring their communities at different spatial scales.

The first level examined is the microscale (or small-scale), i.e. the variation of assemblages within a river reach, in an approximate area of 10x10 m. From the analysis of communities of Parma, Enza and Nure Streams, during summer and winter, we have drawn some main conclusions. At first, we found that the variability of metrics is explained almost completely by local environmental factors, with the amount of BOM being a general and powerful driver for taxa richness, abundance and biomass (likely representing both a refugia and a food resource). Nevertheless, this relation is not so strong considering single taxon or community matrices. Indeed, as second main outcome, we highlighted that the space (and spatial arrangement of the systems) can be a strong driver of macroinvertebrate communities also at the small-scale, up to explain most of the total variance, and therefore should not be neglected, even when studying distributions among near microhabitats. Indeed, considering this spatial scales, the significance of space and therefore the resemblance of communities close to each other, is reflected in an autocorrelation among them (e.g. in the first study of Section I we proved a range of autocorrelation of 4.45 m, in which data are not independent). Whatever is the cause of this autocorrelation (biotic interactions or limiting factors), its presence must be considered and community studies should be done according to techniques that are able to manage this autocorrelation, in order to avoid type I errors (Dray et al. 2012). Based on all these findings, we can hypothesize a sort of carrying capacity of the amount of BOM for the abundance of organisms: to a given amount of organic matter, we found a corresponding number and diversity of organisms, but in this ratio, the relative proportion among different taxa may change, as highlighted by results of variance partitioning and single taxa. This variable ratio, imply the possibility of taxa replacements, and the presence of drivers different from environmental ones, like biotic interactions that regulate the fine scale assemblages of macroinvertebrates. Another remarkable outcome of this section is that main drivers can change depending on which is the studied variable: metrics are more affected by the environment, while for community matrices and single taxa also space is needed in order to explain their variability. This first section provides useful finding for two different application fields. The relations that we found represent a good step toward the creation of predictive models, able to estimate the distribution and the abundance of organisms based on values of driving variables. Moreover, our results contribute to the improvement of biomonitoring protocols, since the goodness and precision of biotic indexes is strictly related to the small-scale patchiness of aquatic invertebrates (Laini et al. 2014). Nevertheless, further research is needed in order to evaluate this small-scale approach in different conditions, like in other longitudinal sections or in other biogeographic regions and to test reliability of the found relationships also in other systems.

The second spatial resolution considered here was the mesoscale, with an analysis of the spatio-temporal variability of communities among three mesohabitats (main channel, secondary channel and pool) in eight braided river reaches. We found that in these systems the biodiversity is unequally divided, with marginal habitats hosting a number of taxa not present in the main channel. This habitat specificity of communities is remarkable, especially considering that biomonitoring protocols only account for the main channel, and therefore they are not representative of the total diversity inside these rivers. Based on our results, mesohabitats represent a good classification unity for lotic systems (and in particular for the braided ones), with the same mesohabitat located in different rivers being more similar than different mesohabitats located in the same river or even in the same sampling station. Again, we can state that macroinvertebrate community organization is strictly related to local scale variables (in this case the mesohabitat category) which dictate the structuration of assemblages. In addition, we found that during the summer phase, with the decrease of water level, there is also a decrease of the dissimilarity among mesohabitats, which imply a homogenization of main channels, secondary channels and pools, with the loss of more sensitive taxa. Despite this dissimilarity reduction, we still found a prevalence of taxa turnover among mesohabitat, suggesting the presence of specialized taxa, with resistance adaptations to flow reduction (Datry et al. 2014). In addition to the biomonitoring implications, our results offer valuable insight also considering BR conservation and restoration. Maintaining the high environmental heterogeneity, that is characteristic of these environments, should be a main goal of environmental policies, in particular in the regime shift perspective. Furthermore, another improvement in this field could be to assess how much is the variability within mesohabitat compared to the variability between mesohabitats, maybe also applying the method that we used in Section I.

The third level examined in this PhD thesis concerned the large-scale distribution of macroinvertebrate, assessing their diversity in an area of approximately 3500 km^2. We focussed the attention on two main topics: the differentiation of assemblages between permanent and intermittent watercourses and the differential importance of driver variables belonging to three hierarchical spatial scale in shaping communities of watercourses with these two hydrological regimes. Main results highlighted a strong influence of the hydrology (permanent versus intermittent) on community metrics, with higher values of diversity in permanent watercourses. Moreover, we found that communities of intermittent watercourses exhibit greater levels of variability: in permanent streams we found a common pool of organisms with assemblages from different system similar to each other, whereas for intermittent streams we found a high level of heterogeneity. Such difference could be the result of the hydrological history of these systems, characterized by a frequent alternation of floods and droughts, which leads aquatic organisms to cope with very harsh conditions (Larned et al. 2010). This pattern filters the larger pool of macroinvertebrate that can be found in perennial regimes, generating a patched distribution and selecting a number of subsets of organisms that are well adapted to the local environmental conditions. We also highlighted a prevalence of local-scale variables in explaining community patterns, both for intermittent and permanent watercourses, with BOM selected for the two hydrological conditions. This has confirmed our previous findings about the relevance of the amount of organic matter, broadening this relation over the large-scale. A future objective of this work is to evaluate the recovery of communities of these watercourses after the summer drought, assessing the effect of a particularly dry year (as it was in 2017) on aquatic invertebrate persistence. Moreover, the quantification of the hydrological regime, in term of duration and frequency of the dry events will help to improve our knowledge about intermittence and its effect on lotic systems and carry out better strategies of environmental management and preservation.

CONCLUSIONS

The outcomes of the present PhD thesis offer appreciable insights into the variability of macroinvertebrate communities and factors affecting this variability, especially considering the small-scale drivers, for which the informations were scarce and focused only on environmental features of the microhabitats.

We also paid special attention to hydrology and hydraulic variables, explaining their relation with macroinvertebrate at several spatial scales and remarking their position as master variables in riverine systems. However, our work also revealed the striking role played by the organic matter and, more in general, by trophic factors, for aquatic invertebrates. Thus, an insight into the relationship among hydrology, hydrological history and organic matter could really ameliorate the understanding of macroinvertebrate distribution patterns.

With this work, a wide range of spatial scales, explanatory and dependent variables has been analysed, highlighting, among the various results, also a difference in the predictability of dependent variables and going one-step further in understanding the dynamics of lotic ecosystems.

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