

LAND-USE INFLUENCE ON HYPORHEIC BIOTA FROM MEDITERRANEAN STREAMS IN CENTRAL SPAIN

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ABSTRACT. Detailed knowledge of hyporheic zone (HZ) biota response to change in land use is crucial for understanding the ecohydrological functioning of communities within the river corridors. This paper investigates the response of hyporheic crustacean communities in relation to spatial heterogeneity in water conditions under changes in land use of the alluvial floodplain of the Jarama basin in central Spain. The study is conducted in four streams of the basin under distinct local land-use and water resource protection conditions: i) preserved forested natural sites at river headwaters where critical river ecosystem processes were unaltered or less altered by human activities, and ii) sites with different degrees of anthropogenic impact from agriculture and urban/industrial activities in the lowland. The results indicate that streams draining forest and semi-natural areas were characterized by cold and pristine hyporheic waters and crustaceans' communities harbor a well-developed stygobite fraction. Conversely, intensive agricultural practices in the lowland cause nutrient enrichment of hyporheic waters. Thus, this type of land use increases the diversity and abundance of non-stygobites, whereas the abundance of stygobites is decreased. Mixed activities, industrial and urban development and agricultural cause an extreme decline of the crustacean community and/or species loss due to a combined effect of increase of nitrites, ammonia, trace metals and volatile organic compounds, and a deleterious decline of dissolved oxygen reaching hypoxic and/or anoxic hyporheic water conditions. The combined information of spatial variability of hyporheic biota has the potential to improve the understanding of impacts caused by changes in land-uses on HZ water conditions.

Key words: *land-use, hyporheic zone, crustacean, river management, Spain.*

INTRODUCTION

The hyporheic zone (HZ), located at the interface between surface and groundwater and plays a significant role as ecosystem service provider for river ecosystems (Boulton, 2008). This zone is an important component of river ecosystems controlling the water exchanges between surface and groundwater and ensures the cycling of carbon, energy, and nutrients and provides a habitat for benthic and hyporheic invertebrates (Danielopol, 1989; Dole-Olivier et al., 2009; Iepure et al., 2013). Furthermore, it serves as buffer and bioremediation zone for distinct pollutants mainly organics (Dole-Olivier et al., 2009). If changes in land use occur in alluvial plains, the hyporheic habitat may undergo severe disturbance reflected in loss of ecosystem services (Boulton, 1997, 1998; Hancock, 2002). This occurs via direct contamination of the surface/hyporheic waters or by polluted groundwater discharge in stream bed sediments (Conant et al., 2004; Chapman et al., 2007; Kalbus et al., 2007, 2009) by loading suspended sediments (Kuhnle et al., 2001), phosphorous (Cooke and Prepas, 1998), organic matter (Strayer et al., 2003) and trace metals (Mösslacher, 1988; Plénet et al., 1995; Moldovan et al., 2011; Iepure et al., 2013). In addition, overexploitation in the alluvial plain causes a disruption of hydrological exchange pathways between surface, groundwater, and the riparian zone of river ecosystems (Ward et al., 1998; Gibert et al., 1990; Tockner et al., 2000). Overall, the alteration of both river channels and their alluvial plain functionality endorses changes of the main functional role of the hyporheic zone of rivers: to warrant a good quality and 'health' for lotic ecosystems (Boulton et al., 2010).

The water quality in river ecosystems impacts the hyporheic community composition and the abundance of different groundwater dwellers (Dole-Olivier et al., 2009). Recent exhaustive studies in remote lotic ecosystems have shown that increasing agricultural practices (i.e. pasture, logging), intensive deforestation and rivers channel alterations cause a decline in hyporheos diversity and/or abundance (Gibert, 1991; Gibert et al., 1995; Notenboom et al., 1994; Boulton et al., 1998; Mösslacher and Notenboom, 1999; Illyová et al., 2011; Di Lorenzo and Galassi, 2013). They also revealed that the strong modification of alluvial floodplains for agricultural purposes causes a decline of obligate groundwater species (stygobites) that are mainly present in pristine streams draining native forested areas (Boulton et al., 1998). Intensive urban industrial activities also contribute to the impairment of hyporheic waters (especially with trace metals and volatile organic compounds) that will promote a reduction in groundwater communities' diversity further (Mösslacher, 1988; Plénet and

Gibert, 1994; Gibert et al., 1995; Plénet, 1995; Plénet et al., 1995, 1996; Claret et al., 1999; Marmonier et al., 2000; Iepure and Selescu, 2009; Steube et al., 2012; Iepure et al., 2013).

The Jarama basin located in central Spain (a tributary of the river Tago from northwest) has an extended alluvial floodplain and has a great economic significance for agriculture and industry (Bastida, 2009). The basin has been subject to a variety of land-use changes during the last century (Llamas, 2007). After '30 almost all headwaters streams of the basin were affected by geomorphological changes through construction of large dams, or the channelization of rivers for flow regulation purposes. This has altered the downstream flows and discharges and has changed the erosion processes and sedimentary dynamics of their tributaries. During the '50 the alluvial floodplains were prone to alterations from the primary use of soil for forestry and rural shifted to agricultural and industrial purposes (Vizcaíno et al., 2003; Llamas, 2007). Furthermore, the intensive industrialization in the '50' in the region caused an increase of urban development, an extension of residential areas and of large-scale industrial and commercial areas, and an enhanced infrastructure for transport (Alcolea and Garcia Alvarado, 2006).

Nowadays, the main pressures that pose risks to the Jarama basin occur in the lowland alluvial floodplain and stem from both industry and agricultural practices (Bastida, 2009). River water is directly affected by emissions from wastewater treatment plants (WWTPs) (approx. 5000) that process the water discharged from large urbanizations and the metropolitan area of Madrid (with > 6.5 mil. inhabitants) and from industrial plants. Besides a large number of contaminants from industry (i.e. trace metals, hydrocarbons and volatile organic compounds) or contaminants emerged from personal care products and micro drugs (recently detected for > 80 groups of such products) (Martínez-Bueno et al., 2010; Hernando et al., 2011) alters the rivers water quality and their associated aquifers.

The intensive practice of gravel extractions (for conglomerates or mining) puts additional pressure on alluvial aquifers and triggers large fluctuations (± 3 m) of the shallow aquifers (Llamas, 2007; Bastida, 2009). These practices also cause a direct impact on discharge of river channels by removing water and in-stream sediments. 2/3 of a total of 50 gravel bar extractions in the Madrid area in 2003 were located in the Jarama River only (Blanco-Garcia et al., 2004). Moreover, some of them have changed their initial exploitation locations leaving a large number of scattered gravel pits and residual lagoons, which were left with minimal or inadequate management and restoration measurements (Martínez-Pérez and Sastre, 1999). Additional risks upon the river-alluvial aquifer system are also triggered by intensive groundwater withdrawal for irrigation and illegal aquifers exploitation (Bastida, 2009).

These long-term and persistent activities in the Jarama river and the alluvial floodplain sum up to a mosaic of different land-use terrains which nowadays alternate from pristine areas at the headwaters to areas with mixed anthropogenic activities from agriculture and industry in the lowlands (Bastida, 2009). They affect the river ecosystems further, by changing the water quality at both the surface and the hyporheic zone, and with negative consequences for the surface and subterranean aquatic life (Camargo and Jimenez, 2007; Camargo et al., 2011; Iepure et al., 2013). The ecological response of benthic (Camargo and Jimenez, 2007; Camargo et al., 2011; Rasines, 2011) and hyporheic invertebrates (Iepure et al., 2013) to changes in physico-chemical conditions of the waters at local level is well known and clearly reflects a decline of populations diversity and abundance with decrease of water condition in the lowland.

