Macroinvertebrate variability in mediterranean and temperate streams: unimpacted and impacted river basins

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MACROINVERTEBRATE VARIABILITY IN MEDITERRANEAN AND TEMPERATE STREAMS: IMPACTED AND UNIMPACTED RIVER BASINS

Memory presented by

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1. ABSTRACT

Mediterranean-climate streams are characterised by predictable and seasonal floods and droughts. These characteristics have resulted in high diverse communities with a myriad of species adapted to survive floods and droughts or to recover from them. In contrast, in temperate-climate streams, droughts are rare and floods can occur throughout the year. Seasonal differences are thus supposed to be higher in mediterranean streams. The few existing studies carried out separately in mediterranean and temperate streams suggested that interannual variability is another important driver to explain differences in community composition. Inteannual differences are thus supposed to be higher in mediterranean streams, with significant differences between wet and dry years. On the other hand, mediterranean and temperate streams are both subjected to many types of human disturbances. This could have led to the homogenization and simplification of riverine communities, with the consequent potential loss of the uniqueness of both stream types. Our main aim was to analyse the effects of disturbances due to land use changes in macroinvertebrate taxonomic (structure) and biological traits (function) composition in mediterranean and temperate streams, as well as, to assess seasonal and interannual variability between regions and the effects of disturbances in both patterns. Two natural unimpacted and two agriculturally impacted basins were sampled in both mediterranean (Catalonia) and temperate (Luxembourg) regions. In each basin, macroinvertebrate quantitative and qualitative samples were collected in 5 sites with different stream order. Samples were taken in spring and autumn over 3 years. Results showed that despite structural and functional composition differed between unimpacted and impacted basins, climatic effects were much more important. Thus, a higher variability was found between climatic regions, followed by effects of land use changes. Seasonal and interannual differences were much less important. Our results suggest that climatic effects are more relevant than previously expected, indicating that large-scale environmental filters (climate) are much more important than basin scale ones (land-use) during the species sorting process that drive community assembly.

RESUM

Els rius de clima mediterrani estan caracteritzats per inundacions i sequeres estacionals i predictibles. Aquestes característiques han resultat en una gran diversitat de comunitats amb innumerables especies adaptades a sobreviure o recuperar-se de les sequeres i les riuades. Per contra, en els rius de clima temperat les sequeres són rares i les riuades poden ocórrer tot l'any, de manera que s'espera que les diferències estacional siguin més grans als rius mediterranis. Alguns estudis fets separadament en ambdós climes suggereixen que la variabilitat interanual és un altre factor per explicar la composició taxonòmica. Així, als rius mediterranis s'espera una major variabilitat entre les comunitats presents en anys secs i anys plujosos. Per altra banda, els rius mediterranis i temperats estan subjectes a una gran varietat de pertorbacions de tipus humà. Això, podria haver permès l'homogeneïtzació i la simplificació de les comunitats, amb la conseqüent pèrdua de singularitat d'ambdós tipus de rius. El principal objectiu d'aquest treball es analitzar els efectes de les pertorbacions provocades per canvis en els usos del sòl en la composició taxonòmica de macroinvertebrats (estructura) i els trets biològics (funció) en els rius de clima mediterrani i temperat, així com, avaluar la variabilitat estacional i interanual entre ambdues regions i els efectes de les pertorbacions sobre aquests patrons. Dos rius naturalitzats, no impactats i dos rius pertorbats per agricultura van ser mostrejats en ambdues regions. A cada un, es van fer mostrejos qualitatius i quantitatius en 5 punts, en trams de diferents ordre. Els mostrejos es van dur a terme a la primavera i a la tardor durant 3 anys. Els resultats mostraren que la major variabilitat era trobada entre les regions, seguida per la variabilitat generada pels efectes dels diferents usos del sòl. Les diferencies estacionals i interanuals eren molt menys importants. Els nostres resultats suggereixen que els efectes climàtics són més rellevants del que s'esperava, indicant que els factors ambientals a gran escala (clima) són molt més importants que els factors a escala de conca (usos del sòl) com a conductor del procés d'assemblatge de les comunitats de macroinvertebrats.

2. INTRODUCTION

Temperate and mediterranean-climate regions have different characteristics that confer particular environmental conditions. Hot and dry summers and wet winters characterize the mediterranean climate (Aschmann, 1973), whereas summers are milder and winters are colder and wetter in temperate regions (Bonada *et al*., 2007). These climatic characteristics act as large-scale factors and have triggered many evolutionary adaptations on organisms, for example, geophytes in mediterranean-climate regions, or summer perennial plants in temperate-climate regions (Dallman, 1998).

Streams in mediterranean regions have highly seasonal discharge patterns, with predictable torrential floods and severe droughts, with very frequent intermittent conditions (Bonada & Resh, 2013). In contrast, discharge is less variable in temperate regions, where riffle-pool sequences along the year are common and permanent conditions are usually the rule (Bonada *et al*., 2007). Besides these seasonal differences, interannual variability is also likely to be higher in the mediterranean region, with dry and wet years usually well differentiated (Gasith & Resh, 1999; Bêche & Resh, 2007a).

These differences between both regions together with other large-scale factors, such as geological or evolutionary processes, are supposed to affect the structure and function of biological communities (Bonada *et al*., 2007). Regions with similar climatic and ecological characteristics should support organisms with similar biological traits, whereas regions differing in climate or ecology should have organisms with different ones. Hence, species in mediterranean streams would require particular adaptive mechanisms to survive drought and flood conditions or to recover. In contrast, these adaptations are not expected in temperate streams. In fact, research suggests that communities in mediterranean streams are characterized by a dominance of traits to cope with seasonal droughts, whereas temperate rivers have communities with traits adapted to cope better with flood and perennial conditions (Bonada *et al*., 2007).

On the other hand, freshwater ecosystems are considered the most endangered ecosystems in the world (Dudgeon *et al.*, 2006). Besides the natural drivers and stressors that disturb freshwater ecosystems, like the above-mentioned sequences of floods and droughts (Stendera *et al.*, 2012), streams worldwide are highly disturbed as a consequence of the high human population density, and the consequent competition between water need for agriculture and domestic use (Bonada & Resh, 2013). Anthropogenic stressors such as overexplotation, water pollution, flow modifications, destruction or degradation of habitat, and invasion by exotic species, cause changes in hydrologic processes, food resources, nutrient dynamic, riparian vegetation, and many other factors that intimately affect the structure and function of stream ecosystems (Gasith & Resh, 1999; Dudgeon *et al.*, 2006). All these anthropogenic stressors are also expected to homogenize stream assemblages across the landscape. In particular, land use changes increase temperature and pollutant inputs on streams promoting the extirpation of sensitive species and favouring the presence of tolerant ones (Ricart *et al.*, 2010). For example, with increasing catchment development, richness, density, and biomass of the highly sensitive Ephemeroptera, Plecoptera and Trichoptera (EPT) decrease, whereas those of Diptera and the non-insects Nematoda, Oligochaeta, Hirudinea, or Mollusca increase (Waite *et al*, 2010). Land use changes often increase scouring flood flows, removing and transporting invertebrates downstream and fostering the dominance of quick-colonizing, r-selected species, with immediate or short-term effects on invertebrate densities, species composition, and richness (Gasith & Resh, 1999). At the other extreme, land use changes can decrease stream flows and increase the duration of dry periods during dry season, selecting for species that can withstand or recover quickly from droughts (Lake, 2011). Finally, in some cases, return flows from agricultural and urban areas can increase dry season flows, both disrupting the life cycles of species adapted to seasonal droughts and increasing the survival and densities of exotic species, sometimes resulting in the demise of native species through their interactions with exotic species (Riley *et al.*, 2005).

Both disturbance types, the natural and the anthropogenic, make mediterranean streams the most stressed streams worldwide (Bonada & Resh, 2013)**.** Despite temperate streams are also affected by anthropogenic disturbances, the effects on stream ecosystems are more exacerbated in mediterranean streams because of the large seasonal and interannual variability in stream flow. Land use changes have exaggerated hydrological variation in mediterranean streams, increasing peak flood flows and decreasing dry season flows (Konrad & Booth, 2005). In addition, during the dry season, pollutant concentrations may become high, with sublethal and lethal effects on stream organisms (Prat & Munné, 2000).

Our study assesses differences in macroinvertebrate communities between mediterranean and temperate-climate regions and considering taxonomic (structure) and biological traits (function) composition. Studying communities combining taxonomic and trait characteristics is important to consider not only biogeographical and ecological factors involved but also ecological functions that can be relevant for the ecosystem function (i.e., biological traits are directly or indirectly related to ecological functions; Statzner *et al.*, 2004). Furthermore, we also assessed the effects of land use changes in the community composition of mediterranean and temperate streams. Finally, seasonal and interannual differences were also assessed between mediterranean and temperate streams, and compared how these differences varied in impacted and unimpacted streams in both climatic regions.

We tested 6 main hypotheses: (1) climate effects should be more important than impacted-unimpacted effects because climate is a larger-scale factor filtering traits and taxonomy (Poff, 1997), (2) climate differences would be higher in unimpacted (forested) than in impacted streams because anthropogenic disturbances are expected to homogenize and simplify communities in freshwater ecosystems worldwide (Cooper *et al*, 2013), (3) seasonal differences should be higher in mediterranean streams due to climate characteristics and the predictable seasonal floods and droughts (Bonada & Resh, 2013), (4) these seasonal differences should be more evident in unimpacted than impacted streams, following the second hypothesis, (5) interannual differences between macroinvertebrate communities should be more evident in mediterranean-climate regions because climatic and environmental variability between years is higher than in temperate-climate regions (McElravy, 1989; Bêche & Resh, 2007b), and interannual and seasonal variability determine the temporal and spatial dynamics of these systems, controlling the structural biological communities (Gasith & Resh, 1999), and (6) these patterns would be more evident for taxonomic than for trait composition because traits are less affected by the biogeographical differences of the two climate regions and vary relatively little across large spatial and temporal scales (Statzner *et al.*, 2001a, 2004, 2005).

