

Bridging community and metacommunity perspectives in benthic photosynthetic organisms (Serranía de Cuenca, Central Spain)

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ABSTRACT

Bridging community and metacommunity perspectives in benthic photosynthetic organisms (Serranía de Cuenca, Central Spain)

We analysed summer assemblages and the metacommunity structure of benthic microalgae and Cyanobacteria (BMC) living in pristine ecosystems in the Serranía de Cuenca (Central Spain), whose flora is not well-known. Our analyses were focused on both overall samples and some site subgroups (two large catchments, lotic vs lentic environments, plant vs mineral substrates). 379 taxa were recorded, with Diatoms, Chlorophytes and Cyanobacteria being the richest groups. The flora composition depended on conductivity and the percentage of forests in each sub-basin; even so, the composition was spatially homogeneous, reflecting the relatively low variability of the landscape. Taxonomic richness did not differ between inventory subgroups, but it was vulnerable to the somewhat higher values of low concentrations of nitrogen and phosphorus compounds. Two groups of BMC assemblages emerged in the territory: one of cosmopolitan Diatoms and Cyanobacteria, and another of Cyanobacteria, Diatoms and Chlorophytes associated with travertine. The structure of the metacommunity was governed by the contributions of space, catchment features and the local environment (chemical and biological); the relevance of biological factors, which are not usually analysed, was highlighted. Finally, we suggest the joint study of local ecology and metacommunity that is feasible with the many occurrence databases of many benthic organisms. Soon artificial intelligence and new taxonomic tools will allow faster and more comprehensive processing of the huge number of freshwater datasets already in existence, which will be a huge leap in ecological knowledge of benthic assemblages in the coming years.

Key words: microalgae, Cyanobacteria, karst environments, taxonomic richness, control factors, environmental gradients, metacommunity

RESUMEN

Uniendo las perspectivas de comunidad y metacomunidad en organismos fotosintéticos bentónicos (Serranía de Cuenca, Centro de España)

Se analizan aquí las asociaciones estivales y la estructura de la metacomunidad de microalgas y Cianobacterias bentónicas (MCB) en ecosistemas prístinos situados en la Serranía de Cuenca y de flora semidesconocida. El análisis se centró en el total de las muestras y en subgrupos de ellas (cuencas del Júcar o del Tajo, ambiente lótico o léntico, sustrato mineral o vegetal). Se inventariaron 379 taxones, siendo Diatomeas, Clorofitas y Cianobacterias los grupos más relevantes. La composición de la flora dependió de la conductividad y del porcentaje de bosques en cada subcuenca; aun así, la composición fue homogénea espacialmente, reflejo de la relativamente escasa variabilidad del paisaje. La riqueza taxonómica no difirió entre los subgrupos de muestras, pero resultó vulnerable al incremento de las bajas concentraciones de compuestos de nitrógeno y fósforo. Hubo dos grupos de comunidades de MCB: uno de Diatomeas y Cianobacterias cosmopolitas, y otro de Cianobacterias, Diatomeas y Clorofitas asociadas al travertino. La metacomunidad la estructuraron el espacio, las características de la cuenca hidrográfica y el ambiente local (tanto químico como biológico); el factor biológico, que habitualmente no se emplea en esos análisis, destacó también. Como conclusión, sugerimos el estudio conjunto de la ecología local y la metacomunidad bentónica, factible

con las numerosas bases de datos de presencia-ausencia para muchos grupos de organismos. Pronto la inteligencia artificial y las nuevas herramientas taxonómicas permitirán un procesamiento más rápido y completo del enorme número de datos de agua dulce ya existentes, lo cual supondrá un gran salto en el conocimiento ecológico de las comunidades bentónicas en los próximos años.

Palabras clave: *microalgas, Cianobacterias, ambientes kársticos, riqueza taxonómica, factores de control, gradientes ambientales, metacomunidad*

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INTRODUCTION

For many years, attempts have been made to characterize plant assemblages in order to describe their own biology and ecology. This is the classic case of phytosociology which, using procedures similar to those of taxonomic nomenclature, defines plant assemblages. An aquatic example was the efforts of Margalef (1949, 1951), who typified assemblages of Catalan inland algae on the basis of the Braun-Blanquet phytosociological criteria, something which was laudable at the time but which hardly had any continuity. Inventories of aquatic organisms have continued to be published, although without attempting a description of them that would allow comparisons with others recorded in different areas of the globe, despite the efforts of the Central European Limnosociology (Šrámek-Hušek, 1962; Botosaneanu, 1979; Illies & Botosaneanu, 1963). Another older, yet interesting approach is the ecological characterization of the local biota, analysing its composition and diversity indicators, and using multivariate statistical tools to establish both their assemblages and the relationship with the environmental factors which could govern these structures (Rojo et al., 2000). Regionally, local assemblages can be compared, which allows the discovery of, among other things, which flora or fauna are regional, which species are cosmopolitan or rare, which associations may be specific to a given environment and what their spatial heterogeneity (or beta-diversity) is (Rojo et al., 2012).

In recent years, a plethora of statistical methodologies have appeared that enrich the study of biological assemblages (see, for example, Borcard & Legendre, 2018), analysing them both at the local and regional level, and including their

organization in metacommunities in order to understand their ecology. As we have mentioned, having information on assemblages from many different sites will allow us to compare local characteristics among communities, but if the populations under study can also be dispersed among these sites, we can consider all of them as a metacommunity. If so, we could try to characterize it and analyse how this distribution is, i.e. more or less homogeneous, dependent on the environment of each site, randomly distributed or if the different assemblages are formed with those that arrive first, etc. (Heino et al., 2015). The joint description and comparison between communities and the analysis of the metacommunity will provide a better view of the ecology of the studied group of organisms. Since dispersion is a key factor in the structuring of metacommunities, it is not surprising that streams and their catchments where local sites are typically more ecologically connected by discharge have extensively been studied (Soinen & Teittinen, 2019).

The main objectives in the study of benthic metacommunities (e.g. Vilmi et al., 2017; Rodriguez-Alcalá et al., 2020) are usually to describe their features such as beta diversity, the underlying explanatory models of their spatial distribution (Baselga 2012; Leibold et al., 2004) and the variance explained by their possible controlling factors (i.e. variance partitioning techniques; Borcard et al., 2018). The notion of metacommunity (Leibold et al., 2004), which is associated with biogeographic hypotheses (Leibold & Chase, 2018), broadens our point of view concerning the biology of assemblages; in the case of aquatic ones, the number of these studies has increased (Heino et al., 2015) and has dominated ideas about the ecology of inland benthic algae

over the last two decades. A multitude of meta-community studies have appeared, dedicated –as far as algae are concerned– almost exclusively to Diatoms. From the metacommunity perspective, taxon richness (Soininen & Kongas, 2012; Virta & Soininen, 2017), rare species (Soininen, 2008; Tornés & Ruhí, 2013), groups of taxa according to their occurrence (Jamoneau et al., 2018), and the absolute and relative abundances of taxa (Potapova & Charles, 2002; Passy, 2007; Urrea-Clos & Sabater, 2012), etc., have been considered.

