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Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia, realizada sob a orientação científica da Professora Doutora Manuela Abelho (Instituto Politécnico de Coimbra - Escola Superior Agrária) e da Professora Doutora Cristina Canhoto (Universidade de Coimbra)

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Abstract

Urbanization has deep impacts in streams affecting all aspects of the ecosystem. The distinctive impacts of urbanization include the increase in impervious surfaces which in turn alters the hydrology and geomorphology of streams. Thus, urbanization affects not only water quality but also habitat characteristics. Biological indicators may reveal spatial-temporal effects of stressors and their cumulative effects on stream biota and biotic indices based on intolerance to disturbance and taxonomic richness are effective and widely used to assess ecological health. However, the multiple, co-occurring and interacting stressors of urban streams, namely habitat alterations, may be better revealed by macroinvertebrate traits - the species adaptations to environmental conditions. The aim of this study is to compare traditional quality assessment approaches related to macroinvertebrate community structure and widely used biotic indices with information provided by the study of aquatic macroinvertebrate traits in order to establish the ability of species traits to assess ecological integrity of urban streams. The results obtained during four sampling occasions at four sites in Ribeira dos Covões, a small peri-urban stream in Coimbra revealed more temporal than spatial variability. The combination of five traits related to macroinvertebrate biological (duration of the life cycle, resistance form and feeding method), physiological (respiration form) and ecological (locomotion and relationship to the substrate) adaptations was able to separate sampling dates whose environmental variables were distinct but not sites. In conclusion, the use of species traits allows distinguishing samples that are clearly associated different environmental characteristics.

Key words: urban streams; stream health; macroinvertebrates; ecological traits; Ribeira dos Covões.

Resumo

A urbanização tem efeitos profundos nos rios afectando todos os aspectos do ecossistema. Os efeitos mais distintivos da urbanização incluem o aumento de superfícies impermeáveis que por sua vez alteram a hidrologia e a geomorfologia dos rios, afectando não só a qualidade da água mas também as características do habitat. Os indicadores biológicos podem revelar os efeitos espácio-temporais das pressões exercidas sobre o ecossistema mas também os seus efeitos cumulativos sobre as comunidades bióticas. Índices bióticos baseados na intolerância à perturbação e na riqueza taxonómica são eficazes e amplamente utilizados para avaliar a qualidade ambiental. No entanto, a multiplicidade de pressões co-ocorrendo e interagindo nos rios urbanos, nomeadamente alterações de habitat, poderão ser melhor reveladas recorrendo a *traits* de macroinvertebrados – adaptações das espécies às condições ambientais. O objectivo deste estudo é comparar as abordagens tradicionais de avaliação de qualidade, relacionadas com a estrutura da comunidade de macroinvertebrados e índices bióticos correntemente utilizados, com informações obtidas pelo estudo de *traits* de macroinvertebrados aquáticos, a fim de estabelecer a capacidade da sua utilização para avaliar a integridade ecológica dos rios urbanos. Os resultados obtidos durante quatro amostragens em quatro locais da Ribeira dos Covões, um pequeno rio peri-urbano de Coimbra, revelaram existir maior variabilidade temporal que espacial. A combinação de cinco *traits* biológicos (duração do ciclo de vida, forma de resistência e modo de alimentação), fisiológicos (modo de respiração) e ecológicos (locomoção em relação ao substrato) permitiu separar datas de amostragem cujas variáveis ambientais foram distintas mas não locais. Concluindo, o uso de *traits* permite distinguir amostras associadas a características ambientais distintas.

Palavras-chave: rios urbanos; qualidade ambiental; macroinvertebrados; *traits*; Ribeira dos Covões.

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1. Introduction

1.1. Importance and Functioning of Flowing Waters

Fresh water is a finite natural resource that can only be replenished through the hydrological cycle in which water from seas, lakes, forests, land, rivers, and reservoirs evaporates, forms clouds, and returns as precipitation (Corvalán et al., 2005). Fresh water represents only 0.01% of the world's water but it holds approximately 6% of all described species; and the biodiversity decline rates of freshwaters are among the most concerning (Dudgeon et al., 2005). Discharge of toxic chemicals, over-pumping of aquifers and long-range atmospheric transport of pollutants are the major causes for water quality degradation (Bartram et al., 1996). While water quality is diminishing the world's population increases, further increasing water demands for irrigation, drinking, household uses and industrial production (Rosegrant et al., 2005). Furthermore, water resources are not evenly distributed around the globe intensifying competition for water (Watkins et al., 2006).

Pristine rivers shaped the development of ancient civilizations. Cities emerged in the fertile areas downstream where agricultural resources were ensured and transport costs were lowest (Algaze, 2008). Nowadays, running waters are shaped by human needs and subjected in a world-wide scale by a variety of water-management practices, overexploitation, flow modification, destruction or degradation of habitat and invasion of exotic species (Dudgeon et al., 2005), all of which affects stream ecosystems.

Stream ecosystems are complex and, from source to mouth, a gradient of morphological and physical conditions shape the dominant processes and the biotic communities (Vannote et al., 1980). Low order streams are narrow and shaded by the surrounding riparian canopy, limiting primary production in water. Riparian vegetation plays an important role structuring the ecosystem as it heavily contributes to the energy flux when primary production is insufficient (Allan 1995, Imberge et al., 2008). The communities on those headwater streams rely on materials coming from outside the channel, such as leaves and twigs (CPOM – coarse particulate organic matter), as the main energy source. Shredding invertebrate detritivores, by breaking leaves into smaller pieces will convert CPOM into smaller particles - fine particulate organic matter (FPOM), which is used downstream as food by other organisms, the collectors and the filterers. When moving downstream the stream channel widens reducing shading from riparian canopy, increasing the amount of incident sunlight and allowing for more primary production and thus more phytoplankton and algae in water that serve as food source for scrapers and collectors. Close to the mouth the influence of riparian vegetation lowers to a minimum and the macroinvertebrate communities are mainly composed of collectors and predators. (Vannote et al., 1980).

1.2. Effects of Human Activities on Streams

Human activities have been causing increasing alterations in surface waters and the hydrological cycle. Those activities include the alteration of the land cover of river basins, the regularization of the water fluxes, the construction of dams for irrigation or other purposes, soil drainage, and groundwater extraction, among others (Kuchment, 2004).

Regarding urbanization, the replacement of the natural land cover by the urban impermeable surfaces results in the most significant alterations, greatly reducing infiltration and evapotranspiration, and increasing surface runoff (Kuchment, 2004). The removal of riparian vegetation, the decreased groundwater recharge and the “heat island” effect associated with urbanization affect stream temperature increasing the mean temperature during the summer while decreasing in winter (Pluhowski, 1970), altering stream ecosystem processes such as leaf decomposition (Webster and Benfield, 1986) and invertebrate life history (Sweeney, 1984). Depending on the type and extent of urbanization, the presence of wastewater treatment plants, effluents and/or combined sewage overflows, and the extent of storm water drainage, the chemical effects are less predictable than hydrologic or geomorphic effects

(Paul and Meyer, 2001). Therefore, all aspects of the stream aquatic habitat are altered by urbanization: the ‘urban stream syndrome’. The urban stream syndrome describes the recurrently observed ecological degradation of streams draining urban land, including symptoms as a flashier hydrograph, increased temperatures, concentration of nutrients (organic and inorganic), contaminants, and siltation and altered channel morphology (Paul and Meyer, 2001, Meyer et al., 2005).

The responses of the macroinvertebrate communities to these alterations can be summarized as follows: (i) decreased biotic richness and diversity in response to contaminants, temperature change, siltation and organic nutrients; (ii) decreased abundances in response to toxins and siltation and (iii) increased dominance of tolerant species in response to inorganic and organic nutrients (Resh and Grodhaus, 1983, Wiederholm, 1984, Paul and Meyer, 2001, Meyer et al., 2005). Leaf breakdown can also be indirectly affected by urbanization when indigenous riparian species are replaced by exotic species that differ in quality, quantity or even seasonality (Abelho and Graça, 1996, Miller and Boulton, 2005, Ryder and Miller, 2005). The riparian vegetation assemblage may be altered due to various processes such as enrichment of riparian soils (Riley and Banks, 1996), drying of riparian soils following stream incision (Groffman et al., 2003) or simply deforestation and replanting. These alterations in leaf litter may affect the macroinvertebrate community (Abelho and Graça, 1996), especially detritivore shredders, which can have bottom-up effects on the food web (Gulis and Suberkropp, 2003, Lepori et al., 2005) especially on low order streams were shredders are of crucial importance in the energy cycle. Agriculture effects include altered water chemistry that may result in increased levels of nitrates and phosphates (which may lead to eutrophication), increased conductivity, food web modification with increased Chironomidae densities and descreased Ephemeroptera and Plecoptera abundances , biocide leaching and increased suspended loads from soil erosion (Welch et al., 1977, Moss, 2008).

1.3. Stream Health Assessment

The awareness that rivers supporting rich and diverse fauna are valuable natural resources led to an increased concern towards stream ecological health and to the use of ecological indicators for water quality assessment (Abel, 1996, Wenn, 2008). Ecological indicators integrate the condition of resources, the magnitude of stresses, and the exposure of biological components to stress, related impacts and consequences (Manoliadis, 2002).

Conventionally, water quality was assessed by measuring chemical parameters, which are efficient and allow precise measurements of pollutant concentrations. However, water quality in flowing waters oscillates rapidly and chemical analysis may fail to identify peak of pollutant concentrations. Stream biota may respond to extremely low levels of pollutants and reflect water quality over an extended period of time, thus biological methods my reveal information that is not accessible via punctual chemical sampling (Abel, 1996, Ziglio et al., 2006).

To assess the ecological condition in urban ecosystems, it is vital to define a target condition for management. However, the complexity of restorable benchmarks increases where multiple stressors influence ecosystem quality (Davies and Jackson, 2006), such as in urban streams. In addition, restorable benchmarks may also vary along the river continuum where not all sites or river reaches have the ability to attain a pristine condition. Therefore, a continuum approach (Carter and Fend, 2005) that sets the realistic minimum condition for ecological restoration based on the level of urbanization provides a context for evaluating both the current condition and the potential for recovery of impacted waters. This context can allow for more realistic management targets and prioritization of sites for restoration and protection (Stoddard et al., 2006). Reference sites are commonly used in bioassessment studies to identify undisturbed or pristine conditions, as a means of comparison between degradation levels and therefore management targets (Hughes, 1995, Prins and Smith, 2007). However, the continuous urban development often results in the absence of reference sites in urban streams (Chessman and Royal, 2004) and it may be thus difficult to define a target condition for restoring urban stream sites (Meyer et al., 2005).

1.4. Macroinvertebrates as Assessment Tools

In 23 October 2000 the European Parliament and the Council established a framework for community action in the field of water policy named The Water Framework Directive 2000/60/EC (WFD). This framework overhauls existing policies on European water quality management, and establishes the requirement for ecological assessment of water quality (Bell and McGillivray, 2006). The purpose is ‘to prevent further deterioration and to protect and enhance the status of aquatic ecosystems’ (European Council, 2000). Member states must achieve ‘good ecological and chemical status’ in all surface waters by 2015, where ‘good’ is defined with reference to pristine environments.