3. MATERIAL AND METHODS

3.1 STUDY AREA

We initially considered 4 river basins in Luxembourg (temperate region) and in Catalonia (mediterranean region). Streams were selected with contrasting land uses in each region and included unimpacted basins (hereafter, **forested**), and agricultural impacted basins (hereafter, **impacted**) (fig. 1). Unimpacted basins included well-developed forests and woody riparian vegetation, which are important to supply matter and energy to stream ecosystems, influencing geomorphological processes of river basins, or regulating water temperature among other factors (Naiman *et al.*, 1993). All basins were located over calcareous geology to reduce heterogeneity of macroinvertebrate composition. Geology clearly discriminates macroinvertebrates communities in large-scale studies (Nijboer *et al*., 2004), and particular species characterize calcareous, siliceous, and sedimentary stream reaches (Bonada *et al*., 2005; Bonada *et al.*, 2008). In Luxembourg, Schwaarzbaach and Consdorferbaach were selected as unimpacted streams, and Hemeschbaach and Sauerbaach as impacted ones mainly by agricultural and cattle. In Catalonia, Talamanca and Vall d'Horta were selected as unimpacted streams, and Pontons and Múnia as impacted ones by agricultural and wineyards.

Basins were also selected as similar as possible in terms of morphological and riverbed characteristics. In Catalonia, the unimpacted Talamanca and Vall d'Horta had a V-shaped valley and a sinuated channel form. The main riverbed substrate varied between mesolithal, macrolithal, and megalithal (different grain size) depending on the reach. Riparian vegetation varied between 60% and 80% of coverage along the stream, it was permanently present and the riverbed was medium shaded in all sampling sites by its canopy. On the other hand, the impacted Pontons and Múnia had a U-shaped valley and a sinuated channel form. The main riverbed substrate varied between microlithal and mesolithal depending on the reach. Riparian vegetation was also present along the stream but its coverage was poorer than in the unimpacted streams (between 5% and 20%). The riverbed was not regularly shaded. In both streams, the first station, which were close to the source, were medium shaded but the others were opened.

In Luxembourg, the unimpacted Schwaarzbaach and Consdorferbaach had 100% of woody riparian vegetation permanently along the stream and permanent shade. A V-shaped valley form and a sinuate channel form were also characteristics of both streams. The main channel substrate was mesolithal. On the other hand, the impacted Hemeschbaach and Sauerbaachhave had similar characteristics than unimpacted ones, with a V-shaped valley form, sinuated channel form, and a mesolithal substrate, whereas woody riparian vegetation varied between 5% and 60% and it was also permanent along the stream. The riverbed was medium shaded in the first reaches of both streams but the other reaches are opened.

In order to know if our a priori classification of river basins was correct, we compared the physicochemical parameters collected in each basin for the whole sampling period. Results indicated that the Múnia stream was much more impacted than the other impacted streams, and therefore, it was omitted the rest of our study. Furthermore, the Consdorferbaach stream, which was initially selected as unimpacted stream, showed high physicochemical parameter values, and therefore, it was considered as impacted.

Thus, we ended up with 1 unimpacted and 3 impacted streams in Luxemburg and 2 unimpacted and 1 impacted streams in Catalonia.

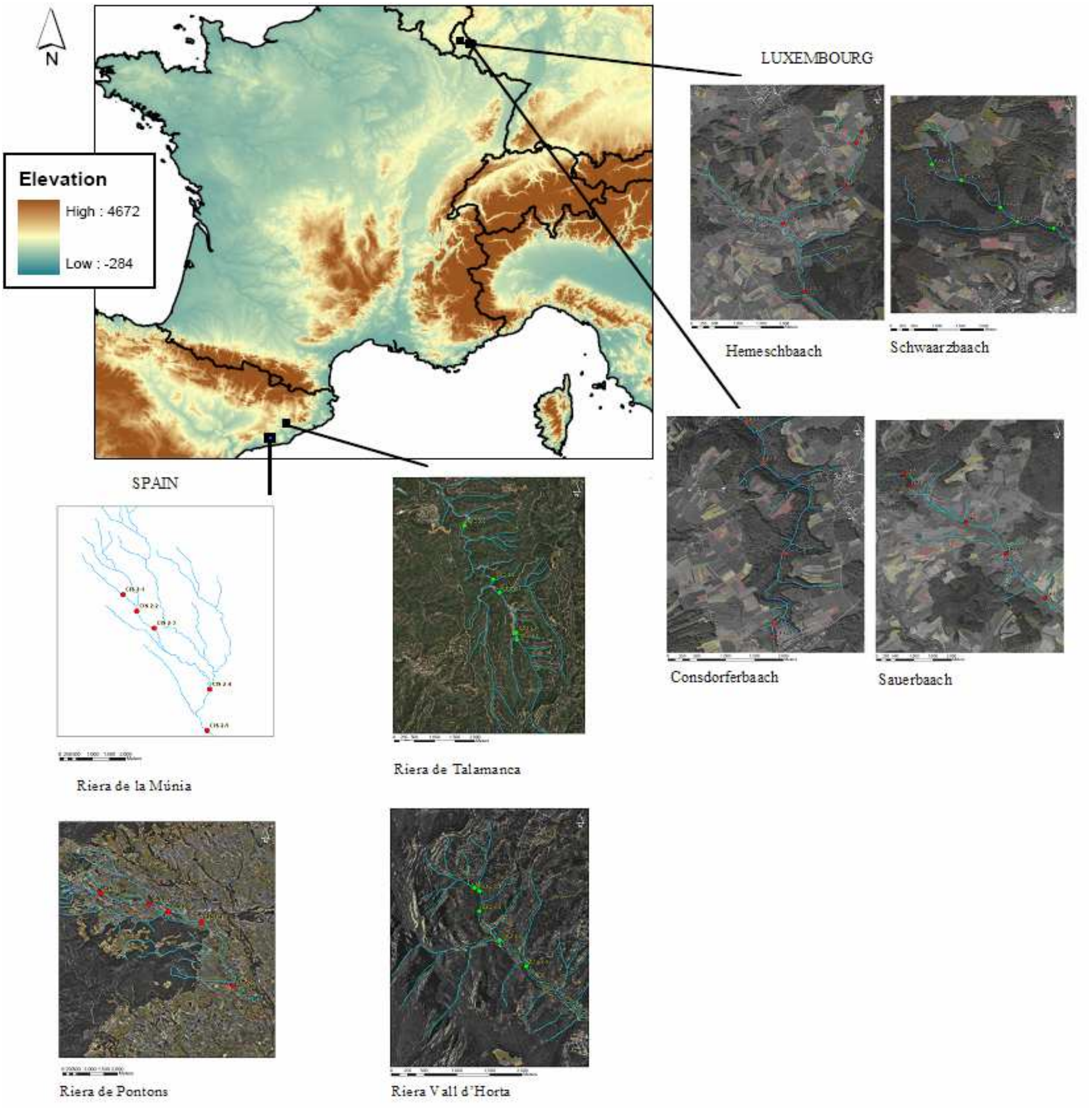


Fig.1 Map of the study area including areal pictures of the 4 original study streams of both Catalonia and Luxembourg regions. Green points show unimpacted stream sampling sites, while red points show impacted stream sampling sites.

3.2 SAMPLING CHARACTERISTICS

Macroinvertebrates samples were collected in 40 sites along the 8 streams; 5 sites by stream. Sites within each stream were distributed along the main river channel before and after the stream order changed (fig. 1). Sampling was conducted during 3 years (2010, 2011, 2012), in spring and autumn. Thus, the total number of samplings was 240, 210 without the Múnia stream. Some of the sampling sites of mediterranean streams were dry, so the analysis was finally done with 203 sampling sites.

Spring and autumn seasons were selected over other seasons to assess the differential effect of the summer drought in the mediterranean and temperate regions, and because of the life cycle development of aquatic macroinvertebrates. For instance, early spring sampling of benthic invertebrates may be very important according to the affluence of water flowed abruptly during winter and early spring and the influence of temperature on their larval development; and autumn samples can reflect effects of recovery from the drought period in the mediterranean-climate region (Haines, 1988).

3.3 ENVIRONMENTAL VARIABLES

Several physicochemical parameters were measured in the field (*in situ*) and in the laboratory (Table 1). Temperature, pH, conductivity, and O_2 were measured with multisensors at the moment of the macroinvertebrate sampling. Water samples were collected and brought to the laboratory for the correspondent analysis of TOC (Total Organic Carbon) and nutrient concentration. This environmental information is required to identify direct anthropogenic pressures, which potentially affect macroinvertebrate composition.

Table 1 List of physicochemical parameters from each sampling site classified between field or laboratory measurements.

Field measurements (*in situ)*	**Laboratory measurements**	
Temperature (ºC)	PO_4^{3-} (mg/l)	Na (mg/l)
pH	PO_4^-P (µg/l)	NH_4^-N (mg/l)
Conductivity (µSm/cm)	Cl^- (mg/l)	K (mg/l)
O_2 (mg/l)	NO_2N	Mg (mg/l)
O_2 (%)	NO_3N	Ca (mg/l)
	SO_4 (mg/l)	TOC (mg/l)

3.4 MACROINVERTEBRATE SAMPLES

Three quantitative random samples and one qualitative sample were collected in each site. Quantitative samples were collected using a Hess Sampler with a mesh size of 250 µm and a diameter of 40 cm, which allow a better quantification of the invertebrate abundance. Qualitative samples were collected sweeping all available habitats for five minutes using a sweep net (hand net) with a mesh size of 250 µm, which provide information of the total richness of the site. Both sample types were preserved separately in alcohol 96% and brought to the laboratory where all benthic invertebrates were sorted, counted, and identified to genus level. Dipterans were not considered because the taxonomic difficulties to get the genus level and the lack of detailed trait information (Pfenninger *et al*., 2007). The mean of the 3 Hess samples was obtained for each sampling data and site. Qualitative data was quantified according a rank value and classified as *rank 1* (1-2 individuals), *rank 2* (3 to 5 individuals), *rank 3* (6 to 25 individuals) and *rank 4* (>25 individuals). For each rank we obtained a median value: 1.5 for *rank 1*, 3.5 for *rank 2*, 15 for *rank 3*, and 50 for *rank 4*. The higher numerical value between qualitative and quantitative data was selected to build up the taxonomic matrix.