Unfortunately, a similar analysis has rarely been carried out taking all the microscopic photosynthesizing organisms of the benthos (algae as a whole and Cyanobacteria) into account, as has been done in this study. Using only Diatoms, and at the catchment scale (that is, comparing the results with those of other remote regions or even continents), most studies conclude that the communities found are the result of interactions between the locality (or geographical position), and interactions between local or regional factors (including climatic ones). Obviously, the importance of all these sets of factors changes according to the geographical extension (from local basins to continents, Soininen et al., 2016) and the spatial scale of the analysis (in other words, its spatial resolution; Passy, 2001; Grenouillet et al., 2008).

In classical botany and zoology, it is common to register taxa based on their presence or absence in a given territory. In addition, it is often the case that quantifying the density of organisms is not possible, as happens when floating filamentous algae are involved (Álvarez Cobelas, 2020) and therefore the researcher has to rely only on qualitative information. The statistical tools developed in recent years are perfectly applicable to presence-absence data; in fact, some of the aforementioned databases have been analysed following the metacommunity criteria (Jamoneau et al., 2018, Potapova & Charles, 2002).

As for inland benthic algae, studies have been limited almost exclusively to Diatoms, a fact possibly favoured by: 1st) better taxonomic knowledge, and 2nd) the existence of a complete database that facilitates the taxonomic classification of the species while at the same time reporting information concerning their habitat (OMNIDIA, Lecointe et al., 1993), all of which makes them

the benthic organisms which are the preferential indicators of biological quality. There are, however, calls to consider all algae and benthic Cyanobacteria together in these issues (Poikane et al., 2016), an approach that we advocate here and that Thomas et al. (2015) have also taken.

This article aims at recording benthic microalgae and Cyanobacteria present in the Serranía de Cuenca, an area that –despite being one of the Iberian territories least affected by human impact at present– has barely received the attention of algologists (see Álvarez Cobelas & Rojo, 2022). The number of studies concerning them is surprisingly low, and these studies date back many decades (González Guerrero, 1940, Caballero, 1942). They are based on the collections of Arturo Caballero (first alone) and his daughter Francisca during the 1930s and 1940s when they both worked at the Real Jardín Botánico of Madrid (Álvarez Cobelas, 2020). Fortunately, the present contribution has been carried out in a limestone area of the Peninsula and there are some previous studies in limestone environments with which to compare our results (for example, those of Aboal, 1989, 2006; Sabater, 1989; Cantonati & Spitale, 2009; Špoljar et al., 2012; Ciorca et al., 2017).

In addition, the purpose of this study has been to describe the ecology of these organisms, from their integration into local assemblages to the factors that structure their distribution in the aquatic ecosystems of the region. This approach, which brings together information concerning local assemblages and the comparison between these assemblages and the metacommunity, is not at all a common one, since it can be seen in the literature on these topics that when microalgal assemblages are analysed their metacommunity is not described, and vice versa (see Rojo, 2020). To achieve this, well-known descriptors and statistical tools have been used on a presence-absence database, so that results are easily comparable with those of other data sets of the same type.

STUDY AREA

The area studied in the Serranía de Cuenca covers 3588 km², and the waters drain towards the Tajo and Júcar basins, both of which are similar in surface area within the territory. The substrate of

both is fundamentally limestone. In the water, the dominant formation in these karst landscapes is composed of travertine, whose abundance and impact are greater in high areas, with steeper slopes and forests, as occurs at the source of the Cuervo river and in the Guadiela river gorge in Beteta. In very localized areas, there may be conglomerates, as in the Cabriel downstream Boniches, or boulders produced by snow erosion, as in the Guadarojo River, upstream from Valdemoro Sierra. The valleys appear between a river valley, flanked by large limestone rock formations, partly due to geochemical dissolution. The towns can be located at the bottom of the valley or in the middle of the hillside, especially if they had a strategic interest in the Middle Ages, such as the towns of Beteta, Cañete and Moya.

As for the vegetation, the dominance of Scots pine (*Pinus sylvestris* L.) forests can be seen in the highest areas, and of black pine (*P. nigra* J.F. Arnold) in the others. On the moors there were many junipers in the past, but most of them disappeared due to logging. In areas with less soil

and more slopes, boxwood (*Buxus sempervirens* L.) predominates, while in the small mountain valleys there are grasslands, perhaps of human origin, for livestock feeding. Holm oaks, cleared (pasture) or not, are residual. Oak groves, too. The river valleys are dominated by riparian vegetation of large willows (*Salix* spp.) in the upper areas, interspersed with ash, lime, maple and hazelnut trees; in the middle and lower portions of the rivers, the willows have been replaced by plantation poplars (*Populus nigra* L.). Another very conspicuous landscape is that generated by the wicker plantations (*Salix eriocephala* Michx.), an irrigated crop present in many valleys in the upper part of the Serranía, but now almost abandoned and growing naturally in many areas.

The most remarkable thing about the studied territory is the effect of towns on the landscape. Around them, in a perimeter of 1-5 km, the area is intensely deforested, but then it is reforested until the next town downstream of the hydrographic basin. In the deforested environment, whose agricultural (cereal or wicker) and livestock uses are