Macroinvertebrates are one group that the WFD requires member states to monitor. Because macroinvertebrate families are diverse in their pollution sensitivity, their relative abundance is used to understand the nature, load and severity of contamination (MacNeil et al., 2002). As the group is heterogeneous it is likely that some members respond to pollution. Some members have long life histories which allow the observation of temporal changes in communities and the pollution to which they are responding (Abel, 1996, Ziglio et al., 2006). For instance, stoneflies (Plecoptera) are highly sensitive to organic pollution due to their high oxygen requirements (Mason, 2002), while mayflies (Ephemeroptera) are sensitive to environmental stress. However, the ephemeropterans Baetidae are reasonably tolerant of nutrient enrichment (Hall and Lenwood, 2006) and dominate in poorer environmental conditions than other mayflies. Caddisflies (Trichoptera) are sensitive to environmental stress, though some families such as Limnephilidae and Hydropsychidae are relatively tolerant (Hall and Lenwood, 2006). Chironomidae (Diptera) are widely tolerant to organic pollution, although Stuijfzand et al. (2000) claim that the success of this group is more related to the use of organic food sources, rather than “tolerance” to pollution. Aquatic worms (Oligochaeta) are extremely tolerant to organic pollution and able to survive anoxic conditions due to the presence of hemoglobin (Mason, 2002). However, factors such as drift and seasonality may camouflage the effects of water quality on the aquatic communities in urban streams (Paul and Meyer, 2001, Wenn, 2008).

1.5. Species Traits

Traits are the attributes of species related to physiological, morphological and life-history adaptive features that are intrinsic to the organism and consequently can be measured on the individual level without making reference to the environment (Violle et al., 2007). Macroinvertebrate adaptations to environmental conditions are characterized by their species traits.

As trait classifications are not bound to taxonomy (Menezes et al., 2010), trait-based assessments are not limited by the spatial-temporal specificity of traditional assessment methods. Instead, trait-based approaches rely on the commonality of traits instead of species identity (Verberk et al., 2013), offering advantages as (i) direct transferability to distant geographic locations, (ii) direct comparability of biologically determined quality standards (Statzner et al., 2001, 2008, Horrigan and Baird, 2008), and (iii) enhanced understanding of species-environment relationships (Kearney and Porter, 2009). Because certain traits influence the organism performance they also reflect the ecosystem functioning and therefore can be used as measures of community functional diversity (Petchey and Gaston, 2006, McGill et al., 2006).

Tachet (1996) showed that the genus and even the family level are sufficient to describe the functional diversity (e.g., traits) of lotic invertebrate communities (Usseglio-Polatera et al., 2000). Species preferences and adaptations described by their traits can reveal certain effects of urbanization such as alterations on the river bed or river flow. However, species performance is a combination of natural selection and species sorting which do not apply on the level of single traits but on the whole organism carrying multiple traits. Therefore, is the combination, rather than single traits, that represents the adaptive response to the environment (Stearns, 1976, Grime, 1977, Southwood, 1977, Winemiller and Rose, 1992, Verberk et al., 2008).

1.6. Objectives

The objective of this study is to compare traditional quality assessment approaches related to macroinvertebrate community structure and widely used biotic indices with information provided by the study of aquatic macroinvertebrate traits in order to establish the ability of species traits to assess ecological integrity of urban streams.

2. Material and Methods

2.1. Study Area

The study stream was Ribeira dos Covões, a left tributary of the Mondego River located on the outskirts of Coimbra – Central Portugal (40° 12′ 41″ North, 8° 25′ 45″ West). Ribeira dos Covões is a 4th order small peri-urban stream draining a 7 km^2 basin (Pato et al., 2011). Its source is located at an elevation of 177 m, it flows 4.5 km before reaching its mouth at an elevation of 22 meters (Pato, 2007), conferring a 3.4% gradient. In the last three decades, the area has been exposed to continuous urbanization. In 2001 the catchment area had an estimated resident population of 7000 inhabitants in an irregular distribution, with 25% of the basin urbanized in 2002 (Ferreira, 2008). The basin is covered by 55.5% forest, 13.0% farmland and 31.5% artificial surfaces (Ferreira et al., 2011).

The area is characterized by a humid Mediterranean climate with an average annual temperature of +15 ºC and with a total rainfall of 980 mm during an average year, with strong seasonal and inter-annual variability (Ferreira et al., 2011). During the study period (Table 1) mean daily temperature ranged

9.8ºC to 20.9ºC and total precipitation ranged 0 mm to a maximum of 204.6 mm during the heavy precipitation events of December-January.

Table 1. Meteorological data during the study period from September 2013 to April 2014; data collected at the nearest meteorological station located in Bencanta (Escola Superior Agrária), Coimbra (max=maximum; min=minimum).

	Temperature (°C)			**Precipitation (mm)**	
Month	Average	Average max	Average min	Total	Daily max
September	20.9	29.8	14.3	59.8	31.6
October	17.5	22.8	13.6	164.0	46.4
November	11.5	17.2	7.0	17.2	5.2
December	9.8	15.3	5.5	183.8	61.5
January	11.2	14.4	8.3	204.6	45.6
February	10.6	14.2	7.2	213.8	26.8
March	12.4	18.4	7.6	58.8	16.4
April	15.4	20.4	11.1	78.0	23.4

2.2. Sampling Sites

Four different locations along the river longitudinal profile were chosen based on easiness of access and on earlier studies (Ribeiro, 2004; Fernandes, 2005; Soares, 2009). The sites were numbered 1 to 4 from source to mouth as L1, L2, L3 and L4.

The first sampling site (L1; Figure 1) is located 1605 m from the source, below a hospital center and above a factory. There is a riparian gallery upstream of the sampling site, composed of shrubs and deciduous trees. The channel is relatively natural, with a “v” section, and the substrate is mainly composed of pebbles, gravel and boulders. The second sampling site (L2; Figure 1) is located 640 m downstream L1. The riparian vegetation is dominated by small shrubs and herbaceous plants with a few dispersed trees. At the end of the summer, the channel was covered by aquatic plants. The stream channel has an open “u” section, and above the sampling site there is a bridge which fell down during the heavy precipitation events of December. The terrains around the site have been profoundly modified due to the construction of roads. The substrate is a mixture of fine sediment and gravel, the surrounding area is agricultural and pastures with nearby habitations. The third sampling site (L3, Figure 1) is located 493 m downstream L2, has a prairie on the left margin and a dense Mediterranean bush on the right margin which composes a closed riparian gallery. It is located upstream a small dam, with a wide “u” section and accumulates fine sand which is the only substrate. The fourth sampling site (L4, Figure 1) is located 790 m downstream L3, inside ESAC (Escola Superior Agrária de Coimbra). The construction of roads and pathways led to highly modified riparian vegetation that doesn’t exceed one tree in width with the rest being mainly annual shrubs. Upstream there is a small dam and the channel is mostly artificial. The substrate is composed of pebbles and gravel. The surrounding area is mainly for cattle and agricultural uses.

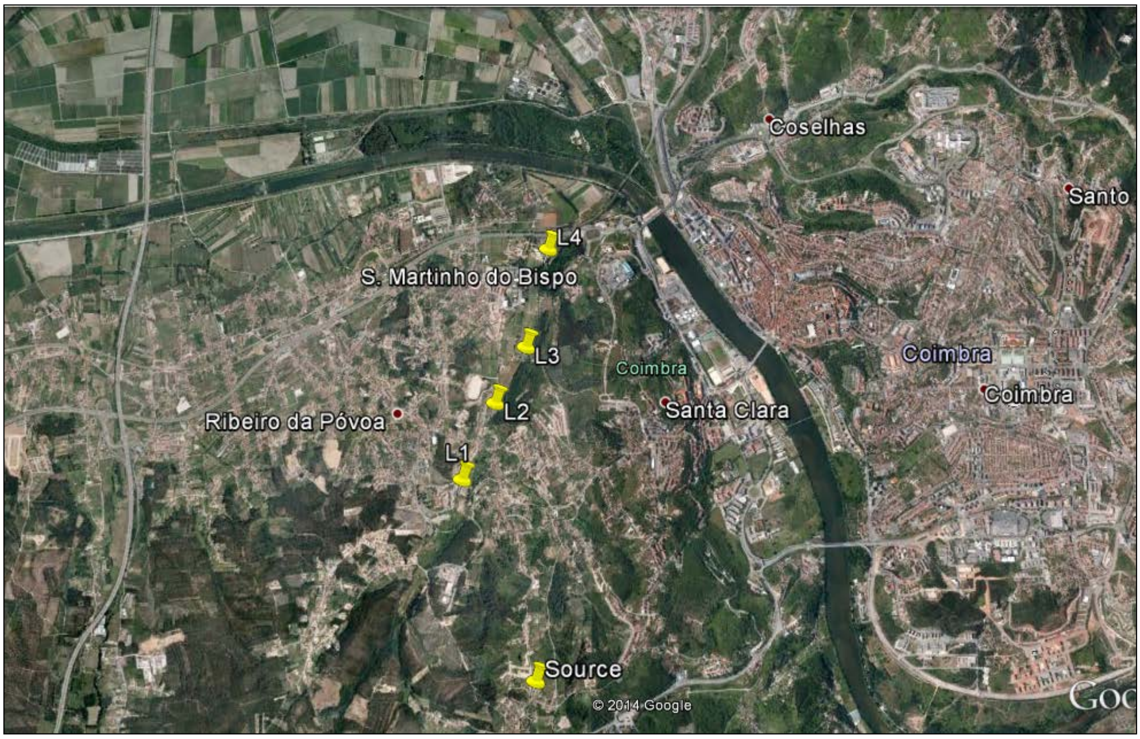

Figure 1. **Location, pinpointed in yellow, of the source and the study sites (L1 to L4) along Ribeira dos Covões. (Earth satellite image from June 2012 of the Ribeira dos Covões catchment; Google ™)**

2.3. Sampling

The four study sites were sampled on four occasions (11 September and 12 November 2013, 29 January and 9 April 2014, D1, D2, D3 and D4 respectively).

On each sampling occasion, water temperature, pH, TDS, conductivity and dissolved oxygen were measured *in situ* with field instruments (pH 3110 SET 2 incl. SenTix® 41, Oxi 3210 SET 1 incl. CellOx® 325). Water samples were collected, transported in an ice-chest, filtered (Whatman® Glass microfiber filters, Grade GF/F) and the water was analyzed to determine orthophosphate (PO_4-P), nitrate (NO_3-N) and ammonia (NH_4-N) concentrations (Hach® Lange DR 3900).

After sampling, channel width was measured on five transects along a reach of circa 10 m, depth was measured at five points along each transect and surface current velocity was determined by measuring the time taken by a floating object to travel along the reach. Discharge was calculated as the cross-sectional area of the portion of the channel times the average current velocity. The physicochemical parameters of the study sites ate shown in Table 2.

Biological sampling was carried out on all habitats using a Surber net (0.0929 m^2, 500 μm mesh) with the same effort for each replicate with a total of six replicate samples per site. Samples were collected from downstream to upstream at each sampling site, individually allocated to plastic bags, carried out in an ice-chest and refrigerated (5ºC) until processing. In the laboratory the content of each bag was washed through a 500 μm sieve, placed into a tray and all macroinvertebrates were collected and preserved in 70% alcohol.

Table 2. Physicochemical characteristics of the sampling sites during the study period. Values are the average of all sampling dates ± standard deviation with ranges in parenthesis.