3.5 BIOLOGICAL TRAITS

Macroinvertebrate communities were characterized using 11 biological traits divided into 61 categories (Tachet *et al.*, 2002; Bonada *et al.*, 2007). These 11 traits describe morphology and physiology (maximum size, respiration), life-cycle features (life-cycle duration, reproductive cycles per year, aquatic stages), types of dispersal, locomotion and substrate relation, resistance forms, and feeding behaviour (food and feeding habitats) (table 2).

Each genus was coded according to its affinity to each category of the trait using a fuzzy coding approach. This method synthesizes various sources of numerical data obtained from literature or collected in the field over a long time period giving a degree of affinity of each genus to each trait (*Chevenet et al*., 1994).

Table 2 Table containing the 11 biological traits divided by 61 categories obtained from Tachet et al., 2002.

Trait	Category	Trait	Category
Maximal size	≤ 2.5 mm	**Respiration**	Tegument
	>2.5-5 mm		Gill
	>5-10 mm		Plastron
	>10-20 mm		Spiracle (aerial)
	>20 -40 mm		Hydrostatic vesicle (aerial)
	>4-8 cm	**Locomotion and substrate relation**	Flier
	>8 mm		Surface swimmer
Life cycle duration	≤ 1 year		Swimmer
	>1 year		Crawler
Potential number of reproduction cycles per year	<1		Burrower (epibenthic)
	1		Interstitial (endobenthic)
	>1		Temporarily attached
Aquatic stages	egg		Permanently attached
	larva	**Food**	Fine sediment + microrganisms
	nymph		Detritus < 1 mm
	imago		Plant detritus ≥ 1 mm
Reproduction	Ovoviviparity		Living microphytes
	Isolated eggs, free		Living macrophytes
	Isolated eggs, cemented		Living microinvertebrates
	Clutches, cemented or fixed		Living macroinvertebrates
	Clutches, free	**Feeding habits**	Absorber
	Clutches in vegetation (endophytic)		Deposit feeder
	Clutches, terrestrial		Shredder
	Asexual reproduction		Scraper
Dissemination	Aquatic passive		Filter-feeder
	Aquatic active		Piercer (plant or animal)
	Aerial passive		Predator (carver/engulfer/swallower)
	Aerial active		Parasite, parasitoid
Resistance form	Eggs, statoblasts, gemmules		
	Cocoons		
	Cells against desiccation		
	Diapause or dormancy		
	None		

3.6 STATISTICAL ANALYSIS

First of all, 3 matrixes were done with all data: matrix A was a log-transformed taxonXsite matrix of the abundance of genera en each site, matrix B was a taxonXtrait matrix and matrix D was an environmental matrix, envXsite. Matrix A and B were multiplied obtaining the proportion of each trait category in each site in a traitXsite matrix called matrix C (fig. 2) (Elosegui & Sabater, 2009). Matrixes A, C and D were used for statistical analysis.

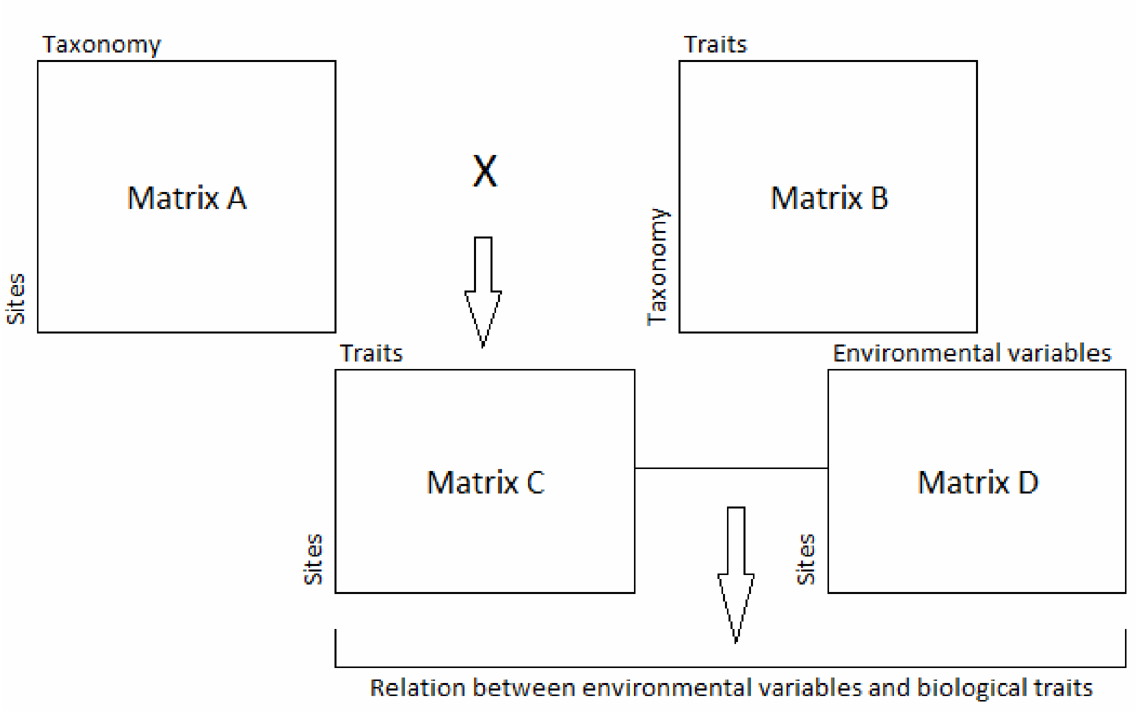


Fig. 2 Drawing of the construction of the matrix C (traitXsite) that combines biological traits of the taxonomy (matrix B) with the relative abundance of the taxonomy per site (matrix A), obtaining a matrix of the proportion of traits per site (matrix C), to combine with environmental variables (matrix D) in statistical analysis.

A Principal Component analysis (PCA) was done to analyse the physicochemical parameters in order to distinguish unimpacted (forested) and impacted streams. A PCA is a simple, non-parametric, standard method in data analysis for extracting relevant information from data sets. PCA provides a roadmap for how to reduce a complex data set to a lower dimension to reveal simplified structures (Shlens, 2014) and transforms the original variables into new, uncorrelated variables (axes), called the principal components, that are linear combinations of the original variables. The new axes lie along directions of maximum variance (Sârbu & Pop, 2004).

Despite taxonomic richness and abundance vary along headwaters, midstream, and lowland reaches in response to changes in the stream environment (Lamouroux *et al.*, 2004), the selected streams were not too much long and it was expected to find less sampling site differences than seasonal and interannual differences. A Generalized Linear Model (GLM) was applied to reject the effect of the distribution of sampling sites in regional community composition. A GLM is a flexible generalization of ordinary linear regression that allows for response variables that have error distribution models other than a normal distribution. Given that there was no effect of the reach, sampling sites of each stream were used as replicas.

The effects of climate, human impact, seasonality and interannuality on taxonomic composition were also analysed with method PCA on the log-transformed taxonXsite matrix (matrix A), whereas effects on trait composition were analysed using a Fuzzy Principal Component Analysis (FPCA) on the traitXsite matrix (matrix C). A FPCA is a more robust algorithm than simple PCA and less sensitive to outliers, missing data and poor lineal correlation between variables due to poorly distributed variables (Sârbu & Pop, 2004). Significant differences of each factor were assessed with Monte-Carlo test, a non-parametric randomness test based on permutation test on the PCA and FPCA results.

In addition, abundance, richness, Simpson index and ratio EPT/EPTOCH were calculated for taxonomic data, and trait richness and Rao's diversity coefficient were calculated for trait data. The Simpson index is a commonly used diversity index that calculates the probability that two organisms, randomly taken, belong to the same group (species, genus…) (Simpson, 1949). The ratio EPT/EPTOCH assesses the presence and absence of Ephemeroptera (E), Plecoptera (P) and Trichoptera (T), related to the presence and absence of Odonata (O), Coleoptera (C) and Heteroptera (H). EPT are commonly found in cold and lotic systems whereas OCH are more abundant in warm and lentic habitats (Bonada *et al.*, 2006). Finally, Rao's diversity coefficient is an index of functional diversity based on quadratic entropy of Rao (1982) that incorporates both the relative abundances of species and a measure of a pair-wise functional differences between species (Botta-Dukát, 2005). It is therefore comparable to the Simpson index for taxonomic composition.

These metrics were done in order to analyse some of the effects of impacted streams in temperate and Mediterranean-regions streams, and some differences between both regions. Differences were analysed with non-parametric kruskal-Wallis tests.

Finally, we looked for indicator genus and indicator biological traits for the factors that showed the largest differences in composition. Restricted genus to one or a few habitat types potentially represents a better ecological indicator of environmental change than a habitat generalist, owing to the greater susceptibility of the specialist to local or regional extinction (de Caceres & Legendre, 2009). Indicators were analysed with the Dufrene-Legendre Indicator Species Analysis, that calculates the indicator value and a p-value considering fidelity and relative abundance to clusters or types. Genus and biological traits selected as indicators were those that are significant for a particular cluster but not for any other cluster. Thus, indicators genera and biological traits were analysed in order to find out what kind of genus and biological traits are characteristic of a type of environmental and ecological stream state, so it can possibly be used as an indicator of that kind of state or stream condition. In this case, we tried to find genera and biological traits that were only found in particular conditions produced by different climate regions and land use. Additionally, we also looked for those genus or biological traits that were characteristic of spring or autumn.

All analyses were computed using R (R core development team, 1996) and the libraries vegan (Oksanen *et al.*, 2013), ade4 (Thioulouse *et al.*, 1997) and labdsv (Roberts, 2013).