The present study aims at the quantification of the response of hyporheos diversity and ecological community structures to alterations in hyporheic river water conditions linked to land-uses changes at local (habitat) and regional (hydrographic basin) scales in the Jarama basin alluvial floodplain (central Spain). Our main goal was to evaluate which type of land use affects the hyporheic environmental conditions and hyporheic crustacean communities the most. To achieve this goal, we conducted an intensive field-survey in the hyporheic zone of the main Jarama channel and three main tributaries. Here we tracked changes in the structure of hyporheic crustacean assemblage and water physico-chemical features associated with distinct types of land-use: 1) undisturbed sites at headwaters (forested and semi-natural zones) and 2) perturbed areas in the lowland (agricultural, urban and industrial development). We start from the hypothesis that intensive and mixed land-uses within the alluvial floodplain of the basin will reduce the diversity and abundance of hyporheos and will change the ecological structure of the crustacean community (stygo-bite vs. non-stygo-bite species). Due to the scattered pattern of regional faunal composition and the restricted geographic distribution of some hyporeobiotic taxa we hypothesize that the observed responses might vary between distinct rivers.

MATERIAL AND METHODS

Location of the investigation area

The Jarama catchment is the largest discharge basin of Tajo River (6,000 km²) and drains several rivers crossing the Madrid and Guadalajara provinces from central Spain (figure 1). Jarama River and three tributaries

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(Manzanares, Henares and Tajuña) were considered for the present study. The landscape is characterised by higher slope at the headwaters (maximum of 28.5%) and moderate slope in the lowland (maximum of 7.68%). The natural flow regime of the investigated rivers is by groundwater discharge in-stream bed sediments and rainwater (Fennessy, 1982).

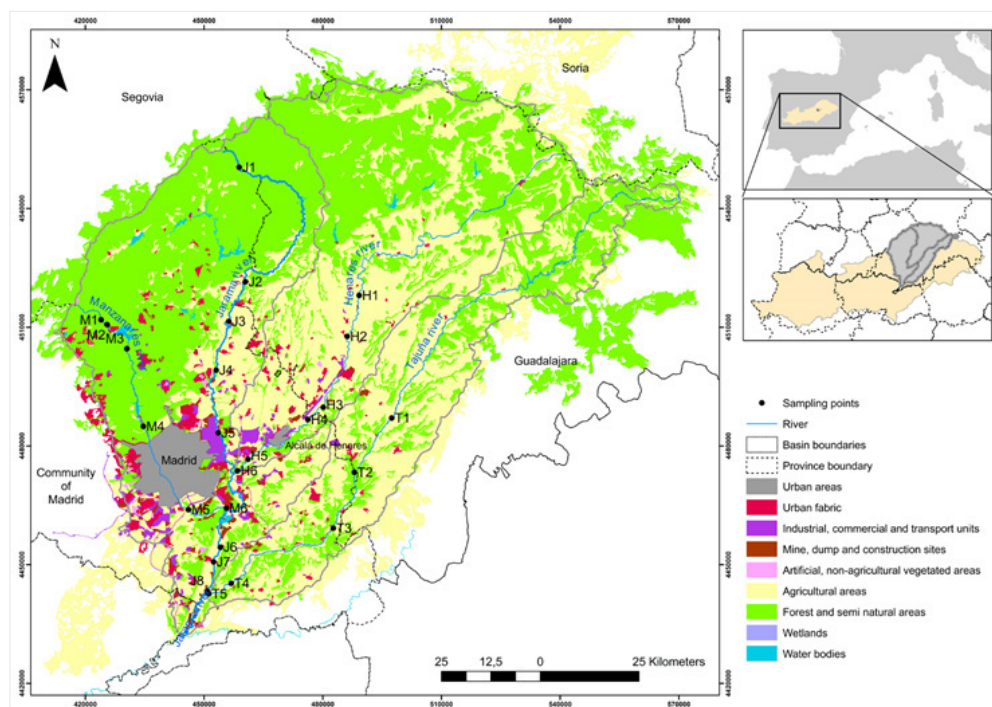


Fig. 1. Location of the hyporheic sampling sites in the Jarama basin (central Spain)

The climate in the region is Mediterranean with hot (average temperature in the hottest month $>22^{\circ}\text{C}$) and warm summers (average temperature in the hottest month $\leq 22^{\circ}\text{C}$), and with four months or more with average temperature $>10^{\circ}\text{C}$) and relatively dry winters (AEMET, 2011). The rainfall in the region is low with a mean annual precipitation averages between 400 mm and 500 mm/yr (AEMET, data for the last 10 years), which causes a high variability of river discharges throughout the year (www.chtajo.es).

The river processes and associated ecosystems at the headwaters of the Jarama River and the Manzanares River are unaltered by human activities and consist of semi-natural zones of uncultivated lands and forests.

These areas are protected by national legislation, i.e. they are located in the national park of Sierra de Rincon and the Biosphere Reserve Cuenca Alta de Manzanares. Both streams in the upper parts flows through hilly slopes and semi-natural forests dominated by *Populus nigra*, *Salix* spp., *Fraxinus angustifolia*, *Quercus pyrenaica*, *Betula pendula*, *Frangula alnus* and *Crataegus monogyna* (Almodóvar et al., 2006).

The mid- and lower courses of the investigated rivers are characterised by distinct human activities of diverse intensity (e.g. agricultural and urban development) (Bastida 2009). The agricultural land use areas are represented by arable land (permanent irrigated land and rice fields), permanent crops (vineyards, fruit trees and berry plantations and olive groves), pasture and heterogeneous agricultural cultures (annual crops associated with permanent crops, complex cultivation patterns, land principally occupied by agriculture with significant areas of natural vegetation) (see figure 1 for specific location of the sites and Appendix 1). Artificial surfaces are present in urban and rural areas, where a mixture of activities can be found: urban fabrics, and industrial, commercial and transport units (road and rail networks and associated land, airports); mine, dump and construction areas and artificial, non-agricultural and vegetated areas (with green urban areas and sport and leisure facilities).

Within the lowland courses, the riparian zone is well-developed at specific sites as leisure parks (mainly in the Henares and Jarama Rivers). The vegetation is dominated by *Populus nigra*, *Salix* sp., *Rosa canina*, and/or is mixed with hydrophilic plants, shrubs, grasses, and emergent plants.

The Madrid community established a legal framework to protect seven sites from the Jarama and Henares Rivers through Nature 2000 Network (Boletín Oficial de la Comunidad de Madrid, 2011). Additionally, some sectors of the Manzanares and Jarama Rivers downstream Madrid were subject to punctual restorations and are protected through natural parks (Parque Regional del Sur-Este).

Sampling design and land-use types classification

The survey was conducted in spring 2011 (March-April) during high flow at 25 sampling sites in the Jarama River and the three tributaries (figure 1). The sampling sites were selected according to land-use heterogeneity: 1) forested areas, 2) agricultural crops and 3) artificial surfaces with urban and intensive industrial development (table 1). For all streams there are distinct land uses to various extents present in the area adjacent to the sampling points (figure 1). However, in some cases the crops or artificial surfaces do not reach the stream edge due to the presence of a riparian zone or leisure parks.

A geographical information system (GIS) (ArcGIS 9.3, ESRI) was used to quantify the land cover/use at each sampling point. The information's were based on the 2006 Corine Land cover data set derived from satellite images produced by Landsat TM (1990), Landsat7 (2000) and SPOT4 (2006) (table 1). A buffer polygon with a diameter of 500 m was placed around the study site, and the percentage of each type of land use present in this area was calculated (Appendix 1). We used actual aerial photography and Google Maps for a verification of the Corine Land cover classification nearby our 25 measurement sites. In 10% of the cases we had to conduct minor corrections. At each site we appraise the abundant type of vegetation. This serves as surrogate to infer the development of the according riparian corridor.

The distance of each site to the headwaters was considered to establish if spatial patterns of species distribution (abundance/diversity) are natural or induced by anthropogenic activity.