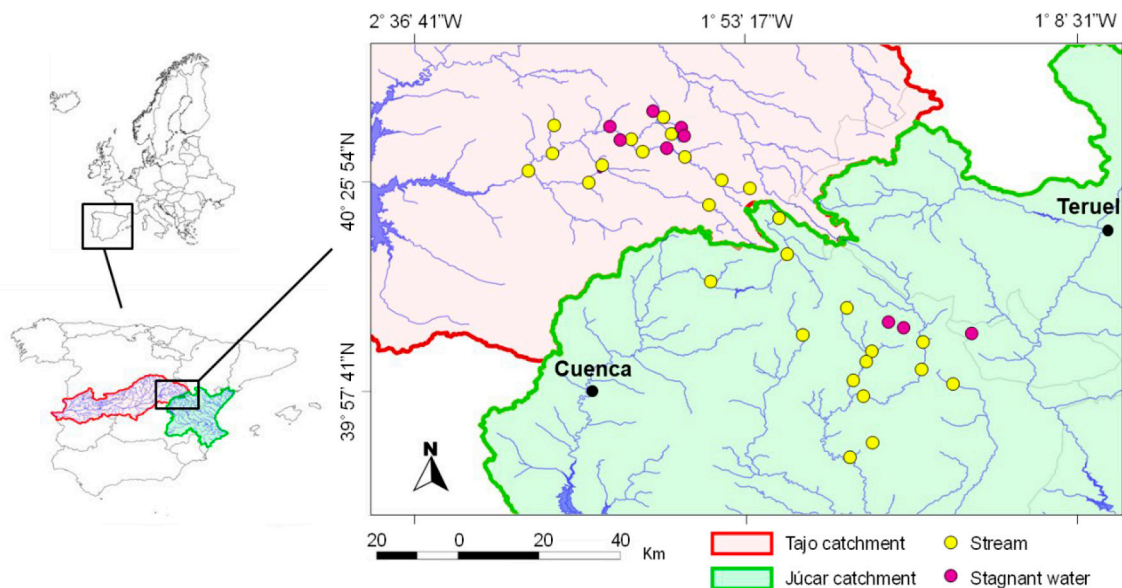


Figure 1. Map of the study area, showing the sampling sites located in the upper parts of two grand catchments (Júcar and Tajo rivers). Coordinates and more information on these sample sites in Tables S2-S3 (Supplementary Material available at <http://www.limnetica.net/en/limnetica>). Mapa de la zona de estudio, donde figuran los lugares de muestreo situados en la parte más alta de las dos grandes cuencas hidrográficas (Júcar y Tajo). Se ofrece más información sobre las coordenadas geográficas y los lugares de muestreo en las Tablas S2 y S3 del material suplementario albergado en <http://www.limnetica.net/en/limnetica>.

almost abandoned today, wild rose bushes (*Rosa* spp.) and hawthorn (*Crataegus monogyna* Jacq.) predominate.

The highest parts of the Serranía are relatively low (< 1800 m). With few exceptions, the slopes are modest, that is, there are hardly any torrent. Chemically, its waters are calcium-bicarbonated and mostly oligotrophic (Table S1 in Supplementary Material, available at www.limnetica.net/en/limnetica; see also Álvarez-Cobelas et al., 2020). Given the depopulation, the impact of urban wastewater is negligible almost everywhere, although it is sometimes noticed, as in the Cabriel river downstream from Boniches and in the Masegar stream, downstream from the Beteta dairy farm. Agricultural pollution does not seem important either, thanks to the abandonment of most of the farmland, once rainfed. There is only an appreciable extension of farmland in some towns in the lower Serranía, such as Cañete and Boniches. Regarding livestock, its impact seems greater in the case of the permanently housed animals (pigs and chickens) than those which are occasionally let loose (lambs and cows, which are much less abundant). The local impact of erosion due to livestock can be seen in some river environments like the Masegar creek.

More environmental information on this territory can be found in Cava (1994) and Álvarez Cobelas et al. (2020).

MATERIALS AND METHODS

Sampling and spatial and environmental databases

During the month of August 2017, samples were taken in all the environments of the Serranía that housed water. In total, we took into consideration 32 stations in fluvial environments and 10 in stagnant ones (Fig. 1). The coordinates of each station, its hydrographic basin (Júcar or Tajo) and the substrate on which the benthic photosynthetic organisms grew were recorded, specifying the host plant group when we were dealing with epiphytes. These data can be consulted in Table S2, in Supplementary Material, available at www.limnetica.net/en/limnetica.

In situ, we measured discharge, water tem-

perature, dissolved oxygen, pH and conductivity, using sensors recalibrated before each sampling, as well as taking a free water sample for chemical analysis. This sample was immediately frozen and then analysed in the Biogeochemistry laboratory of the National Museum of Natural Sciences. Chemical analyses using APHA (2005) procedures were undertaken shortly after water collection. Nitrogen (nitrate, nitrite and ammonia) compounds and SRP were measured on filtered water with a Seal-3 auto-analyzer whereas organic carbon (total, TOC hereafter, and dissolved, DOC hereafter) and total nitrogen were with a TOC-VCSH Shimadzu equipment on raw samples. Raw samples were digested with strong acids to mineralize all phosphorus forms to render total phosphorus as SRP, which was measured as above. More details on the followed methodology are available in Álvarez Cobelas et al. (2020).

Some biological variables which could affect the assemblages of microalgae and Cyanobacteria were qualitatively registered, such as riparian vegetation or not (0: with, 1: without), the presence of fish herbivores (0: no fish, 1: fish present) and human impact (0: none, 1: low, 2: moderate). The data of the fish species present in the different areas of the Serranía were extracted from Buil et al. (1987). To geographically locate each inventory, we used its geographic latitude and longitude coordinates in their decimal version. We also estimated other variables related to the river basin located upstream of each inventory from SIGPAC data (www.sigpac.mapama.gob.es/feqa/visor/), such as the altitude, the extension, the joint percentage of agricultural land and abandoned cultivated areas, the percentage of forests, the average slope, the existence (or not) of stagnant ecosystems upstream a given site that might enhance water quality downstream and the existence (or not) of towns upstream of a given site that might impair water quality downstream.

Taxonomic databases

At each station we tried to take samples of benthic microalgae and Cyanobacteria in all the existing mineral and plant substrates, which meant a total of 136 samples of organisms. The samples were fixed with 4 % formalin. Diatom valves were treated by

the method recommended by Blanco et al. (2008) for cleaning, observation and determination. The latter was carried out with a Nikon Eclipse Ci-L microscope, mainly using the Süßwasserflora Mitteleuropa literature (Büdel et al., 1984-2013), although the British flora was also used (John et al., 2002). All formalin-treated samples and Diatom slides were stored at the Biogeochemistry Dept. collection of the National Museum of Natural History (CSIC) in Madrid. We identified to species level whenever possible; when not, our identification, based on morphologically distinct traits, allowed us to consider populations as separate taxa within a given genus, which is a common practice in inventories of all taxonomical groups (e.g. Virta & Soninen, 2017).

In the present work, we also considered Cyanobacteria because, even though they are not eukaryotes, they photosynthesize and form part of the same biological assemblages of benthic algae. Although in the determination of Cyanobacteria we tried to be very careful and only based on morphology, we consider that our results are useful to typify the communities of benthic organisms. The morphology of vegetative forms has been proposed for other groups of algae which are difficult to classify, such as *Oedogonium* taxa and filamentous Desmids, whose taxonomy continues to be based on the fruiting forms, which –unfortunately– only occur at isolated times of the annual cycle and rarely in summer. For this reason, we could

not identify the species in this group and had to be content with the recommendation of Hainz et al. (2009). The occurrence matrix of the taxa, and the places where they were found, can be looked up in Table S3, in Supplementary Material, available at www.limnetica.net/en/limnetica.