4. RESULTS

4.1 PHYSICOCHEMICAL PARAMETERS

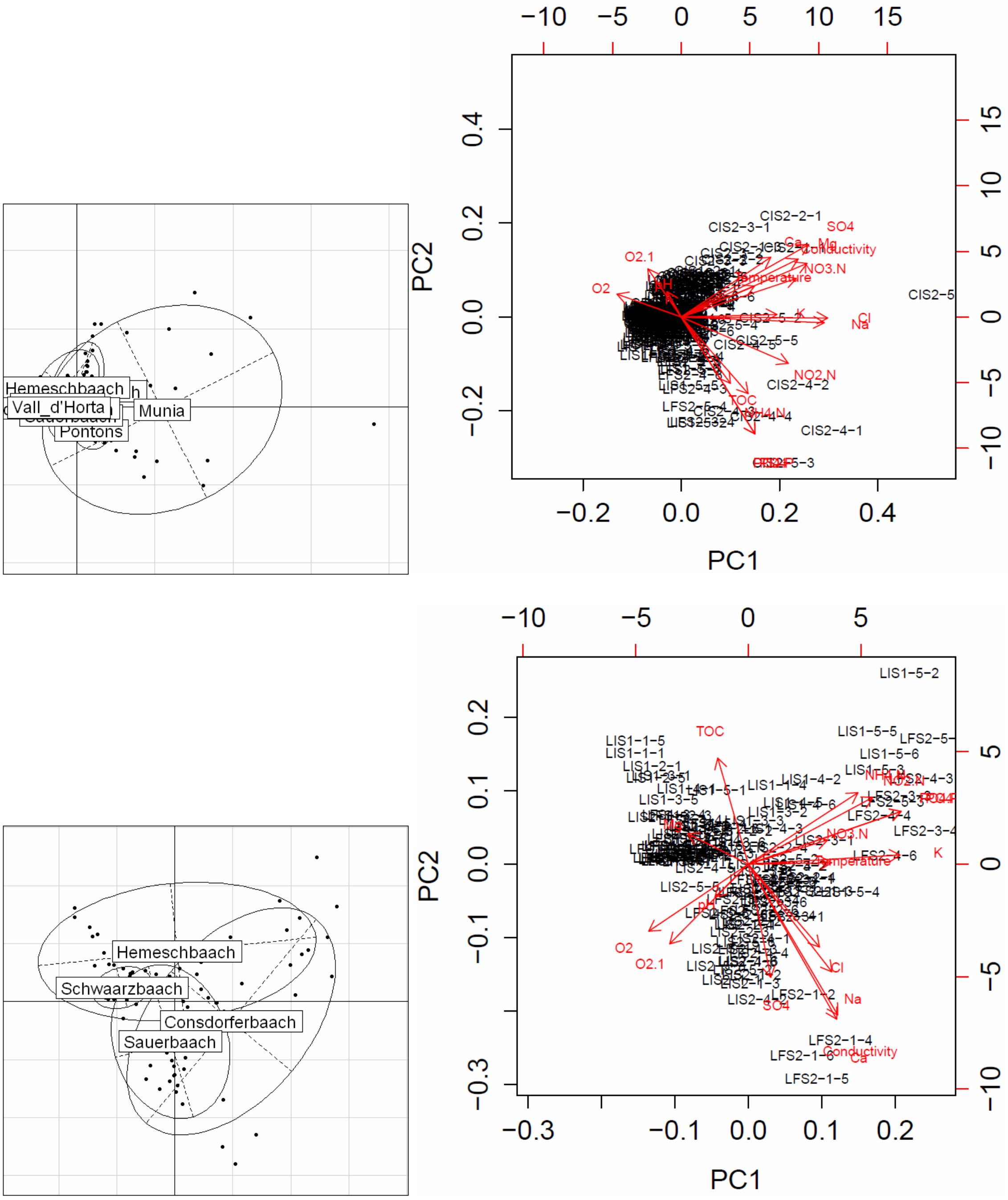


Fig. 3 Principal Component Analysis (PCA) on the physicochemical parameters of all 8 streams (upper plots) and streams in Luxembourg (lower plots), including both plots of factorial maps. For all streams, axes 1 and 2 explained 34.1% and 14.3% of the total variability, respectively, and for Luxembourg, axes 1 and 2 explained 25.2% and 18.5% of the total variability, respectively. Samplings on the left are grouped by streams with each ellipse enveloping 70% of samplings of a particular stream.

Results on physicochemical data assessed by the PCA showed that the Múnia stream was much more impacted than any other stream, included the impacted ones (fig 3 upper plots). Furthermore, PCA on physicochemical data of Luxembourg showed that the Consdorferbaach stream had physicochemical values more similar to other impacted streams, as Sauerbaach and Hemeschbaach (fig 3 lower plots), leaving Schwaarzbaach as the only unimpacted stream from Luxembourg.

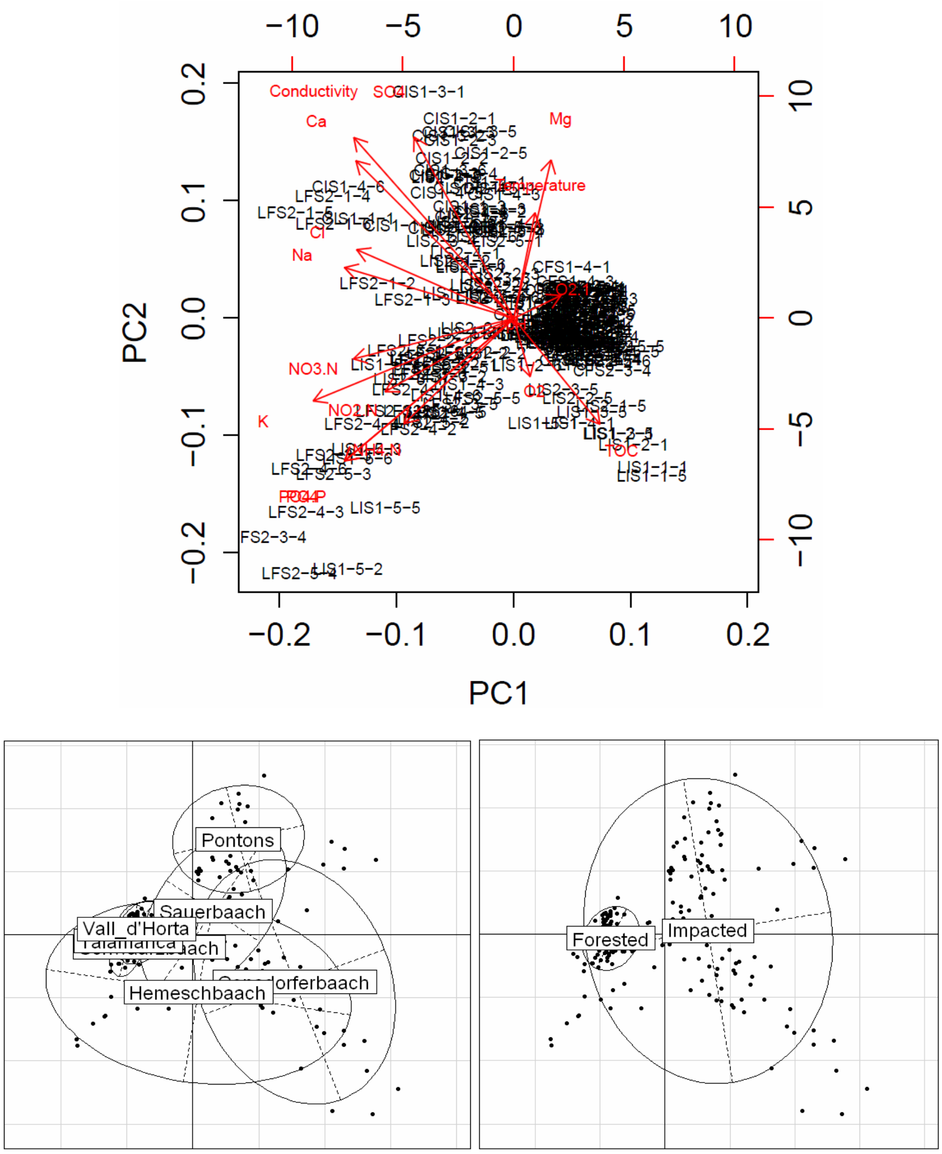


Fig. 4 Principal Component Analysis (PCA) on the physicochemical parameters of 7 streams (without the Munia stream), including plots of factorial maps grouping sampling by streams (lower left) and unimpacted/impacted (lower right). Each ellipse envelops 70% of samplings of a particular category. Axes 1 and 2 explained 25.6% and 19.7% of the total variability.

After taking the Munia stream out, differences in physicochemical parameters between unimpacted and impacted streams were clearly showed in the PCA and factorial maps (fig. 4). Moreover, impacted streams differed in the kind of impact, as can be shown in the biplot. Thus, Pontons in Catalonia and Sauerbaach in Luxembourg had high values of conductivity, SO4, Ca and water temperature, whereas, in Luxembourg, Consdorferbaach had high values of N, P, and K, while Hemeschbaach, that also had quite high values of N, P and K, was the stream with higher O_2 and total organic matter (TOC) values.

4.2 GENERAL MACROINVERTEBRATE PATTERNS

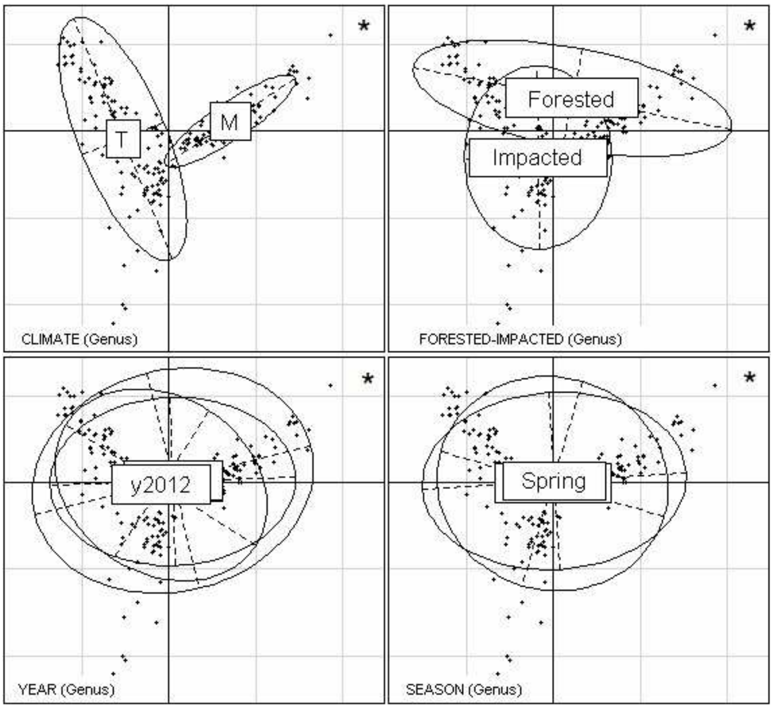


Fig.5 Principal Component Analysis (PCA) on macroinvertebrate taxonomic composition (genus level) of all data. Axes 1 and 2 explained 10% and 6% of the total variability, respectively. Differences between categories were assessed with Monte-Carlo test, a non-parametric randomness test and significant values at $p < 0.05$ are indicated with a (*). Each ellipse envelops 70% of samplings of a particular category. M= Mediterranean climate; T= Temperate climate.