Table 1. Land use codes for the selected 25 sampled sites in the Jarama basin (central Spain)

Land Covers used in this study	Classes	Code
Artificial surfaces	Urban fabrics	Urb
	Industrial commercial and transport units	Ictu
	Mine dump and construction sites	Mdcs
Agricultural areas	Arable land	Ar
	Heterogeneous agricultural areas	Haa
Forested areas	Forest	F
	Scrubs and/or herbaceous vegetation associations	Shva
	Open spaces with little or no vegetation	Osv

Environmental variables

Temperature (°C), dissolved oxygen (in % and mg/l), electrical conductivity (µS/cm) and pH were measured *in situ* by means of field sensors in triplicates samples from the river and hyporheic zone. No a-priori treatment was applied to water samples that were transported to the laboratory and were stored refrigerated (4°C) before the analyses. Water samples were analysed for 27 quantitative chemical variables (Iepure et al., 2013).

The particle size from hyporheic was determined using the standard protocol and described in Iepure et al. (2013). Four sediment size categories were identified at each hyporheic site and categorized as: silt/clay (< 63 µm), fine sand (63-250 µm), medium coarse sand (250 µm-1 mm) and coarse sand (1-2 mm).

Hyporheic faunal sampling

A perforated steel pipe with 2-3 cm in diameter and 1.5 m in length, and with 10 cm with holes of 0.5 mm diameter at one end was installed at a depth of 20-40 cm in-stream river bed sediments of unconsolidated detrital deposits represented by a mixture of non-homogeneous fine sand, boulders, and stones. The interstitial material (water, sediment, and fauna) was sampled by pumping 12 l using a Bou-Rouch sampler (Bou and Rouch, 1967; Malard et al., 2002). The macroinvertebrates and meiofauna was sieved through a 63 µm mesh net, together with the water and sediments. Three faunal replicates in the hyporheic habitat per each sampling site have been collected at each site (about 0.5-1.0 m distance). The fauna samples were preserved in the field in 96% ethanol, then stored in the laboratory at 4°C and kept until laboratory analyses.

The hyporheos was pre-sorted into major taxonomic groups under a 20-40 x magnification and then counted. Cyclopoids, harpacticoids, and ostracods were identified to the lowest taxonomic level (Meisch, 2000; Dussard and Defaye, 2006). Amphipods, isopods and syncarids were identified at family level, whereas other metazoan were just counted.

The crustacean species were classified according to their ecological preferences: in stygobites - obligate groundwater species (s) and non-stygobites - commonly found in groundwater (ns) as stygophiles (species able to survive temporary in subsurface) and stygoxenes (species accidentally drifted from the surface habitats) (Stoch and Galassi, 2010).

Data analysis and statistics

For multivariate statistical analysis of the environmental hyporheic water conditions, we pooled the triplicates in a single data set. Most of the variables do not show a normal distribution, and they were log-normalized ($\log_{10}(x+1)$) before any further statistical analyses. A principal component analysis (PCA) of the covariance matrix of the following attributes was performed: two groups of geographical variables (elevation and slope); water environmental data e.g. nitrites, nitrates, phosphates, non-purgeable organic carbon (NPOC), non-purgeable total organic carbon (NTOC), inorganic carbon (IC), total carbon (TC), trace metals (Cu, Zn, Ni, Mn, Pb, Cd), volatile organic compounds (VOCs) and endosulfan sulphate. The PCA directions thus indicate common environmental conditions. Loadings over 0.4 were considered significant (Hair et al., 1987) and hence only these parameters were retained. The resulting ordination was evaluated in agreement with land use data by labelling sites regarding the highest % of use (i.e. artificial surface/agricultural use/forest). The analysis was performed with the PAST software.

The changes in community structure pattern of hyporheic crustaceans were evaluated by quantifying total crustacean abundance (total number per site) and diversity (Shannon index, H') using the subprogram DIVERSE of the PRIMER v.6 package software (Clarke and Gorley, 2006; Clarke and Warwick, 2006). These indexes are generally used to identify the environmental stress. Additional estimation of stygobites (H'_s) and non-stygobites diversity (H'_{ns}) was assessed for each site in order to use their bio-indicative status for water disturbance in hyporheic communities.

A one-way analysis of variance (one-way ANOVA) was computed on biotic and abiotic data to test whether there are significant differences among and within the sites using PAST software. Multivariate analyses were furthermore applied to depict the pattern of physico-chemical variables and crustacean's attributes (abundance and H') among the sites. Canonical analysis of principal components (CAP) based on square root transformation for the abundance of biota was performed using subprogram PERMANOVA+ Ltd, 2009 of PRIMER v. 6 (Clarke and Warwick, 2006). In figure 3b, CAP axes 1 and 2 and overlaying vectors indicating Pearson correlation between species (only species with correlation > 0.4 were considered) is illustrated. Furthermore, the SIMPER routine was used to estimate the contribution of each species to characterize the hyporheic waters under the influences of distinct land use types.

A regression tree analysis (Berk, 2008) was additionally completed to determine if certain environmental, geographical parameters (e.g. elevation or slope) and water physico-chemical features are important for the prediction of abundance and diversities (H' , H'_s and H'_{ns}) of hyporheic crustacean communities. This method does not need the specification of the link function between predictors and response (e.g. linear or polynomial) and is thus suitable for the assessment of their rather complex and unknown relationships. In a second step, we linked the environmental parameters from each site with the associated type of land cover (e.g. % of forests and semi-natural areas) and land use (e.g. % of arable land, urban fabrics, industrial commercial and transport units; and mine dump and construction sites) and could thus relate land cover and land use types to the response of biotic component. The regression tree analysis was performed with the *r* part package of the software R (R Development Core Team, 2013).

RESULTS

Land-use pattern

The spatial arrangement and percentage of forests and semi-natural areas, agriculture and artificial surfaces varied considerably among streams at the selected scale (Table 1). It showed that the Jarama and Manzanares

headwaters sites had > 80% forest and < 20% agriculture and/or artificial surfaces within the 500 m round buffer zone. The lowland sites are governed by agricultural practices with > 60% arable crops and the rest of lands is fragmented between several urban activities (up to 34% of urban fabrics, industrial commercial and transport, mine dump) and forested riparian zones (up to 30%). Within Tajuña and Henares Rivers all investigated sites were entirely dominated by agricultural practices (Appendix 1). At five sites scrub and/or herbaceous vegetation associations (< 31%) and urban fabrics (< 16%) are recognized. Within the lowland of the Jarama basin, the artificial surfaces with urban fabrics are identified at ten nearby sites and attain almost 35% (Appendix 1).

Environmental condition of the hyporheic waters

The physical variables showed to vary in relation to the site location and to the land-cover/use patterns (see Iepure et al., 2013). The percentage of medium pebbles and coarse gravel (> 0.5 mm) is higher at forested sites. Sand particles (0.05-0.1 mm) increase at sites governed by agricultural land-use and are slightly negatively related with elevation ($r=-0.47$, $p<0.05$) and slope ($r=-0.41$, $p<0.05$).

Temperature shows the highest variability among sites. Mean hyporheic temperature decreases with increasing altitude ($r=-0.75$, $n=25$, $p<0.005$) indicating that forested and semi-natural headwaters were on average the coldest habitats (< 12.03°C). In contrast the warmest sites and more stable at the time of sampling (approximately 15.9°C on average) were located in the lowland where agricultural and urban land-use governs. Maximum temperatures in the lowland were mainly observed at sites without a riparian zone or with high discharge and low water flow (Appendix 1).

Dissolved oxygen (DO) exceeds 9 mg/l at forested sites and slightly decreases with increasing percentage of agricultural land use (~ 7.2 mg/l). Dissolved oxygen exceptionally decreases to hypoxia (< 3 mg/l) at sites located in agricultural areas and/or intensive urban development areas (e.g. urban fabrics, industrial commercial and transport units and/or mine dumps). The total carbon (TOC) is significantly lower at forested sites (2.7-3.77 mg/l) and increase consistently where agricultural practices dominate (18.2 mg/l on average) (Iepure et al., 2013).

