Final Project

Master's degree in Ecology, Environmental Management and Restoration

EFFECTS OF FLOW CESSATION ON MACROINVERTEBRATE COMMUNITIES ALONG A MEDITERRANEAN TEMPORAL STREAM: STRUCTURAL AND FUNCTIONAL ASPECTS

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September 2015

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1 Abstract

This study examined the effect of flow cessation in macroinvertebrate communities along a Mediterranean temporal stream. Spatial heterogeneity was analyzed at three nested scales: macrohabitat (stream), mesohabitat (pool or riffle) and microhabitat (different substrate); whereas temporal variation corresponded with aquatic regimes and sampling season. The results indicated that flow cessation during the dry season increased nutrient and lowered oxygen concentration. Moreover, a reduction and fragmentation of the different habitats was observed. Temporariness of sites and microhabitat were the main factors for macroinvertebrate taxonomic and trait composition. Several organisms were associated to the different aquatic regimes, except to ephemeral site, which presented nested-subsets of other invertebrates found in other sites. Moreover, several organisms were associated to different microhabitats, Biological traits varied according to the temporariness of different sites. Ephemeral site had traits related with resilience strategies (e.g. aerial dispersal); intermittent site had resistant traits to deal with drought (e.g. diapause), and permanent sites had traits related with flow conditions (filter feeder). Finally, flow cessation caused a loss in functional diversity in ephemeral site.

Resum

Aquest estudi va examinar l'efecte de la interrupció de cabal en les comunitats de macroinvertebrats presents en un riu temporal mediterrani. La variació espaial es va considerar en tres escales jeràrquiques: macrohabitat (tram), mesohabitat (basses o ràpids) i microhabitat (diferent substrat); mentre que la variació temporal va ser estudiada en funció dels règims aquàtics i del període mostral. Els resultats obtinguts mostren que: la interrupció de cabal va fer augmentar la concentració de nutrients i va disminuir l'oxigen. Durant el període sec es va observar una reducció i fragmentació dels diferents habitats. La temporalitat dels diferents trams i els microhabitats van ser els principals factors de la variabilitat taxonòmica i d'adaptacions dels macroinvertebrats presents. Varis invertebrats van ser representatius dels diferents règims aquàtics excepte efímer, els quals eren organismes presents en altres estacions. A més, alguns organismes es van trobar associats a diferents microhabitats, Les adaptacions biològiques van variar en funció de la temporalitat. El tram efímer va presentar adaptacions relacionades amb la resiliència (p. ex. dispersió aèria); el tram intermitent es van trobar adaptacions per a resistir la sequera (p. ex. diapausa), mentre que als trams permanents, els organismes presentaven adaptacions relacionades amb el flux continu (filtradors). Finalment, la interrupció de cabal va causar la pèrdua de diversitat funcional en el tram efímer.

2 Introduction

Stream ecosystems are characterized by a great hydrological variability that can be manifested at different spatial and temporal scales. Hydrology, especially flow regime, is one of the most important drivers that determine physical, chemical and biological processes in streams (Allan & Castillo, 2007), shaping geomorphology, substrate stability, habitat suitability, thermal regulation, metabolism, biogeochemical cycles, matter and energy fluxes and aquatic community composition. At the spatial scale, water flow creates a hierarchical structure that shapes the different habitats available for the biota (Frissell et al., 1986). Processes occurring in upper levels of this hierarchical organization -i.e suitable riparian condition- control features at lower levels, but not vice versa –i.e shredding invertebrates abundance at microhabitat scale - (Poff et al., 1997). The difference in the riverbed composition as well as flow regime at the reach scale creates a mosaic of habitats with different depth and water velocity (pool/riffle system) which in turn can contain several different microhabitats, such as sand-silt patches, fine gravel or detritus accumulation. At the temporal scale, variability in flow regime is related with the annual and suprannual variation in flow magnitude, frequency, and rate of change (Poff et al., 1997), which is controlled by the precipitation regime and the evapotranspiration of the basin. Many studies have attempted to disentangle the relative importance of each scale for various aquatic organisms (Lamoroux et al., 2004; Williams, 2006; Lake, 2011), however there is a lack of knowledge in temporal mediterranean streams (García-Roger et al., 2013).

Temporary streams are defined as waterways that cease to flow or complete dry across their channel (Datry et al., 2014), which encompass broad terms such as temporary, ephemeral, periodic, episodic or seasonal (William, 2006; Acuña, 2014). Flow cessation can be caused by different factors such as transmission loss, evapotranspiration, downward shifts in groundwater tables, hillslope runoff recession and freeze-up (Larned et al., 2010). Temporary streams constitute the most common freshwater ecosystem in the Mediterranean and arid regions, representing more than 50% of river network (Datry et al. 2014); and their extension will increase due human activities and climate change (Larned et al., 2010, Lake, 2011).

Temporary rivers can be classified based on flow permanence and predictability of the dry season (Gallart et al., 2012). According to these variables, streams can be (a) permanent, if water flows regularly all the year, (b) intermittent-pool, if during the dry season discontinued pools remain, (c) intermittent-dry, if cessation of flow is usual and streams normally dry out in dry season, and (d) ephemeral, if water flows only occasionally.

The dynamics of a flowing stream to a completely dry channel is well known and has been described by different authors (Boulton, 2003; Gallart et al., 2012). In flowing conditions surface water favours a high variability of pool-riffle habitat as well as different patches of microhabitats (*eurheic* state). Floods (above bankfull flow) can also occur in temporal streams (*hyperheic* state), acting as a pulse disturbance (Lake et al., 2003) and indiscriminately cleaning of most species present. The reduction of water leads a constriction and habitat fragmentation, breaking surface water contact between the stream and its riparian zone, as well as reducing the heterogeneity of flow (*oligorheic* state). During this phase, some riffles are lost and pools can be formed. If the flow cessation intensifies, the surface discharge is null, and depending on the severity of the drying as well as basin geology, connectivity is lost, and only pools can remain (*arheic* state). The complete loss of water concentrates the remaining biota, deteriorates their water quality, and stimulates algal blooms, predation and competition (Gasith & Resh; Lake et al., 2003; Boulton, 2003; Acuña, 2014). Finally, the surface water can be lost and the remaining pools disappear (*hyporheic* state). However, despite the absence of surface water, hyporheic zone can serve as a refuge from biota during the drought period (Robson et al., 2011; Datry et al., 2014a).

Mediterranean climate streams have a predictable flow regime, with high flow in winter and very low flow in summer and autumn (Gasith & Resh, 1999; Bonada et al., 2006; Bonada et al. 2007). The more complex and species-rich macroinvertebrate assemblages occur in spring to early summer and the more species-poor assemblages occur in mid to late summer, when a succession of isolated pools is formed (Gasith & Resh, 1999; Bonada et al., 2006; Munné & Prat, 2009, 2011). Drought has been described as a ramp disturbance (Lake et al., 2003) that can greatly affect the assemblage of macroinvertebrate community. The creation of isolated pools in these streams has a major impact shifting lotic conditions into lentic conditions. Therefore, rheophilic species such as Ephemeroptera, Plecoptera, Trichoptera and Simuliidae would be absent during the drought phase, whereas lentic species such as Heteroptera and Odonata can increase their abundance (Bonada et al., 2007; Munné & Prat, 2011; Bogan et al., 2013). Also, species that complete their life cycle in the water, such as *Gammarus* sp. and snails can also be found in the remaining pools. Following flow resumption, early colonizers such as mayflies or Chironomidae and Simuliidae can be found again in a short period of time (Acuña et al., 2005; Williams, 2006).

In the context of the River Habitat Template (Townsend et al., 1997), the assembly of local communities is the result of a process where multiple habitat filters act hierarchically, selecting those organisms that possess a set of biological traits, that allow them to survive, grow and reproduce under increasingly constraining factors (Poff et al., 1997, Statzner and Bêche, 2010). As a consequence, macroinvertebrates living in temporary streams have acquired different adaptations to deal with extreme hydrological disturbance (floods and droughts), present in these ecosystems, as different authors have shown (Acuña et al., 2005; Williams, 2006; Bêche et al., 2006; Bonada et al., 2007; Arscott et al., 2010; Robson et al., 2011; García-Roger et al., 2013; Bogan et al., 2013; Cid et al., in press). These traits can explain how organisms respond to the environmental restrictions imposed by flow cessation and therefore formulate a priori predictions (Statzner & Bêche, 2010). The spectrum of responses dealing with flow cessation encompasses two main strategies: resistance and resilience. Resistance refers to the capacity of the biota to withstand the stress, whereas resilience refers to the capacity to recover from the disturbance (Lake, 2011). Some authors have highlighted that macroinvertebrates communities have low resistance to flow cessation (Acuña et al., 2005; Arscott et al., 2010; Robson et al., 2011, Bogan et al., 2013), although some species may use refuges (Sheldon et al., 2010) or have drought resistant forms (eggs, cysts or diapause in adults) (Williams, 2006; Lake, 2011). Refuge-use strategies vary depending on the different taxa (Robson et al., 2011) as well as availability limitations imposed by the flow cessation (García-Roger et al., 2013). Nevertheless, common refuges used by macroinvertebrates include surviving in the remaining pools, under moist habitats (under leaf litter and below the stones), burrowing to the hyporheic zone, having desiccation resistant forms and migrating to other permanent water bodies (Robson et al., 2011; Lake, 2011). On the other hand, resilience is strong, as recovery is fast and occurs through flying adults present in persistent pools or permanent streams, with Chironomidae and Simuliidae being an early dominant group (Acuña et al., 2005; Williams, 2006; Arscott et al., 2010; Lake, 2011).