A total of 130 genera were found in all sampling (Catalonia + Luxembourg). More abundant genera were Coleoptera (29 genera) and Trichoptera (28 genera), followed by Ephemeroptera (15 genera), Odonata (13 genera), Mollusca (12 genera), Plecoptera and Heteroptera (10 genera), Hirudinea and Crustacea (4 genera), Turbellaria (3 genera), and finally Megaloptera and Coelenterata (1 genera).

Climate, land use pressures, interannual, and seasonal differences were assessed for taxonomy and biological traits data. The results of the PCA for taxonomic composition showed that climate was the main factor separating communities (inertia: 0.077, p-value: 0.01), followed by land use effects (inertia: 0.038, p-value: 0.01), whereas, interannual (inertia: 0.013, p-value: 0.03) and seasonal (inertia: 0.017, p-value: 0.01) differences were less important albeit significant (fig. 5).

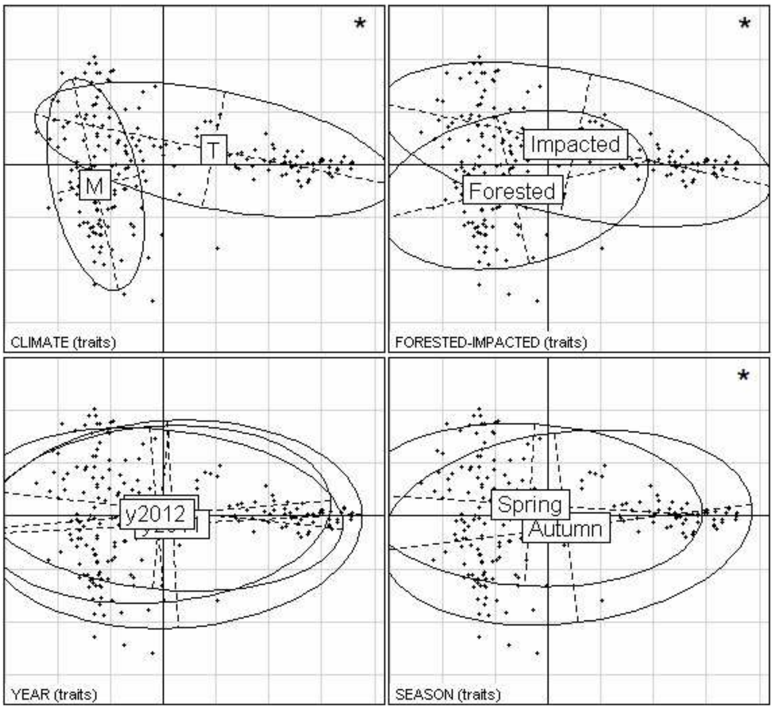


Fig.6 Fuzzy Principal Component Analysis (fPCA) of macroinvertebrate biological trait composition of all data. Axes 1 and 2 explained 61% and 16% of the total variability. Higher differences were assessed with Monte-Carlo test, a non-parametric randomness test and significant values at $p < 0.05$ are indicated with a (*). Each ellipse envelops 70% of samplings of a particular category. M= Mediterranean climate; T= Temperate climate.

Fuzzy Principal Component Analysis (fPCA) on biological traits composition (fig. 6) showed similar results than PCA on taxonomic composition. Climate was the factor that explained higher variability between communities (inertia: 0.20, p-value: 0.01) followed by land use effects (inertia: 0.078, p-value: 0.01) and seasonal variability (inertia: 0.017, p-value: 0.01). Interannual differences were not significant for biological traits (inertia: 0.012, p-value: 0.22).

4.3 CLIMATIC AND LAND USE PATTERNS

Further, PCA and FPCA on the taxonomic and trait data for unimpacted and impacted streams separately, showed that climatic differences observed in unimpacted streams for taxonomic data (inertia: 0.14, p-value: 0.01) were quite maintained in impacted streams (inertia: 0.07, p-value: 0.01) (fig. 7). The same was observed for biological traits, between unimpacted streams (inertia: 0.27, p-value: 0.01) and impacted ones (inertia: 0.23, p-value: 0.01). However, variability is both climate regions, higher in unimpacted streams than impacted ones. Moreover, these differences appeared to be higher for taxonomic composition than for biological traits (more overlapping in mediterranean and temperate categories).

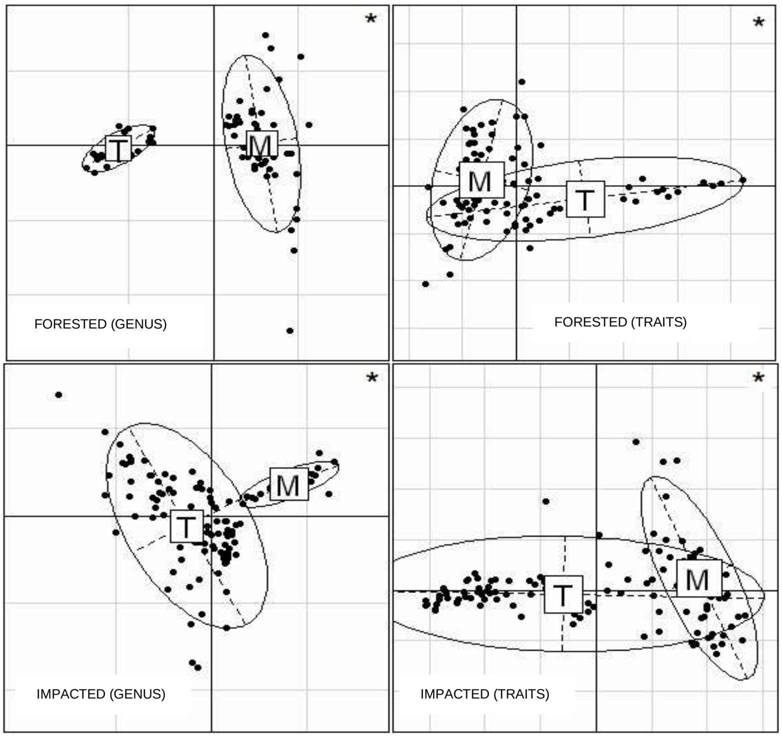


Fig. 7 Principal Component analysis (PCA) for macroinvertebrate taxonomy (left) and Fuzzy Principal component analysis for biological traits (right) comparing climate differences depending on unimpacted (forested) streams (up) and impacted streams (down). Axes 1 and 2 explained 15% and 6% of total variability for forested genus, 9% and 8% for impacted genus, 49% and 17% for forested traits and 72% and 10% for impacted traits. For each plot, (*) indicates significant differences between climates assessed with Monte-Carlo test, a non-parametric randomness test. Each ellipse envelops 70% of samplings of a particular category. M= Mediterranean climate; T= Temperate climate.

The assessment of different community metrics (fig. 8) showed that genus richness was significantly lower in impacted streams than in unimpacted (forested) streams in mediterranean (M mean: FS= 16.8; IS= 9.3; p-value = 10^{-6}) and temperate-climate regions (T mean: FS= 18.4; IS= 11.5; p-value = 10^{-10}). However, genus richness did not vary significantly between regions in unimpacted streams (M mean: 16.8; T mean: 18.4; p-value = 0.085) and in impacted streams (M mean: 9.3; T mean: 11.5; p-value = 0.055). Despite that, p-value was close to be significant in both cases. Genus abundance showed significantly much higher values in temperate climate than mediterranean climate in unimpacted streams (M mean: 85.7; T mean: 938.7; p-value = 10^{-14}) and in impacted ones (M mean: 72.5; T mean: 877.5; p-value = 10^{-13}).

Nevertheless, when we assessed impact of land uses in macroinvertebrate abundance, there were not significant differences between unimpacted and impacted streams in mediterranean (M mean: FS= 85.7; IS= 72.5; p-value = 0.15) and temperate streams (T mean: FS= 938.7; IS= 877.5; p-value = 0.51).