Parameters	**L1**	**L2**	**L3**	**L4**
Width (m)	1.55 ± 0.61 (0.74-2.17)	1.77 ± 0.47 (1.66-2.17)	2.62 ± 0.88 (1.49-3.36)	1.35 ± 0.50 (0.96-2.00)
Depth (m)	0.12 ± 0.07 (0.05-0.19)	0.14 ± 0.07 (0.06-0.11)	0.14 ± 0.06 (0.10-0.24)	0.15 ± 0.10 (0.04-0.28)
Current velocity ($m\ s^{-1}$)	0.31 ± 0.19 (0.06-0.52)	0.25 ± 0.18 (0.05-0.44)	0.09 ± 0.07 (0.03-0.19)	0.54 ± 0.50 (0.13-1.25)
Discharge ($m^3\ s^{-1}$)	0.082 ± 0.080 (0.002-0.178)	0.092 ± 0.099 (0.003-0.222)	0.050 ± 0.069 (0.005-0.153)	0.214 ± 0.323 (0.005-0.690)
Temperature (°C)	15.35 ± 2.40 (12.50-18.30)	15.25 ± 2.48 (12.50-18.50)	14.05 ± 1.81 (12.10-16.40)	14.02 ± 2.08 (12.00-16.80)

Parameters	**L1**	**L2**	**L3**	**L4**
Dissolved oxygen (mg L^{-1})	9.57 ± 2.43 (7.49-13.06	8.94 ± 2.68 (6.30-12.57)	8.52 ± 2.74 (5.42-12.11)	10.07 ± 2.27 (8.08-13.33)
Conductivity (μS cm^{-1})	329.5 ± 126.4 (232.0-500.0)	383.2 ± 103.5 (295.0-504.0)	378.5 ± 145.3 (210.0-536.0)	402.2 ± 107.5 (292.0-526.0)
TDS (mg L^{-1})	155.2 ± 57.8 (111.2-234.0)	181.9 ± 47.3 (142.2-238.0)	192.2 ± 50.5 (150.7-254.0)	190.5 ± 49.7 (140.0-249.0)
pH	7.54 ± 0.19 (7.37-7.81)	7.60 ± 0.12 (7.44-7.70)	7.56 ± 0.14 (7.36-7.70)	7.89 ± 0.27 (7.54-8.17)
PO_4-P (mg L^{-1})	0.035 ± 0.032 (0.008-0.072)	0.047 ± 0.025 (0.013-0.071)	0.053 ± 0.051 (0.005-0.114)	0.092 ± 0.098 (0.032-0.235)
NH_4-N (mg L^{-1})	0.82 ± 0.47 (0.21-1.31)	1.31 ± 0.47 (0.89-1.99)	1.08 ± 0.39 (0.60-1.52)	1.21 ± 0.31 (0.75-1.42)
NO_3-N (mg L^{-1})	0.081 ± 0.085 (0.024-0.205)	0.286 ± 0.444 (0.019-0.128)	0.304 ± 0.407 (0.001-0.892)	0.610 ± 1.190 (0.000-2.395)

2.4. Identification and Trait Classification

Identification was carried out to the lowest practicable taxonomic level using the identification key of Tachet et al. (2000). From the 21 species traits provided in this book (Appendix Table 1), five traits were selected for the study: three biological (life cycle duration, resistance form and feeding), one physiological (respiration) and one ecological (locomotion-relationship to the substrate). The *taxa* abundances per trait were calculated by summing all organisms that shared affinity for a certain modality of a trait. When one *taxon* had the same affinity for various modalities of a trait, its abundance was evenly distributed among them.

The trait concerning life cycle duration had only two modalities, (i) ≤ 1 year and (ii) > 1 year. The trait concerning resistance form had four different modalities represented by (i) eggs, gemmules, statoblasts, shells; cocoons; (ii) diapause or quiescence; and (iii) none, but there was no information for some of the *taxa*. The trait concerning feeding method had six different modalities represented by (i) eater of fine sediment; (ii) shredder; (iii) scraper-grazer; (iv) filter feeder; (v) piercer; and (vi) predator. The trait concerning respiration form had four different modalities represented by (i) tegument; (ii) gill; (iii) plastron; and (iv) stigmata. Finally, the trait concerning locomotion-relationship to the substrate had five different modalities represented by

(i) surface swimmer; (ii) swimmer in open water; (iii) crawler; (iv) burrower; and (v) temporary fixation.

The community was characterized in terms of density (nº of individuals m^{-2}), diversity (Shannon diversity index and Margalef community index; PRIMER® version 6.1.13) and eveness (Pielou's evenness index; PRIMER® version 6.1.13). For quality assessment purposes, %EPT (Ephemeroptera, Plecoptera and Trichoptera) abundance and richness, the biotic indexes IBMWP and ASPT were calculated (Alba-Tercedor et al., 2002).

IBMWP is an adaptation of the original BMWP to the Iberian Peninsula ecosystems. The BMWP (Biological Monitoring Working Party) score is an index for assessing the river biological quality using macroinvertebrate species on a presence – absence basis, each family has as a score ranging from 1 (tolerant) to 10 (intolerant) according to its intolerance to pollution (Alba-Tercedor et al., 2002). The final score is the sum of the scores of all families in the sample (Table 3). In order to account for sample size, as larger sample sizes are likely to include more taxa biasing the results, the Average Score Per Taxon (ASPT; Table 4) can be calculated by dividing the total BMWP scores by the number of *taxa*.

The %EPT index (Table 4) is based on the fact that macroinvertebrates from the families Ephemeroptera, Plecoptera and Trichoptera have low tolerance to pollution and represent various functional groups: predators, scrapers and shredders (Lenat, 1988).

Table 3. IBWP classification and interpretation according to the different scores.

IBMWP score	Water quality	Interpretation
> 101	Very good	Unpolluted or no sensitive alterations
61-100	Good	Slightly altered or polluted
36-60	Moderate	Altered or polluted
16-35	Poor	Very polluted or altered
≤ 15	Very poor	Heavily contaminated or altered

Table 4. ASPT and %EPT classification and interpretation according to the different scores.

ASPT score	Water quality	% EPT score	Water quality
≥ 5	Excellent	> 10	Undisturbed
4.9 – 5.4	Very good	6 – 10	Lightly disturbed
4.4 – 4.8	Good	2 – 5	Disturbed
3.7 – 4.3	Moderate	0 – 1	Heavy disturbed
3.0 – 3.6	Poor		
1.0 – 2.9	Very poor		

2.5. Statistical Analysis

Data was analyzed by MDS (multidimensional scaling), cluster analysis and ANOSIM (analysis of similarities) with the software PRIMER® version 6.1.13 and the probability level set at α=0.05. In MDS, the goal of the analysis is to detect meaningful underlying dimensions that allow explaining observed similarities or dissimilarities (distances) between the investigated objects. Cluster analysis is an exploratory data analysis tool which aims at sorting different objects into groups in a way that the degree of association between two objects is maximal if they belong to the same group and minimal otherwise. ANOSIM provides a way to test statistically whether there is a significant difference between two or more groups of sampling units.

The biological data was transformed with log(x+1) and converted to a similarity matrix using the Bray-Curtis coefficient before analysis. MDS achieved by a maximum of 500 iterations, a 0.01 minimum stress and a Kruskal fit scheme 1. For the cluster analysis, agglomeration was achieved with group averages. A two-way (sampling site and occasion) crossed ANOSIM with no replicates was performed with 999 permutations. The transformed matrix was tested with a one-way SIMPER (Similarity Percentages) analysis using the Bray-Curtis coefficient and a cut-off percentage of 90% to examine the percentage contribution of each *taxon* or trait to the similarity within the cluster and to the difference between two clusters. Environmental data was transformed as above and normalized (the values for each variable had their mean subtracted and then divided by their standard deviation) before using the Euclidean distance to calculate the similarity matrix. A Spearman correlation was used to create vectors of the environmental data into the MDS spatial distribution of the biological data.

3. Results

3.1. Macroinvertebrate Community Structure

Macroinvertebrate density across all sampling occasions (Appendix Table 2) ranged from 119 individuals m^{-2} at L1 on date 3 to 66054 individuals m^{-2} at L4 on date 4 (Figure 2, top). Dates 2 and 3 registered the lowest densities while dates 1 and 4 registered the highest. Overall, L3 attained the highest while L1 attained the lowest density. Richness ranged from 6 *taxa* at L1 on date 2 to 30 *taxa* at L1 on date 2 (Figure 2, bottom). Dates 2 and 3 registered the lowest macroinvertebrate richness while dates 1 and 4 the highest richness. Overall, L1 and L2 had the highest while L4 had the lowest richness.

The Shannon diversity index ranged from 0.356 at L3 on date 4 to 2.076 at L1 on date 4, varying both along the longitudinal and the temporal gradients (Figure 3, top) with no specific pattern. Globally, L1 showed the highest while L3 showed the lowest index value. Margalef community index (Figure 3, middle) ranged 1.306 at L1 on date 2 to 4.655 at L1 on date 1. Dates 2 and 4 registered the lowest values while dates 1 and 3 the highest index values. Overall, L1 attained the highest while L4 the lowest value. Pielou’s evenness index (Figure 3, bottom) ranged from 0.128 at L3 on date 4 to 0.908 at L1 on date 3. Dates 3 and 2 attained the highest values while dates 1 and 4 the lowest index values. Globally, L1 registered the highest while L3 the lowest value.

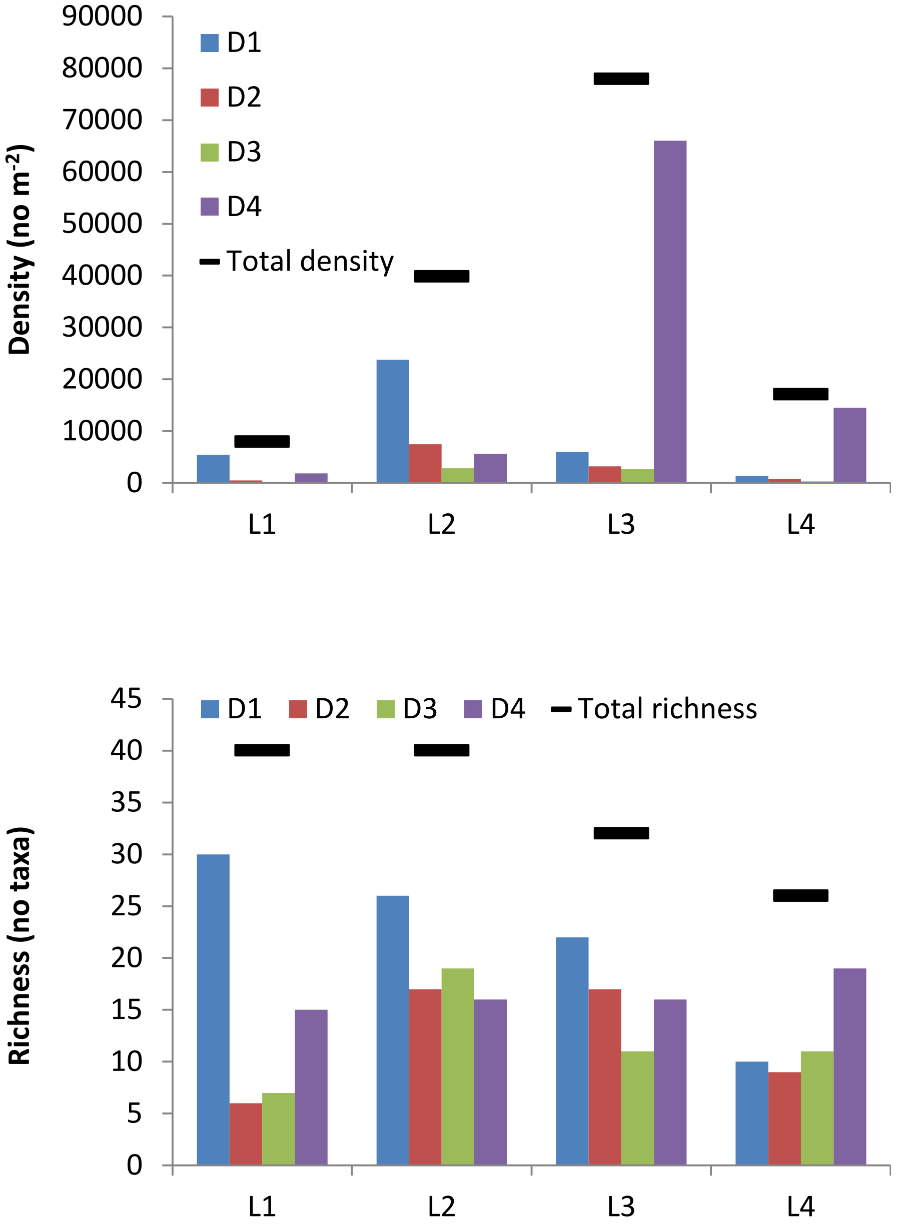

Figure 2. Density (top) and richness (bottom) of the macroinvertebrate community at the four sampling sites. D1, D2, D3, and D4 refer to the sampling dates; Total refers to the total of all four sampling dates at each sampling site.

