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Comparison of benthic diatoms from Mediterranean and Atlantic Spanish streams: community changes in relation to environmental factors

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Abstract (250)

Water and benthic diatom samples were collected in different climatic and geological areas of Spain. Seventy-two sites were sampled in Atlantic Galicia (NW mainland) and forty-seven sites in the Mediterranean Balearic Islands (NE) in different seasons during2004 and 2006 to identify the most important environmental factors influencing water composition. Furthermore, spring samples from 2004 and 2006 were explored to assess differences among diatom assemblages. Streams were selected to cover a wide range of environmental variability. Overall, the Atlantic streams had higher discharge and the Mediterranean streams higher conductivity. In second instance, in both areas water chemistry was most importantly influenced by diffuse agriculture and local point source organic inputs, leading to high contents of nitrate, ammonium and phosphate. Two-way indicator species analysis (TWINSPAN) produced five diatom groups with different species composition in each study area. The unpolluted streams in the Balearic Islands were characterized by the presence of *Cymbella microchepala* and *Cymbella vulgata*, while in Galicia *Eunotia subarcuatoides, E. intermedia* and *Surirella roba* characterized minimally disturbed streams. *Achnanthidium minutissimum sensu lato* appeared in high abundance in both studied areas. Taxa inhabiting organic polluted Mediterranean streams were *Cocconeis euglypta*, *Navicula veneta*, *Nitzschia inconspicua* and *Planothidium frequentissimum*, while organic loading led to a dominance of *Cocconeis euglypta* and *Eolimna minima* in Atlantic streams. The first two CCA axes explained 82% and 69% of total variance in diatom distribution in Galicia and the Balearic Islands, respectively. In spite of the presence of different diatom communities across Mediterranean-Atlantic streams in undisturbed conditions predictable changes in diatom assemblages do occur in response to organic and nutrient loading gradients.

Keywords: *Diatoms, Mediterranean, Atlantic, streams, environmental gradients, TWINSPAN.*

1. Introduction

Periphyton communities in streams and rivers are an important component of aquatic ecosystems. Benthic algae assimilate dissolved nutrients and use of solar energy to produce available biomass, being key organisms because their position at the base of the grazer's food web, constituting food resources for invertebrates, and fish (Finlay et al., 2002). Periphyton composition and distribution is influenced at local scales by environmental factors such as water chemistry, light, temperature, flow and type of substrate (Stevenson, 1996; Dodds and Biggs, 2002). At larger scales, regional differences in climate characteristics and geology determine distinctive attributes in geomorphology, biogeochemistry and hydrology, influencing water physical conditions and chemical composition and, consequently, the biogeography and species composition of benthic algal assemblages (Pan et al., 2000).

Human activities have caused a range of alterations to the biota in the majority of freshwater ecosystems, and the management of water resources insisting for precise and accurate tools (European Union, 2000) to measure the biological integrity of aquatic ecosystems (Cao et al., 2007). Environmental degradation is any change or disturbance to the environment that is perceived to be deleterious or undesirable for biotic communities, in this sense human activities have degraded watersheds, generating awareness and the increase in the scientific development of biomonitoring programs to assess the status of aquatic systems (Kelly and Witton, 1995, Hering et al., 2006). Direct sewage inputs, runoff from fertilized soils, and land erosion through logging activities, had a major impact on water resources over the last century (Billen et al., 2001; Wunsan et al., 2002; Ducharne et al., 2007). The increasing availability of nutrients such as nitrate and phosphate (through fertilizers or sewage) in freshwaters, commonly associated with eutrophication, is affecting the productivity and community structure of primary producers in aquatic ecosystems (Leira et al., 2009).

Benthic diatoms have been used in monitoring studies due to their short life cycles and their rapid response to different stressors (Bona et al., 2007). There is an ample body of knowledge on the ecology of diatoms and their optimal environmental conditions and tolerance ranges to water chemistry (Van Dam et al., 1994; Potapova et al., 2004; Schneider et al., 2013). Diatoms are often used for the assessment of nutrient enrichment due to their high sensitivity and specific abilities to respond quickly to this particular environmental change (Hering et al., 2006).