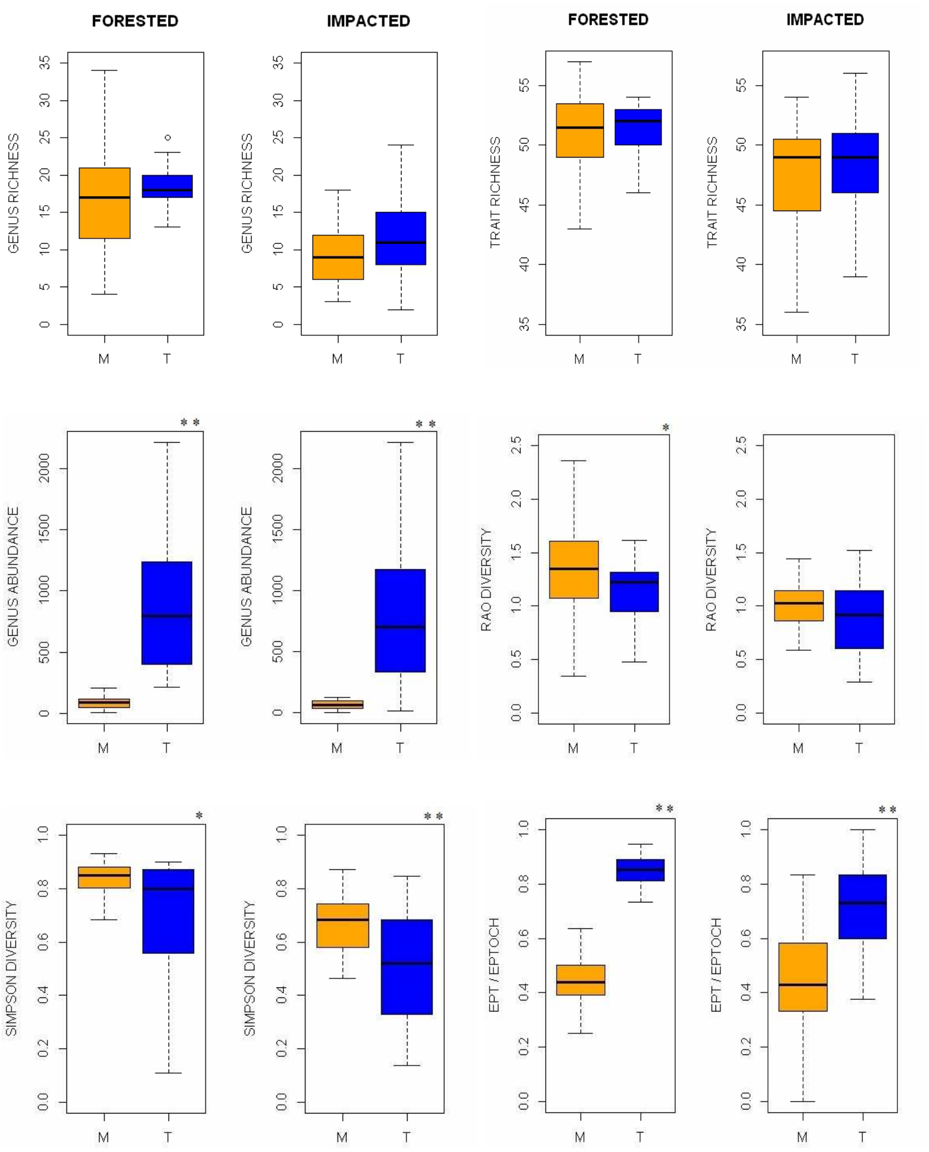


Fig. 8 Box-Plots of different metrics comparing mediterranean and temperate-climate streams for two unimpacted (forested) and impacted streams. Analysed metrics were: genus richness, trait richness, genus abundance, Rao's diversity, Simpson diversity, and the ratio EPT/EPTOCH. Differences were assessed with non-parametric Kruskal-Wallis test. For each plot, (*) indicates significant differences between climates (p-value < 0.05) and (**) indicates significant differences between climates (p-value < 0.002) after Bonferroni correction. In orange: mediterranean-climate region (M); in blue: temperate-climate region (T).

Simpson diversity showed higher values in unimpacted streams that in impacted ones in both, mediterranean (M mean: FS= 0.82; IS= 0.66; p-value = 10^{-8}) and temperate region (T mean: FS= 0.69; IS= 0.51; p-value = 10^{-5}). Furthermore, mediterranean streams had higher Simpson diversity values than temperate ones in unimpacted (M mean: 0.82; T mean: 0.69; p-value = 0.01) and impacted streams (M mean: 0.66; T mean: 0.51; p-value = 0.001). On the other side, the ratio EPT/EPTOCH was higher in temperate streams than in the mediterranean ones in both unimpacted streams (M mean: 0.45; T mean: 0.85; p-value = 10^{-14}) and in impacted ones (M mean: 0.44; T mean: 0.77; p-value = 10^{-7}). Thus, comparing unimpacted and impacted streams, there were not differences in mediterranean streams (M mean: FS= 0.45; IS= 0.43; p-value = 0.87), but there were differences in temperate streams for this EPT/EPTOCH metric (T mean: FS= 0.85; IS= 0.77; p-value = 10^{-5}).

Trait richness had higher values for unimpacted streams than for impacted streams in the mediterranean (M mean: FS= 50.9; IS= 47; p-value = 10^{-4}) and in the temperate region (T mean: FS= 51.3; IS= 48.4; p-value = 10^{-4}). Whereas, there were not significant differences between climate regions in unimpacted streams (M mean: 50.9.; T mean: 51.3; p-value = 0.89) and impacted ones (M mean: 47; T mean: 48.4; p-value = 0.43). Finally, Rao’s diversity showed higher values in unimpacted streams than in impacted stream, in mediterranean (M mean: FS= 1.45; IS= 1; p-value = 10^{-4}) and temperate climate-regions (T mean: FS= 1.12; IS= 0.88; p-value = 10^{-4}). Furthermore, Rao’s diversity was significantly higher in mediterranean unimpacted streams (M mean: 1.45; T mean: 1.12; p-value = 0.01) but not in impacted ones (M mean: 1; T mean: 0.88; p-value = 0.10), although it was close to be significant.

4.4 SEASONAL VARIABILITY

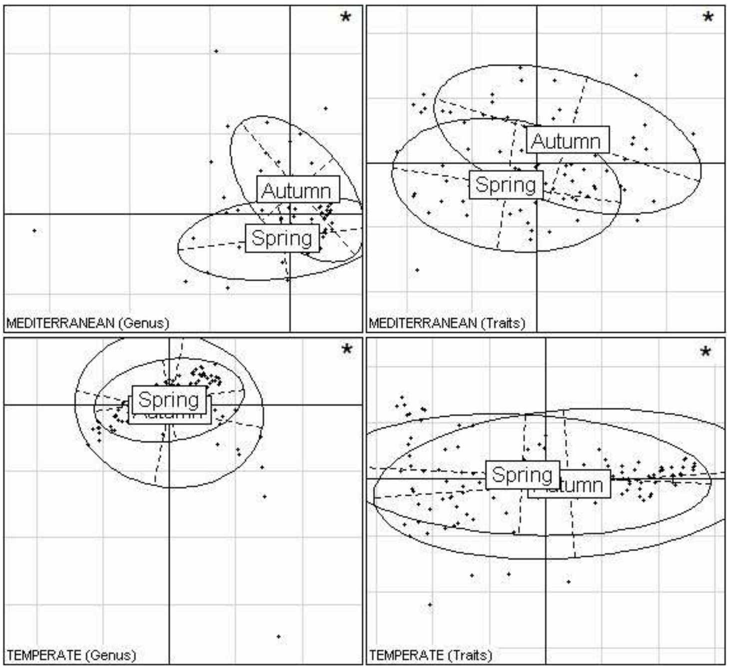


Fig. 9 Principal Component Analysis (PCA) for macroinvertebrate genera and Fuzzy Principal Component Analysis (fPCA) for biological traits showing communities composition differences between seasons (spring (S) and autumn (A)) in temperate and mediterranean-climate regions. In mediterranean-climate region, axes 1 and 2 explained 9% and 6% of total variability for genus and 40% and 50% for biological traits. In temperate-climate region, axes 1 and 2 explained 14% and 8% of total variability for genus and 78% and 9% for biological traits. For each plot, (*) indicates significant differences between climates assessed with non-parametric randomness test.

Seasonal differences on taxonomy and traits appeared to be more important in mediterranean than in temperate streams when pooling together all sites (fig. 9), for taxonomic composition (M inertia: 0.045, p-value: 0.01; T inertia: 0.03, p-value: 0.01), and biological traits (M inertia: 0.135, p-value: 0.01; T inertia: 0.048, p-value: 0.01). Nevertheless, seasonal differences were significant in all stream types. Furthermore, differences seemed as important for genus as for biological traits.

When differentiating between land use patterns within climate regions (fig. 10 and 11), seasonality was also an important factor for taxonomic variability in unimpacted temperate streams but less in impacted ones. In contrast, seasonal variability in impacted streams was as important as in unimpacted streams in the mediterranean region. Thus, seasonality was also important for taxonomic composition in unimpacted streams (inertia: 0.06, p-value: 0.01), as in impacted ones (inertia: 0.07; p-value: 0.01) in mediterranean streams, while, in temperate streams, unimpacted streams had higher seasonality (inertia: 0.06, p-value: 0.01), than impacted ones (inertia: 0.03, p-value, 0.02). Even though, it was significant in all cases.