The chemical response of hyporheic waters was also related to land cover and land use patterns. The specific conductivity (EC) varies among sites from < 470 $\mu\text{S}/\text{cm}^{-1}$ in forested and semi-natural areas up to > 2700 $\mu\text{S}/\text{cm}^{-1}$ in agricultural areas and artificial surfaces (Appendix 1). EC was strongly

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positively related to the majority of cations and anions and negatively related to elevation. Trace metals highly varied among sites with high concentrations for Mn (0.9 – 3.28 µg/l) and Zn (0.02-0.26 µg/l) in sites located downstream industrial and urban development areas. Similarly, VOCs are higher at these sites and attain 3,462.6 µg/l. Endosulfan sulphates appears locally at agricultural governed sites and exceeds 40 ppm (Iepure et al., 2013).

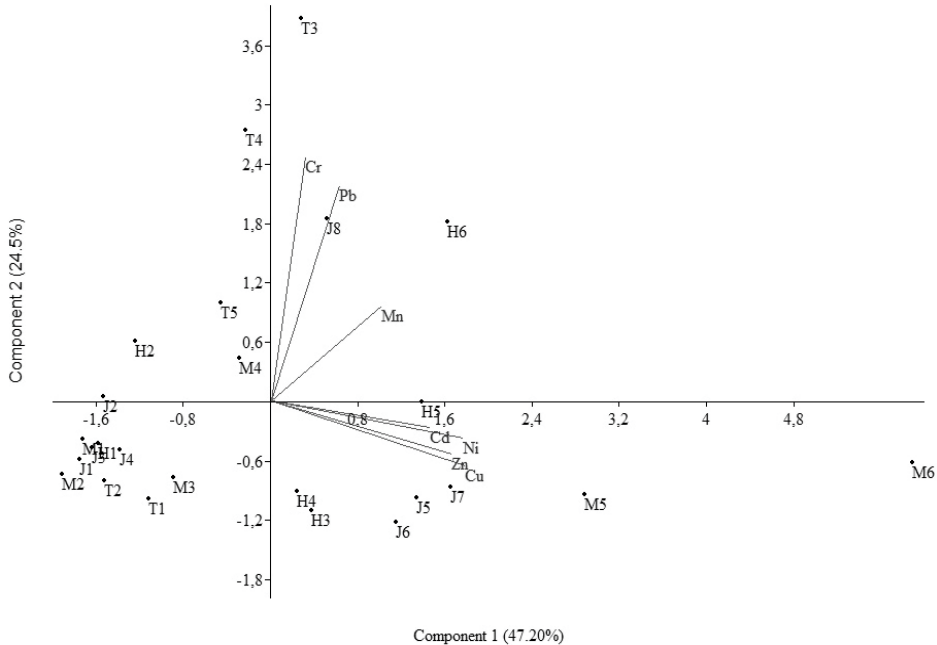


Fig. 2. Principal component analysis (PCA) ordination plot showing sampling sites distribution based on trace metals concentration in hyporheic waters.

The distribution pattern of the sites in the PCA ordination reflects the distinct type of land-uses around a hyporheic site. PCA analysis (based on covariance matrix) indicates a strong separation along the first axis (99.83% of the variance) of the hyporheic waters located in areas of mixed agricultural/artificial surfaces (figure 2). Elevation and slope have high positive loadings for this PC, whereas IC, Ni, Zn, Cu, Pb and VOCs have high negative loadings. These variables are presumed to be key benchmarks to classify the sites. The second axis (0.10%) separates mainly sites governed by agricultural practices. For the second PC axis, slope, NPOC, TOC and TC have high loadings.

Hyporheic crustacean assemblage structure among sites and environmental conditions

There was a marked distinction between hyporheic crustacean taxonomic compositions among the sites. The most common crustacean taxa across all sites were: ostracods (36% of total abundance), cyclopoids (27%), harpacticoids (26%) followed by the rest of the groups in < 5% (cladoceran, calanoids, sincarids amphipods and isopods). The rarest crustaceans were calandoids, sincarids and isopods (in 1% of the sites). Of the 42 species identified in total 19 taxa occurred in one sample and the rest in more than two. Only nine locations host stygobites whereas the rest contain exclusively non-stygobites. Hyporheic crustacean abundance varied among sites from complete absence to > 2000 specimens/site (table 2).

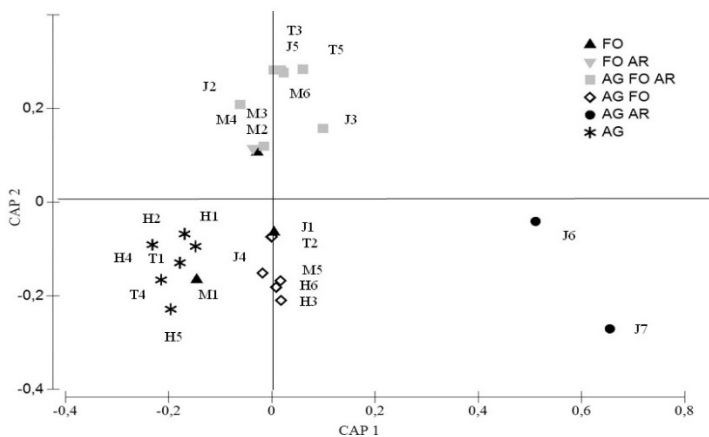
Table 2. *Pearson correlation coefficients between physico-chemical parameters and biota (with bold $p < 0.005$ and $(p < 0.01$; H' - Shannon Wiener, H'_s – stygobites diversity, H'_{ns} – non-stygobites diversity)*

Axis	Eigenvalue	Cumulative percent
1	301763,3	99.83
2	30.94	0.10
Variables	Factor 1	Factor 2
Elevation (m asl)	1	-0.0007
Slope (%)	0.59	0.80
log NPOC	0.001	0.45
log TOC	0.0004	0.44
log TC	-0.21	0.54
log IC	-0.73	-0.08
log Ni	-0.58	-0.085
log Cu	-0.43(-0.10
log Zn	-0.51	-0.004
log Pb	-0.46(-0.12
log VOC	-0.62	-0.13

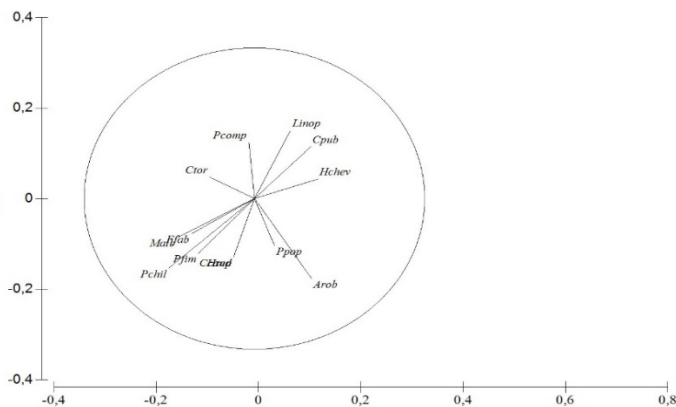
Faunal data have been classified in multidimensional space using CAP analysis to maximize differences between the groups of sites governed by distinct land uses. CAP results (figure 3a) indicate a similar distribution of the sites with respect to the pattern of hyporheic crustacean assemblage composition as the PCA chart based on environmental data (figure 2). The first CAP axis (95.3%) indicates a separation of the hyporheic waters with medium (right upwards) or low diversity (right downwards) of hyporheic crustaceans (figure 3a). These sites are associated with high levels of ammonia (up to

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13.64 $\mu\text{g/l}$) and trace metals (Cu, Cd, Zn, Ni and Mn). The second axis markedly separates highly diverse hyporheic communities (left downwards) with nitrate level of 22 $\mu\text{g/l}$ from the sites less diverse and exposed to elevated concentrations of TC and Cr (left upwards) (figure 3 a).