Flow cessation can eliminate some species, and, as a consequence, some unique traits will be lost. Therefore, the resilience of the ecosystems as well as other ecological processes could be affected (Schriever et al., 2015), depending on the complementarity and redundancy of species present. Nevertheless, it is assumed that the loss of species could affect functional diversity (Lake, 2011) as redundancy in macroinvertebrate communities is usually common (Bogan et al., 2013) and the effect of singular traits loss and species would be minimized.

Temporary rivers have been neglected by current stream paradigms (Larned et al., 2010; Datry et al., 2014a) and by water legislation –i.e. European Water Framework Directive and US Federal Water Pollution Control Act (Prat et al., 2014)-, neglecting the fact that cessation of flow represents a critical stage for the river ecosystem processes, habitat availability and as a consequence, diversity of aquatic biota (Boulton, 2003). Therefore, there is an urgent need for the development of monitoring tools (Prat et al., 2014, Cid et al., in press) and the evaluation of ecological status in temporary rivers (Munné & Prat 2009, 2011).

In this study, the effect of flow cessation was analysed for environmental variables, habitat composition, macroinvertebrate composition and biological traits, as well as functional diversity considering different spatial and temporal heterogeneity along a Mediterranean stream. For operational purpose, we performed a spatial nested analysis where (i) macrohabitat corresponds to streams, (ii) mesohabitats corresponds to pool or riffle within a reach and (iii) microhabitat corresponds to different substratum types within a mesohabitats, as described by Frissell et al., (1986). Temporal heterogeneity was taken account according with aquatic regimes of each site as well as two sampling season corresponding to wet (late March) and dry (mid June) periods.

The main hypotheses tested were: (a) flow cessation in an non-impacted intermittent site will have similar effects in water quality than in human-impacted permanent site, (b) microhabitat and flow cessation are the major responsible of variance in macroinvertebrates assemblages and trait composition, (c) there are specialist taxons associated to a particular aquatic regime and microhabitat (d) there is a significant association between biological traits and flow conditions, and (e) there is some degree of loss in functional diversity caused by flow cessation. As shown in Table 1, in regard to biological traits, it is expected that (d.1) resilience traits will predominate in ephemeral condition to avoid the complete absence of water, such as aerial dispersal, short life cycle or burrowing in hyporheic zone; (d.2) resistance based strategies will be associated to in intermittent conditions, such as a drought resistant forms or adaptation to low oxygen such a spiracle, and, finally, (d.3) traits related with flowing conditions such as aquatic dispersal as well as attachment to the substrate will predominate in permanent conditions.

Table 1 Biological traits expected to be dominant or more abundant in ephemeral, intermittent and permanent sites (either impacted or not). These hypotheses are based on results of Bêche et al., 2006, Bonada et al., 2007, Statzner and Bêche, 2010 and García-Roger et al., 2013.

Trait	Category	Predicted	Reason
Maximal size	0.25 - 0.5 cm	Intermittent	Overcrowding in isolated pools lead an increase of stress condition, limiting their grow
	0.5 - 1 cm	Intermittent	(Same as above)
	1 - 2 cm	Permanent	More stable flow condition favourish bigger grow of macroinvertebrates.
	2 - 4 cm	Permanent	(Same as above)
Life cycle	< 1 year	Ephemeral	Rapid flow cessation favorish fast generation cycles
	> 1 year	Intermittent	Resilent organisms in pools can complete the reproductive cycle in pools as adults
Aquatic stages	adult (imago)	Intermittent	Organisms with low dispersal (f.ex. Snails) can be confined in the remaining pools.
Dispersal	aquatic passive	Permanent	The presence of water conectivity facilitates dispersion
	aerial active	Ephemeral	Drying favourish flying to other permanent waters
Resistant form	eggs / cocoons / diapause	Ephemeral / Intermittent	Unestable water conditions may need some adaptations to dessication.
	none	Permanent	Stable water flow conditions may eliminate the need of resistant forms.
Respiration	spiracle / plastron	Intermittent	Confinament in pools deplers oxigen, requiring an additional way to obtain oxigen.
	gills	Permanent	Continuos flowing water may facilitate the intake of oxigen.
Locomotion	burrower	Ephemeral	Aquatic biota may use the hyporheic zone as a refugia during complete drying of riverbed
	flier	Intermittent	Migration to other permanent water may be favourished during oligorheic state
	temporarily attached	Permanent	Resistant adaptation to flood may be necessary for dealing with floods
Feeding habits	deposit filterers	Permanent	Deposit filterers needs continous flow to feed themselfs.

3 Methods

3.1 Study sites

This study was conducted in a temporary river of the Thau Lagoon basin located in the region of the Languedoc-Roussillon (SE France). The catchment area (290 km^2) consists of Jurassic karstified limestone and Miocene marls (Fouliand et al., 2012). The basin is drained by ten small intermittent rivers that have a long dry period between May and September and have flash floods during the wet season in spring (Perrin & Tournoud, 2009). The main water course in the catchment is the Vène River that drains an area of 67 km^2 and is the only fed by karstic springs (Figure A1).

As described by David et al., 2011, the catchment area of this river can be differentiated into two zones (Figure A1): (1) the central part of the basin is a flat marl plain dedicated mainly to the vineyards (21% of the area) and other agricultural activities (market gardening, orchard and cereals). The population living in this central area is sparse and is distributed in three villages (total of 12 400 habitants that represents a 3% of the total area). (2) At both sides there are limestone massifs highly karstificated covered by natural garrigue and pines used for poultry and sheep farming. Wineries and poultry have their own sewage treatment or are connected to the sewage treatment work. The main inputs of pollutants come from two cooperative wineries and three sewage treatment plants (STPs) using wastewater stabilization ponds (WSP) that discharge directly into the river.

The Vène River drains from 323 to 2 msl and has a regular slope of 0.4%. During its 12 km course, is fed by two intermittent karstic springs: Cournonsec spring upstream and Issanka spring in the lower basin. The cross-sections are about 5m wide and present dense riparian vegetation, with abrupt banks (35%), straight-walled banks (15%) or a mixed pattern (Tournoud et al., 2005). Its riverbed is composed by stones and gravels with a small proportion of fine sediments. Vegetation consists in bryophytes on rock's surface and terrestrial macrophytes near the karstic springs and a developed biofilms in rocks near the discharge of SWP (David et al., 2012).

The annual precipitation measured at the Montbazin rain gauge station (1994-2004) varied from 520 mm to 890 mm, with an average of 659 mm ± 94 mm. The potential evapotranspiration for the same period is far superior, ranging from 1268 to 1386, with a mean of 1338 ± 24 mm (Perrin & Tournoud, 2009). The low flow period (< 60L/s) can last from 60 to 200 days and even up to 315 days in the driest years. In this case, the main river is dry except for a few hundred meters downstream where only pools remain (David et al., 2012).

3.2 Aquatic macroinvertebrate sampling

Aquatic macroinvertebrates were collected from the Vène stream during two hydrological periods. The sampling dates were established after examining hydrological conditions where two differentiated periods were most likely to occur. Four sites along Vène stream were chosen to collect benthic macroinvertebrates community (Figure A1). The first sampling station was situated downstream Cournonsec spring, named ephemeral). This spring fed this site during the wet period but during summer this site was totally dry (Figure 1a). For this reason, summer macroinvertebrate samples were collected from the nearest downstream stream reach with pools and, therefore was considered a different sampling sites (intermittent) (Figure 1b). The third site was located downstream of a STP that treats poultry and domestic sewage waters therefore modifying their natural aquatic regime from temporal to permanent, and therefore referred as Impacted (Figure 1c). Finally, the last sample station was situated at the upwelling of Issanka spring, a permanent karstic spring with no significant reduction of discharge during the study period (Figure 1d). Therefore, this site was named Permanent and was considered to have no significant impacts. Samples were collected from representative stream reaches (20-35m length) from each site.