For most statistical analyses, the data from all sampling stations were analysed together, but also separated by subgroups of samples. The ones that stood out were the large basins (Júcar vs Tajo), the habitats (stagnant vs fluvial) and the main types of substrate (mineral vs vegetal, except for RDA, see below). The methods applied to our data of the taxon presence-absence follow the scheme in Figure 2.

Indices and multivariate analysis of assemblages and metacommunity

Local and regional composition.– The following aspects were considered (Fig. 2):

A) From each sample, its taxonomic composition was obtained, which also constituted a measure of its alpha diversity, such as taxon richness.

B) In order to know what factor could be influencing the richness of each place, its correlation with the variables of the sub-basin and the local physico-chemical and biological variables was estimated. Spearman's correlation was used since we assumed that the statistical distributions involved were not parametric (Siegel & Castel-

	LOCAL COMPOSITION	REGIONAL COMPOSITION	SPATIAL HETEROGENEITY
Structural analysis of communities and its likely control factors	Microflora/Flora/Fauna Alpha diversity (richness)	Microflora/Flora/Overall fauna Taxon occurrence (% presence) Rare taxa (<20% all samples) Cosmopolitan taxa (>80% all samples) Associations (cluster similarity) Overall taxa richness Maximal estimated value (rarefaction)	Beta diversity (Harrison index) Gradient ordination of samples (CCA flora & environment)
CONTROL FACTORS Spatial location Catchment Chemical Biotic	Correlations		Partial RDA (explained variability by each factor group)

Figure 2. Methodological scheme followed in this work, useful for the occurrence analysis of any set of botanical, zoological or microbiological inventories. CCA: canonical correspondence analysis; RDA: redundancy analysis. *Esquema metodológico seguido en este trabajo, útil para análisis de presencia-ausencia de cualquier conjunto de inventarios, sean botánicos, zoológicos o microbiológicos. CCA: análisis canónico de correspondencias; RDA: análisis de redundancias.*

lan, 1988). These analyses were also performed for the subgroups of samples of the large basins, habitat and substrate.

C) Taking into account the totality of samples, it was possible to quantify the presence of each taxon as the percentage of those in which it appears. A rarity analysis was also performed, quantifying the relative frequency of each taxon in a subgroup of samples; those species that appeared in less or more than 30 % of the samples were considered rare, or, on the contrary, cosmopolitan.

D) The set of samples makes it possible to obtain a dendrogram that groups together the taxa that coexist, so that the most common taxa associations emerge. To do this, we used hierarchical agglomeration cluster analysis, based on the Jaccard similarity matrix and the UPGMA clustering method (Unweighted Pair Group Method with arithmetic Average clustering; Legendre & Legendre, 1998). The degree of confidence of each node, which implies the probability that a cluster of taxa will be found or the percentage of replicates supporting each node, was evaluated by a bootstrap repetition process ($N = 500$), thus showed on the resulting dendrogram and being used as a criterion to select the relevant clusters (Rojo et al., 2017). Given the large number of taxa present in few inventories, those whose frequency of appearance was less than 20 % of the total were eliminated before the analysis.

E) We also studied the rarefaction of taxa for the total inventories, measured as Mao's Tau (Colwell et al., 2004), which allows us to estimate the maximum number of taxa that could occur in the territory at the time of sampling, since it is assumed that not all existing taxa would be inventoried.

Spatial heterogeneity. - Regarding this, we studied the following facets (Fig. 2):

A) Calculation of beta diversity. This is a measure of spatial heterogeneity based on how many taxa are common between sites and how many are different. It is clear that the more samples included, the greater the beta diversity could be; therefore, it is appropriate to weigh it taking into account the number of samples, and for this the Harrison index is used (Koleff et al., 2003). The beta diversity is thus obtained for the set of samples and for the already mentioned subsets (large

basin, type of environment and type of substrate).

B) To find the relationships between the main taxa (dependent variable) and the main environmental factors (independent variables) which could control their distribution, we use canonical correspondence analysis (CCA), which orders taxa according to axes that are a function of combinations of environmental variables, that is, it is an analysis of environmental gradients. The latter encompass local chemistry and biology and those of the subcatchment already mentioned above. All of them were chosen taking into account that they were not significantly correlated with each other, in order not to generate collinearity (Burnham & Anderson, 2002), and that they represented the main aspects to be considered. Thus, the altitude, the percentage of each basin with forests, the conductivity, the total phosphorus (an urban pollution indicator) and the existence or not of riparian vegetation and the possible fish herbivory were used. The variables were transformed by logarithmic transformation when their statistical distribution was not normal. For the matrix of organisms, those whose frequency was less than 20 % in the total of the inventories were discarded.

C) In order to discriminate whether space, the environmental conditions of each place, the catchment features, the local factors or the mediation of space on these three latter sets of variables are the conditioning factors of benthic assemblages in a metacommunity, we used partial redundancy analysis (Borcard et al., 1992; Redundancy Analysis or RDA). The results would enable to distinguish the effect (explained variance) of the pure factors (space, catchment, chemical and biological matrices) without the other groups of factors and their overall variability (Maestre et al., 2008). Since inventories on all available substrates were recorded for a given site, we pooled all of them to produce a whole presence-absence assemblage for each site, thus discarding the substrate effect on the local flora. Therefore, as a dependent variable for the RDA, we calculated a Jaccard similarity matrix using such a site approach, working only with taxa that appeared –at least– in 20 % of the total sites.

As independent variables we used four matrices: one for geographic coordinates (locality), one for chemistry, one for biology, and one for the

characteristics of the upstream subcatchment. A preliminary correlation analysis was undertaken to discard strongly correlated variables that might

inflate the explained variance by multicollinearity (Borcard et al., 2018). The chemistry matrix included water conductivity, total phosphorus,

Table 1. Composition of benthic microalgal and Cyanobacteria, recorded in Serranía de Cuenca (Central Spain) during summer. Numbers of total taxa, and of each large taxonomic group depending on the selected subgroup of samples, are reported. The numbers of common taxa and the coefficient of similarity (Jaccard index) between subgroups of samples are also indicated. The richness values weighted by the number of samples (richness per sample) and the average richness of sample groups, as well as their standard deviation, the coefficient of variation (CV), and the maximum and minimum values reached are shown. *Composición de microalgas y Cianobacterias bentónicas estivales de la Serranía de Cuenca. Número de taxones totales y de cada gran grupo taxonómico dependiendo del subgrupo de muestras seleccionado. Se indica el número de taxones comunes y el coeficiente de similitud (índice de Jaccard) entre subgrupos de muestras. Se ofrecen los valores de riqueza ponderados por el número de muestras (riqueza/muestra) y el promedio de la riqueza de los grupos de muestras, así como su desviación típica, el coeficiente de variación (CV), y los valores máximo y mínimo alcanzados.*