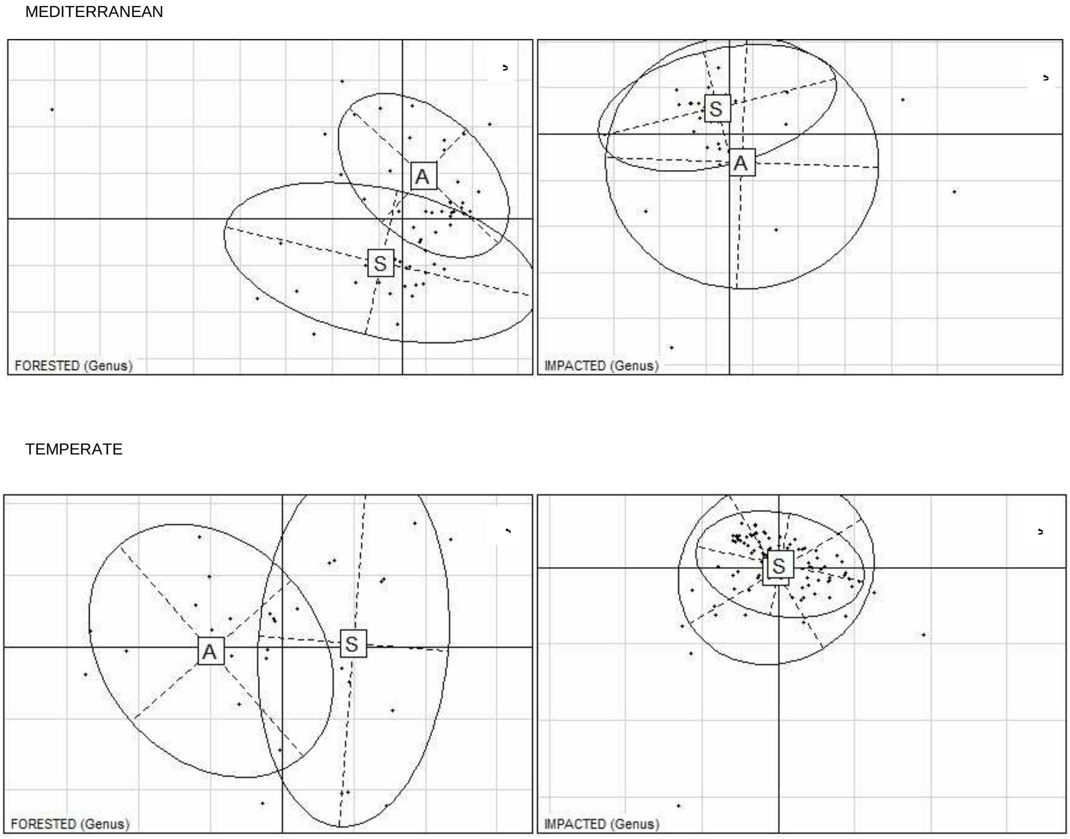
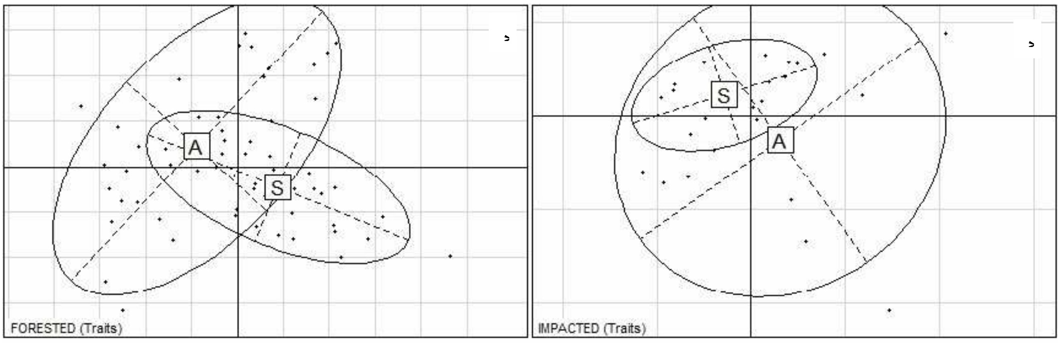


Fig. 10 PCA of macroinvertebrate taxonomy for mediterranean (up) and temperate-climate (down) regions, comparing community composition between climates and differentiating between unimpacted (left) and impacted streams (right). Axes 1 and 2 explained 9% and 8% of total variability for mediterranean-climate unimpacted streams genus, 16% and 13% for mediterranean-climate impacted streams genus, while 15% and 12% of total variability was explained for temperate-climate unimpacted streams genus, and 12% and 9% was explained for temperate-climate impacted streams genus. For each plot, (*) indicates significant differences between climates assessed with Monte-Carlo, non-parametric randomness, test. A = autumn; S = spring.

Looking at biological traits (fig. 11), they were also important in impacted streams (inertia: 0.09, p-value: 0.01) as in unimpacted streams (inertia: 0.11, p-value: 0.01) in mediterranean regions. On the other hand, traits were significant only in unimpacted streams (inertia: 0.07, p-value: 0.04) in temperate streams, but not in impacted ones (inertia: 0.02, p-value: 0.08).

MEDITERRANEAN



TEMPERATE

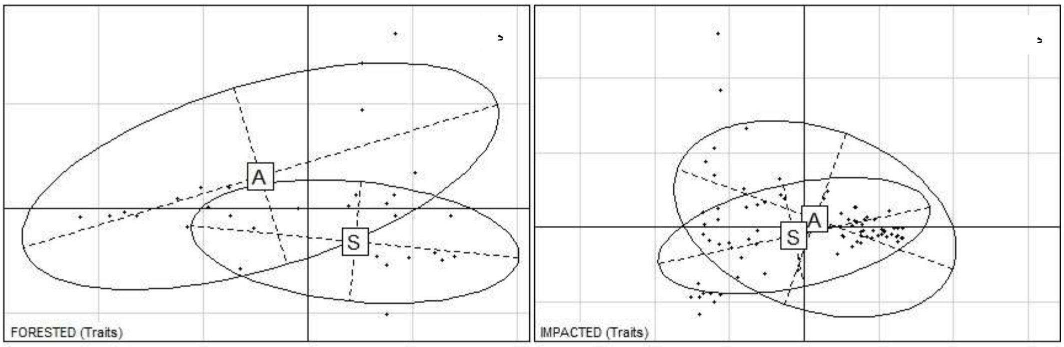


Fig. 11 FPCA of macroinvertebrate biological traits for mediterranean (up) and temperate-climate (down) regions, comparing communities composition between climates and differentiating between unimpacted (left) and impacted streams (right). Axes 1 and 2 explained 21% and 13% of total variability for mediterranean-climate unimpacted streams genus, 22% and 13% for mediterranean-climate impacted streams genus, while 53% and 15% of total variability was explained for temperate-climate forested streams genus, and 49% and 12% was explained for temperate-climate impacted streams genus. For each plot, (*) indicates significant differences between climates assessed with Monte-Carlo, non-parametric randomness, test. A = autumn; S = spring.

4.5 INTERANNUAL VARIABILITY

Interannual variability showed significant differences between years only in mediterranean-climate streams (fig. 12), for taxonomic composition (inertia: 0.038, p-value: 0.01) and biological traits (inertia: 0.048, p-value: 0.01). The year 2012 in the mediterranean region had the most different taxonomic community, while there were not significant differences between 2010 and 2011. Whereas, there were not significant interannual differences between years in temperate climate streams neither for taxonomy (inertia: 0.02, p-value: 0.26) nor for biological traits (inertia: 0.023, p-value: 0.33).

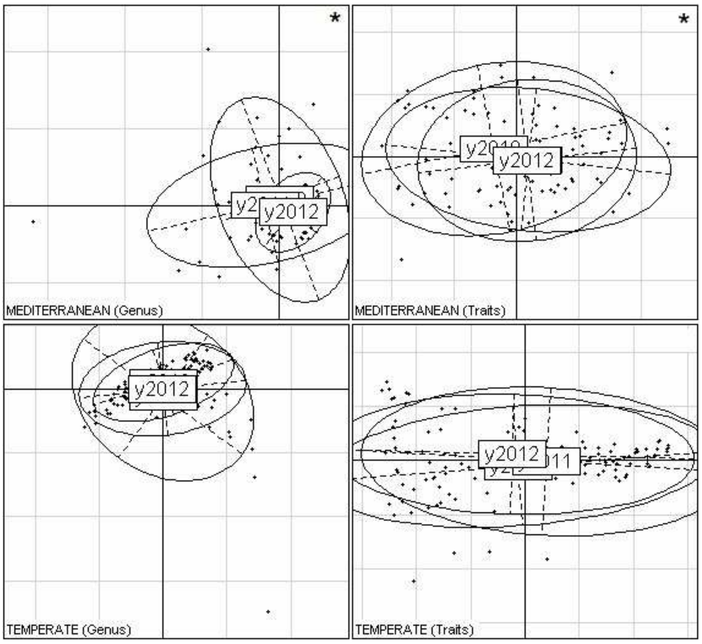


Fig. 12 Principal Component Analysis (PCA) for macroinvertebrates genus and Fuzzy Principal Component Analysis (fPCA) for biological traits showing communities composition differences between years (2010, 2011, 2012) in temperate and mediterranean-climate regions. In mediterranean-climate region, axes 1 and 2 explained 9% and 6% of total variability for genus and 40% and 50% for biological traits. In temperate-climate region, axes 1 and 2 explained 14% and 8% of total variability for genus and 78% and 9% for biological traits. For each plot, (*) indicates significant differences between climates assessed with non-parametric randomness test.

4.6 INDICATOR GENERA AND BIOLOGICAL TRAITS

Results for indicator genera in mediterranean and temperate streams showed that the group OCH (Odonata, Heteroptera, and Coleoptera) was more indicator for mediterranean streams, with genera as the Coleoptera *Stictonectes* in unimpacted streams and the Heteroptera *Nepa* in impacted ones, while the group EPT (Ephemerotera, Plecoptera and Trichoptera) was more indicator for temperate streams, with the Trichoptera *Odontocerum* and *Agapetus*, and the Ephemeroptera *Leuctra* in unimpacted streams, and the Trichoptera *Hydropsyche* in impacted ones (Table 3). Besides that, some Coleoptera was indicator for temperate streams, with the genus *Elmis*, in impacted temperate streams, and some Ephemeroptera and Plecoptera were indicator for mediterranean streams, with the genera *Baetis* in impacted streams and

Nemoura in unimpacted ones. On the other hand, Hirudinea was present as indicator group in impacted streams of both mediterranean and temperate streams.

Table 3 Land use pressures indicator genus and biological traits according to each climate region. Three indicator genus and three biological traits are represented for each land use analysed (forested and impacted). Furthermore each indicator is joined to and indicator value and a p-value.

Climate	Land use	Group	Genus	Indicator value	p-value
M	Unimpacted	**Mollusca**	***Ancylus***	0.65	0,001
		Plecoptera	***Nemoura***	0.67	0,001
		Coleoptera	***Stictonectes***	0.56	0,001
	Impacted	**Ephemeroptera**	***Baetis***	0.5	0,012
		Heteroptera	***Nepa***	0.28	0,002
		Hirudinea	***Dina***	0.38	0,002
T	Unimpacted	**Trichoptera**	***Agapetus***	0.93	0,001
		Trichoptera	***Odontocerum***	0.60	0,001
		Ephemeroptera	***Leuctra***	0.84	0,001
	Impacted	**Coleoptera**	***Elmis***	0.8	0,001
		Hirudinea	***Erpobdella***	0.72	0,001
		Trichoptera	***Hydropsyche***	0.36	0,012

		Trait	Indicator value	p-value
M	Unimpacted	**size: 0.25-0.5 cm**	0.74	0,001
		Resistance form: diapause or dormancy	0.79	0,001
		Surface swimmer	0.66	0,002
	Impacted	**Life cycle duration ≤ 1 year**	0.59	0,001
		Reproduction: clutches fixed	0.63	0,001
		Resistance form: eggs, statoblasts, gemmules	0.61	0,009
T	Unimpacted	**Reproduction: isolated eggs, free**	0.72	0,001
		Food: Fine sediment + microorganisms	0.83	0,001
		Life cycle duration > 1 year	0.73	0,001
	Impacted	**Feeding habits: Piercer**	0.65	0,001
		Reproduction: clutches free	0.77	0,001
		Food: living macroinvertebrates	0.60	0,002

On the other hand, results for indicator biological traits showed that resistance forms as diapause in unimpacted streams and as eggs in impacted ones were significant indicator traits in mediterranean streams. Small sizes and surface swimming organisms were characteristics of unimpacted mediterranean streams, and reproduction by fixed clutches in impacted mediterranean streams. In temperate streams, feeding and reproduction patterns were important indicator traits. Thus, fine-sediment and microorganisms feeding were important in unimpacted streams, while living-macroinvertebrates feeding was important in impacted ones. Moreover, reproduction by isolated and free eggs and long cycle life were important indicator traits in unimpacted streams, and reproduction by clutches free was and indicator of impacted conditions.