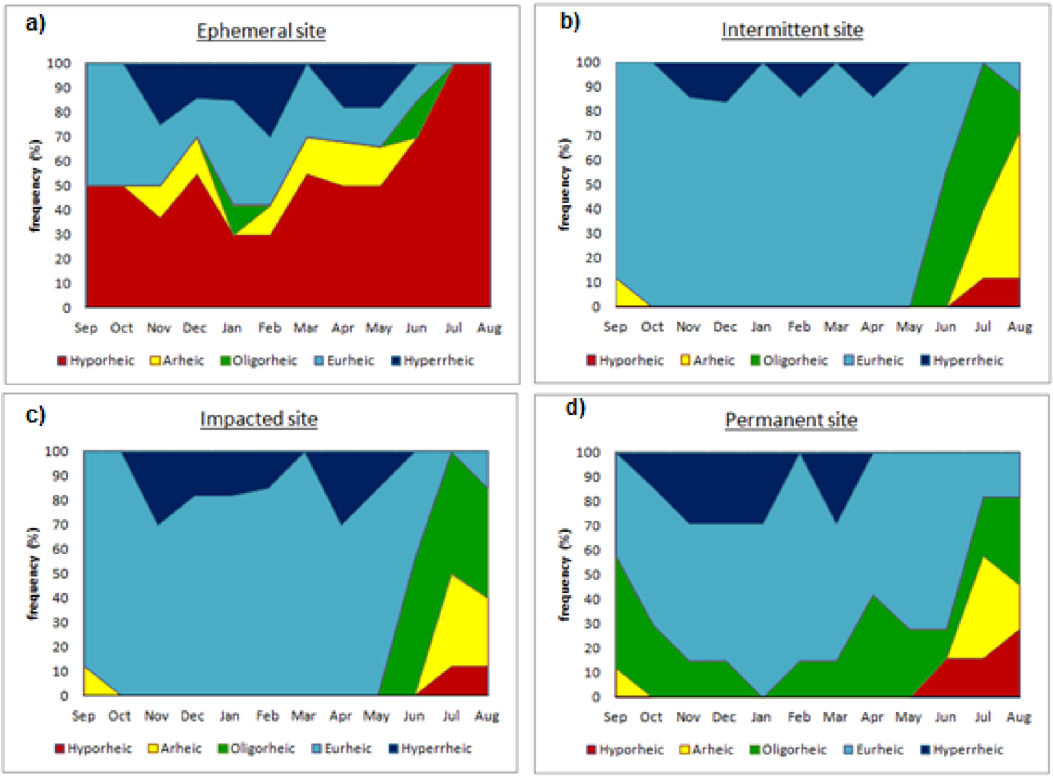

Figure 1 Aquatic states frequency graphs of the sites of this study (a-d). Adapted from Gallart et al., 2012.

A total of 20 surber samples (15 x 15 cm, 250 µm mesh) were taken from every stream reach (20 to 35m length) and sampling period. Samples were distributed within the reach based on the relative percentage of mesohabitats (pool and riffle) and the relative proportion of microhabitats (different mineral and organic substrate). The different microhabitats found were similar to those described by the AQEM project protocols (Hering et al., 2004), as shown in Table A1.

Macroinvertebrates samples were preserved in the field with formaldehyde (4%) and taken to the laboratory for identification. Macroinvertebrates were examined using a stereoscope. All individuals were sorted and identified to the lowest taxonomic level possible, most of them at the genus level or even species level. However, early stage larvae were more difficult to identify to this taxonomic resolution, and therefore they were referred to family level (some dipteral larvae and Oligochaeta). Microcrustacea (Ostracoda, Copepoda, Cladocera), Nematoda and Hydracarina were kept at class, phylum or suborder level, respectively. Identification was possible using taxonomical guides such as Tachet et al., 2010.

3.3 *Environmental variables*

Water samples were collected in order to characterize the concentration of chemical pollutants at every site and sampling period (wet and dry). Water temperature (ºC), conductivity (µS cm^{-1}), pH and dissolved oxygen (% and mg L^{-1}) were measured *in situ* at each site using portable multi-variable probes. Water samples of 250 mL were collected from each site and transported in a cooler for lab processing. In the laboratory, were filtered through 0.45 µm filters and frozen. Concentration of nitrates (mg NO_3^- L^{-1}), nitrites (mg NO_2^{2-} L^{-1}) and soluble phosphorous (mg PO_4^{3-} L^{-1}) were measured by spectrometry according to the Standard Methods (APHA, 1998). Daily average discharge (m^3 s^{-1}) was recorded every day during 9 months from Montbazin gauge station in the Vène catchment. Depth of the channel (cm) and flowing velocity (m s^{-1}) were measured for each site and sampling period.

Habitat quality at each site was calculated by using the Fluvial Habitat Index (IHF; Pardo et al., 2002) and the Riparian Corridor Quality Index (QBR; Munné et al., 2003). The IHF evaluates the diversity of mesohabitats and microhabitats available that could sustain a rich and diverse fauna. This index analyzes seven different characteristics: embeddedness, riffle frequency, substrate composition and particulate size, velocity regimes, degree of shade on the riverbed, presence of heterogeneity elements and the degree of aquatic vegetation cover. The QBR index assesses the conservation status and the integrity of the riparian corridor. This index quantifies the riparian corridor quality analysing the river channel naturalness, the degree of vegetation cover, structure and quality.

3.4 *Data analysis*

3.4.1 *Environmental variables and habitat composition*

A Principal Component Analysis (PCA) was performed to assess the environmental heterogeneity associated with each sampling site at each season. Prior to analysis, environmental variables were standardized due the different scales of the variables. At lower spatial scale, Fisher's exact test of independence was performed to statistically evaluate inter-seasonal shifts in mesohabitats and microhabitat composition. Fisher's exact test is a more robust option than similar tests (Barnard test, chi-square independence) when small numbers are expected (McDonald, 2014).

3.4.2 Biological data

Macroinvertebrate abundance data were reported as density values (individuals m^{-2}). Macroinvertebrate abundance data were log-transformed to downweight the contributions of the most abundant taxa, and then was converted in a Bray-Curtis dissimilarity matrix. In order to determine similarities among samples in macroinvertebrate data, non-metric multidimensional scaling (nMDS; Kruskal, 1964) was performed on Bray-Curtis dissimilarity matrix. The minimum stress level of the ordination (a measure of goodness of fit) and r^2 values (a measure of total variance explained) was determinate after 100 random starting configurations and then running 999 iterations to the final solution. Individual samples were labelled in each ordination solution according to different spatial scales and season to visualize differences. Permutational multivariate analysis of variance using Bray-Curtis distance matrix was performed to test differences assemblages in a nested design: (a) microhabitats (substrate type) within (b) mesohabitats (pools or riffles) within sampling sites, all crossed against season and including all their interactions. Finally, a total of 9999 permutations were made to calculate the pseudo p-value.

The Indicator Value method (IndVal) (Dufrêne & Legendre, 1997) was used to determine the representative macroinvertebrate taxa based on abundance data matrix for each factor. The IndVal is based on the relative frequency of taxa in the samples of one group and the mean abundance of taxa in the samples of that group compared with all groups. Following the criteria defined by Dufrêne and Legendre (1997), a threshold level of 25 was considered for the index to be accepted as relevant. This threshold means that a given taxa is present in >50% of the sample from one group and with a relative abundance in that group of at least 50%.

3.4.3 Biological trait analysis

The traits characteristics of macroinvertabrate taxa were obtained from Tachet et al., 2010 which includes 11 categories with a total of 61 traits. These traits are related to different biological features which include life-cycle features, reproduction, resistance and resilience potential and feeding behaviour. Each taxon (normally at genus level) is coded by fuzzy code according to its affinity to each taxon and category (Chevenet et al., 1994), where greater values mean a greater affinity for a specific trait. This method synthesizes various sources of numerical data obtained from literature and from field work.

The fuzzy coding matrix (64x61) was transformed to a percentage of each trait within each category. Afterwards, the log-transformed abundance data (120x64) was multiplied with the traits matrix (64x61), obtaining a new matrix (120 x 61), which contains the pondered abundance of each trait in every sample (Bonada et al., 2007; Dray & Dufour, 2007). Fuzzy Correspondence Analysis was performed to assess overall differences in trait assemblage for every sampling site. The overall difference was tested by MonteCarlo permutation test against simulated values obtained after 999 permutations. Also, Indicator Value analysis was performed in order to reveal which traits were representative of each site.

3.4.4 Functional diversity indices

Functional diversity measures the distribution and the range of the function of the organisms present that accomplishes in communities and ecosystems, and thus considers the complementarity and redundancy of co-occuring species. Several indices have been proposed in the last decades as a descriptors for functional diversity (Villéger et al., 2008; Schleuter et al., 2010; Mouchet et al., 2010), however not a great consensus has been achieved for the best metric for functional diversity. Instead of quantify the functional diversity in a single index, Mason et al., 2005 decomposed functional diversity into three independent components – i.e. functional richness, functional evenness and functional divergence-. These components could be computed separately and are orthogonal (independent) between them (Villéger et al., 2008). Functional richness (FRic) represents the niche space filled by the species present and is calculated using the minimal convex hull that includes all species and quantifies the volume occupied by the community traits. Functional evenness (FEve) describes the distribution of traits within a community -i.e. whether they are distributed evenly within occupied trait space-. Functional divergence (FDiv) describes the degree of niche differentiation and thus resource competition. These indices were standardized by species trait ranges from all communities together to restrict index values between 0 and 1. This standardization is useful when comparing different communities harboring similar trait spans albeit at different mean trait values (Schleuter et al., 2010).
Finally, Rao's diversity coefficient is an index of functional diversity based on quadratic entropy of Rao that incorporates the relative abundances of species, as well as a measure of pair-wise differences between species (Botta-Dukát, 2005). Therefore, this index can provide an overall value of functional diversity and is closely related to functional divergence index.