The Iberian Peninsula has two main dominant climatic regions: the Atlantic and the Mediterranean ones, arising by the differences in latitudinal position and the orography. One difference between these hydro-ecoregions is the mean annual precipitation higher in the Atlantic region (Wasson et al., 2007). The area under the Atlantic climate in Spain has permanent streams, with maximum water levels during autumn and winter (Martínez et al., 2000, Pardo and Álvarez, 2006) and mild temperatures due to the proximity of the sea. In contrast, the Mediterranean Islands climate shows irregular rainfall patterns, particularly concentrated in autumn and spring (Pardo and Álvarez, 2006; Sabater et al., 2008), with the result that Mediterranean streams tend to show a marked seasonality in their hydrology (Gasith and Resh, 1999). Many temporary streams in the Balearic Islands have their origin in springs, which are refugia zones for the flora and fauna that colonize the streams when water flow initiates each rainy season (Álvarez & Pardo, 2007: Delgado et al., 2013). Several factors, varying with season, are responsible for the differences found in periphytic algal biomass between Mediterranean and Atlantic streams, such as higher radiation, temperature and water nutrient contents in Mediterranean streams (Pardo and Álvarez, 2006).

Diatom metrics are commonly used to evaluate the ecological status of Spanish rivers (Blanco et al., 2008, Martín et al., 2010; Delgado et al., 2012; Álvarez-Blanco et al., 2013), to integrate and summarize the complex ecology of stream diatom communities (Kelly, 2002; Atazadeh et al., 2007). Meanwhile, this is the first study aiming to understand the changes in diatom communities' composition and abundance occurring in response to different environmental conditions that characterize streams and rivers from contrasting different climatic regions.

The purpose of this study was to identify the most important ecological relationships between stream environmental conditions of Mediterranean and Atlantic climates and their diatom communities. The classification the sampling sites was based on their diatom flora. Direct ordination methods were further used to identify key environmental factors related with the diatom-based stream classification. Finally, we tested whether regional differences in diatom community structure, if any, were more evident in reference sites than in variously impacted streams.

Our objectives were: i) to explore environmental factors that characterize natural and disturbed streams and their diatom composition in Atlantic and Mediterranean streams; ii) to identify diatom groups along the most relevant organic/nutrients loading gradients in water quality; iii) to compare the relationship between diatom groups and environmental factors along an increasing gradient of environmental degradation in water quality, and iv) to ecologically characterize species groups using the analyzed ranges of environmental stream conditions.

2. Materials and methods

2.1 Study area

The Northwest of the Iberian Peninsula (42.5°N 8.1°W) is characterized by a rainy weather with mild temperatures throughout the year, influenced by Atlantic climate. The studied area comprises all the river basins rising within the Galician region (Fig. 1). Most abundant bedrock materials are granite and schist rocks and granite reliefs dominate the orography, where hills alternate with valleys. The mountain geomorphology and regular precipitation influences rivers and streams leading to regular discharge throughout the year (Delgado et al., 2010).

The Balearic Islands (39°30′N 3°00′E) are located in the western part of the Mediterranean Sea (Fig. 1), influenced by the Mediterranean climate, characterized by moderate to low rainfall levels, hot dry summers and cool to cold winters. The seasonal patterns are relatively predictable, but interannual differences can be very large. Annual mean temperatures are around 12.5ºC in winter and 25ºC in summer (Font Tullot, 2007) in this area. In particular, the streams stop flowing during the hottest and dry summer months. This is due to the combination of seasonal rainfall variation, the predominance of a calcareous lithology which promotes infiltration (water stored in subterranean aquifers), and the relief in the island of Majorca, with steep slopes that favors a strong surface runoff.