3a



3b

Fig. 3 (a). Canonical Analysis of Principal Coordinates (CAP) showing sites distribution based on species presence in hyporheic waters (symbols are land-uses types: grey squares – arable land/forest; black triangle – forest; grey triangle – forest/arable land; rhombus – scrubs and herbaceous vegetation/arable land/forest; black dots - scrubs and herbaceous vegetation/arable land; stars – arable land);
(b) – Vectors pointing species based on Pearson correlation (Ostracoda: *Linop* – *Limnocythere inopinata*, *Cpub* – *Cypris pubera*, *Hchev* – *Herpetocypris chevreuxi*, *Hrep* – *Herpetocypris reptans*, *Ffab* – *Fabaeformiscandona faba*, *Ctor* – *Cyprideis torrosa*, *Pcomp* – *Pseudocandona compressa*; Cyclopoida: *Arob* – *Acanthocyclops robustus*, *Ppop* – *Paracyclops poppei*, *Pfim* – *Paracyclops fimbriatus*, *Pchil* – *Paracyclops chiltoni*, *Malb* – *Macrocyclus albidus*)

Species showing a high correlation with the first two axes are those identified by the SIMPER analysis as characterizing particular sites with similar types of land use (figure 3b). The ostracods *Fabaeformiscandona fabaeformis* and *Herpetocypris reptans* and the cyclopoids crustaceans *Macrocylops albidus*, *Paracyclops fimbriatus* and *P. chiltoni* were primarily confined to sites governed by agriculture practices. All these species are non-stygobites with large ecological valence. The cluster of non-stygobites species formed by *Limnocythere inopinata*, *Cypris pubera* (ostracods) and *Acanthocyclops robustus* (cyclopoid) was most highly related to sites governed by mixed land use types (especially agricultural and artificial surfaces). A group of typical hyporheos with mixed ecology are highly correlated with forested areas, e.g. *Pseudocandona eremita* (stygobite) and *Cryptocandona vavrai*, *Pseudocandona albicans*, *P. compressa* gr. (non-stygobites). There are few shared species between the forested and less forested sites and only two are stygobites (*Acanthocyclops* sp. new and *Darwinula stevensoni*) whereas the majority of species which occur at sites with distinct land use types are cosmopolitan species, which are tolerant to various environmental conditions, e.g. *Diacyclops languidoides* s.l., *Paracyclops poppei* and *Pseudocandona albicans*.

Hyporheic crustacean pattern land-use predictors

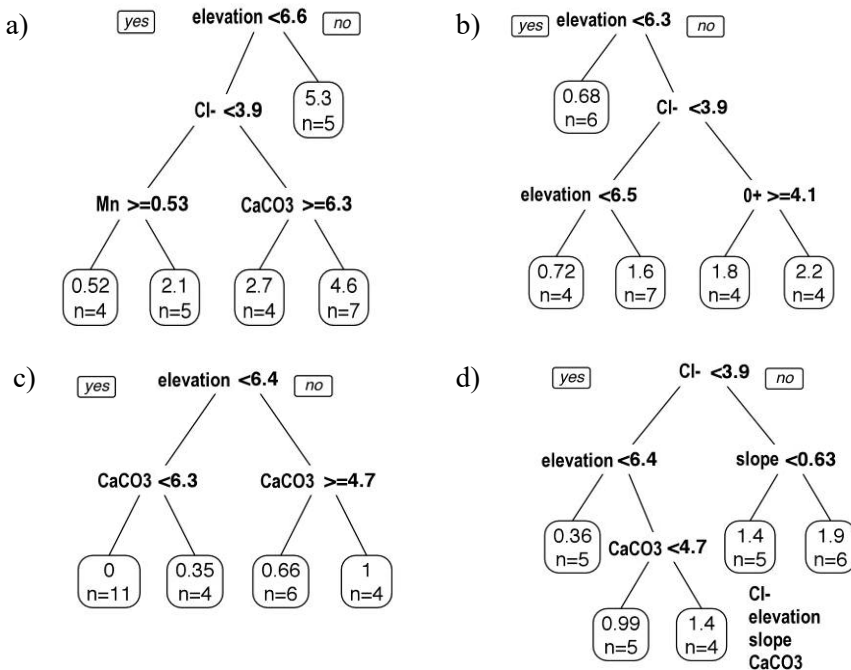


Fig. 4. Regression trees **a)** total abundance of hyporheic crustaceans; **b)** Species diversity (H'); **c)** Stygobites diversity (H'_s); **d)** Non-stygobites diversity (H'_{ns})

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The one-way ANOVA does not indicate significant differences between the sites in terms of abundance and diversity (H') ($F=2.75$, $p<0.1$), whereas significant differences have been found when the ecological community structure is considered (stygobite vs. non-stygobite) ($F=21.38$, $p<0.0002$). The most important predictors for the total abundance were primarily elevation (ANOVA analysis, significance level 0.1) and secondly TC, which are both associated with forested areas (figure 4). The Pearson correlation returns similar results and indicates a slightly positive correlation of hyporheos abundance with elevation and, a negative correlation with temperature, NO_2^- and Ni (at 0.01 significance level) (table 3).

Table 3. Pearson correlation coefficients between physico-chemical parameters and biota (with bold $p < 0.005$ and * $p < 0.01$; H' - Shannon Wiener, H'_s – stygobites diversity, H'_{ns} – non-stygobites diversity)

Variables	Unit	Crustacean density (individ./ sample)	H'	H'_s	H'_{NS}
Elevation	m	0,36	0,31	0,56	0,09
Slope	%	0,06	0,31	0,47*	0,15
Temperature	° C	-0,18	-0,19	-0,50	0,01
Dissolved oxygen	%	0,43*	0,69	0,38	0,64
NO_2^-	mg/l	-0,41*	-0,45*	-0,35	-0,38
NO_3^-	mg/l	0,13	0,12	-0,15	0,23
NH_4^+	mg/l	-0,49*	-0,69	-0,39	-0,63
NPOC	mg/l	-0,02	0,13	0,27	0,04
TOC	mg/l	-0,02	0,10	0,26	0,01
TC	mg/l	-0,08	-0,03	-0,02	0,00
IC	mg/l	-0,09	-0,15	-0,46*	0,10
Cr	mg/l	0,07	-0,01	-0,02	0,05
Mn	mg/l	-0,28	-0,50	-0,29	-0,43*
Ni	mg/l	-0,63	-0,59	-0,50	-0,46*
Cu	mg/l	-0,33	-0,40*	-0,31	-0,34
Zn	mg/l	-0,32	-0,26	-0,44*	-0,13
Cd	mg/l	-0,35	-0,40*	-0,17	-0,38
Pb	mg/l	0,05	0,09	-0,16	0,20
VOCs	mg/l	-0,38	-0,54	-0,54	-0,39
Endosulfan sulfate	mg/l	0,33	0,38	0,22	0,41*
% Agricultural areas & forest and seminatural areas		89(16-138)	7 (5-9)	2(0-4)	5.66(3-7)
% Agricultural areas & forest and semi-natural areas & artificial surfaces		361.2(1-2700)	3(2-4)	2(0-3)	2.4(1-4)
% Agricultural areas & artificial surfaces		32.16(0-129)	0.5(0-1)	0	1(0.1)

River ecosystems draining non-irrigated and permanently irrigated croplands were characterized by relatively well-developed sclerophyllous vegetation in the riparian zone of the selected sampling sites and they display furthermore a high species diversity mainly non-stygobites (table 3). However, ANOVA (linear regression) finds no significant predictor for total species diversity (H') but the exploratory analysis suggests a link in polynomial form of second order for TOC and elevation, and a linear relation for carbonates (figure 5). The Pearson correlation indicates a positive correlation of the diversity of non-stygobite species with dissolved oxygen, and a negative correlation with NO_2^- and NH_4^+ (table 3).