	All sites	Stagnant waterbodies	Streams	Júcar catchment	Tajo catchment	Mineral substrate	Vegetal substrate
NUMBER OF SAMPLES	136	26	110	71	65	45	91
TAXA							
BACILLARIOPHYTA	154	89	130	106	127	114	130
CHLOROPHYTA	133	46	106	76	84	52	113
CYANOBACTERIA	84	39	68	58	54	49	68
DINOPHYTA	2	0	2	0	2	1	1
EUGLENOPHYTA	6	4	3	3	5	4	4
RHODOPHYTA	1	0	1	1	1	1	1
Overall taxa	380	178	310	244	273	221	317
Common taxa		106		137		158	
Jaccard similarity		0.28		0.36		0.42	
WEIGHTED DATA BY SAMPLE NUMBERS							
BACILLARIOPHYTA	1.13	3.42	1.18	1.49	1.95	2.53	1.43
CHLOROPHYTA	0.98	1.77	0.96	1.07	1.29	1.16	1.24
CYANOBACTERIA	0.62	1.50	0.62	0.82	0.83	1.09	0.75
DINOPHYTA	0.01	0.00	0.02	0.00	0.03	0.02	0.01
EUGLENOPHYTA	0.04	0.15	0.03	0.04	0.08	0.09	0.04
RHODOPHYTA	0.01	0.00	0.01	0.01	0.02	0.02	0.01
All taxa	2.79	6.85	2.82	3.44	4.20	4.91	3.48
STATISTICS							
Average	19	22	18	17	22	19	19
Standard deviation	7	8	7	7	6	7	7
CV	36	34	36	40	28	35	37
Min value	1	7	1	1	7	9	1
Max value	40	40	36	34	40	36	40

nitrate, ammonia and dissolved organic carbon. The biological one comprised the existence (or not) of riparian forest, fish herbivory (or not) and human impact (see above). The catchment matrix encompassed altitude, the percentage of range- and croplands (which was complementary to that of forests), the average slope in the catchment upstream of sites, and the existence (or not) of upstream towns and stagnant environments. RDA was applied to all sites and to the subgroups of catchments (Júcar and Tajo) and riverine habitats. Stagnant environments could not be tested in this way because of their low numbers that precluded meaningful statistical results.

All the analyses were carried out using the PAST statistical package (Hammer et al., 2001) version 3.0 (2018), except for the RDA, for which we used the R language algorithms that appear in the Vegan package (Oksanen et al., 2019).

RESULTS AND DISCUSSION

Composition of local and regional flora

The total number of taxa found in the study is 379. The Tajo basin exhibited more taxa than that of Júcar, and if we take into account the number of samples, a lower richness is clearly observed in stagnant environments compared to fluvial ones (Table 1). More taxa appeared on plant substrates than on mineral ones (see Tables S2-S3), something which Špoljar et al. (2012) also noted in a karst river in Croatia. The average richness, and its variability, varied very little between the different subgroups studied (Table 1).

Analysing the rarefaction of the taxa over the territory, whose adjustment function was taxonomic richness = $(526 * \text{No. samples}) / (59 + \text{No. samples})$, we were able to estimate a maximum value of richness of 431 taxa. This means that doubling the number of samples analysed would have only increased overall taxon richness by 14 %.

Regarding the environmental factors that could be affecting richness, of all the variables analysed (a total of 31), only ammonia showed a weak ($r = -0.41$, $p < 0.05$) inverse relationship with total richness, thus suggesting that even low ammonia concentrations, as recorded in Serranía de Cuenca waters (Table S1) could be slightly detrimental to

the richness. And this is consistent with what is observed in samples from stagnant places, where we observed an inverse relationship with ammonia and total phosphorus ($r = -0.74$ and -0.70 , respectively, $p < 0.05$). A statistically significant relationship ($p < 0.05$), albeit a weak one, was also found when the samples on plant substrate were analysed, again showing an inverse relationship with nitrogen compounds such as nitrate and ammonia ($r = -0.41$ and -0.48 , respectively), but it also seems that richness was enhanced by water temperature ($r = 0.41$). A relationship between the richness of benthic Diatoms and Cyanobacteria and local variables has also been found in sub-arctic stagnant environments, although in this case the most important controlling variable was pH (Teittinen et al., 2017).

Regarding the flora composition, Diatoms were the group with the highest number of taxa, followed by Chlorophytes and Cyanobacteria (Table 1). Despite being in a limestone landscape, the presence of several species of Desmids, belonging to the genera *Cosmarium*, *Hyalotheca*, *Pleurotaenium* and *Staurastrum*, could be observed, something which has also been verified in other non-acidic landscapes (Coesel, 1982; Osório et al., 1982). Interestingly, *Oocardium*, the travertine-encrusting desmid (see Moreno Alcaraz et al., 2013), has not been recorded despite our search in the area, but it must not be forgotten that ours was not an approach to maximize biodiversity knowledge, but to describe ecology of assemblages and this may overlook rare taxa which could otherwise be present. Anyway, the Serranía de Cuenca algal flora is suggestive because it reflects conditions of little environmental alteration, increasingly rare in our country; the current composition of the flora could serve as the basis for subsequent determinations of environmental changes, whether or not derived from global change.

From the point of view of algal and Cyanobacterial morphotypes, the territory is quite varied. In addition to the large arbuscular forms (*Chara vulgaris*, Table S2), there is an abundance of filamentous organisms, which can be branched (*Cladophora glomerata*, *Chaetophora* spp.) or not (Oscillatoriales, Oedogoniales, Ulotrichales, Zygnetmatales), Cyanobacteria that embed lime-

stone (*Phormidium incrustatum*, *Rivularia*), nitrogen-fixing Cyanobacteria that form macroscopic gelatinous colonies (*Calothrix*, *Nostoc*, *Stigonema*), arbuscular Diatoms whose gelatinous sheaths fasten onto the substrates (*Cymbella* spp., *Gomphonema angustatum*), Chlorophytes attached to the substrate (*Aphanochaete*, *Coleochaete*, *Gongrosira*), and Diatoms, which can or cannot move on their own, etc.

The dominant taxa in the associations were always Diatoms (*Achnanthydium minutissimum*, *Gomphonema angustatum*, *Cocconeis placentula*) and species of Cyanobacteria of the genus *Heteroleiblenia*, often occurring both as epiphytes and on mineral substratum; this can be seen for all subgroups of the samples (Table 2). Obviously, the order of dominant taxa varies depending on the group of samples studied (Table 2), but the dominant group throughout the territory is invariable, which suggests that we are facing relatively homogeneous environmental conditions (Álvarez Cobelas et al., 2020). As for their ubiquity, the number of taxa found in only one sample was 169, these being rare species. Altogether only 25 taxa were present in 20 % or more of the samples. It is, therefore, a flora where rare taxa predominate, which is defined by very

stable environments where the specialization of the niches is very high. Related to this, Tornés & Ruhi (2013) conclude that unstable environments (intermittent flow in their case) reduce the number of rare taxa in the regional flora of Catalan fluvial benthic Diatoms.