2.2 Sampling design and selection of reference sites

Databases from the regional governments of Galicia and Balearic Islands were used to evaluate the existence of point and diffuse sources of pollution in the studied areas. Land use cover was evaluated as percentages within the basins draining each site and extracted from level I of CORINE Land Cover (2000) expressed as the percentage of three land-use types (artificial surfaces, agricultural areas and forest and semi-natural areas). A network of minimally disturbed sites, named reference sites, was selected *a priori.* These sites were thought to lack significant pressures such as artificial surfaces (< 0.4% of catchment area), agricultural land (< 35% of catchment area), sewage effluents, hydromorphological alterations of the stream bank or significant flow regulation following the criteria described in Delgado et al. (2012). Similarly, a number of sites were selected because they were influenced by different degrees of organic and nutrient enrichment degradation, aiming to cover the whole gradient of impairment in the studied areas (Table 1). The criteria used to select the reference sites were those of the Central/Baltic Geographical Intercalibration Group based on the absence of significant pressures (C/B GIG; van de Bund, 2009; Kelly et al., 2009; Pardo et al., 2012).

2.3 Data collection and laboratory procedure

A total of 119 sites were sampled in spring in the two studied areas. 72 sites were sampled once in Galicia in spring 2004 (April and May) and 47 sites were sampled once in Balearic Islands in spring 2006 (May). The sampling network in the Balearic Islands included those streams that have water for period equal or greater than 5 months (more information in Delgado et al., 2012). The sampling design used in this study fulfil the WFD's scientific and technical requirements to evaluate the ecological status of rivers in these two areas (Delgado et al., 2010; Delgado et al., 2012).

2.3.1 Physico-chemical variables

Water temperature, pH, dissolved oxygen and electrical conductivity were measured *in situ* with portable meters. Temperature and oxygen were measured with a WTW Oxi 197 oxymeter, conductivity with an Orion Model 115 at 25ºC, and pH with a Thermo Orion 290+. Current velocity was measured three times in one transect of the stream with a portable current meter (Flow probe, model FP101; Global water instrumentation, Gold River, California). Water discharge in each site was derived from the multiplication of the wetted perimeter by mean velocity. To analyze water composition, stream water was collected into polypropylene bottles from flowing-waters near the center of the stream; bottles were stored at 4ºC in darkness, and transported to the laboratory. Nutrients (phosphate, nitrate, nitrite and ammonia) and sulphate were analyzed with a continuous-flow autoanalyzer (Bran+Luebbe Auto Analyzer 3, Germany) conforming to the specific ISO standard. The chemical data analysis also included alkalinity, Ca^{2+}, Cl^- , Na^+, K^+ and Fe^{2+}. The biological oxygen demand (BOD_5) was measured from the difference between two measurements of dissolved oxygen before and after an incubation period of 5 days with the OXITOP IS 12 WTW.

2.3.2 Land use variables

Land use variables were calculated as the percentage of: artificial surfaces, agricultural areas and forest and semi-natural areas, in each catchment area, from the level I categories of CORINE Land Cover 2000. Different basin characteristics such as drainage area, geology and altitude were also obtained from a Digital Terrain Model (DTM; Instituto Geográfico Nacional, 2004-2008).

2.3.3 Periphytic samples

Five rocks with similar sizes (approximately 10-20 cm^2), were randomly sampled from riffles and collected from a section of flowing water in well-lit parts of the stream. Each epilithic sample was pooled by scraping the surfaces with a small toothbrush. Immediately after collection, diatom samples were fixed with formaldehyde (4%v). The sampling, treatment of samples and diatom identification from periphyton was based on European standards (CEN 2003; CEN, 2004). A subsample of the preserved periphyton suspension was treated with H_2O_2 30% and acid-cleaned.

Permanent slide mounts were prepared for each sample with Naphrax® (r.i. 1.74) to enumerate diatom species. Diatoms were observed and identified at the lowest taxonomic level possible using an optical microscope (Olympus BX40); a minimum of 400 diatom valves were counted at 1000X magnification from each slide. The identification and nomenclature were based on Krammer and Lange-Bertalot (1986-1991) and Lange-Bertalot (1993).