All statistical analysis was performed with packages from R 3.2.1 statistical software (R Core Team, 2013). The packages used for these analysis were *ggplot2* (Wickham, 2009) graphical representation, *vegan* (Oksanen et al., 2015) for PCA, nMDS, permanova, and taxonomic indices, *indicspecies* (De Cáceres & Legendre, 2009) for Indicator Value analysis, *ade4* (Dray & Dufour, 2007) for the computation of taxonomic distances and the fuzzy correspondence analysis and *FD* package (Laliberté et al., 2014) for functional diversity indices.

4 Results

4.1 Spatial and environmental heterogeneity

PCA summarized environmental differences between sampling sites and season (Figure 2). The first axis explained 56.98% of total variance and was positively related with temperature, nutrients and conductivity and negatively correlated with oxygen, water depth and water velocity. This first axis clearly separated sites from wet period, which had more water flow and oxygen, whereas in the dry period, due a reduction of water flow, an increase in nutrient concentration and conductivity can be observed. The second axis explained 26.92% of total variance and is related with pH and negatively related with QBR and IHF. The second axis of the PCA highlighted that the impacted site is affected not only by nutrient enrichment, but also highlights problems associated with the riparian corridor. Low values of QBR in Impacted site as well as nutrient enrichment from WTP, led a problem of eutrofication; which is more severe in summer due the reduction of water availability. pH is also higher in this site, as a result of eutrofication present in this site.

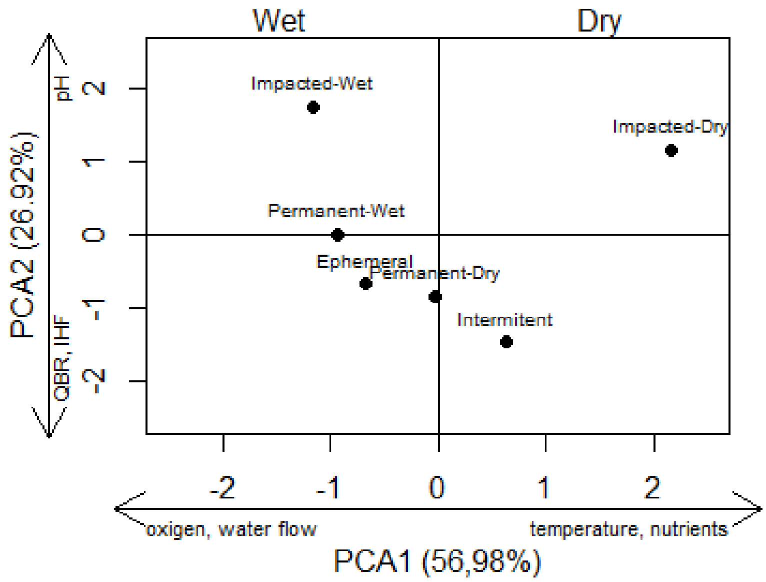

Figure 2 Distribution of each sampling site along the space defined by the first two axes of Principal Component Analysis. Correlation coefficients of the first axis: temperature (0.76), nitrate (0.72), nitrite (0.70) ammonium (0.68) and phosphate (0.72) and conductivity (0.48), oxygen concentration (-0.77), average depth (-0.75) and average velocity (-0.59). Correlation coefficients of variables on second axis: pH (0.578), QBR (-0.71) and IHF (-0.73).

Seasonal differences were also observed at mesohabitat and microhabitat scale (Figure 3). During the wet season, the relative frequency of pools and riffles was the same in all sampling sites. However, during the dry session a reduction of riffles was observed in all sampling sites (p-value = 0.0021), although there was no significant reduction for permanent and impacted site (p-value = 0.5231 and 0.3332, respectively). At microhabitat scale, the total relative frequency of organic substrates (greenish colours) was very similar to mineral substrates (brownish colours) during the wet season (Figure 3). In the dry season a major dominance of organic substates was observed (p-value = 0.0145) , which is likely to be a consequence of the shift from erosional to depositional conditions. Significant differences between seasons was found in impactacted site, were a clear shift to organic substrates was found (p-value = 3.15×10^{-3}), due an increase in algal (AL) , woody debris (XY) which is likely to be related with an increase of nutrients in this site.

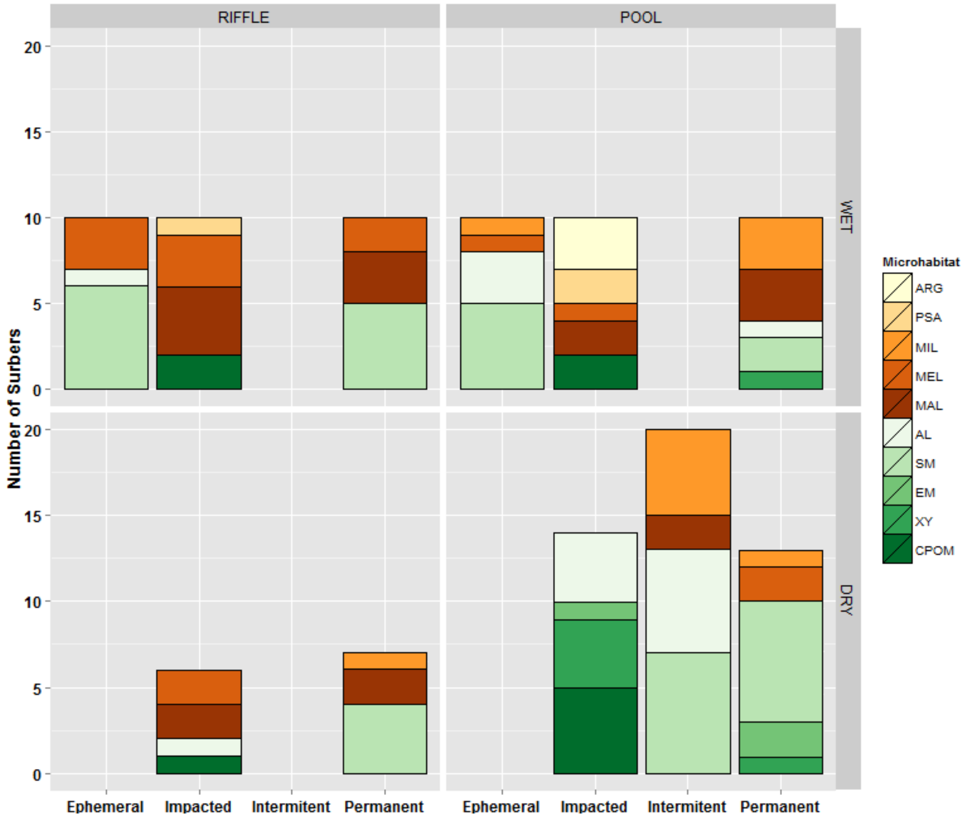

Figure 3 Mesohabitat and microhabitats relative frequency in wet and dry season in the Vène stream. Brownish colours correspond to mineral substrates. Substrates colored in green correspond to organic substrates. Abbreviations used for microhabitats are the same as described in Table A1.

4.2 Aquatic macroinvertebrate taxonomic composition

More than 44.000 specimens were identificated to 73 different taxons, most of them at genus or even at specie level. Densities ranged from 0 to 80 177 individuals m^{-2} with Orthocladidae, Tanitarsiini and Naididae being the most abundant and ubiquitious taxon in all sites and seasons.

nMDS solution based was computed to reveal patterns in the community structure among different spatial scales (Figure 4). Stress value of the two-dimension ordination was 0.2035, which indicates an acceptable representation (Legendre & Legendre, 2012) and the explained variances were also high (r^2 = 0.959). The wet-season ordination (Figure 5a) revelated differences of macroinvertebrates assemblages among the first nMDS axis, which highlights that seasonality is an important driver for macroinvertebrate structure. According to sampling site ordination (Figure 4b) there is a clear differenciation also according to the different temporariness condition of the different sites. The second axis on the representation moderately sepates sampling sites position. Permanent site is tighly situated in the bottom right of the figure, indicating that macroinvertabrate structure is very similar in this site during the different seasons. In contrast, ephemeral site is situated in an extrem of the representation, which indicates that macroinvertebrates assemblages is very different from the other site. Finally, impacted and temporal sites are closely situated in the nMDS representation. At lower scale (mesohabitat and microhabitat), there is not a clear pattern that clearly permits differenciate more between macroinvertebrates assemblages (Figure 4c and 4d).