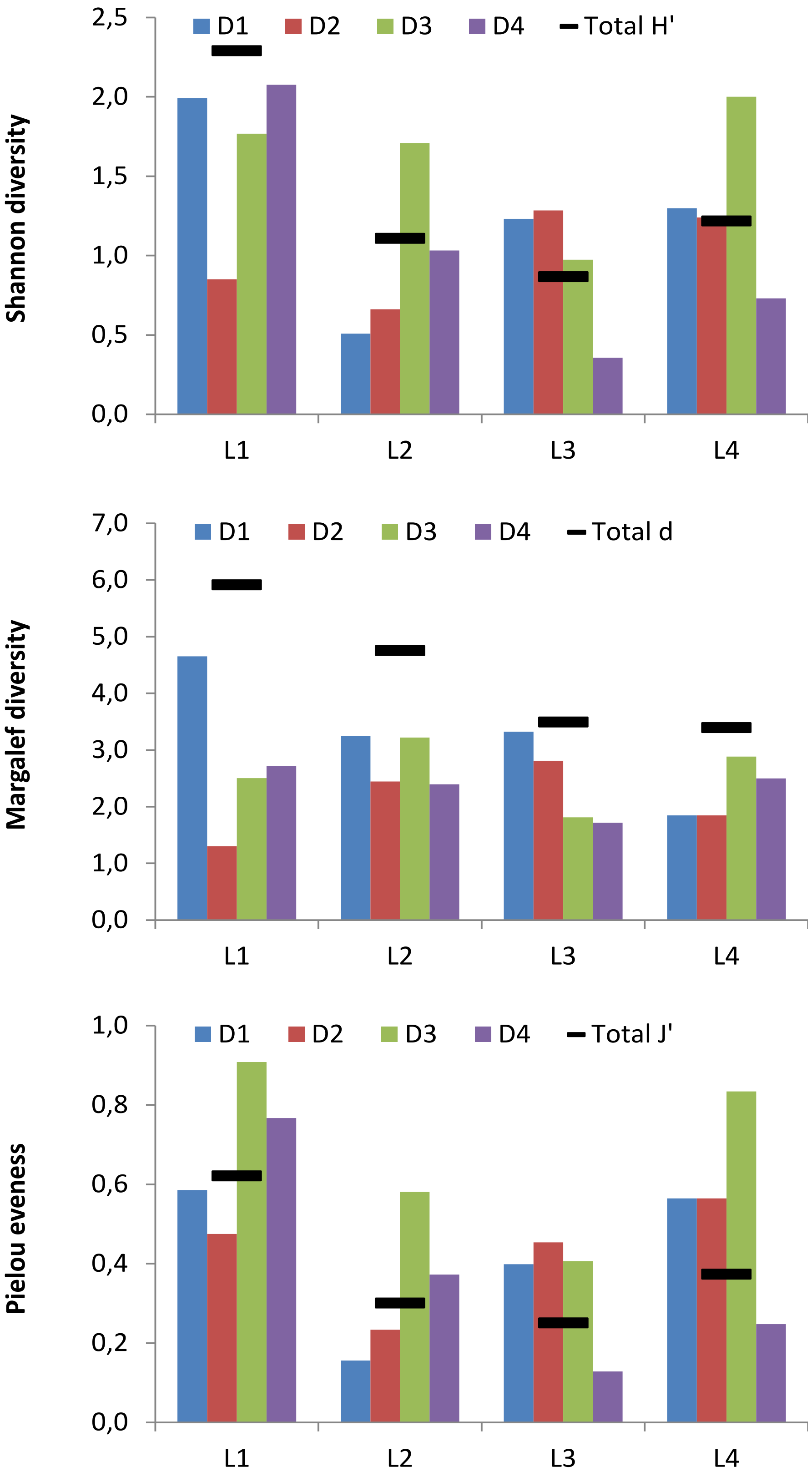

Figure 3. Diversity (Shannon, H' (top) and Margalef, d (middle)) and eveness (Pielou, J' (bottom)) of the macroinvertebrate community at the four sampling sites. D1, D2, D3, and D4 refer to the sampling dates; Total refers to the total of all four sampling dates at each sampling site.

3.2. Environmental Health Assessment

3.2.1. %EPT and Biotic Indices

The percentage abundance of Ephemeroptera, Plecoptera and Trichoptera (EPT) ranged from 0.04% at L2 on date 1 to 68.42% at L4 on date 2 (Figure 4, top). The percentage richness of EPT *taxa* ranged from 3.85% at L2 on date 1 to 33.33% at L1 on date 2 (Figure 4, bottom). EPT percentages showed both longitudinal and temporal variability, with higher values on dates 2 and 3 and on L1 and L4. Based on %EPT scores (Appendix Table 4), the sites L1 and L4 are classified undisturbed while L2 and L3 as heavily disturbed.

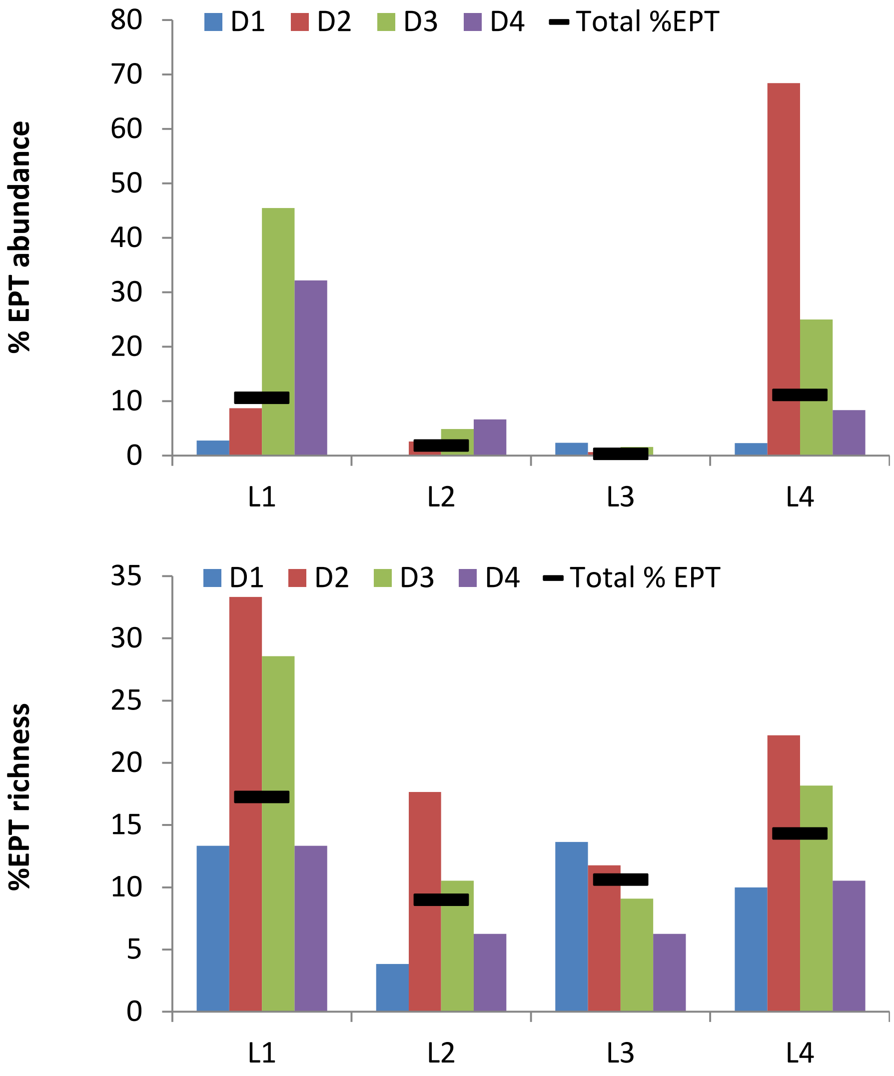

Figure 4. Percentage EPT abundance (top) and richness (bottom) of the macroinvertebrate community at the four sampling sites. D1, D2, D3, and D4 refer to the sampling dates; Total refers to the total of all four sampling dates at each sampling site.

The IBMWP score ranged from 22 at L3 on date 3 to 99 at L2 on date D1 (Figure 5, top). Dates 1 and 4 had the highest while dates 2 and 3 the lowest scores. Overall, L2 had the highest while L4 the lowest score. ASPT score ranged from 3.1 at L3 on date 3 to 4.9 also at L3 on date 1 (Figure 5, bottom). Globally, L2 had the highest while L1 the lowest ASPT score. According to IBMWP scores (Appendix Table 4), the sites L1, L2 and L3 are classified as very good, unpolluted or with no sensitive alterations while the site L4 is classified as good, with slight effects of pollution or disturbance. The ASPT results (Appendix Table 4) classified site L1 with moderated quality and sites L2, L3 and L4 as good quality.

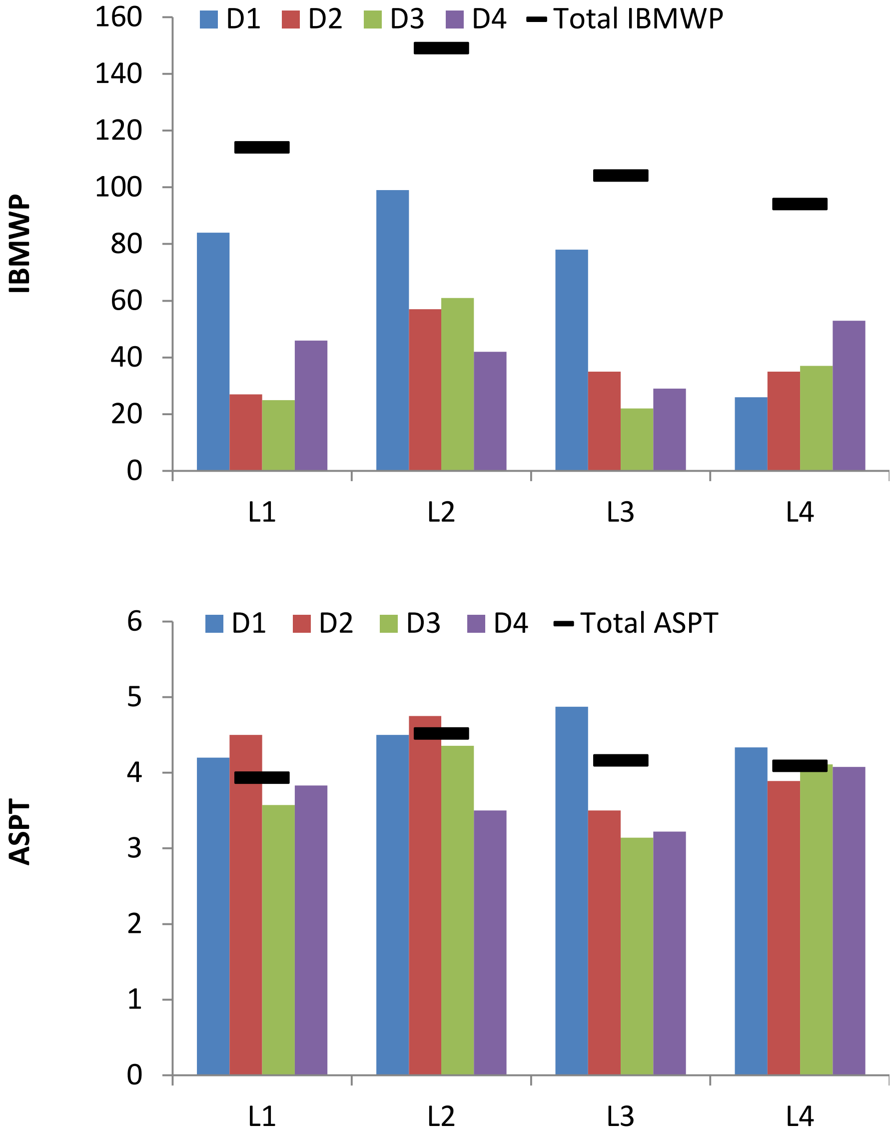

Figure 5. IBMWP (top) and ASPT scores (bottom) of the macroinvertebrate community at the four sampling sites. D1, D2, D3, and D4 refer to the sampling dates; Total refers to the total of all four sampling dates at each sampling site.