The stygobite diversity (H') decreases in sites with a low percentage of forest and/or riparian zone development. The ANOVA determines as best predictors for the diversity of stygobites, elevation ($p < 0.01$, the higher the altitude, the higher the H'_s) and temperature ($p < 0.05$, the lower the temperature the higher the H'_s).

Furthermore, regression tree analysis has been performed to confirm these results by allowing for non-linear relationships, which are most probably present in the data. Due to the limited number of available data, the minimum number in a node is restricted to 4 and the minimum number to split is 6. Thus, prediction trees with 4-5 terminal nodes are produced (figure 4). The few data available causes the regression tree analysis results not to be significant. However, they give valuable insight in the process dynamics and serve thus for interpretation purposes. As depicted in figure 4 a) and b), elevation shows to be a good classifier for species diversity and abundance in general. Moreover, elevation is as well the most important variable to determine stygobite and non-stygobite diversity (see figure 4c) and d). Slope is also an important classification variable to predict non-stygobites diversity. Thus, results are in line with the ANOVA and Pearson correlation assessment.

The regression tree analysis serves also to classify and predict species abundance and diversity (figure 6), even with a quantitative error approximation. It shows that, except for stygobite diversity (figure 6 c), the model estimates the observations reasonably well (small differences between the observations (black circles) and regression tree node values (black dots) are envisaged), given the few data available. It also shows that medium Cl^- values (~ 60 mg/l) causes a high total (H') and non-stygobites (H'_{ns}) species diversity, and total abundance and a high CaCO_3 value (> 150 mg/l) causes high diversity rates of stygobites.

Some of the site classes (see x axes) show a clear correspondence to land use types (figure 3a). For all four response variables (abundance, H' , H'_s/H'_{ns}), the sites dominated by agricultural activities are put in different classes than the forest dominated sites. Moreover, the class with highest stygobite

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diversity is forest dominated sites, whereas the class with highest non-stygobite diversity comprises sites dominated by agricultural practices. This confirms our theses on the influences that land use types on the species diversity and especially of stygobites have.

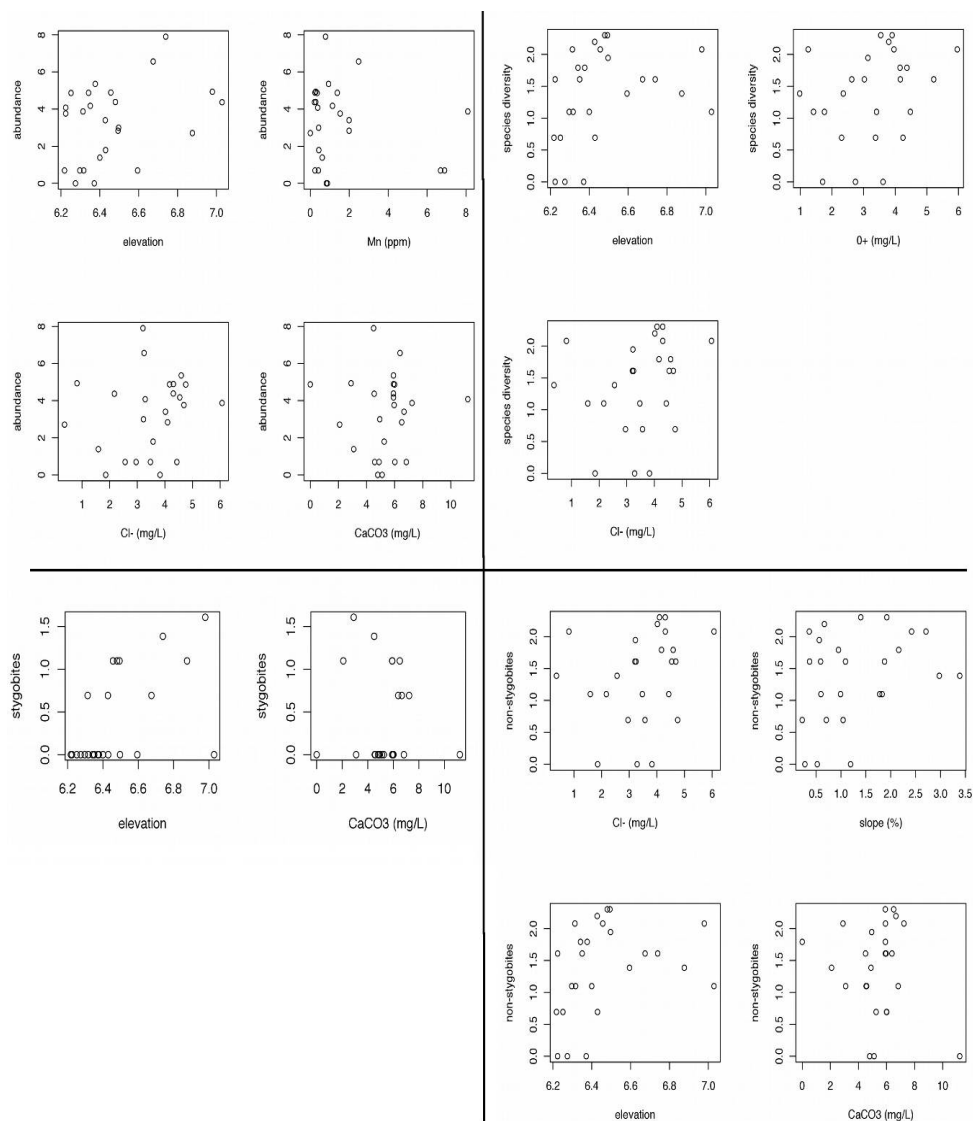


Fig. 5. Scatterplots of abundance, species diversity, stygobites and non-stygobites versus their respective best predictors (according to ANOVA).

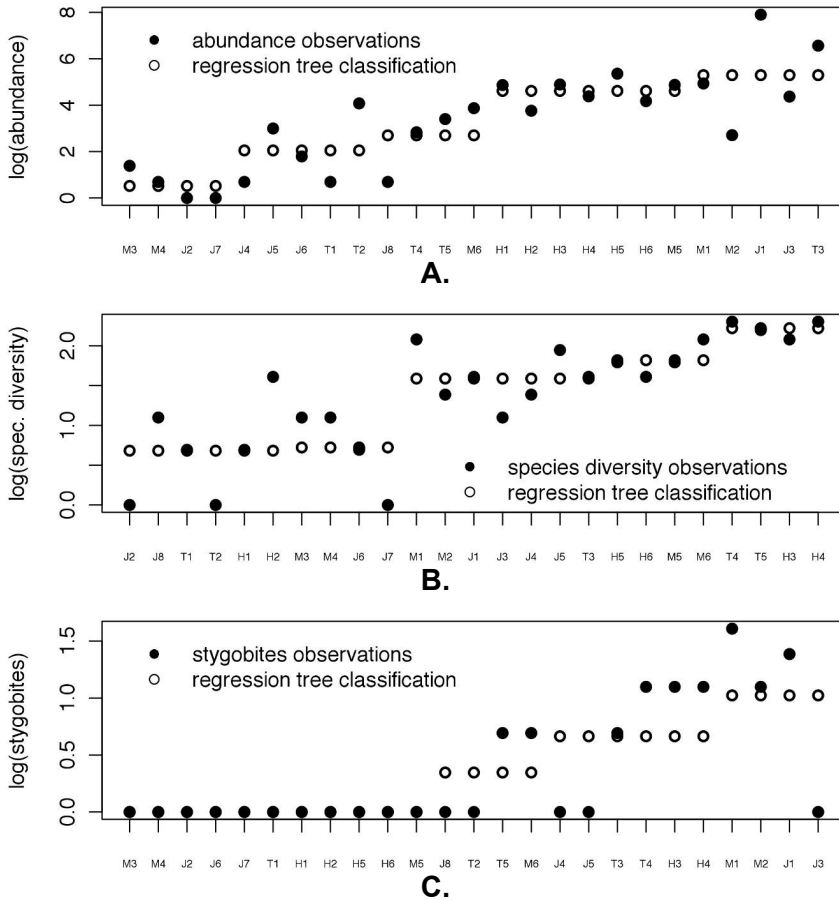


Fig. 6. Regression tree classifications: **a)** Total hyporheic abundance; **b)** Species diversity (H'); **c)** Stygobites diversity (H'_s); **d)** Non-stygobites diversity (H'_{ns})

DISCUSSIONS

Environmental water conditions differences among hyporheic waters

Results from our study of the hyporheic zone of four streams in the Jarama basin (central Spain) indicate significant changes of physico-chemical properties of hyporheic water, which are associated with physico-geographical features (e.g. elevation and slope) and land use types at local scale in the river watersheds.