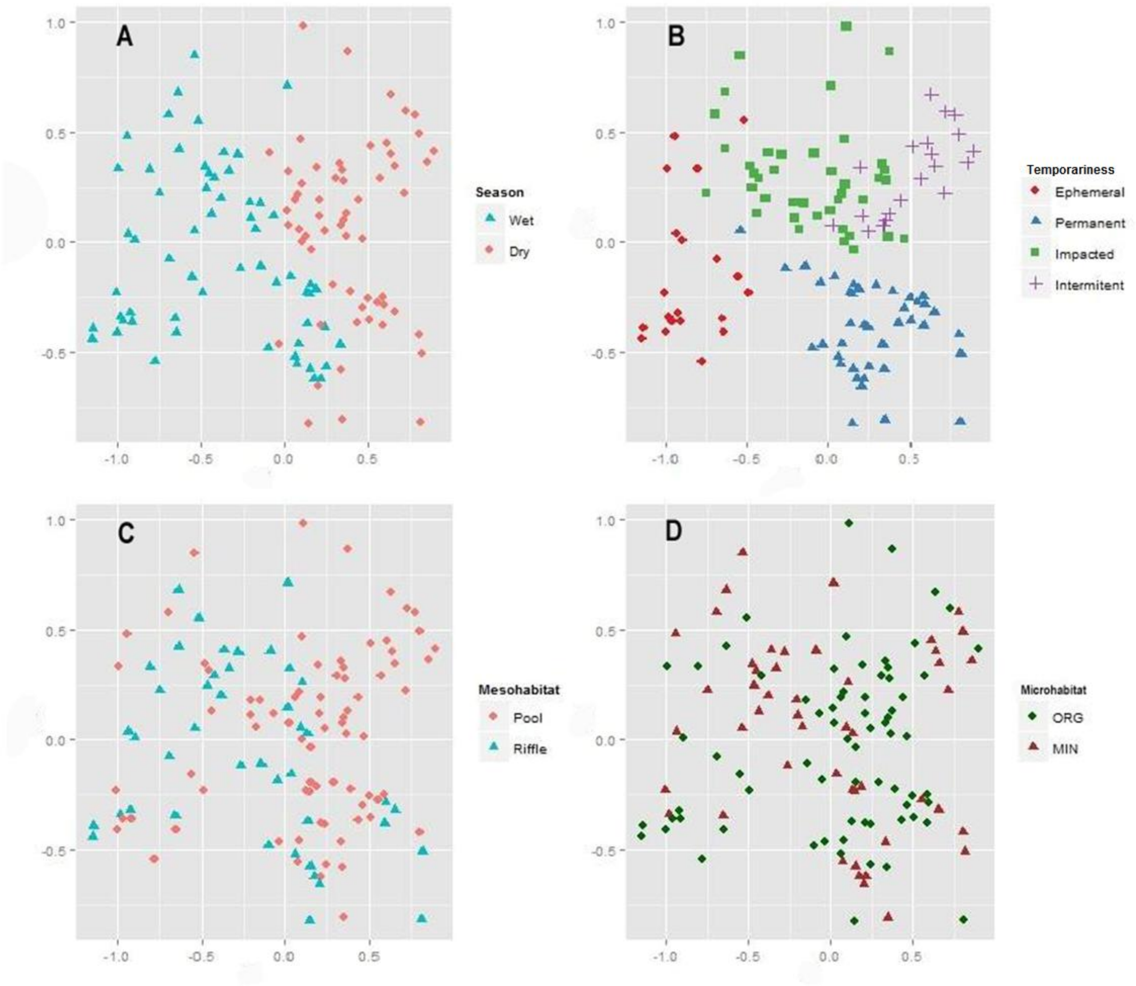

Figure 4 Results of the nMDS performed on log transformed Bray Curtis dissimilary matrix of abundance macroinvertebrates for each different factor considered in this study. (A) season, (B) temporariness, (C) mesohabitat and (D) microhabitat.

A Permanova analysis was performed in order to summarize the significance and contribution of each factor to the total variance as in shown in Table 2. The nested factors (temporariness, mesohabitat within temporariness and microhabitat within mesohabitat) were all crossed against season and include the maximum number of interactions allowed by the model. The hierarchical-crossed model explained a total of 75.60% showing that macroinvertebrates assemblages were affected by all the factors. The highest percentages of variance was refered to temporariness which explained a total of 41,58% of the variance. Microhabitat was also an important factor explaining a total of 15,87% of variance. Season and mesohabitat explained less than a 10% each (7,20% and 5,06%, respectively). Interactions between factors were also significantive, however they only explained together a total of 5.89%.

Table 2 Summary of the nested-crossed permanova performed on Bray Curtis dissimilarity matrix of the log-transformed macroinvertebrates abundance matrix.

Factor		df	Sums of Sqs	Mean Sqs	F. Model	r^2	p-value (MC)
Temporariness		3	10.734	3.578	43.168	0.4158	0.0001
Season		1	1.859	1.859	22.431	0.0720	0.0001
Mesohabitat	*Mesohabitat(Temporariness)*	3	1.305	0.435	5.249	0.0506	0.0001
Microhabitat	*Microhabitat(Mesohabitat(Temporariness))*	28	4.097	0.146	1.766	0.1587	0.0001
Interaction between factors							
Season x Temporariness		1	0.563	0.563	6.796	0.0218	0.0001
Season x Mesohabitat		2	0.478	0.239	2.886	0.0185	0.0001
Season x Microhabitat		2	0.478	0.096	1.156	0.0185	0.001
Residuals		76	6.299	0.083		0.24401	
Total		119	25.815			1	

Indicator species analysis revealed a number of different taxa that were representative of temporariness of sites (Table 3). A great diversity of taxa was indicative of permanent condition samples, including mayflies (*Baetis rhodani*), several midges (Tanytarsiini and *Corynoneura sp.*), leeches (*Erpobdella sp.*), tricladids (*Dugesia tigrina* and *D. gonocephala*), crustaceans (*Gammarus sp.*) and several hydrobiid snails (*Potamopyrgus antipodarum* and *Bythinella sp.*). Indicator species of impacted site was less diverse; however contained, mayflies (*Cloeon dipterum*), several taxons of diptera (*Prosimulium sp.*, *Simulium sp.* and *Thienemaniella sp.*) and two families of oligochaeta (Naididae and Tubificidae). Indicator species of intermittent site were also diverse. The taxons found included several coleoptera (*Oulimnius sp.* and *Haliplus sp.*), several snails (*Lymnaea peregra*, *Ancylus fluviatilis* and *Gyraulus sp.*), midges (Chironomiini) and oligochaeta (*Pristina sp.*). Finally, no indicator taxa were found for ephemeral condition, suggesting that the taxons present are commonly found in the other sites.

Table 3 Indicator species analysis results for the temporariness of sites with each taxon's indicator value (IV) and their associated stadistical significance (P). Ephemeral site has no significative taxon associated.

Permanent			Impacted			Intermittent		
Taxon	IV	P	Taxon	IV	P	Taxon	IV	P
Gammarus	100	0.0001	Naididae	85.9	0.0001	Chironomiini	79.5	0.0001
Tanytarsiini	80.7	0.0004	Pentaneuriini	70.4	0.0001	*Oulimnius sp.*	78.4	0.0001
Bythinella	80.6	0.0001	*Prosimullium*	66.8	0.0001	*Lymnaea peregra*	77.5	0.0001
Dugesia tigrina	61.2	0.0001	Tubificidae	65.1	0.0001	*Gyraulus*	69.5	0.0001
Corynoneura	54.9	0.0227	*Simullium*	58.5	0.0005	*Ancylus fluviatilis*	62.2	0.0001
Baetis rhodani	47.9	0.0095	*Cloeon dipterum*	54.7	0.0009	*Pristina*	46	0.0023
Erpobdella	47.4	0.0014	*Thienemaniella*	44.7	0.0216	*Haliplus*	43	0.0065
Dugesia gonocephala	46.3	0.0054						
Potamopyrgus antipodarum	44.7	0.0037						

Indicator species analysis revealed different taxa that were representative of either organic or mineral substrate (Table 4). A total of 4 taxons were indicative of organic substrates, including mayflies (*Cloeon dipterum*), isopods (*Asellus aquaticus*), snails (*Lymnaea peregra*) and odonata (*Chalcolestes viridis).* Two of these species, were also found representative of different sites (Table 3). *Cloeon dipterum* was found representative of impacted site, whereas *Lymnaea peregra* was representative of intermittent site. Several taxa were found representative of mineral substates. Indicator species of this group of microhabitat, were two midges: the chironomid *Thienemaniella*, and ceratopogonid *Bezzia sp.* as well as nematoda.

Table 4 Indicator species analysis results for the microhabitat, with each taxon's indicator value (IV) and their associated stadistical significance (P).