3.2.2. Species Traits

The macroinvertebrate community (Appendix Table 3) was dominated by short life-cycles on almost all sites and sampling dates, with the exception of date 4 at L2, L3 and L4 where life-cycles > 1 year where more abundant than life-cycles ≤ 1 year (Figure 6).

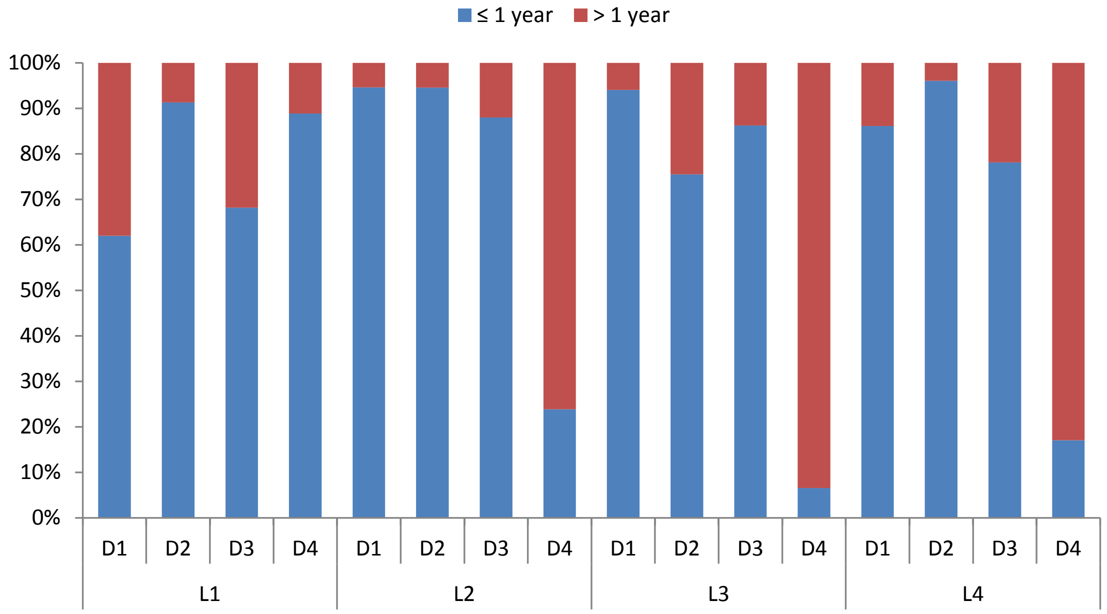

Figure 6. Duration of the life cycle: percentage of macroinvertebrates with life-cycles ≤ 1 year or > 1 year at the four sampling sites. D1, D2, D3, and D4 refer to the sampling dates.

In relation to the resistance form, the macroinvertebrate *taxa* using diapause or quiescence were the most abundant while macroinvertebrate *taxa* using cocoons as resistance forms were the least abundant (Figure 7). There was a tendency for the increase in resistance form using eggs, gemmules, statoblasts or shells along the time. Overall, *taxa* using diapause or quiescence were more abundant at L2, *taxa* using eggs, gemmules, statoblasts or shells at L4, *taxa* using cocoons at L4 and *taxa* without a resistance form at L1.

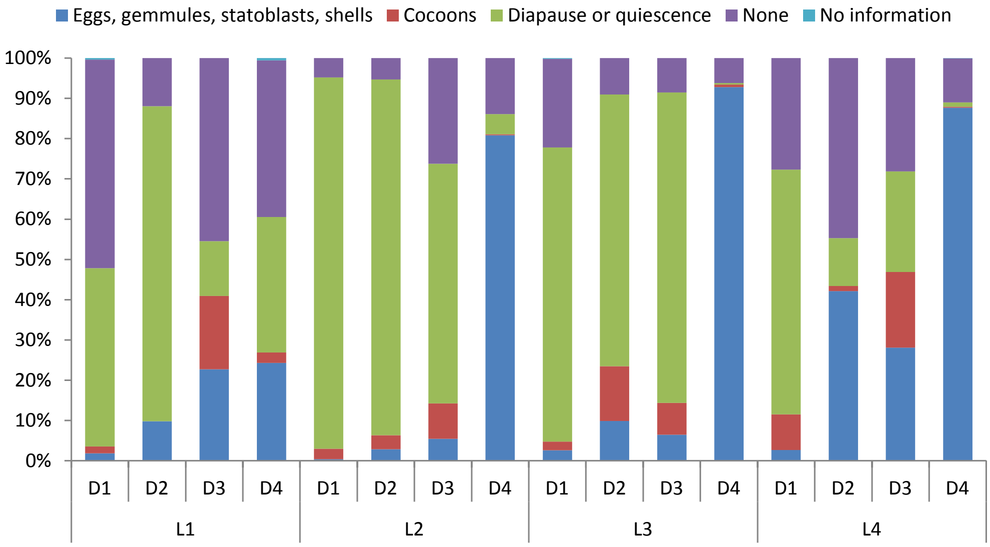

Figure 7. Resistance form: percentage of macroinvertebrates with eggs, gemmules, statoblasts and shells, diapause or quiescence, cocoons or no resistance form at the four sampling sites. D1, D2, D3, and D4 refer to the sampling dates.

Shredders were the most abundant feeding group followed by eaters of fine sediment and grazers-scrapers (Figure 8), except for L4 where grazers-scrapers were dominant. Eaters of fine sediment tended to increase with time, especially at L3 and L4, while shredders and predators decreased. Overall, eaters of fine sediment were most abundant at L3, shredders at L2, and scraper-grazers and filter feeders at L4.

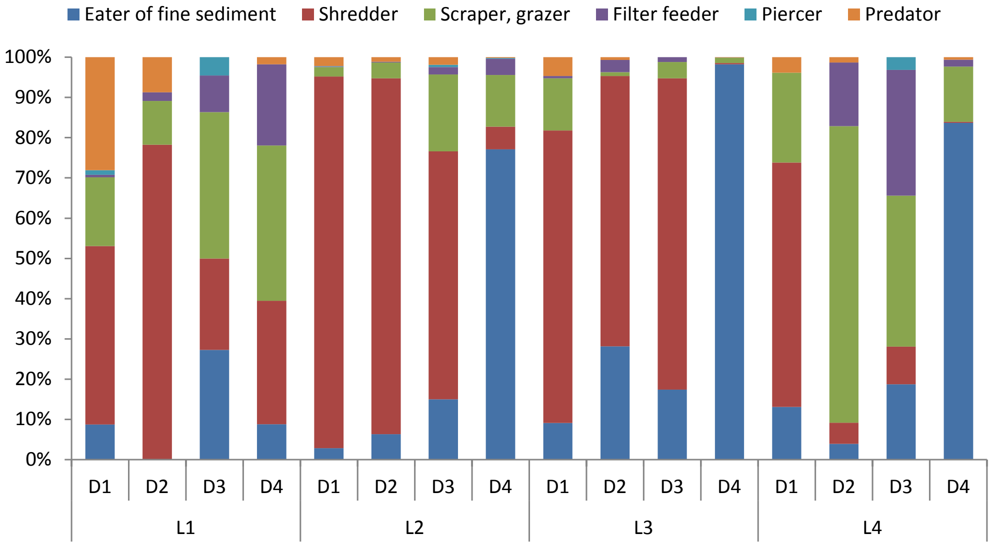

Figure 8. Feeding method: percentage of macroinvertebrates eaters of fine sediment, shredders, scraper-grazers, filter feeders, piercers or predators at the four sampling sites. D1, D2, D3, and D4 refer to the sampling dates.

Gills were the dominant respiration form followed by tegument, which tended to increase downstream and with time (Figure 9). Respiration by tegument, gill and plastron were most abundant on dates 1 and 4 and by stigmata on dates 3 and 4. Overall, respiration by tegument was most abundant at L3, by gill at L2 and by plastron and stigmata respiration at L1.

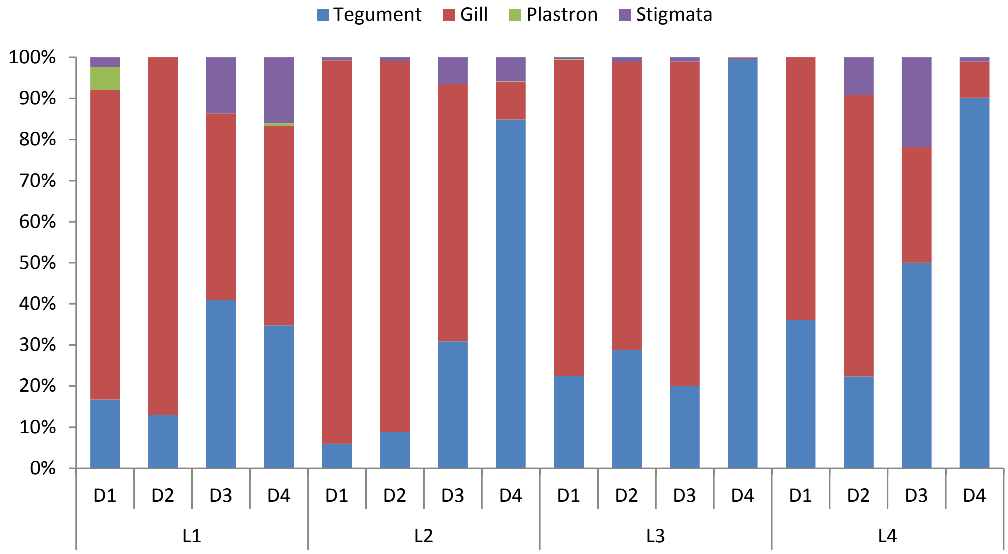

Figure 9. Respiration form: percentage of macroinvertebrates using tegument, gill, plastron or stigmata at the four sampling sites. D1, D2, D3, and D4 refer to the sampling dates.

Regarding locomotion – relation to the substrate, swimmers in open water were the dominant group, and were more abundant at L2 and L3 (Figure 10). Crawlers dominated the community on dates 3 and 4 at L1 and on dates 2 and 3 at L4. Temporary fixation was most abundant at L4, especially on date 3 while burrowers and surface swimmers were most abundant at L1.