Three rocks, with similar size to the other five, were randomly sampled from riffles, their upper surface divided into two halves (n=3 for Chl *a* and AFDW) to provide a total of six-replicated samples of periphyton (20 to 60 cm^2) to estimate chlorophyll *a* (Chl *a*) and ash free dry weight (AFDW). The periphyton samples were collected by scraping the surface with a toothbrush collected periphyton communities samples and stored at 4ºC and transported to the laboratory into darkness. Periphyton samples were filtered through Whatman GF/F glass fiber-filters and the chlorophyll *a* (Chl *a*) was extracted with acetone (90%) for 48h at 4ºC, kept in the dark. Chl *a* concentration was measured spectrophotometrically (Hitachi Model U-2001 UV/Visible Spectrophotometer: 665nm), and corrected for degradation products using the equations given by Lorenzen (1967) without pheopigments. Samples for AFDW were filtered on to glass-fiber filters (pre-ashed and weighed), dried to constant mass at 105ºC for 24h, and reweighed. Then, filters were placed in a muffle furnace at 505ºC for 1.5 h to estimate the AFDW that was determined as the difference between initial and ash masses (APHA, 1989).

2.4 Data analysis

2.4.1 Environmental variables

A total of 25 environmental variables were measured in the two studied areas. The physico-chemical variables and Chl *a* and AFDW values were ^{10}log (x+1) transformed prior to data analysis, except for pH, and the percentages of land use were transformed with the arcsine as needed.

A Principal Component Analysis (PCA) was used to obtain major patterns of variation within the dataset of reference sites and environmental variables from the two study areas. PCA analysis was performed on log transformed environmental variables and analysed with the CANOCO 4.5 (ter Braak and Smilauer, 1998).

2.4.2 Diatom data

Diatom taxa with relative abundance < 1% in only one sample were eliminated from the analysis. Diatom abundance data were previously ^{10}log (x+1) transformed in order to stabilize variances and give more weight to larger species that are often found at low relative abundance in benthic diatom communities and which can be important for defining assemblages (ter Braak and Verdonschot, 1995; Tison et al., 2005). We used two-way indicator species analysis (TWINSPAN analysis; Hill, 1979) to define diatom assemblage's types of PcOrd software. Then, we tested the significance of between-groups differences at each TWINSPAN division with an ANOSIM analysis. In the global test, the null hypothesis of no difference between groups was rejected at a significant level of $p < 0.1\%$. For TWINSPAN we used four samples as the minimum group size.

2.4.3 Species-environment relationships

The transformed diatom abundances and environmental variables were analysed individually for each of the two studied areas, using CANOCO version 4.5 (ter Braak and Smilauer, 1998). Diatom data were initially ordinated with Detrended Correspondence analysis (DCA), and canonical correspondence analysis (CCA) (ter Braak and Verdonschot, 1995), to determine the length of the gradients for the first two axes, and to elucidate patterns in assemblage structure without incorporating data on environmental variables. DCA indicated that the gradient length was greater than two standard deviation units in both areas of study; therefore, the use of unimodal ordination techniques was appropriate (ter Braak, 1986). CCA analysis was used to relate diatom assemblages with the environmental variables to explore their relationships. CCA ordinated the samples based on the transformed relative abundance of diatom species, while constraining the solution based on regressions with environmental variables. CCA was used to explore the distribution of the diatom taxa and diatom assemblage groups defined by TWINSPAN along major environmental gradients. We used a stepwise variable selection procedure with Monte Carlo permutations that included only 10 environmental variables after 499 permutations.

3 Results

3.1 Characterization of the sampling sites

The environmental dataset, used only for the PCA analysis, consisted of 119 water samples from both areas. A total of 17 environmental variables and 3 percentages of land use cover in the basins were analyzed in the two studied areas. The mean, standard deviation and range for each environmental variable and area of study are summarized in table 2.

The Mediterranean streams have in general higher values of alkalinity, SiO_2, SO_4 and higher contents of dissolved salts, expressed by higher values of Cl^-, Ca^{2+}, Na^+, K^+, pH and electric conductivity (EC), but also higher mean values of periphytic biomass, expressed as Chl a and AFDW, than the Galician streams (Table 2). Nutrients (N_NO_2, N_NO_3, NH_4 and P_PO_4) and land use cover in the basins were similar in the two areas. The Mediterranean climate was reflected in the higher water temperatures recorded in the Balearic Islands (14.85-26.65ºC), while the higher flow levels in the Galician streams corresponded with higher rainfall (Table 2).