Table 4 Seasonal indicator genus and biological traits according to each climate region. Three indicator genus and three biological traits are represented for each season (spring and autumn). Furthermore each indicator is joined to and indicator value and a p-value.

Climate	Season	Group	Genus	Indicator value	p-value
M	Spring	**Heteroptera**	***Microvelia***	0.61	0.001
		Ephemeroptera	***Baetis***	0.74	0.001
		Ephemeroptera	***Habrophlebia***	0.7	0.001
	Autumn	**Crustacea**	***Atyaephyra***	0.37	0.011
		Ephemeroptera	***Cloeon***	0.46	0.016
		Ephemeroptera	***Caenis***	0.34	0.002
T	Spring	**Plecoptera**	***Amphinemura***	0.22	0.001
		Trichoptera	***Chaetopteryx***	0.69	0.001
		Plecoptera	***Protonemura***	0.33	0.001
	Autumn	**Coleoptera**	***Hydraena***	0.32	0.001
		Plecoptera	***Nemoura***	0.37	0.001
		Coleoptera	***Elodes***	0.36	0.017

Climate	Season	Trait	Indicator value	p-value
M	Spring	**Maximal size: ≤ 0.25 cm**	0.61	0.001
		Resistance form: eggs, statoblasts, gemmules	0.73	0.001
		Reproduction: isolated eggs, cemented	0.65	0.001
	Autumn	**Reproduction: ovoviviparity**	0.69	0.001
		Respiration: Plastron	0.54	0.012
		Substrate relation: burrower (epibenthic)	0.55	0.002
T	Spring	**Substrate relation: permanenty attached**	0.51	0.003
		Aquatic stages: nymph	0.64	0.003
		Maximal size: >0.5-1 cm	0.54	0.011
	Autumn	**Feeding habits: parasite, parasitoid**	0.12	0.035
		Respiration: spiracle (aerial)	0.50	0.001
		Locomotion: flier	0.5	0.032

Indicator genera for seasonality show the presence of Ephemeroptera as indicator group of both seasons in mediterranean-climate region, but with different indicator genera in each season. *Baetis* and *Habrophlebia* were indicator genera in spring, and *Cloeon* and *Caenis* in autumn. Furthermore, the Heteroptera *Microvelia* and the Crustacea *Atyaephyra* were indicator genera for spring and autumn, respectively. In temperate-climate region, Plecoptera seemed to be an important indicator group in both seasons, with the genera *Amphinemura* and *Protonemoura* in spring, and the genus *Nemoura* in autumn. Furthermore, Coleoptera were only indicator group in autumn, with the genera *Hydraena* and *Elodes*, while the Trichoptera *Chaetopteryx* was indicator genus in spring conditions.

On the other side, small size, eggs, statoblast and gemmules as resistance forms and reproduction by isolated and cemented eggs were the most significant indicator traits of spring in mediterranean-climate region, while plastron respiration, burrower macroinvertebrates, and ovovivivіparity were significant in autumn. In temperate-climate streams, nymph as aquatic stage, permanently attached organisms and reproduction by free clutches were significant in spring, while aerial respiration by spiracles, flying and parasitism seemed as more important traits in autumn conditions.

5. DISCUSSION

Climate differences between stream types explained the main variability in taxonomy and trait composition, confirming our first hypothesis. Climate is a regional-scale factor that is supposed to affect before other more local factors in the hierarchical process of species sorting (Poof & Ward, 1990). Thus, land use changes, considered a basin-scale factor had a second role. Regarding land use effects, our results also agree with our second hypotheses. Land use impact was great enough to change community composition but differently depending on the climatic context.

Taxonomic richness was significantly higher in unimpacted than impacted streams in both climate regions, confirming also our second hypothesis, that stream invertebrate assemblages have numerous responses to land use changes. The relative and absolute richness, abundance, and biomass of many sensitive stream insect orders decline with increasing catchment and simplifying communities (Cooper *et al.*, 2012)

In agreement with other studies, local richness was similar between mediterranean and temperate streams. Despite regional richness is higher in the mediterranean because of geological events (e.g., glaciations; Ribera and Vogler, 2004) or environmental heterogeneity (e.g., seasonal floods and droughts; Bonada & Resh, 2013) resulting in high rates of endemism (Myers *et al.*, 2000; Smith & Darwall, 2006), local richness is often saturated by local environmental constraints (Bonada *et al*., 2007). Contrarily, Simpson diversity reflects higher values for mediterranean streams than for temperate streams, indicating that abundance play a role. In this sense, the study from Statzner *et al*. (2008), suggests that abundance in mediterranean streams is much lower than in temperate ones, which is confirmed in our study. Temperate streams are usually dominated by very few genera highly abundant (Statzner *et al*., 2008), a pattern that seems very different in mediterranean streams.

The ratio EPT/EPTOCH showed higher values for temperate streams than for mediterranean streams. Permanent sites, with rifle pool sequences along year, common in temperate streams, are characterized by a dominance of Ephemeroptera, Plecoptera and Trichoptera, while intermittent sites, common in mediterranean streams, are dominated by Odonata, Coleoptera and Heteroptera (Boulton & Suter, 1986; Bonada *et al*, 2006, 2007). Assessing biological traits metrics, trait richness, like genus richness, showed no differences between climatic regions, indicating that all trait categories are found in both regions, which agrees with the high heterogeneity of rivers despite the regional effects (Gasith & Resh, 1999). Rao's diversity showed similar results, but with significantly higher values in mediterranean-climate unimpacted streams than temperate ones, which agrees with the higher seasonal variability in mediterranean than in temperate streams allowing a higher diversity of adaptations to cope with a higher seasonal variability of environmental conditions (Bonada *et al*., 2007). However, our results on seasonality showed that seasonal variability was also important in temperate streams but less important than in mediterranean streams, in agreement with our third hypothesis. Seasonal variability in temperate streams, albeit present, is much less marked than in mediterraenan streams.

In mediterranean streams, as well as, in temperate streams, the wet period is dominated by rifle-like taxa, like Ephemeroptera, Plecoptera and Trichoptera. This could explain the presence of Ephemeroptera as indicator group in mediterranean streams, and Plecoptera and Trichoptera in temperate streams in spring. Furthermore, in temperate streams, the presence of nymphs as aquatic stage and permanently attached in the substrate in spring can be related to a higher frequency of floods in temperate streams that have selected organisms with strategies to avoid being drift or with an holometabolous life cycle (with the presence of nymphs) with terrestrial adults to compensate downstream drift (Williams & Williams, 1993). In contrast, different types of resistance forms in mediterranean streams could allow macroinvertebrates in these streams to cope with summer droughts (Bonada *et al*., 2007). However, these resistance forms in mediterranean streams can be complemented with other resilience forms to recover after drought, such us the high frequency of ovovivípar organisms found in our study in autumn and hypothesised by other authors (Bonada *et al.*, 2007).

Confirming our fourth hypothesis, seasonal variability was affected by land use changes and taxonomic and trait variability was lower in impacted streams than in unimpacted ones, in both climatic regions. This could be explained because land use changes like other anthropogenic disturbances simplify communities. Overall, most affected organisms are macrophytes, invertebrates and fishes, causing a change in community structure (Brönmark & Hansson, 2002). Increases in diversion of water for human use exacerbates the impact by the combined effect of reduced dilution capacity (Tuch & Gasith, 1989) altering the presence and distribution of biota (Gasith & Resh, 1990), favouring pollution resistant and unseasonal species (Gasith & Sidis 1984). For instance, mediterranean ecosystems have a remarkable capability of resisting against or to recover from disturbances in comparison with non-mediterranean ones (Fox & Fox, 1986).

Interannual variability was only significant in mediterranean streams for both, taxonomic and biological trait composition, confirming our fifth hypothesis. Although seasonal precipitation pattern is highly predictable in some mediterranean-climate areas, annual rainfall can vary markedly in some regions from year to year; a deviation of 30% or more from a multiannual average is not uncommon (Gasith and Resh, 1999). These patterns of variability promote the existence of different community assemblages in wet and dry years (Bêche & Resh, 2007a).

Finally, effects of climate, land use changes, seasonality, interannuality were higher in taxonomic composition than in biological traits, confirming our sixth hypothesis that traits are less affected by geographical, ecological and by disturbances than taxonomy (Statzner *et al.*, 2004).

6. CONCLUSIONS

Our results suggest that, (1) climate is the main factor acting as a large-scale filter for macroinvertebrates in comparison to temporal variation or other factors known to homogenize communities, such as land use changes, which agrees with the idea that organisms are filtered by a sect of environmental factors acting in a hierarchical way during the species sorting process that drive community assembly. (2) After climate effects, land use changes promote a simplification of macroinvertebrates in stream communities, due to the change of water quality conditions that stimulate the presence of pollutant resistant and unseasonal taxonomy that (3) affect seasonal variability. On the other hand, (4) seasonality and interannual variability are much higher in mediterranean-climate streams, promoting more different macroinvertebrate-communities assemblages, between seasons and years.

(5) Biological traits are less affected by large and small-scale factors than taxonomy due to traits are less dependent on biogeographical aspects and more on environmental conditions.

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