The patterns of sediment deposition vary from site to site. However, an increase of fine sands (0.63 mm) from the forested headwaters to the lowlands with intensive land-use for agriculture is observed. The hyporheic waters of the Manzanares and Jarama sites located at high elevations are dominated by medium pebbles and coarse gravel. The mean substratum of fine particle size was inversely related to altitude indicating that this fraction is associated with agricultural practices around sites (cf. Lenat et al., 1981; Richard et al., 1996).

Past studies reveal a direct relationship between the large amount of fine sediments and the lowering of the dissolved oxygen in the hyporheic zone (Poole and Stewart, 1976; Strommer and Smock, 1989; Bretschko, 1994). Our results do not indicate a significant connection at the time of sampling. However, dissolved oxygen content tends to decrease from the headwaters to the lowlands (see Iepure et al., 2013). Hyporheic waters from the forested sites display remarkably high oxygenated waters (> 9 mg/l). Less oxygenated hyporheic waters are associated with sites located in the lowland. Here artificial surfaces and especially industrial and urban activities dominate the land use. Although a longer residence time of water has been proved to partially explain the variation of dissolved oxygen in hyporheic waters (Findlay, 1995), the used natural tracers (conductivity, Br and Cl⁻) found this pattern at eight sites only.

Intensive land-use for agricultural practice in the lowland of the Jarama basin lead to an increase of nitrates and phosphates in the hyporheic waters of all sites. However, the concentrations do not exceed the established standard limits for surface and ground water according to the Water Framework (WFD 2000/60/EC) and Groundwater Directives (2006/118/EC) (< 50 mg/l). Endosulfan sulphate presence is mainly associated with the presence of agricultural activities at specific sites in the mid-courses of Tajuña and Henares (table 2). According to both directives, endosulfan sulphate is a prohibited substance in water bodies since 1999 however it might be still used punctually in the Jarama basin and not at a larger scale.

The physico-chemistry of hyporheic water declines progressively and accumulates trace metals and VOCs due to changes in land use and growing artificial surfaces and industrial development. Trace metals like Cu, Cd, Zn, Pb, Mn and Ni exceed the standards limits in hyporheic waters at sites where industrial practices are present and located downstream the Madrid metropolitan, industrial polls and large residential urbanizations (i.e. Paracuellos de Jarama, Azuqueca de Henares and Alcala de Henares) (Iepure et al., 2013). Previous investigations indicate that some trace metals are accumulating in the riverbed sediments from the Jarama basin from where they are most probably remobilised into the interstitial hyporheic water (Arauzo et al., 2003).

Differences in hyporheic crustaceans and land-use predictors

The comparison of catchment characteristics and nearby land-use practices (within a 500-m buffer) has revealed the impact of land use on hyporheic water quality and biotic crustacean communities in the Jarama basin. In accordance with these results, we identified three groups of hyporheic communities that differed in crustacean abundance and diversity, but also in the proportion of stygobites/non-stygobites taxa present (according to water conditions): 1) forested sites with medium abundance and species diversity (H') dominated by stygobite species; 2) agricultural sites with high diversity and abundance, and communities conquered by non-stygobites; 3) sites with mixed agricultural and industrial activities with low diversity and abundance and/or loss of crustaceans.

Forested hyporheic waters harbor the richest crustacean communities and crustaceans such are cyclopoids, harpacticoids, ostracods and calanoids which are the most abundant species. Except the later epigeal taxa, whose presence is evidently influenced by the site location downstream of a large artificial lake (Santillana reservoir), the other taxa are typically common hyporheos. Results from the regression tree analysis also indicate that the abundance and high stygobites diversity are predicted by a high percentage of forests at headwater sites. The diversity of stygobites is significantly correlated with high levels of dissolved oxygen and with low temperatures of the hyporheic waters. Forested sites are also characterized by oligotrophic hyporheic waters. Species associated with forested sites are obligate subterranean dwellers (stygobites), i.e. *Parastenocaris* n. sp. 1, *Parastenocaris* n. sp. 2, *Acanthocyclops* sp. 1, *Pseudocandona eremita* gr. and *Darwinula stevensoni*. Boulton (1997) found somewhat similar results in five New Zealand streams where in-stream draining native forests harbored a diverse and mixed benthic with few apparently hyporheic taxa. We suggest that the high diversity and abundance of stygobites at the forested sites indicate that hyporheic zone is sufficiently developed and provide conditions to support subterranean dwellers populations.

Generally, stygobites are ground water-adapted species and their presence in the hyporheic ecotone zone commonly provides information on water conditions. The diversity of stygobites in the Jarama basin was negatively related to specific industrial contaminants present in the lowlands (e.g. NO₂⁻, Ni and VOCs), where land use types are associated with continuous and/or discontinuous urban fabrics. Due to their restricted requirements and being influenced by water physico-chemistry settings (Maurice and Bloomfield, 2012), stygobites may play an important role as forthcoming ecosystem service providers for subsurface water conditions (Tomlinson et al., 2007; Griebler et al., 2010; Iepure et al., 2013).

The hyporheic waters exposed to agricultural practices in the Jarama basin lowland display a large amount of nutrients, as indicates the enrichment of total carbon (TC), inorganic carbon (IC) and nutrient content in form of nitrates which are higher at sites exposed to agriculture than at forested sites (see Iepure et al., 2013). Consequently, the hyporheic crustaceans develop a highly diverse community formed by a mixture of species with distinct ecological traits. The regression tree analysis supports these observations and indicates that the abundance and non-stygoxene diversity might be predicted by the agricultural land use practices. Our results are consistent with previous studies reporting that land-cover is a good predictor for in-stream nutrients (Omernik, 1976; Johnson et al., 1997) and that agricultural activities cause an augmentation of diversity in hyporheic faunal assemblage as results of nutrient enrichment (Boulton et al., 2008).

Non-stygoxite species in hyporheic waters, which are exposed to agricultural practices, prevail upon stygoxites, but this observed pattern is likely due to an increase of in-stream productivity. Sufficient nutrients in the hyporheic zone support the development of a large array of non-stygoxite taxa, but also favor the invasion of benthic species like *Paracyclops chiltoni* and *P. imminutus*. The stygoxites *Acanthocyclus* sp. 1 and *Darwinula stenvensoni* are also present however, they appear in low density. We suggest that the reduction or in some cases disappearance of the stygoxites fraction at agricultural sites might be due to cumulative factors such as the enrichment of water organic content and the competitiveness of the non-stygoxites. Our statement agrees with other studies showing that stygoxites are more sensitive to organic contamination and stygoxenes replaced them (Notenboom et al., 1994; Malard et al., 1994; Di Lorenzo & Galassi, 2013).

Our survey also indicates that the presence of riparian vegetation in riverbanks with mixed land use may contribute to an increased diversification of non-stygoxites hyporheos. An example is two sites of the Henares River, which are located close to residential urbanization but surrounded by leisure parks (downstream of Alcalá de Henares city). Here the classification of the land cover/use indicates primarily agricultural practices with non-irrigated and permanently irrigated crops, and secondarily natural grasslands and sclerophyllous vegetation and industrial & commercial units (Appendix 1). It is worth mentioning, that nitrites and nitrates are missing from the two Henares sites, whereas they were present in the hyporheic sites of both Rivers Henares and Tajuña with no riparian zone. This fact may suggest the significant contribution of the riverine vegetation to guaranteeing effective processes of denitrification and nitrification, which consequently lead to a local improvement of the hyporheic habitat quality. The relationship between riparian vegetation and water quality is well known. However, most studies were oriented on

benthic habitats and macroinvertebrates (Peterjohn and Correl, 1984). Previous recent assessment of macroinvertebrate associations and benthic habitats at these sites indicate a slight enhancement of the surface water quality and an increase of the diversity of benthic macroinvertebrates (Rasines-Ladero, 2011; Fuentes-Alvarez, 2011).