A total of 20 environmental variables were included in the PCA analysis. The eigenvalues for PCA axes 1 and 2 were 0.834 and 0.112, respectively, thus capturing 94.6 % of the total variance (Figure 2). The first component was identified as a gradient of nutrients (N_NO_2 and N_NO_3) related with the agricultural activities in the river basins of the two studied areas (Figure 2).

The second component identified the natural variation in climatic and geological variables between the two study areas separating Galician streams towards the positive part of axis 2, and the Balearic Islands towards its negative part (Figure 2). Samples from the Balearic Islands streams were characterized by their higher values of pH, silicon dioxide and electric conductivity, and higher ions concentrations, algae biomass (Chl *a* and AFDM), higher water temperature, and percentage artificial surfaces and BOD_5. Meanwhile Galician streams have a water composition characterized by lower dissolved ions contents, low algae biomass, and generally lower temperatures but higher discharge levels and dissolved oxygen.

3.2 Diatom communities

A total of 114 diatom taxa were identified in the Balearic Islands and 99 in Galicia. These numbers were reduced to 82 and 80, respectively, when rare taxa (with relative abundances < 1%) were eliminated for statistical analyses. Approximately 25% of the diatom taxa were common to both studied areas.

3.2.1 Galician streams

TWINSPAN analysis divided the diatom assemblages of 72 study sites in spring into eight groups but only 5 groups were validated by ANOSIM: GAL_I, GAL_II, GAL_III, GAL_IV and GAL_V (Figure 3). The first TWINSPAN division separated oligotrophic, electrolite-poor streams (groups I and II) (Table 3) with acidophilic indicator species such as *Eunotia paludosa* (EPAL) and *E. intermedia* (EUIN), from eutrophic streams (groups III-V) having *Cocconeis placentula* var. *euglypta* (CPLE), *Navicula lanceolata* (NLAN) and *Fragilaria vaucheriae* (FCVA) as indicator species (Fig. 3).

A further major division (level 2) in the left-hand branch of the TWINSPAN tree separated the groups GAL_I (indicated by *Eunotia paludosa*) from GAL_II indicated by eg. *Gomphonema exilissimum* (GEXL), *Diatoma mesodon* (DMES), *Meridion circulare* (MCCO), *Fragilaria virescens* (FVIR) and different taxa of *Fragilaria* corresponding to higher values of nutrients (P_PO_4, N_NO_2+N_NO_3) in water and higher agriculture ocupation in the basin (Table 3) than the group I.

On the right-hand branch of the hierarchy the mesotrophic rivers (groups GAL_III and GAL_IV), were separted from the more eutrophic rivers of group GAL_V (Table 3). The group GAL_V is indicated by *Sellaphora seminulum* (SSEM) and *Luticola goeppertiana* (LGOE) while the groups III and IV are characterised by *Eunotia paludosa* (EPAL).

3.2.2 Balearic Islands temporary streams

The TWINSPAN analysis of temporary stream samples resulted also in eight groups meanwhile only five were validated by ANOSIM (Fig. 4). The first TWINSPAN division primarily separated streams with lower values of P_PO_4 and N_NH_4 (Table 4), groups BI_I, BI_II and BI_III by *Achnanthidium minutissimum* (ADMI), from more eutrophic streams (groups BI_IV and BI_V) where this taxa does not appear. The second division on the right-hand branch split group BI_IV indicated by *Cocconeis placentula* (CPLE) and *Gomphonema parvulum* (GPAR), from BI_V characterized by *Sellaphora seminulum* (SSEM) and *Gomphonema parvulum* f. *saprophilum* (GPAS), both groups showing high contents of P_PO_4. The same division on the left-hand splited group BI_I (*Diploneis oblongella* (DOBL) and *Brachysira vitrea* (BVIT)) from group BI_II (*Achnanthidium biassoletianum* (ADBI) and *Sellaphora stroemii* (SSTM), while

Gomphonema *pumilum* (GPUM) and *Planothidium frequentissimum* (PLFR) were indicators for group BI_III.