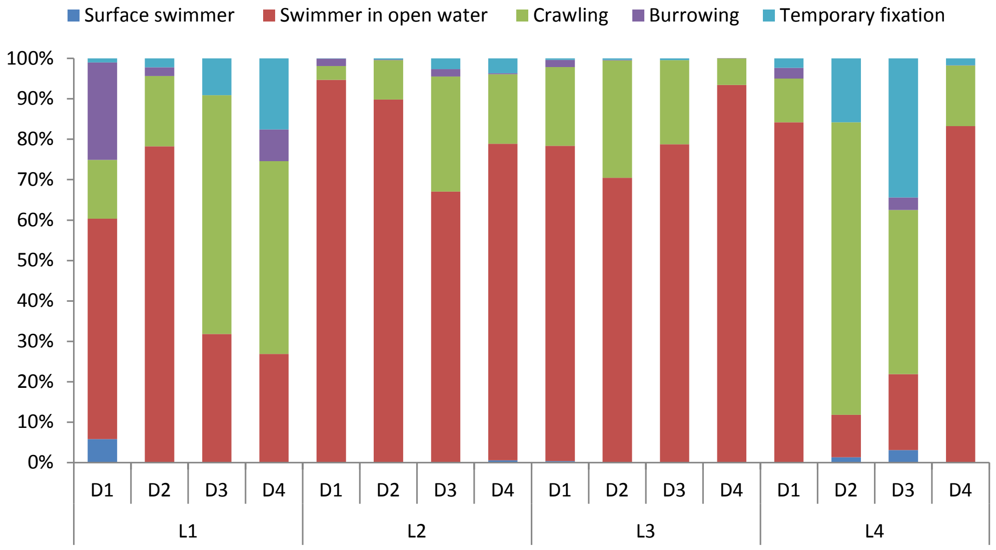

Figure 10. Locomotion – relation to the substrate: percentage of macroinvertebrates surface swimmers, swimmers in open water, crawlers, burrowers or using temporary fixation at the four sampling sites. D1, D2, D3, and D4 refer to the sampling dates.

3.3. Multivariate analyses

3.3.1. *Taxa*

There were no significant differences among sites or dates (ANOSIM, *R*=0.019, *p*=0.388) and *R*=0.190, *p*=0.176, respectively). The MDS distribution shows that the macroinvertebrate community structure is more influenced by sampling date than by site, with samples from date 4 clustered together and most of the samples from date 3 also clustered (Figure 11).

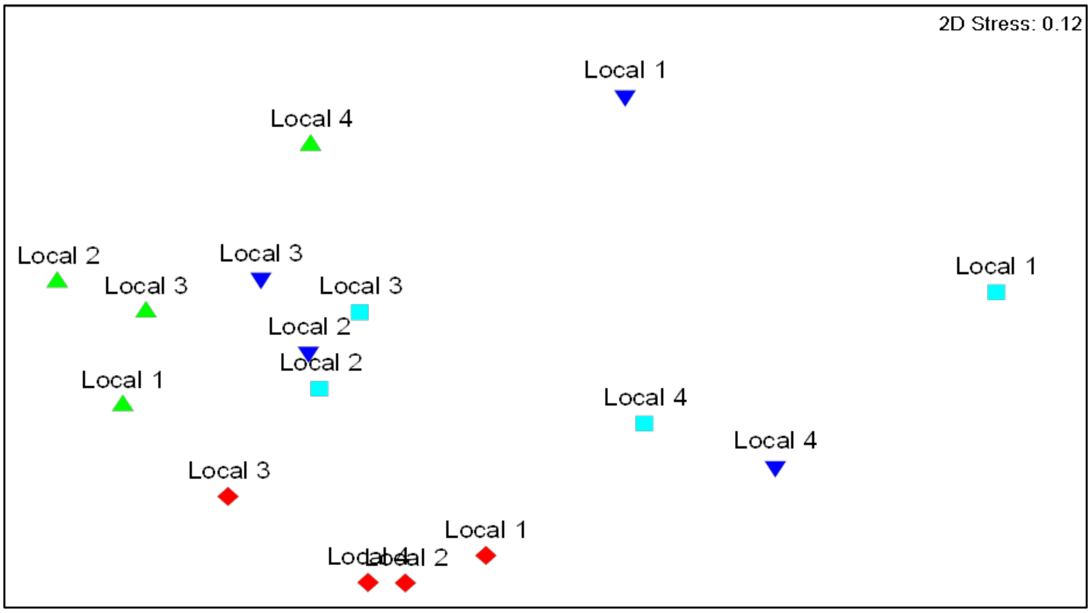

Figure 11. Cluster (MDS) of the similarity matrix of macroinvertebrate abundances per *taxon*. Data was transformed with log (x+1) and the Bray-Curtis coefficient was used to calculate similarity between sites (Local 1, Local 2, Local 3, and Local 4) and sampling dates (▲ D1, ▼ D2, ■ D3, and ♦ D4).

3.3.2. Traits

There were no significant differences (ANOSIM) among sites or dates for any of the individual traits: life cycle duration (Site: *R*=-0.067, *p*=0.618; Date: *R*=0.305, *p*=0.054), resistance form (Site: *R*=0.086, *p*=0.324; Date: *R*=0.229, *p*=0.125), feeding method (Site: *R*=0.057, *p*=0.335; Date: *R*=0.171, *p*=0.195), respiration (Site: *R*=-0.019, *p*=0.479; Date: *R*=0.314, *p*=0.056) nor locomotion and relationship to the substrate (Site: *R*=0.048, *p*=0.379; Date: *R*=0.162, *p*=0.198). When the five traits were analyzed together, there were no significant differences among sites (ANOSIM, *R*=-0.029, *p*=0.540) but there were significant differences between among dates (*R*=0.371, *p*=0.048). At 65% similarity the samples cluster around two groups (Figure 12). At 80% similarity three groups are formed, one containing three samples from date 4, other containing two samples of site 4 and another containing almost all other samples (Figure 12). Table 5 shows the trait modalities which contributed to at least 50% of the similarity among sampling dates.

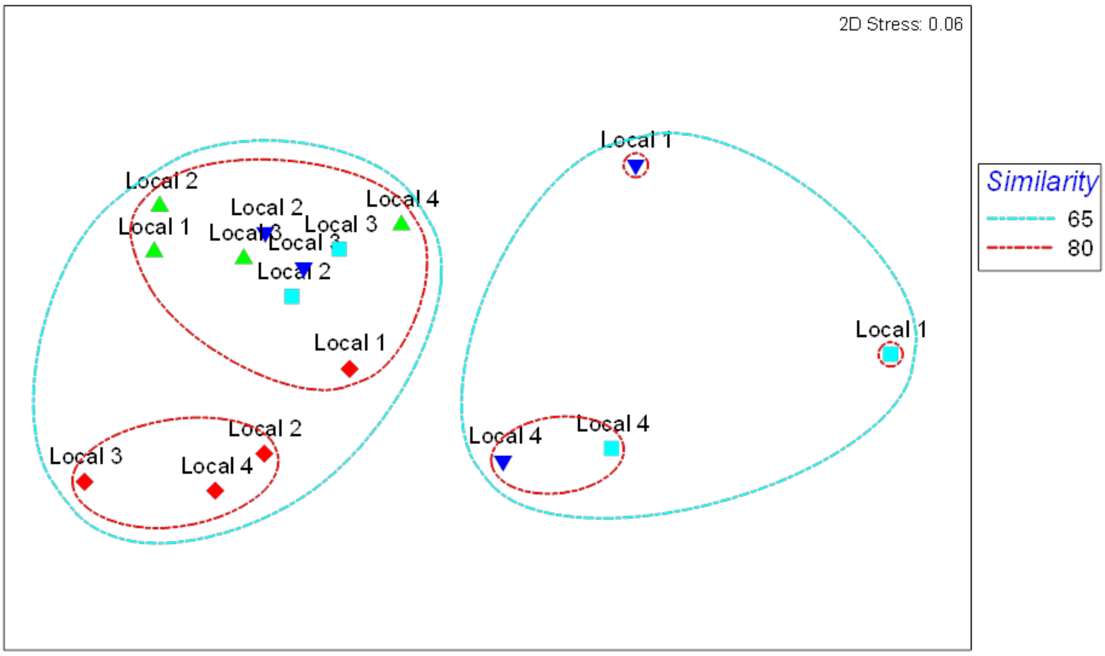

Figure 12. Cluster (MDS) of the resemblance matrix of macroinvertebrate abundances per trait. Data was transformed with log (x+1) and the Bray-Curtis coefficient was used to calculate similarity between sites (Local 1, Local 2, Local 3, and Local 4) and sampling dates (▲ D1, ▼ D2, ■ D3, and ♦ D4).

Table 5. Traits accounting for most of the similarity (≥50%) in the comparison of the dates by cluster analysis.

Date 1 vs. Date 2		**Date 1 vs. Date 3**		**Date 1 vs. Date 4**	
Trait Modality	**Cumulative Contribution (%)**	**Trait Modality**	**Cumulative Contribution (%)**	**Trait Modality**	**Cumulative Contribution (%)**
Burrower	7.82	Predator	8.21	Eggs, shells, gemmules, statoblasts	10.79
Shredder	14.61	Shredder	16.38	Eater of fine sediment	18.22
Swimmer in open water	21.08	Diapause or quiescence	24.25	Shredder	25.28
Predator	27.35	Swimmer in open water	32.01	> 1 Year	32.05
Diapause or quiescence	33.58	Gill	39.42	Diapause or quiescence	38.43
Eater of fine sediment	39.52	Burrower	46.17	Tegument	44.14
Cocoons	45.07	≤ 1 Year	52.57	Burrower	49.38
> 1 Year	50.62			Filter Feeder	54.59

Date 2 vs. Date 3		**Date 2 vs. Date 4**		**Date 3 vs. Date 4**	
Trait Modality	**Cumulative Contribution (%)**	**Trait Modality**	**Cumulative Contribution (%)**	**Trait Modality**	**Cumulative Contribution (%)**
Shredder	8.89	Eater of fine sediment	11.06	Eggs, shells, gemmules, statoblasts	9.97
Diapause or quiescence	17.50	>1 Year	20.92	Eater of fine sediment	18.93
Swimmer in open water	25.68	Eggs, shells, gemmules, statoblasts	30.09	> 1 Year	27.81
Gill	33.47	Tegument	38.72	Tegument	36.05
Eater of fine sediment	40.24	Swimmer in open water	45.61	Swimmer in open water	44.28
≤ 1 Year	46.84	Scraper-grazer	50.62	No resistance form	49.41
Cocoons	53.05			Scraper-grazer	54.22

3.3.3. Abiotic Data

Environmental data was significantly different among dates (ANOSIM, *R* = 0.571, *p*= 0.007) but not among sampling sites (ANOSIM, *R*=0.19, *p*=0.203). Figure 13 shows the MDS spatial distribution. The samples were mostly clustered according to the sampling date and not site. Table 4 shows which variables contributed to at least 70% of the similarity among dates.

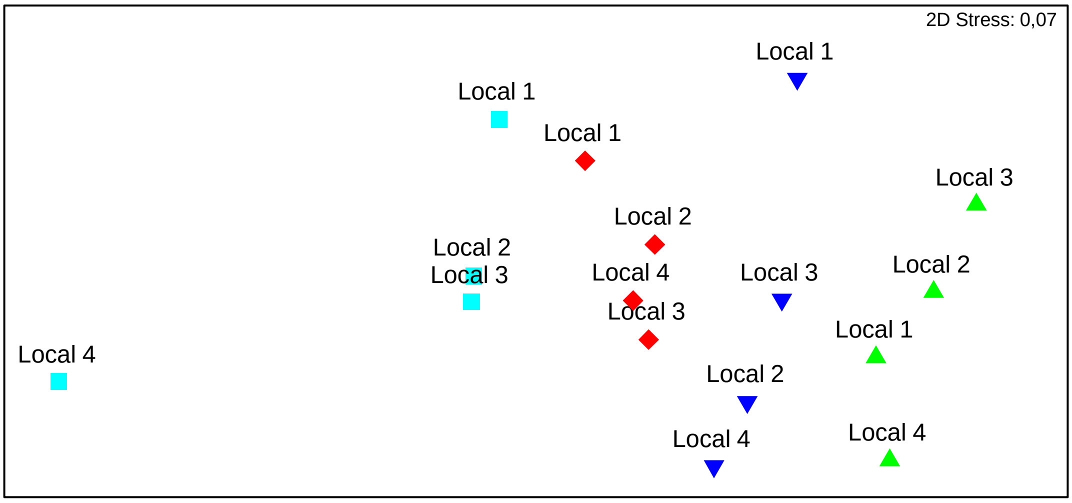

Figure 13. Cluster (MDS) of the resemblance matrix of abiotic data. Data was transformed with log (x+1), normalized, and the Euclidian distance was used to calculate similarity between sites (Local 1, Local 2, Local 3, and Local 4) and sampling dates (▲ D1, ▼ D2, ■ D3, and ♦ D4).