It is not easy to compare our results in terms of composition and richness with the oldest recorded in the area. González Guerrero (1940) worked with samples from a single locality, collected in the Puente de Vadillos in June 1935, although it is unclear whether the samples were from the Cuervo River, the Guadiela River, some stagnant environment in the surroundings or from all of these places. In his work, apart from mentioning some common Diatoms (*Cymbella lanceolata*, *Melosira varians*, *Ulnaria ulna*), he cites several *Spirogyra* and *Zygnema* taxa, as well as the green algae *Draparnaldia glomerata* and *Hydrodictyon reticulatum*. However, of all those he mentions, we have only found *Ulnaria* in that locality. We did not detect *Rivularia* there either, but we did detect *Oscillatoria subbrevis*, while the Badajoz algologist also pointed out the presence of *O. amphibia*, *O. brevis* and *O. irrigua*. González Guerrero's acolyte, Fernanda Caballero (1942), cited numerous Desmidiaceae and Chlorococcales in

Table 2. Algal and Cyanobacterial taxa that appear with a frequency greater than 50 % in all summer samples and their subgroups in the Serranía de Cuenca. They are arranged by frequency in each column. *Taxones de algas y Cianobacterias que aparecen con una frecuencia superior al 50 % en todas las muestras estivales y subgrupos de las mismas en la Serranía de Cuenca. En cada columna se ordenan por frecuencia.*

All samples	Stagnant waterbodies	Streams	Júcar catchment	Tajo Catchment	Mineral substrate	Vegetal substrate
<i>Gomphonema angustatum</i>	<i>Achnanthydium minutissimum</i>	<i>Gomphonema angustatum</i>	<i>Cocconeis placentula</i>	<i>Achnanthydium minutissimum</i>	<i>Gomphonema angustatum</i>	<i>Achnanthydium minutissimum</i>
<i>Achnanthydium minutissimum</i>	<i>Cymbopleura amphicephala</i>	<i>Achnanthydium minutissimum</i>	<i>Gomphonema angustatum</i>	<i>Cymbopleura amphicephala</i>	<i>Heteroleiblenia</i> spp.	<i>Gomphonema angustatum</i>
<i>Heteroleiblenia</i> spp.	<i>Gomphonema angustatum</i>	<i>Heteroleiblenia</i> spp.	<i>Heteroleiblenia</i> spp.	<i>Gomphonema angustatum</i>	<i>Achnanthydium minutissimum</i>	<i>Heteroleiblenia</i> spp.
<i>Cocconeis placentula</i>	<i>Cymbella helvetica</i>	<i>Cocconeis placentula</i>	<i>Achnanthydium minutissimum</i>	<i>Heteroleiblenia</i> spp.	<i>Cocconeis placentula</i>	<i>Cocconeis placentula</i>
<i>Cymbella affinis</i>	<i>Heteroleiblenia</i> spp.	<i>Cymbella affinis</i>	<i>Cymbella affinis</i>	<i>Cymbella affinis</i>	<i>Cymbella affinis</i>	<i>Cymbella affinis</i>
<i>Cymbopleura amphicephala</i>	<i>Eunotia arcus</i>	<i>Cymbopleura amphicephala</i>		<i>Cocconeis placentula</i>	<i>Cymbopleura amphicephala</i>	<i>Cymbopleura amphicephala</i>
	<i>Cocconeis placentula</i>			<i>Cymbella helvetica</i>		<i>Diatoma vulgare</i>
	<i>Navicula radiosa</i>					