3.3 Diatom groups-environment relationship

The environmental dataset consisted of 119 diatom samples from the two studied areas. Twenty-two environmental variables and 3 percentages of land use cover in the basins were analyzed. The environmental variables with significant influence on the diatom species distribution are depicted as arrows in the CCA ordination plot (Figs 5 and 6).

3.3.2 Galicia

The CCA on Galician data was performed with 72 samples, 80 diatom taxa, 22 environmental variables, and 3 percentages of land use. The step-wise forward selection and Monte Carlo Permutation test ($p < 0.01$) identified 10 chemical and physical variables of water quality (dissolved oxygen, SiO_2, Ca^{2+}, Cl^-, DIN, N_NO_2+N_NO_3, N_NH_4, K^+, pH, percentage of artificial surfaces). The eigenvalues for CCA axes 1 and 2 were 0.401 and 0.117, respectively, thus capturing 82% of the total variance explained by the factors. Axis 1was related to SiO_2, pH, Ca^{2+} and nitrate and nitrite forms, whereas axis 2 is related to dissolve oxygen, N_NH_4, Cl^- and percentage of artificial surfaces. Axis 1 separated groups GAL_I, GAL_II and some samples of GAL_III, on the right of the graph from the samples from other GAL_IV and GAL_V groups on the left (Figure 5). Axis 2 differentiated the most eutrophic samples from group GAL_V highly related with higher values of K^+, $N\_NH_4^{-,}$ Cl^- and artificial surfaces.

3.3.1 Balearic Islands

The CCA ordination from the Balearic temporary streams (Figure 6) in spring used a matrix formed by 47 samples, 82 diatom taxa and 22 environmental variables and 3 percentages of land use cover. The step-wise forward selection and Monte Carlo Permutation test (p < 0 .01) identified 10 chemical and physical variables related with water quality: P_PO_4, BOD_5, water temperature, alkalinity, Cl^-, oxygen, pH, AFDW, Chl *a* and $N\_NO_4^+$. The eigenvalues for CCA axes 1 and 2 were 0.515 and 0.307, respectively, thus capturing 69% of the total variance explaining by the factors. Axis 1 was strongly related to BOD_5, P_PO_4, alkalinity, N_NH_4 and Chl *a* in opposition to dissolved oxygen. Axis 2 was mainly related with pH and AFDW. Axis 1 separated progressively the groups of TWINSPAN samples defined for the Balearic Islands. Most samples from groups BI_I, BI_II and BI_III being located on the left of the graph, while samples of the groups BI_IV and BI_V are placed on right part of Fig. 6. Axis 2 separated group BI_IV by its relationship with Cl^- and AFDW, while samples from group BI_V, on the positive part of axis 2 were associated to high levels of DBO_5, N_NH_4 and P_PO_4 (Figure 6). As for the Galician CCA ordination, the first two axes identified a general nutrient enrichment and organic gradient as indicated by the resulting relationship between diatom composition and environmental variables.

4 Discussion

The joint analysis of water samples from Galicia and the Balearic Islands streams revealed clear differences on particular geology and hydromorphological settings, identified by the surrogate variables water conductivity, discharge and water temperature. The Galician streams were mainly characterized by higher discharges, related to their high annual regional rainfall levels promoting annual flow permanence. The temporary streams from the Balearic Islands had higher contents of dissolved salts (high electric conductivity values, Ca^{2+}) and periphytic AFDW and Chl *a*. These environmental differences have been considered relevant in understanding stream community differences in previous studies (Pardo and Álvarez, 2006). The calcareous nature of Mediterranean stream substrates is responsible for the higher mean contents of dissolved salts than in Atlantic streams. The ionic composition of stream water is extremely important for diatom assemblages, with Ca^{2+}, conductivity and pH strongly influencing diatom communities (Soininen, 2007; Smucker and Vis, 2009) and our results confirm the relevance of these factors in structuring diatom communities on a regional scale. The PCA analysis showed a clear distinction in water chemistry in the sites from the two study areas. This analysis is validating the reference abiotic conditions identified in this study, used to assess the water quality of these streams (Delgado et al., 2010; Delgado et al. 2012).
The TWINSPAN classification identified stream sample groups on the base of their different diatom assemblages for both areas, a statistical tool used successfully in similar studies in