Table 6. Abiotic factors accounting for most of the similarity (≥70%) in the comparison of the sampling dates by cluster analysis..

Date 1 vs. Date 2		Date 1 vs. Date 3		Date 1 vs. Date 4	
Variable	**Cumulative Contribution (%)**	**Variable**	**Cumulative Contribution (%)**	**Variable**	**Cumulative Contribution (%)**
pH	25.07	Dissolved O_2	15.25	TDS	24.75
Nitrate	48.65	Temperature	30.17	Conductivity	44.73
Temperature	71.66	Conductivity	42.12	pH	57.89
		Discharge	53.62	Dissolved O_2	66.84
		Water Velocity	64.67	Temperature	75.68
		TDS	75.40		

Date 2 vs. Date 3		Date 2 vs. Date 4		Date 3 vs. Date 4	
Variable	**Cumulative Contribution (%)**	**Variable**	**Cumulative Contribution (%)**	**Variable**	**Cumulative Contribution (%)**
Discharge	15.40	Nitrate	25.89	Ammonia	24.51
Ammonia	29.56	TDS	42.92	Discharge	40.74
Orthophosphate	43.62	Conductivity	57.48	Water velocity	53.93
Water velocity	54.72	pH	71.18	Orthophosphate	66.51
Conductivity	64.67			Dissolved O_2	77.58
Nitrate	74.30				

3.3.4. Abiotic Data vs. Traits

Figure 14 shows the Spearman correlation between the abiotic factors and traits in the MDS spatial distribution. Orthophosphate, nitrate and pH are more related to the group containing three sites from date 4; discharge, water velocity and dissolved oxygen are more related to the groups containing two dates from L4 and total dissolved solids (TDS), temperature, conductivity and ammonia are more related to the remaining samples.

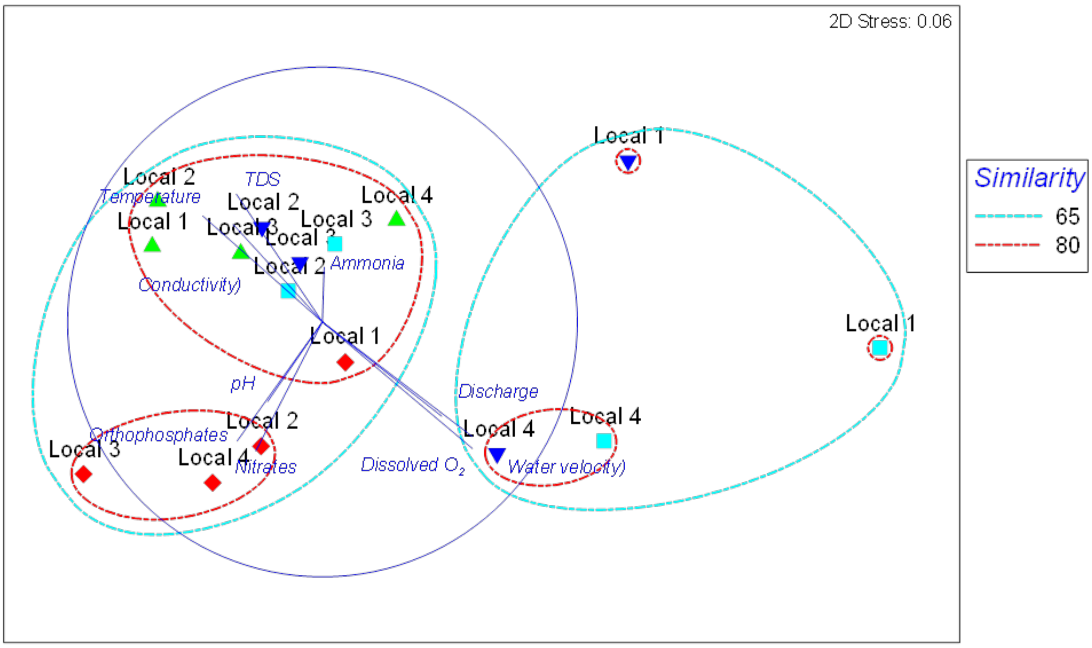

Figure 14. Cluster (MDS) of the resemblance matrix of macroinvertebrate abundances per trait plus the Spearman correlation of the abiotic data (dark blue lines). Data was transformed with log (x+1) and the Bray-Curtis coefficient was used to calculate similarity between sites (Local 1, Local 2, Local 3, and Local 4) and sampling dates (▲ D1, ▼ D2, ■ D3, and ♦ D4).

4. Discussion

Trait-based approaches provide clear advantages compared to the use of taxonomic-based approaches, such as important insights into structure and functioning of stream communities (Usseglio-Polatera et al., 2000) or even dual advantages of direct transferability to distant geographic locations and direct comparability of biologically determined quality standards (Statzner et al., 2001, 2008; Horrigan and Baird, 2008). However, there are still issues needing clarification which difficult their application, such as the lack of adequate understanding of how individual traits are intercorrelated (Poff et al., 2006) and the alternative trade-offs between traits combinations (e.g., Resh et al.,1994, Usseglio-Polatera et al., 2000).

The objective of the present work was to compare trait-based results with those obtained with conventional tools related to community structure (community density, diversity and evenness) and environmental health assessment (IBMWP, ASPT, %EPT) in order to allow some insight into the way how species traits may be used to identify urban stream disturbance. The results related with community structure indicated that the macroinvertebrate community was more diverse and the abundances were more evenly distributed at L1, the sampling site closer to the source and less modified, with a natural channel shape and good riparian vegetation. L2 was the second more diverse but one of the worst in relation to evenness, which is probably related to the high environmental variation observed during the four sampling occasions. In this site, at late summer the stream channel was filled with macrophytes while during winter, and especially after the heavy rains of December-January, the channel was filled with debris of a fallen bridge. Also, the high abundance of snails (*Potamopyrgus antipodarum*) contributed to the uneven abundance distribution. L3 was the second less diverse site and the worst in terms of evenness. This was related to the slower current velocity (due to the downstream dam), the fine sediment composing the substrate, and consequent high abundance of Oligochaeta, mainly Naididae. L4 was the less diverse but had one of the highest evenness. The low diversity is probably related to the fact that this is the sampling site farther away from the source and heavily modified both in terms of river channel and riparian vegetation. However, the evenness of the community suggests that, despite the habitat alterations, a well-established macroinvertebrate community is thriving in that environment.

Community richness was higher at the less disturbed site (L1) and decreased along the longitudinal gradient – where multiple stressors contributed to increasingly disturbed habitats. These results show an opposite trend to the ones obtained by McCabe and Gotelli (2000), who found higher richness disturbance treatments than in undisturbed controls, and also contradict the pattern predicted by the dynamic-equilibrium model of Huston’s (1979, 1994) for communities with the species populations growing rapidly and having high rates of competitive exclusion. However, the results obtained in this study seem to support the intermediate-disturbance hypothesis, with diversity peaking at an intermediate level of disturbance (L1) and decreasing with increasing disturbance downstream. Evidences for the intermediate-disturbance hypothesis have been found at communities with high rates of competitive displacement (Huston, 1994).

The three indexes differed on the quality classification attributed to each site. The %EPT showed the existence of heavy disturbance on L2 and L3 while both IBMWP and ASPT classified both sites as very good quality or with no sensitive alterations (IBMWP) or as good quality status (ASPT). L1 ranged from good quality (ASPT), to unpolluted with no sensitive alterations (IBMWP) and to undisturbed (%EPT), mainly due to the abundance of intolerant taxa. In fact, it was the only site where Plecoptera were found (*Nemoura* sp.). L2 had the best IBMWP and ASPT scores but %EPT abundance and richness were among the lowest, granting a classification of heavily disturbed. This may be related, as discussed above, with the drastic seasonal variation observed at this location. Similarly, L3 was also well classified in terms of IBMWP and ASPT scores, with good to moderate quality but the %EPT abundance was the lowest granting a classification of heavily disturbed. As discussed above, the characteristics of the river bed and the reduced flow might have prevented the existence of a more diverse macroinvertebrate community, especially the members of the EPT group. According to IBMWP and ASPT, L4 had moderate to good quality status with some slight effects of pollution or alteration but had the highest percentage of members of the EPT group and the second best richness among them. These results are in agreement with the general objectives of the indices, with IBMWP and ASPT mainly assessing the effect of organic pollution (Hawkes, 1998) while several EPT members are reasonable tolerant to nutrient enrichment (Hall and Lenwood, 2006).

As in other studies where taxonomic data suggested only a weak and inconsistent response of biodiversity to hydromorphological impact in the lotic environment (e.g. Feld et al., 2014, Gerisch et al., 2011), in the present study taxonomic data did not distinguish habitat alterations due to either temporal or spatial variability. The same was true for the single trait analyses which revealed no significant differences among sampling sites or dates. Regarding the feeding method, the community was dominated by shredders in almost all sampling dates at sites L1 to L3, revealing that although this feeding group may be abundant in impacted streams (Tolkkeinen et al., 2013) it does not overcome the multiple stressors related to habitat alteration at L4.

The combined use of the five traits allowed the separation of dates, thus revealing that the combination of traits is more sensitive to environmental variation than its individual counterparts. Environmental variability related to date was more pronounced than environmental variability related to sampling site, which explains the absence of significant differences among the four sampling sites – related to the inexistence of heavy distortions along the longitudinal gradient of the stream, and the significant differences among the sampling occasions – related to the high seasonal variability during the study period, especially regarding precipitation events and consequent effects in the stream habitat.

The high precipitation events which occurred before sampling date 3 caused alterations in water chemistry (ammonia, nitrates and orthophosphates), as well as pH, TDS and conductivity, which clustered the combination of traits in the MDS and cluster analysis. Additionally, the occurrence of high densities of *taxa* such as *Naididae* sp. in only the last sampling occasion introduce a strong temporal rather than a spatial trend. Those highly abundant but temporally concentrated *taxa* were dominated, in terms of traits, by resistance in the form of eggs, gemmules, statoblasts, and shells, by feeders of fine sediment, by tegument respiration, and by swimmers in open water. Thus, these traits also contributed significantly for the separation of the data by sampling date instead of sampling site.

The multi-trait MDS analysis (Figure 12) provided no insight into the relation of species traits and conventional community and water quality assessment methods, since the groups included highly variable classifications of water quality based on %EPT, IBMWP and ASPT and also community diversity and evenness. The biggest group formed by 80% similarity corresponded to quality classifications of the %EPT and the biotic indices ranging from poor to very good and to community diversity and evenness ranging from low to high. The second biggest group formed by three samples from date 4 (L2, L3 and L4) ranged from poor to moderate quality with medium to high diversity compared and the worst abundance distributions. The group containing the remaining samples at 65% similarity (Figure 12) ranged poor to good quality and from medium to high diversity and the best abundance distribution of species.

In conclusion, although the use of the five traits did not allow a spatial separation of the study sites, it clearly separated sampling dates whose environmental variables were also distinct. Thus, the use of traits allows distinguishing samples that are clearly associated to variables related to habitat characteristics. The lack of a pronounced quality gradient along the longitudinal stream profile limited the possibility of finding significant differences among sites. Moreover, because sampling was carried out only four times, and did not complete a full seasonal cycle, some of results obtained could not be attributed to a natural pattern or to the effect of perturbation, such as urbanization effects or land use. A more complete data set collected at sites with a more pronounced quality gradient is necessary to assess the utility of the trait-based approaches on the evaluation of disturbance in urban streams.