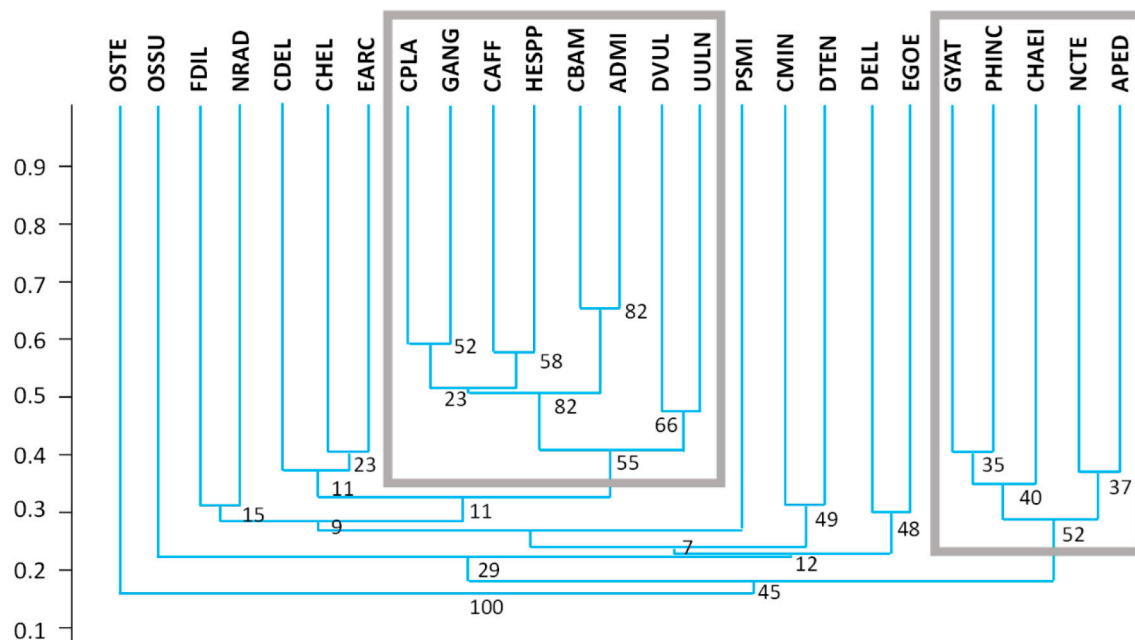


Figure 3. Main groups of benthic algal taxa and Cyanobacteria present in the Serranía de Cuenca, based on Jaccard similarity and the UPGMA algorithm, using taxa whose frequency in the total inventories exceeds 20%. The most remarkable groups are framed. The Omnidia 6.0 package has been followed for Diatom abbreviations. *Grupos principales de taxones de algas y Cianobacterias bentónicas presentes en la Serranía de Cuenca y basados en la similitud de Jaccard y el algoritmo UPGMA, empleando taxones cuya frecuencia en el total de inventarios supera el 20%. Los grupos más destacables están enmarcados. Se ha seguido el paquete Omnidia 6.0 para las abreviaturas de las Diatomeas.* ADMI: *Achnantheidium minutissimum*, APED: *Amphora pediculus*, CAFF: *Cymbella affinis*, CBAM: *Cymbopleura amphicephala*, CDEL: *C. delicatula*, CHAE1: *Chaetophora* sp., CHEL: *C. helvetica*, CMIN: *C. minuta*, CPLA: *Cocconeis placentula*, DELL: *Diploneis elliptica*, DTEN: *Denticula tenuis*, DVUL: *Diatoma vulgare*, EARC: *Eunotia arcus*, EGOE: *Epithemia goeppertiana*, FDIL: *Fragilaria dilatata*, GANG: *Gomphonema angustatum*, GYAT: *Gyrosigma attenuatum*, HESPP: *Heteroleiblenia* spp., PHINC: *Phormidium incrustatum*, PSMI: *Pseudanabaena minima*, NCTE: *Navicula cryptotenella*, NRAD: *N. radiosa*, OSSU: *Oscillatoria subbrevis*, OSTE: *O. tenuis*, UULN: *Ulnaria ulna*.

El Tobar lake, without specifying whether she found them in the plankton or in the benthos, or how many substrates she sampled. Of their rich inventories, only *Cosmarium laeve* and *C. punctulatum* appear in ours. In both studies, it seems that the local algological flora of more than 70 years ago was more eutrophic, perhaps the result of a territory where agriculture was more developed than nowadays.

The grouping analysis of the different taxa according to their coexistence (Fig. 3) allows us to visualize two different groups (bootstrap $p > 50\%$). In the first one (bootstrap $p = 55\%$), we find *Achnantheidium minutissimum*, *Cocconeis placentula*, *Cymbella affinis*, *Cymbopleura amphicephala*, *Gomphonema angustatum* and *He-*

teroleiblenia spp.; this cluster is included in a bigger cluster of very low-probability ($p = 29\%$; Fig. 3) which makes us consider it to be more weakly defined. This is a set of cosmopolitan species, with a broad environmental or eurioic spectrum (Carayon et al., 2019). In the second, better defined group (bootstrap $p = 52\%$), *Amphora pediculus*, *Chaetophora* sp., *Gyrosigma attenuatum*, *Navicula cryptotenella* and *Phormidium incrustatum* appear; this grouping is more restrictive, and *Phormidium* and arbuscular green algae such as *Chaetophora* coexist in it, both involved in the formation of travertine, and *Amphora pediculus* as well, with which they have previously been associated (Freytet & Verrecchia, 1999; Jones & Renault, 2017). Roughly speak-

ing, although their analyses were not oriented in the same way as ours, our assemblages coincide with those of limestone massifs without chlorides (Margalef, 1951) and with those present in the limestone town of Vallfogona (Gerona), analysed by Sabater (1989).

Spatial heterogeneity

Beta diversity in the studied region amounted to 0.14 (Harrison's index). The highest figures for beta diversity occurred in stagnant environments (0.28), followed by communities living on mineral substrate (0.25). The differences between large basins seem negligible (Júcar-0.20 and Tajo-0.18). The range of values calculated for all sample groups was between 0.14 and 0.28, that is, beta diversity was not high. Therefore, it is a fairly uniform area in terms of the composition of

benthic photosynthetic organisms, thus responding to what is expected in a region with a fairly homogeneous environment (Álvarez Cobelas et al., 2020). When comparing our figures with the beta diversity values of Diatoms in river environments throughout France (0.64-0.76; Jamoneau et al., 2018) and although these figures have been normalized by the number of samples, the much greater heterogeneity of the French landscape is obviously decisive in terms of its beta diversity.

However, we have been able to verify that the conductivity gradients of waters and the percentage of forests in each sub-basin, a figure that is complementary to the percentage of abandoned lands, formerly agricultural, were the factors that best explained the presence of the dominant taxa of benthic algae and Cyanobacteria (Fig. 3). A factor that acts locally (conductivity) and another that works at the level of the hydrographic sub-ba-

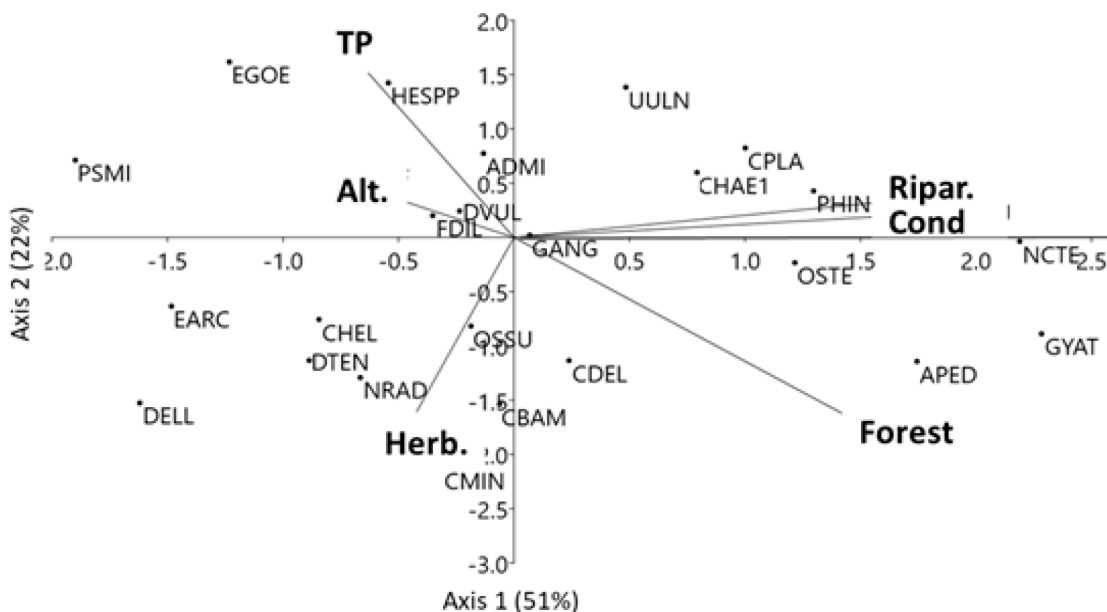


Figure 4. Canonical correspondence analysis of benthic algae and Cyanobacteria in the Serranía Cuenca during summer, using the taxa of Figure 3. The variance explained by each axis and the orientation of the environmental factors are indicated. Alt.: altitude (masl), Forest: forest land in the hydrographic sub-basins (%), Cond: conductivity ($\mu\text{S}/\text{cm}$), Ripar.: presence or absence of riparian forest, TP: total phosphorus (mg P/L), Herb.: possible herbivory by fish or not. *Resultado del análisis canónico de correspondencias de algas y Cianobacterias bentónicas estivales de la Serranía conquense, usando los taxones de la Figura 3. Se indican la varianza explicada por cada eje y la orientación de los factores ambientales. Alt.: altitud (m.s.n.m.), Forest (%): terrenos de bosque en las subcuencas hidrográficas (%), Cond: conductividad ($\mu\text{S}/\text{cm}$), Ripar.: presencia o ausencia de bosque ripario, TP: fósforo total (mg P/L), Herbiv: posible herbivoría por peces o no.*