Europe (Soeninen et al., 2004; Gomá et al., 2005). In both areas, this analysis identified five groups of sites that were distributed along a trophic gradient. There was a succession of sample groups from the more oligotrophic groups GAL_I and II, to nitrate riche groups GAL_III and IV and to the most phosphate rich GAL_V. A similar succession across water quality was observed for the Balearic samples. The most oligotrophic BI_I, II and III had low nutrient contents, although conductivity and several ions concentrations were naturally higher than for the Galician groups. Meanwhile, both nutrients and oxygen levels of the mentioned groups tend to agree with nutrient thresholds estimated has having only minor impact on stream algae communities (Soeninen et al., 2004). The groups BAL_IV and BAL_V showed, in comparison with previous groups, evidence of significant organic/nutrients impacts.

In combination with the CCA ordination, the diatom TWINSPAN groups aligned consistently on the identified eutrophication and organic pressure gradients. The CCA identified a general nutrient enrichment and organic gradient in the two areas, and the identified TWINSPAN groups matched a progressive community change, as for other studies along organic gradient (Soeninen et al., 2004, Gomá et al., 2005; Tornés et al., 2007).

In our study, the diatom groups from eutrophic streams in Galicia were strongly related with artificial surfaces ocupation, demonstrating the diatom assemblage's responsiveness to landscape level variables, but also to local levels of stressors such as nutrients (N_NH_4, N_NO_2, N_NO_3) and dissolved oxygen. Results confirm that their ecological responses can be transferred to stream management practices and conservation of these systems at local and basin scales (Kelly and Whitton, 1995; Martín et al., 2010).

In both areas, the streams are affected by urban sewage and diffuse inputs from agricultural areas. Meanwhile higher solute dilution should be occurring in the fast flowing permanent Atlantic streams of Galicia, in comparison with the low flow of Balearic streams, so the quantity and quality of the stream water may influence the efficiency of stream ecosystems to remove nutrients (Martí et al., 2004). Other explanatory factors correspond to lower temperatures, presence of riparian cover and natural oligotrophic characteristic in Galician streams when compared with the opposite environmental conditions occurring in temporary Mediterranean streams (Pardo and Álvarez, 2006).

Achnanthidium minutissimun was the common species across both areas and disappeared in streams with values of P_PO_4 higher than 0.3 mg l^{-1}, as in the group GAL_V. In Galicia, this taxon is abundant in reference sites together with different taxa of the genus *Eunotia*, defined by Van Dam et al. (1994) as strong indicator for acid, fresh, oligotrophic waters rich in oxygen and poor in organic nitrogen compounds. The high abundance of this genus in the minimally disturbed stream conditions in Galicia supports this ecological assignation. According to Hofmann (1994) and Van Dam (1994), in Galicia the most intolerant taxa to saprobic conditions were those characterizing the oligotrophic unpolluted rivers of Galicia: *Eunotia intermedia, E. paludosa, E. subarcuartoides* and *Surirella roba*. These species were replaced by other species more tolerant to organic/eutrophic stream conditions (Van Dam et al., 1994) such as *Cocconeis euglypta*, *Eolimna minima*, *Navicula gregaria, N. lanceolata*, *Fragilaria vaucheriae*, *Luticola goeppertiana* and *Sellaphora seminulum* in Galicia streams. All those taxa are tolerant to the presence of elevated concentrations of organically bound nitrogen and to up to 50% oxygen saturation in the water; in contrast, Galician rivers have *Karayevia oblongella* that only tolerate very low concentrations of nitrogen in waters (Van Dam et al., 1994). In other studies from Europe, diatom taxa such as *Gomphonema parvulum*, *Navicula gregaria* and *Nitzschia inconspicua* were defined as species typical of organic polluted waters (Kelly and Whitton, 1995; Torrisi et al., 2010).