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6. Appendix

Appendix Table 1. List of the 21 species traits classified by Tachet et al. (2000). The traits ‘duration of life-cycle, resistance form, feeding method, respiration and locomotion and relationship to the substrate were used in the presented study.

Trait	Modalities	Trait	Modalities
Maximum size	• < 2.5 mm • 2,5 - 5,0 mm • 5,0 - 10,0 mm • 10,0 - 20,0 mm • 20,0 - 40,0 mm • 40,0 - 80,0 mm • > 80,0 mm	Trophic extent	• Oligotrophic • Mesotrophic • Eutrophic
Duration of life cycle	• ≤ 1 year • > 1 year	Saprobic value	• Xenosaprobe • Oligosaprobe • Beta mesosaprobe • Alpha mesosaprobe • Polysaprobe
Potential number of reproduction cycles per year	• < 1 • 1 • >1	Salinity	• Fresh water • Brackish water
Aquatic stage	• Egg • Larvae • Nymph • Imago	Biogeographic area (*Limnofauna europaea*)	• 2: Pyrenees • 4: Alps and Jura • 8: Central Massif and Vosges • 13a: lowlands (oceanic) • 13b: lowlands (Mediterranean)
Reproduction (sexual and asexual)	• Ovoviviparity and care of the offspring • Free isolated eggs • Fixed isolated eggs • Free egg clutches • Fixed egg clutches • Endophytic egg clutches • Terrestrial egg clutches • Asexual reproduction • Parthenogenesis	Altitude	• Plain and hill (<1000 m) • Mountain (1000-2000 m) • Alpine (>2000 m)
Dissemination	• Aquatic, passive • Aquatic, active • Aerial, passive • Aerial, active	Longitudinal distribution	• Spring stream • Epirhithron • Metarhithron • Hyporhithron • Epipotamon • Metapotamon • Estuary • Off river hydrosystem

Trait	Modalities	Trait	Modalities
Resistance form	• Eggs, gemmules, statoblasts, shells • Cocoons • Stall against desiccation • Diapause or quiescence • None	Transverse distribution in the channel	• River channel • Banks, side channels • Etangs, ponds, abandoned meander • Marshes, peatlands • Temporary waters • Lakes • Underground habitat
Food type	• Fine sediment and microorganisms • Debris < 1 mm • Vegetable debris > 1 mm • Living microphytes • Living macrophytes • Dead animals > 1 mm • Living microinvertebrates • Living macroinvertebrates • Vertebrates	Preferential microhabitat	• Slabs, blocks, Stones, pebbles • Gravel • Sand • Silt • Macrophytes, filamentous algae • Microphytes • Branches, roots • Litter • Mud
Feeding method	• Absorption through teguments • Eater of fine sediment • Shredder • Scraper, grazer • Filter feeder • Piercer • Predator • Parasite	Locomotion method and relationship to the substrate	• Flier • Surface swimmer • Swimmer in open water (plankton, nekton) • Crawler • Burrower (epibenthic) • Endobenthic (interstitial) • Temporary fixation • Permanent attachment
Respiration	• Tegument • Gill • Plastron • Stigmata (aerial respiration) • Hydrostatic vesicles	Preferential current velocity	• None • Slow (< 0.25 m/s) • Medium (0.25 – 0.30 m/s) • Fast (> 0.50 m/s)
Temperature	• Stenothermal psychrophilic (<15˚C) • Stenothermal thermophilic (>15˚C) • Eurythermal	pH	• < 4 • 4,0 - 4,5 • 4,5 - 5,0 • 5,0 - 5,5 • 5,5 - 6,0 • > 6

Appendix Table 2. Abundance (sum of six replicates; number per 0.557 m^2) of the macroinvertebrates sampled during the study period. L=site; D=sampling date.

Genus / Species	L1D1	L1D2	L1D3	L1D4	L2D1	L2D2	L2D3	L2D4	L3D1	L3D2	L3D3	L3D4	L4D1	L4D2	L4D3	L4D4
Dugesia		3			5				3			1		1		
Enchytraeidae			2							1		1				
Eiseniella tetraedra	6			4	54	11	5	1	8	13	3	28	8		4	1
Naididae				5				396				5679				1112
Tubificidae						1	13		1	3	4	5			1	1
Lumbriculidae	5				4	23	11	1	6	47	25	13	7	2	1	3
Ancylus fluviatilis					5				1				25	6	1	1
Physa	6				26	4	7	1	5	1		1				1
Potamopyrgus antipodarum	218	36	1	38	2018	607	153	13	400	196	189	20	79	2	2	2
Pisidium	1				2				3	8	2					
Gammaridae										1						
Procambarus clarkii	1				20				3							
Baetis	10	3	4	43		16	11	35		1	4	3		50	7	112
Cloëon	1								10							
Pseudocentroptilum pennulatum										1						
Habrophlebia																1
Nemoura			1	12												
Boyeria irene					2											
Calopteryx					4	3										
Coenagrion pro parte	1															
Ischnura									3							
Pyrrhosoma nymphula	3															
Cordulegaster	117	1			13		1		1				3			
Gomphidae									6							
Onychogomphus							1									
Sympecma					1											
Nepa cinerea	1															
Microvelia	1							1								
Velia	1				1										1	
Dryops (adult)	3			2	8			1								
Dryops (larvae)				1												
Dytiscus (adult)					1											
Meladema (larvae)			1				1									
Platambus												1				
Oulimnius (larvae)	18								2							
Oulimnius (adult)	37															
Haliplus (larvae)	4				2											
Haliplus (adult)																
Anacaena (adult)					1											
Laccobius (adult)	1															
Elodes																1
Calamoceras									1							
Diplectrona felix		1												2		
Hydropsyche					1											
Oxyethira	1															
Ylodes						1										
Tinodes						1										
Metalype fragilis	2						2		2				3		1	
Chironomini	14		1		4	8	4	4	22	10	9	290	2	1		2
Tanytarsini	15			5		1	4	1	8	9	2	6				9
Orthocladiinae	12	2		17	17	6	31	31	57		6	75	1		3	70
Tanypodinae	19				13	5	3		11	1		5	1			4
Ceratopogoninae				2						1		1	1			2
Dasyheleinae	3						1			1						
Culicinae									1							
Dixa								2								
Hemerodroniinae	2			1												1
Ephydridae				9												
Limoniini	2															
Eriopterini	1			1	1		2	1								
Psychodidae					1	3	11	16	1	3	2					1
Acanthocnema					1			1								1
Simuliini	2		1	30		1	5	19		1	1			10	10	23
Stratiomyidae					1	2						1		2		
Tabanidae							1									
Tipulidae				1	3	1									1	
Total	508	46	11	171	2209	694	267	524	555	298	247	6130	130	76	32	1348
Richness	30	6	7	15	26	17	19	16	22	17	11	16	10	9	11	19
Density n°/m^2	5474	496	119	1843	23803	7478	2877	5646	5980	3211	2662	66054	1401	819	345	14525
Shannon diversity Index (H')	1.99	0.85	1.77	2.08	0.508	0.66	1.71	1.03	1.23	1.29	0.97	0.356	1.3	1.24	2	0.73
Margalef diversity Index (d)	4.66	1.31	2.5	2.72	3.247	2.45	3.22	2.4	3.32	2.81	1.82	1.72	1.85	1.85	2.89	2.498
Pielou's evenness Index (J')	0.59	0.47	0.91	0.77	0.156	0.23	0.58	0.37	0.4	0.45	0.41	0.128	0.56	0.56	0.83	0.248

Appendix Table 3. Abundance of macroinvertebrate (sum of six replicates; number per 0.557 m^2) per trait modality. L=site; D=sampling date; trait numbers are the same used in Tachet et al. (2000).

Traits	L1D1	L1D2	L1D3	L1D4	L2D1	L2D2	L2D3	L2D4	L3D1	L3D2	L3D3	L3D4	L4D1	L4D2	L4D3	L4D4
1 - ≤ 1 year	315	42	8	152	2091	657	235	125	522	225	213	402	112	73	25	230
2 - ≥ 1 year	193	4	4	19	118	38	32	399	33	73	34	5728	18	3	7	1118
1 - Eggs, gemmules, statoblasts, shells	10	5	3	42	8	20	15	424	15	30	16	5688	4	32	9	1182
2 - Cocoons	9	0	2	5	58	24	24	2	12	41	20	41	12	1	6	4
4 - Diapause or quiescence	225	36	2	58	2037	613	159	26	406	201	191	22	79	9	8	14
5 - None	263	6	5	67	107	37	70	73	122	27	21	380	36	34	9	148
No information	2	0	0	1	0	0	0	0	1	0	0	0	0	0	0	1
2 - Eater of fine sediment	45	0	3	15	63	44	40	404	51	84	43	6022	17	3	6	1128
3 - Shredder	225	36	3	53	2040	614	165	30	404	200	191	22	79	4	3	4
4 - Scraper, grazer	87	5	4	66	52	28	51	68	72	3	10	79	29	56	12	185
5 - Filter feeder	3	1	1	35	3	1	5	21	3	9	3	0	0	12	10	23
6 - Piercer	6	0	1	0	3	0	2	1	0	0	0	1	0	0	1	0
7 - Predator	143	4	0	3	49	8	5	1	26	2	0	7	5	1	0	8
1 - Tegument	85	6	5	60	132	61	83	445	125	86	50	6104	47	17	16	1216
2 - Gill	383	40	5	83	2061	627	167	48	428	209	195	24	83	52	9	118
3 - Plastron	29	0	0	1	3	0	0	0	1	0	0	0	0	0	0	0
4 - Stigmata	12	0	2	28	14	7	18	31	2	4	3	2	0	7	7	14
2 - Surface swimmer	30	0	0	0	2	1	0	3	2	0	0	1	0	1	1	0
3 - Swimmer in open water	277	36	4	46	2091	623	179	411	433	210	195	5726	110	8	6	1123
4 - Crawler	74	8	7	82	75	68	76	90	108	87	52	403	14	55	13	202
5 - Burrower (epibenthic)	123	1	0	14	41	1	5	2	10	1	0	1	4	0	1	1
7 - Temporary fixation	5	1	1	30	1	2	7	19	2	1	1	0	3	12	11	23

Appendix Table 4. %EPT abundance and richness, IBMWP and ASPT scores at the four sampling sites. Total refers to the percentages or scores calculated on basis of the sum of the abundances or *taxa* of all dates.

%EPT abundance	**L1**	**L2**	**L3**	**L4**
Date 1	2,8	0,0	2,3	2,3
Date 2	8,7	2,6	0,7	68,4
Date 3	45,5	4,9	1,6	25,0
Date 4	32,2	6,7	0,0	8,4
Total	10,6	1,8	0,3	11,1
%EPT richness	**L1**	**L2**	**L3**	**L4**
Date 1	13,3	3,8	13,6	10,0
Date 2	33,3	17,6	11,8	22,2
Date 3	28,6	10,5	9,1	18,2
Date 4	13,3	6,3	6,3	10,5
Total	17,2	9,0	10,6	14,3
IBMWP	**L1**	**L2**	**L3**	**L4**
Date 1	84	99	78	26
Date 2	27	57	35	35
Date 3	25	61	22	37
Date 4	46	42	29	53
Total	114	149	104	94
ASPT	**L1**	**L2**	**L3**	**L4**
Date 1	4,2	4,5	4,9	4,3
Date 2	4,5	4,8	3,5	3,9
Date 3	3,6	4,4	3,1	4,1
Date 4	3,8	3,5	3,2	4,1
Total	3,9	4,5	4,2	4,1