Organic			Mineral		
Taxon	IV	P	Taxon	IV	P
Cloeon dipterum	41.7	0.0252	*Thienemaniella sp.*	43.3	0.0214
Asellus aquaticus	40.6	0.0200	*Bezzia sp.*	35.0	0.0039
Lymnaea peregra	38.7	0.0138	Nematoda	33.4	0.0366
Chalcolestes viridis	33.6	0.0186			

4.3 Biological traits composition

Fuzzy Correspondence Analysis (FCA) on biological traits composition showed similar results than Permanova analysis performed with abundance data (Figure 5). driver for macroinvertebrate structure. According to temporariness ordination (Figure 5b).a clear differenciation between different sites, which are ordered along the first axis of the

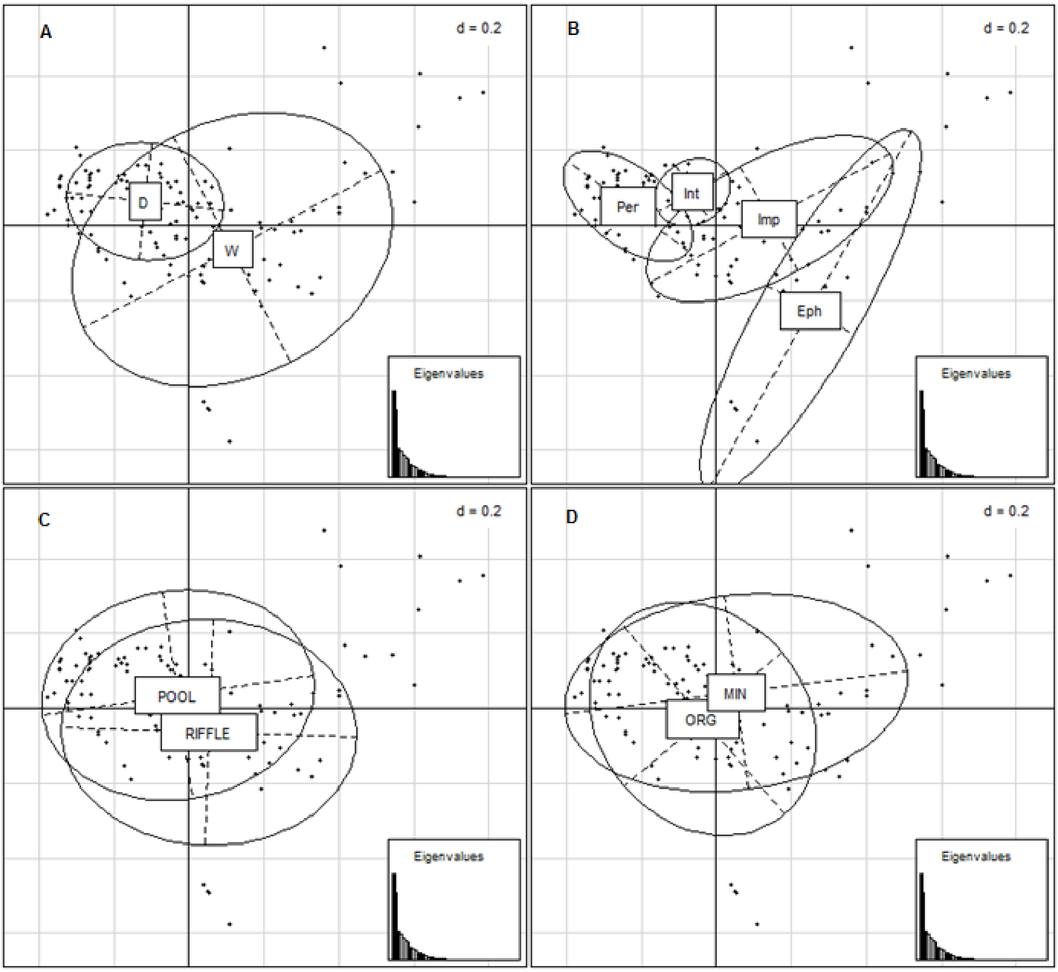

Figure 5 Fuzzy Correspondence Analysis (FCA) on the biological traits matrix, with d indicating the scale of the graphic. Ellipses envelop 70% of the sites of a corresponding condition: (a) represents season ordination (wet or dry period); (b) temporariness of sites: Ephemeral (Eph), Intermittent (Int), Impacted (Imp) and Permanent (Per); (c) mesohabitats (pool or riffle); (d) microhabitat (organic or mineral). The labels indicate the gravity center of the ellipses. Axes 1 and 2 represent 29.91% and 28.40% of total variance respectively.

The wet-season ordination (Figure 5a) revelated differences of macroinvertebrates assemblages among the first nMDS axis, which points that seasonality is an important representation. Intermittent site is situated between permanent and impacted, whereas impacted site is more related with ephemeral site. At lower scale (mesohabitat and microhabitat), there is not a clear pattern that clearly permits differenciate more between macroinvertebrate traits assemblages (Figure 5c and 5d). Temporariness was the main factor explaining the higher variability between communities (inertia: 0.30, p-value=10^{-4}), followed by microhabitat (inertia 0.18, p-value=10^{-4}), season (inertia 0.09, p-value=10^{-4}) and mesohabitat (inertia 0.04, p-value=$4 \cdot 10^{-4}$).

Indicator analysis revealed a number of different traits that were representative of each site (Table A2), which validated most of a priori predictions (Table 5) in Table 1.

All of each categories were represented in at least one trait in one of the sites. A total of 52 traits were found representative of one of the different sites, which represents a total of 85.24% of the total traits. A total of 10 traits were found in ephemeral site. According to indicator analysis, this site is caracterized by small macroinvertebrates (between 0.5 to 1 cm), with fast generation life cycles (more than 1 life/year and life cycles with a duration less than a year), that ovoposit in terrestrial area, and respire directly by tegument. These taxons are found in hyporreic zone due their capacity to burrow in the substratum or live in the intersticial zone. They also did not have any resistance form to deal with dessication. Due the presence of Nematoda in this site, the preferent feeding group found was parasite. 15 indicator traits were found representative of intermittent site conditions. Taxa found in this site were characterized for small size (less than 0.25 cm to 2 cm), with slow generation life cycles (less than 1 life/year and life cycles with a duration more than a year) and adult stage. Adaptations for dessication are diapausing in adults, as well as evasion features as flying adults or surface swimmers. Also, adaptation to low concentration was found in form of plastron. The macroinvertebrates present in this site feed on microphytes and are piercers or scrapers. Impacted site is characterized by big maximal size macroinvertebrates (between 4 cm to 8 cm), with asexual reproduction or ovoposition of free eggs. Spiracle is a common trait shared by these organisms to deal with this low oxygen conditions found in this site. Also, due the presence of great amount of organic matter, the main strategy used to feed is directly from the sediment. Finally, permanent site is characterized by intermidiate macroinvertebrate size (between 2-4 cm), with aquatic passive dispersion and with ovoviviparity and cemented eggs as reproductive characteristics. Also, they resources of food are more diverse (dead animals, macroinvertebrates, plant detritus), as well as their feeding habits (shredder and predator).

Table 5 A priori prediction and results obtained for the different aquatic regimes of Vène stream. For further detail, please consult Table A1.

Trait	**Category**	**Predicted**	**Results**
Maximal size	0.25 - 0.5 cm	Intermittent	Intermittent
	0.5 - 1 cm	Intermittent	Ephemeral
	1 - 2 cm	Permanent	Intermittent
	2 - 4 cm	Permanent	Permanent
Life cycle	< 1 year	Ephemeral	Ephemeral
	> 1 year	Intermittent	Intermittent
Aquatic stages	adult (imago)	Intermittent	Intermittent
Dispersal	aquatic passive	Permanent	Permanent
	aerial active	Ephemeral	--------------
Resistant form	eggs / cocoons / diapause	Ephemeral / Intermittent	Permanent (eggs and coccons) Intermittent (diapause)
	none	Permanent	Ephemeral
Respiration	spiracle / plastron	Intermittent	Intermittent (plastron)
	gills	Permanent	Permanent
Locomotion	burrower	Ephemeral	Ephemeral
	flier	Intermittent	Intermittent
	temporarily attached	Permanent	Permanent
Feeding habits	deposit filterers	Permanent	Permanent

4.4 Functional diversity indices

Within all functional diversity components analyzed, several significative differences could be observed (Figure 6). Functional richness was signitificative lower at the ephemeral site compared to intermittent, impacted and permanent sites with p-value of 0.0011, 0.0204 and 0.0310, respectively. Functional richness mean values for ephemeral site were between two and three times lower than in the other sites. Differences in functional evenness were found between ephemeral and intermittent site (p-value = 0.0110), as well as impacted site (p-value = 0.0023). Moreover, permanent site was significative different from intermittent (p-value=0.0047) and impacted (p-value=0.0001). However, values of functional evenness were high in all Vène river ranging between 0.73 and 0.96. Functional divergence showed a decrease gradient with hydrological conditions, having permanent site lower functional divergence compared to ephemeral (p-value = 0.0043), intermittent (p-value=0.0001) and impacted (p-value=0.0000). Nevertheless, values of functional divergence were also relatively high in all the sites, ranging between 0.649 and 0.917. Finally, quadratic rao index showed a similar result obtained for functional richness. Ephemeral site had a significative lower value than intermittent (p-value=0.0004), impacted (p-value=0.0000) and permanent (p-value=0.0001).