Multiple land-use practices with intense urban and industrial activities cause a reduction of hyporheic crustacean richness with a complete loss of crustaceans at some sites indicating water conditions impoverishment. Here only the non-stygobite *Acanthocyclops robustus* were collected during our survey, which have been most probably drifted from the upstream sites. Particularly, the interactive effects of trace metals, ammonia and VOCs exceeding the standard limits for surface waters (cf. EU-WFD (2000) combined with a lowering of dissolved oxygen down to hypoxia (< 3 mg/l) were detrimental for the hyporheic crustaceans. The univariate ANOVA analysis also indicates that hyporheic crustacean diversity (H') is mainly affected by nitrites, ammonia, Ni and VOCs.

Integrating the hyporheic zone in the management of river ecosystems

The HZ of river ecosystems can provide unique habitat for in-stream invertebrates and refuge for benthic species when surface water conditions decline. Previous studies show the significance of the in-stream biota and the hyporheic zone in ensuring a good functionality of the stream ecosystems as a whole (Boulton et al., 2010). They also show the linkage between the hyporheic zone and the associated alluvial plain of which alteration cause changes in water conditions, sediments deposition in riverbeds and modification in surface/groundwater exchanges to mention few that alter the structuring of hyporheic biota.

Additional conservation of the riparian zone connected with the main channel is also of huge wealth in ensuring a good functionality of the hyporheic water flow and quality and consequently safeguarding the hyporheic communities. Previous researches (Ward, 1989; Tockner et al., 1999; Ward et al., 1999) indicate that the alpha-diversity of macroinvertebrates and biomass increases at moderate connectivity of the riparian zone with the rivers channel, which is likely to occur for hyporheic biota as well.

It is also suggested that for a successful rehabilitation of rivers ecosystems or sectors affected by distinct disturbances a comprehensive understanding and recognition of the hyporheic zone ecology and its capacity to get recovered is required that is often misleading (Danielopol, 1989; Ward et al., 2001; Boulton,

2007; Boulton et al., 2010). For example, the time required for hyporheic biota to recover after persistent disturbances of their habitats is generally high (> 10 years) (Wallace, 1990; Dole-Olivier et al., 2000) and highly depends on the attainment of relatively stable levels of environmental conditions that includes best practices to recover the surface water quality. A more holistic view of river restoration management is compulsory to protect both surface and subsurface associated ecosystems (Boulton et al., 2003; Strayer, 2006; Strayer and Dudgeon, 2010). The large plethora of organisms that have been proved to represent efficient service providers for the subsurface ecosystems they belong should be highly considered in management decisions about river water resource development in the area. In these regards, empirical studies of the transitional surface/ground waters at distinct spatial scales from remote regions are of huge wealth.

CONCLUSIONS

The differences among the investigated hyporheic zone of the Jarama basin in central Spain indicate the consequences of distinct practices occurring in the alluvial floodplain have on the hyporheic zone and selected crustaceans. This study showed important differences in hyporheic waters environmental conditions regarding nutrients (expressed as TOC, NPOC, TC, IC and nitrates) and urban/industrial contaminants (Cu, Zn, Ni, Mn, Pb and VOCs) associated with the land cover and use in the alluvial plain of the Jarama basin. We could also link the land cover and land use types to hyporheic crustacean patterns of the distribution, diversity, and the ecological structure of hyporheic populations. Despite the well-known spatial patchy distribution of hyporheic biota our observations indicate that they are subject to changes significantly, especially in configuration of the communities' ecological structure, when alterations in the associated alluvial aquifer occur.

There was significant evidence that the high overall diversity of hyporheic crustacean communities is linked to agricultural land use, whereas stygobites presence is mainly related to pristine and cold hyporheic waters from forested areas of the investigated Mediterranean rivers. However, we are still lacking the information on changes in hyporheic biota related to the temporal dynamics of land use that impair the prediction of the future impact on this biota by increasing human pressures in the area.

Investigating the effects of land-use at distinct spatial and temporal scales will be critical for a comprehensive understanding and the prediction of the hyporheic community structure in a catchment. Sensitivity tests of certain hyporheos to distinct types of contaminants would also enhance their effective use as bioindicators of the decline of subsurface waters caused by changes in land uses within the alluvial floodplain.

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River unit	Hyporheic water physico-chemistry										Land use cover (%)								
	Codes	Elevation (m)	Distance to the headwaters (km)	Slope (%)	EC (µs/l)	Temp.	pH	DO (mg/l)	NO ₃ (mg/l)	NPOC (mg/l)	NO ₃ (mg/l)	Urb	Ictu	Mdcs	Ar	Haa	T	Shva	OSv
Tajujá River	M3	844	11	1,98	113,17	16,17	5,23	6,47	0,34	3,63	0,34	34,11	10,36	0,0	37,00	0,0	0,0	18,53	0,0
	M4	601	28	1,7	430,00	12,90	4,66	2,77	1,59	15,31	1,59	3,75	0,0	0,0	93,25	0,0	0,0	0,0	0,0
	M5	553	40	5,22	1298,33	18,37	7,19	2,96	5,38	5,26	5,38	0,20	0,0	0,0	99,80	0,0	0,0	0,0	0,0
	M6	530	54	2,3	2744,67	17,70	7,13	2,81	0,00	12,05	0,00	0,30	0,0	0,0	55,50	0,0	0,0	24,21	0,0
	T1	791	85	5,53	552,00	12,40	5,28	7,84	8,14	1,17	8,14	0,0	0,0	0,0	28,92	71,08	0,0	0,0	0,0
	T2	660	97	5,63	1279,67	12,90	7,78	8,79	6,63	1,55	6,63	0,0	0,0	0,0	98,14	0,0	0,0	31,86	0,0
Henares River	T3	618	121	0,95	1076,00	16,33	8,06	9,21	9,18	24,41	9,18	16,89	0,0	0,0	62,72	11,74	0,0	8,65	0,0
	T4	518	144	0,25	1384,33	12,57	8,03	10,46	22,36	1,86	22,36	0,0	0,0	0,0	97,12	2,88	0,0	0,0	0,0
	T5	551	154	0,45	1490,67	18,33	8,01	9,32	5,95	11,00	5,95	0,84	0,0	0,0	89,16	0,0	0,0	0,0	0,0
	H1	636	62	10,24	999,67	13,93	7,94	8,30	0,00	23,96	0,00	0,0	0,0	0,0	79,29	0,0	0,0	0,0	0,0
	H2	651	72	3,05	1066,33	15,10	8,02	9,71	2,86	35,82	2,86	0,0	0,0	0,0	100	0,0	0,0	0,0	0,0
Henares River	H3	587	88	7,68	937,33	16,30	7,73	8,74	5,51	3,15	5,51	0,0	0,0	0,0	11,92	0,0	0,0	25,18	0,0
	H4	572	98	0,81	883,00	13,73	7,41	5,07	5,24	2,59	5,24	0,0	0,0	0,0	80,43	0,0	0,0	0,0	0,0
	H5	567	125	1,59	983,00	17,30	7,88	9,04	0,00	38,34	0,00	0,0	7,27	0,0	92,73	0,0	0,0	0,0	0,0
	H6	551	131	0,44	1838,67	15,03	7,20	11,56	0,00	107,46	0,00	0,0	0,0	0,0	80,11	0,0	0,0	19,87	0,0