Table 3. Partial redundancy analysis. Percentages of explained variance of the distribution of algal and Cyanobacteria assemblages in the Serranía de Cuenca by different groups of factors. Both the overall variance of each matrix (i.e. variable groups) and the variances of the pure matrices (that is, without interaction with the other matrices) are reported. Notice lacking of the stagnant environment subgroup (see main text). *Resultados del análisis de redundancias parciales. Porcentajes de la varianza explicada de la distribución de comunidades de algas y Cianobacterias en la Serranía de Cuenca por los distintos grupos de factores. Se incluye la varianza de cada matriz (grupos de variables independientes) y las varianzas de las mismas matrices sin interacción con las demás matrices. Advértase que no se han incluido los ambientes estancados en el análisis (véase el texto principal).*

Independent variables	Dependent variables: assemblages			
	All sites	Streams	Júcar catchment	Tajo catchment
Space	5	5	0	4
Chemistry	20	24	19	40
Catchment features	6	3	5	12
Biology	7	3	5	15
Pure space	2	3	0	4
Pure chemistry	8	10	7	15
Pure catchment features	3	4	0	11
Pure biology	2	5	0	12
Unexplained variability	79	82	85	52

sin (the percentage of forests) manage to explain 79 % of the total variance of the canonical correspondence analysis. Also, in another study of microscopic benthic flora in an area of scarce human impact (Kiamichi basin, SE Oklahoma; Atkinson & Cooper, 2016), such as the one studied here, it was seen that conductivity and not nutrients, was the most relevant local factor for the benthic flora. However, in an extensive and complex river basin, such as the Guadiana one (Southern Iberia), other local factors (nutrients) and those related to the hydrographic basin (geology) stood out as those that best explained the distribution of benthic Diatoms (Urrea & Sabater, 2009).

Our canonical correspondence analysis highlights the ecosystems located in areas with more forest (positive part of axis 1, Fig. 4), which have well-developed riparian vegetation fringes and match somewhat lower conductivity systems. The species associated with them are those that were shown to coexist in the similarity analysis, i.e. the association typical of travertine environments (for example, *Phormidium incrustatum* as an indicator taxon). On the other hand, there is a set also made up of Cyanobacteria and moder-

ately large Diatoms (*Pseudoanabaena minima*, *Diploneis elliptica*, *Eunotia arcus*) on axis 1, recognized as epiphytes on different parts of macrophytes, such as *Potamogeton* or Nymphaeaceae (Letáková et al., 2016; Arguelles, 2019). The variance explained by axis 2 was not very high, but it distinguishes two environments marked either by nutrients (phosphorus) or by fish herbivory. In the former, another tandem of Cyanobacteria and Diatom (*Heteroleibleinia* spp., *Epithemia goeppertiana*) seems to stand out, both cited in eutrophic environments (Belykh et al., 2017; Schneider et al., 2018). And the presence of herbivorous fish is associated with small Diatoms which benefit when their larger benthic competitors are consumed. So, although it can be said that there is homogeneity in the study area in terms of assemblage composition, there is also heterogeneity as shown by some environmental factors and the groups of microscopic photosynthetic organisms associated with them. Summarizing, in addition to the association of Cyanobacteria and Diatoms in the different environments, this analysis also confirms that local factors are not the only ones that explain the distribution of taxa (see below).

Our RDA study emphasizes the importance of local environments (chemistry and biology) in the conformation of the algal and Cyanobacterial metacommunity in the Serranía de Cuenca, since all environmental factors significantly explained variance (Table 3) even without interactions between them (e.g. without spatial autocorrelation; Leibold & Chase, 2018). Existing studies on Diatom metacommunities do not usually analyse the biological influence on continental benthic algae, but rather the local physico-chemical environment (Potapova & Charles, 2002); these studies show that the local component (e.g. the chemical and biological environment) has more relevance as compared with the spatial one as the area covered by the study is diminished; this is our case (Table 3). More specifically, it has been highlighted that in addition to the pure abiotic components (chemical, catchment) the pure biological component has relevant effects too. In one of the few studies of the metacommunity of benthic Diatoms that included biotic factors (Dolomite Alps; Cantonati & Spitale, 2009) the conditions generated by the riparian forest also turned out to be a relevant structuring factor. Anyway, unexplained variability was high, which often takes place in many metacommunity studies as result of low number of sites, overlooked factors, stochasticity, interactions with taxa not considered and so forth (Leibold & Chase, 2018; Szabó et al., 2019; Gálvez et al., 2020).

We conclude this article by pointing out that combining the tools for the analysis of taxa assemblages with those of the metacommunity analysis, including geographic, local (without forgetting the biotic factors which are much less studied) and the catchment factors, will greatly increase our knowledge concerning the ecology of these organisms. As a corollary, we want to emphasize that the joint analysis of these structures (from assemblages to metacommunities) and their control factors could be carried out on the enormous number of inventories (presence-absence) that already exist. Ultimately, our intention is to suggest a standardized approach for the study of the ecological structure of biological presence-absence inventories (botanical, zoological and/or microbiological) at different scales (from local to regional). This methodology would not only make it possible to

better typify communities in order, for example, to compare them with future ones arising from global change and other environmental changes, but it could also have an applied aspect allowing the inventories of organisms, generated by environmental management (such as the application of the Water Framework Directive) to improve our understanding of the ecology of assemblages and metacommunities. Obviously, this approach could be also useful for any organismic group involved.

The methodology proposed here can be automated with little effort in order to generate results based on inventories of occurrence. On the other hand, morphological analysis for taxonomic determination may soon be automatic (Pedraza et al., 2017), something which will also happen with massive biochemical analysis of nucleic acids, proteins and other metabolites present in organisms (McLean, 2013). This two-fold automation will allow rapid environmental comparison between the many biological communities from which data are being generated. In other words, the availability of Big Data and artificial intelligence will develop all these tasks, which will be of great interest for the basic and applied ecology of any biological community (plants, animals, microbes). And this is going to happen sooner (Hristopulos et al., 2021; Xue et al., 2021) rather than later.

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