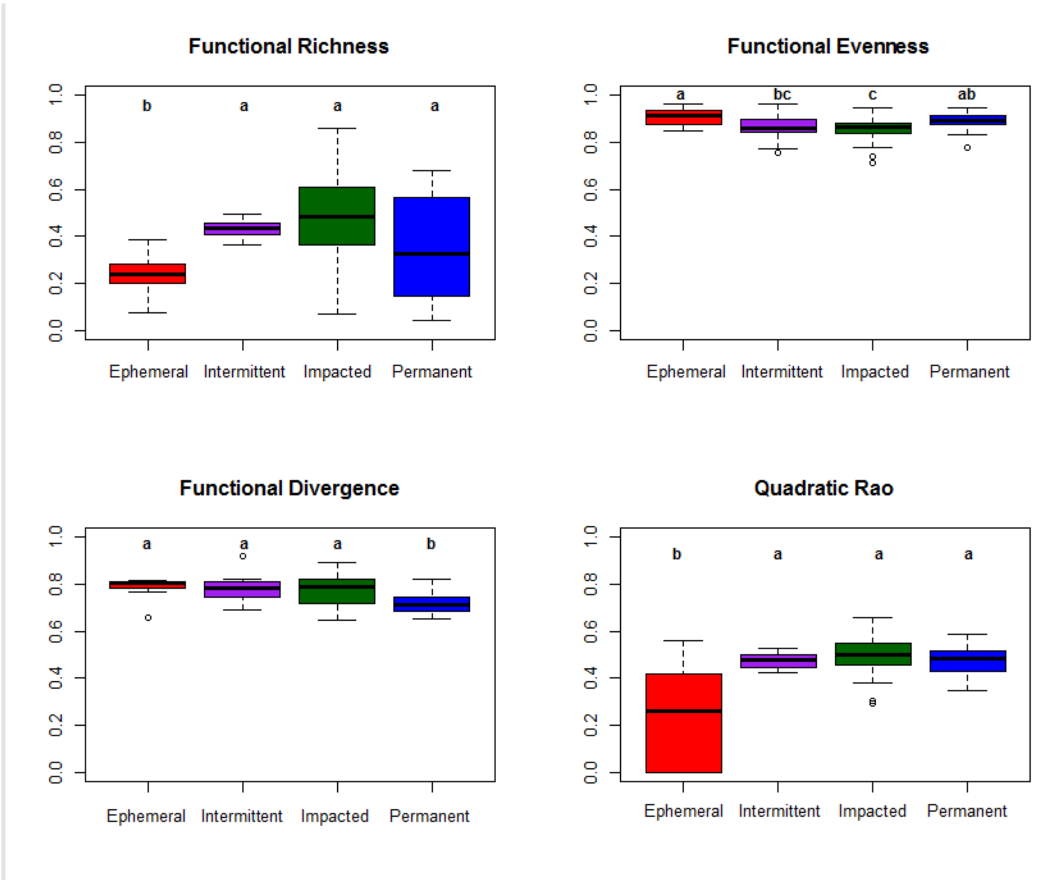

Figure 6 Box-Plots of different standardized functional diversity metrics comparing different sampling sites. Analyzed indices were: functional richness, functional evenness, functional divergence and Quadratic Rao Index. Each box of the box-plot includes data from the percentile 25 and 75 as well as the median and dashed lines include percentiles 10 an 90. For each plot, letters (a, b, c) indicates significant differences obtained by Dunn test after Bonferroni correction.

5 Discussion

5.1 Environmental variables and habitat composition

Environmental variables and habitat features revealed differences between sites with different aquatic regimes along the Vène stream. A clear differentiation between seasons was observed for environmental variables, as highlighted by other authors (Bêche et al., 2006; Bonada et al. 2007; Munné & Prat 2011). In the wet season, all sites have similar physicochemical variables (Figure 3). During the dry season, it was expected a similar water quality reduction between impacted site and intermittent site, because intermittent streams are highly susceptible to rapid heating due solar radiation (Williams, 2006), which lead a concentration of nutrients and depletion of oxygen (Lake, 2011). Also, in intermittent streams with permeable substrate, as in this study, hyporheic upwelling can stimulate locally the production of algae and bacteria (Williams, 2006). Nevertheless, the water quality reduction at the intermittent site was not as notorious as in the impacted site, probably because the better conservation of riparian corridor in this site. The presence of riparian forest could buffer temperature increase due effects of direct solar exposition (Lake, 2011) and minimize changes in water quality.

Despite well established sequences of pool and riffles were observed in all study sites during the wet season, a shift of lotic into lentic conditions among different aquatic regimes were observed in the dry season, as other authors have reported (Gasith & Resh, 1999: Williams, 2006; Bonada et al. 2007; García-Roger et al., 2013; Datry et al., 2014b). Differences in microhabitat composition (patches of substrate) found in this study are similar to those reported by Lind et al., (2006) and García-Roger et al., (2011) where dominance in organic substrates as well as and homogenization of substrates was observed during the dry season in Mediterranean streams, due to a shift in erosional to depositional conditions caused by flow cessation. Nevertheless, the specific microhabitat heterogeneity is very variable even between the same catchment (Arscott et al., 2010; García-Roger et al., 2013) and depends on other factors such as geology of the basin (Munné & Prat, 2011) and local factors (Leitão et al., 2014).

5.2 Taxonomic composition

The combination of fragmentation and habitat contraction over the time as well as the reduction of water quality due flow cessation showed changes in aquatic taxonomic composition of macroinvertebrates. As hypothesized, the most important factors in the assemblage of macroinvertebrate communities were the temporariness and macrohabitat in agreement with the results obtained by other authors (Lamoroux et al., 2004, Bonada et al., 2006, Bonada et al., 2007, García-Roger et al., 2011). In this study, different invertebrates were associated to different aquatic regimes with exception of ephemeral site, as other authors have shown (Bonada et al., 2007, Arscott et al., 2010 and Bogan et al., 2013,) suggesting that no specialist invertebrates live in ephemeral sites, and thus invertebrates present in this particular conditions are nesting subsets of the species that are present in near permanent waterbodies (Datry et al., 2014a). Moreover, other studies have reported no differences between perennial and intermittent macroinvertebrate assemblages (Datry et al., 2013). This can be due to the lack of taxonomic resolution of diverse groups such as Chironomidae and Simuliidae

(Bogan et al., 2013), which is not the case of the present study, where several genus of Chironomidae and Simuliidae were identified.

Microhabitat was reported to be the other major factor responsible of macroinvertebrate composition. Although mixed substrate (organic with mineral substrate) were found, their composition can differ greatly from different catchments and streams (Arscott et al., 2010; García-Roger et al., 2013; Leitão et al., 2014) and can explain intrinsic variation at catchment scale in temporary rivers. Despite some authors have documented that macroinvertebrates are not usually restricted to a specific substrate (Williams, 2006; Sheldon et al., 2010), different macroinvertebrates were associated to organic or mineral substrate. Species in organic substrates had relatively large size and most fed on coarse organic matter (with the exception of *Chalcolestes viridis*). In contrast, species found in mineral substrate have small sizes and flexible and streamlined bodies which enable them to burrow in the small interstices in the bed sediment (Lamourox et al., 2004). The diversity of different microhabitats provides a set of physical refuge from predators and unfavourable environmental conditions and could vary according to the different organism traits as well as their spatial availability (Sheldon et al., 2010; Robson et al., 2011). Identified refuges for macroinvertebrates include persistent pools, moist habitats (either algae and leaf litter; or bellow stones); moving into the hyporheic zone or migrate to permanent water bodies (Williams, 2006; Sheldon et al., 2010; Lake, 2011; Robson et al., 2011).

5.3 Biological traits

Temporariness of sites was also the main factor that explained the variation of biological traits assemblage, consistent with previous studies (Bêche et al., 2006; Bonada et al., 2007; Statzner & Bêche, 2010; García-Roger et al., 2013). However, some differences with the previous studies were found.

The intermittent site had traits associated with smaller body sizes (> 0.25 – 0.5 and 1 – 2 cm) which are similar to the results found by Bonada et al., (2007) and García-Roger et al., (2013). Reduced size is likely to be a consequence of overcrowding and competition in the remaining pools. Also, due to the shift to lentic conditions, air-breathing mechanisms such as plastron can deal with the progressive depletion of oxygen (Stazner & Bêche, 2010). Moreover, taxa found in the intermittent site had diapause state (Beche et al., 2006; Bonada et al., 2007; García-Roger et al., 2013) which is commonly found in different organisms (Robson et al., 2011) and can represent between 74.2-85.7% of taxa remaining in intermittent streams (Williams, 2006). Finally, aquatic adult stage was found to be representative of the intermittent site, which is likely to be a result of organisms without aerial dispersal present in disconnected pools such as the gastropods *Gyraulus sp*, *Lymnaea peregra* and *Ancylus fluviatilis* found in this study.

The ephemeral site was characterized by intermediate size invertebrates (0.5 – 1 cm), that lay clutched eggs. These traits were not previously reported by other authors (Bêche et al., 2006; Bonada et al., 2007; García-Roger et al., 2013). Interstitial and burrower locomotion were also found representative of this site, as previously reported by Bonada et al., (2007). These results highlighted that the main resistant mechanism to survive to habitat desiccation may be burrowing into the hyporheic zone (Robson et al., 2011). Finally, our results suggest that resistant-based strategies, e.g. dormancy (Robson et al., 2011) may be more frequent in intermittent streams, whereas resilience-based strategies (e.g. aerial dispersal) are more common in ephemeral streams (Acuña et al., 2005, Arscott et al., 2010; Bogan et al., 2013 and Datry et al., 2014b).

In contrast to the results found by Bonada et al., 2007, who only found aquatic eggs associated to permanent streams, a large set of biological traits were associated to the permanent sites from this study. Macroinvertebrates from these sites were characterized by large maximum size (2 to more 8 cm), as well as adaptations to flowing conditions such as gills, aquatic passive dispersal, feeding as deposit filterers and temporal attachment to substrate. Large-sized invertebrates and aquatic passive dispersal were associated to permanent sites in our study, as more stable conditions appear to permit the development of large macroinvertebrates (García-Roger et al., 2013; Bêche et al., 2006), and the stability of water flow condition favours mechanisms of aquatic passive dispersal (Statzner & Bêche, 2010; García-Roger et al., 2013). Moreover, temporal attachment to substrate permits avoiding drift (Statzner & Bêche, 2010). Also, gills can be an effective mechanism to increase the oxygen uptake in flowing conditions (Statzner & Bêche, 2010). Moreover, abundant leaf litter accumulation in flowing conditions can provide more diverse food and may explain the abundance of shredders and filter feeders–i.e Simuliidae-. Although not included in our a priori predictions, predators were more abundant in the permanent site as other authors reported (Arscott et al., 2010; Bogan et al., 2013). Therefore, the abundance of predators may be caused by the diversity of available prey, which also diversifies the different predator organisms found. Finally, an unexpected significance in egg resistant form in permanent site was obtained. This result is likely to be due the abundance of *Baetis rhodani* and Simuliidae (*Prosimullium* and *Simuliium*) in this site which are known to have resistant egg forms (Williams, 2006).

5.4 Functional diversity

Functional diversity metrics obtained in this study suggest that communities found in Vène stream have a high degree of functional redundancy (Williams, 2006; Bogan et al., 2013). Functional redundancy in an ecosystem provides protection in ecosystem processes, if some species are lost by a disturbance event (i.e flow cessation) (Díaz & Cabido, 2001; Schriever, et al., 2015). Nevertheless, ephemeral site had lower values of functional richness indicating that some of their functional redundancy was lost due the effect of flow cessation. As a consequence, as indicates lower Rao index, a loss in functional diversity was observed in this site.

6 Conclusions

This study indicated that flow cessation has a major impact in water chemistry, leading to an increment of nutrients and oxygen depletion. However, due to a better conservation state of riparian forest the effects of increase of temperature and in turn in water quality were buffered. Also, a reduction and disappearance of riffles as well as a homogenization of substrates and a dominance of organic microhabitats were observed in the different sites studied.

Temporariness of sites and microhabitat were the main factors responsible of variance for macroinvertebrate taxonomic and trait composition. Several taxa were associated to the different aquatic regimes, except to ephemeral site, indicating that species found in these sites are nested-subsets of other invertebrates found in the other permanent waterbodies. Moreover, several organisms were associated to different microhabitats, which serve as a physical refuge for the different aquatic invertebrates to deal with flow cessation and predation.

Responses of biological traits were different according to the temporariness of the different sites. The ephemeral site presented traits related with resilience strategies such as aerial dispersal and fast generation cycles. Also, present resistant organisms can burrow in the hyporheic zone to survive during the absence of surface water. The intermittent site had resistant traits to deal with drought condition and depletion of oxygen, including lower maximal size, plastron respiration, diapause as resistant form and adult stage. In permanent sites, several traits were associated to continuous flow conditions such as gill respiration, aquatic dispersal, active swimmer or temporary attachment to substrate. Finally, flow cessation caused some degree of loss of functional diversity in ephemeral site.

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APPENDIX A

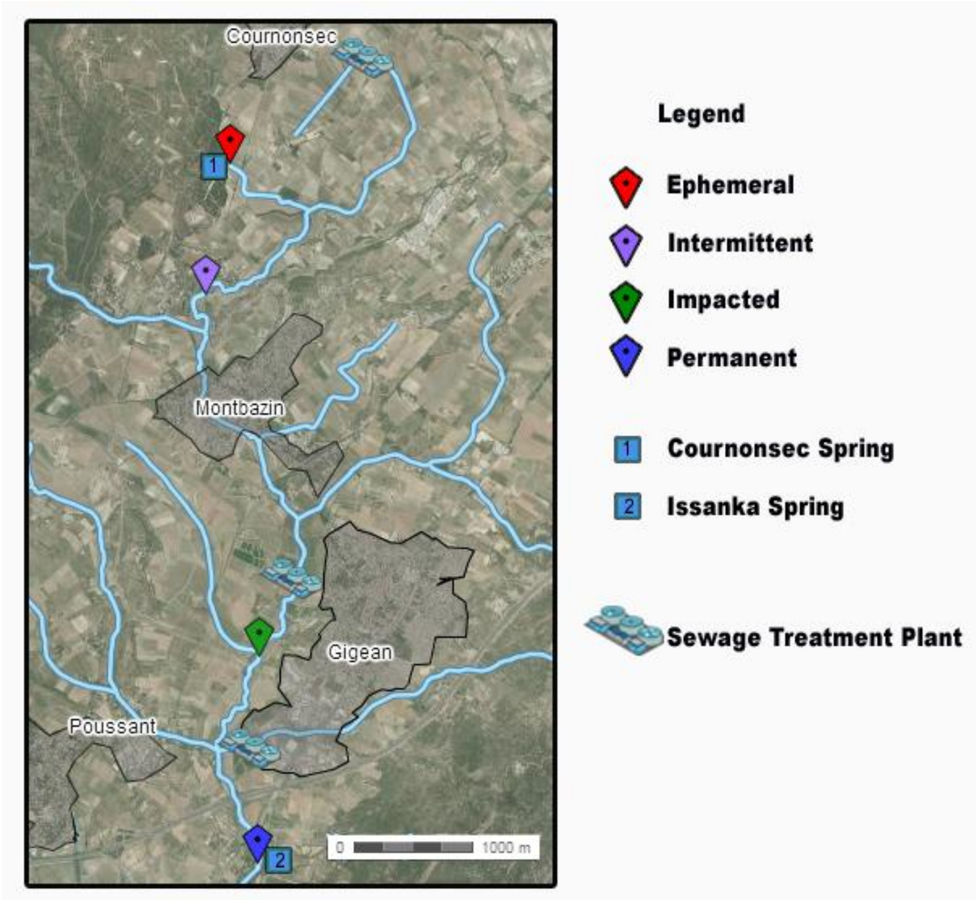

Figure A1 Map of the study area showing the sampling points collected, as well as the localitation of the Sewage Treatment Plant (STP) presents in Vène River.

Table A1 List of microhabitats. Adapted from AQEM protocol (Hering et al., 2004)

Microhabitat		Code
Mineral		
Argyllal	< 6µm	ARG
Psammal	> 6 µm - 2mm	PSA
Microlithal	>2cm - 6cm	MIL
Mesolithal	>6cm -20cm	MEL
Macrolithal	20-40 cm	MAL
Organic		
Algae	Filamentous algae, algal tufts	AL
Submerged macrophytes (including bryophytes)		SM
Emergent macrophytes (*Phragmites*, *Typha*...)		EM
Xylal	Woody debris	XY
CPOM	deposits of coarse particulate organic matter	CPOM

Table A2 Indicator traits analysis results for the temporariness of sites, with each taxon's indicator value (number) and their associated stadistical significance in parenthesis. (*) for signficance at 0.05, (**) for significance at 0.1 and (***) for significance at 0.

Category	Trait	Ephemeral	Intermittent	Impacted	Permanent
Maximal size	*less 0.25 cm*		57.8 (**)		
	between 0.25-0.5 cm		56.8 (***)		
	between 0.5-1 cm	55.1 (***)			
	between 1-2 cm		53.2 (**)		
	between 2 -4 cm				60.7 (***)
	between 4-8 cm			53.3 (**)	
	more 8 cm			38.3 (*)	
Life Cycle	*less 1 year*	54.3 (***)			
	more 1 year		56.1 (***)		
Cycles/year	*1 cycle/year*		56.1 (***)		
	more 1 cycle/year	55.9 (***)			
Aquatic stages	*egg*				52.9 (***)
	larva			52.1 (***)	
	adult		59.3 (***)		
Reproduction	*ovoviviparity*				69.2 (***)
	cemented eggs				63.3 (***)
	clutches cemented		56.2 (***)		
	clutches terrestrial	75.6 (***)			
	free_eggs			58.1 (**)	
	asexual			56.6 (*)	
Dispersion	*aquatic passive*				55.9 (***)
Resistence form	*eggs*				70.5 (***)
	cocoons			56.1 (*)	
	diapause		57.2 (***)		
	none	54.0 (***)			
Respiration	*tegument*	53.0 (***)			
	gill				62.3 (***)
	plastron		74.4 (***)		
	spiracle			50.5 (*)	
Locomotion	*intersticial*	59.6 (***)			
	burrower	58.6 (***)			
	flier		74.9 (***)		
	surface swimmer		68.3 (***)		
	permanently attached		57.4 (**)		
	temporarily attached			57.7 (***)	
	swimmer			53.4 (**)	
	crawler				57.2 (***)
Food	*microphytes*		53.4 (***)		
	fine sediment			62.6 (***)	
	detritus less 1 mm			55.1 (***)	
	dead animal				79.1 (***)
	plant detritus				67.7 (***)
	macroinvertebrates				64.7 (***)
Feeding habits	*parasite*	72.0 (***)			
	scraper		53.5 (**)		
	piercer		43.3 (**)		
	absorber			65.2 (**)	
	filter_feeder			57.6 (**)	
	deposit filterers			55.9 (**)	
	shredder				61.6 (***)
	predator				57.7 (*)