In the unpolluted streams of the Balearic Islands *A. minutissimum* appeared with other species, such as *Cymbella vulgata* and *C. microcephala*, also found in other streams considered minimally disturbed and in calcareous springs in Europe (Sabater and Roca, 1992; Delgado et al., 2013). In this study also appeared *Achnanthidium biassoletianum* in high abundance in group BAL_II indicating the presence of oxygen saturated waters of good quality (Torrisi et al., 2010). The streams most affected by the organic/eutrophic conditions (groups IV and V) were characterized by *Navicula veneta* and *Nitzschia inconspicua.* These taxa and other species of *Nitzschia* were type-specific for rivers affected by intensive agricultural in Mediterranean areas

(Gomá et al., 2005; Tornés et al., 2007). *Eolimna minima*, *Gomphonema parvulum* and *Sellaphora seminulum* having similar ecological preferences with respect to nitrogen because they need periodically elevated concentrations of organically bound nitrogen and high oxygen levels, appearing when oxygen saturation was > 30% (Hofmann, 1994). These taxa appeared in the worst diatom groups from two study areas.

In Galicia, the group GAL_V showed the highest phosphate contents, but was always lower than the levels found in the group V from Balearic Islands that had a mean phosphate value that tripled the value of group GAL_V, and had lower dissolved oxygen than this Galician group.

In this study the mean ratio, DIN:Phosphate values, for the variables characterizing the water composition indicated the prevalence of phosphate limitation for most groups, with the exception of the most polluted group BI_V. This group indicated strong nitrogen limitation; a shift can be attributed to the effect induced by urban sewages of increasing phosphorous contents in stream waters (Passy and Bode, 2004). It calls into question whether some of the diatom taxa exhibit a wider tolerance to nitrogen (eg. *Cocconeis euglyta*) vs. to phosphorous, an interesting approximation to elucidate species limitation in water status assessment.

Nutrient-enrichment and human disturbances have an overriding effect on local and large-scale factors, which are likely to reduce the regional differences (Potapova and Charles, 2003). A consequence is that differences in diatom assemblage composition are more evident among relatively undisturbed sites than among sites severely affected by nutrient enrichment like reported in other studies (Eminson and Moss, 1980; Tornés et al., 2007). Finally, this study confirmed the effectiveness of benthic diatoms to separate, in different geographical areas, similar human organic disturbance signal from natural and environmental variability. We have demonstrated across regions that the variation in diatom assemblages can be explained by human disturbances that influence the concentration of stressors in stream water.

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Table 1. Network of stream sites studied in Galicia and Balearic Islands

Table 2 Mean value and standard error, minimum and maximum values of environmental variables measured in all the studied streams.

Table 3 Mean and standard deviation values for each environmental variable for each TWINSPAN group in Galician streams.

Table 4 Mean and standard deviation values of each environmental variable for each TWINSPAN group in Balearic Islands streams.

Fig. 1. Localization of the two study areas in Europe: Galicia (NW of Spain) and Balearic Islands (Majorca, Minorca and Ibiza) in the Mediterranean Sea (NE of Spain).

Figure 2. Principal components analysis (PCA) ordination diagram showing the localization of the studied samples from the Balearic Islands and Galicia in relation to the measured environmental variables. Stream samples from Galicia (○, reference sites; ●, other sites) and Balearic Islands (□, reference sites; ■, other sites).

Fig. 3. TWINSPAN classification of the Galician study streams. Figures indicate the number of stream samples in each TWINSPAN groups (GAL_I, GAL_II, GAL_III, GAL_IV and GAL_V) and the indicator species of each group.

Fig. 4. TWINSPAN classification of the Balearic Islands stream samples. Figures refer to the number of sites in each TWINSPAN groups (BI_I, BI_II, BI_III, BI_IV and BI_V).

Fig. 5. CCA biplot showing the relationship between the 10 environmental variables selected with Monte Carlo permutation test and the samples from the Galicia grouped by Twinspan groups: GAL_I (○), GAL_II (□), GAL_III (◄), GAL_IV (X) and GAL_V (◊).

Fig. 6. CCA biplot showing the relationship between the 10 environmental variables selected with the Monte Carlo permutation test and the samples of Balearic Islands grouped by TWINSPAN groups: BI_I (□), BI_II (○), BI_III (♦), BI_IV (**X**) and BI_V (▼).

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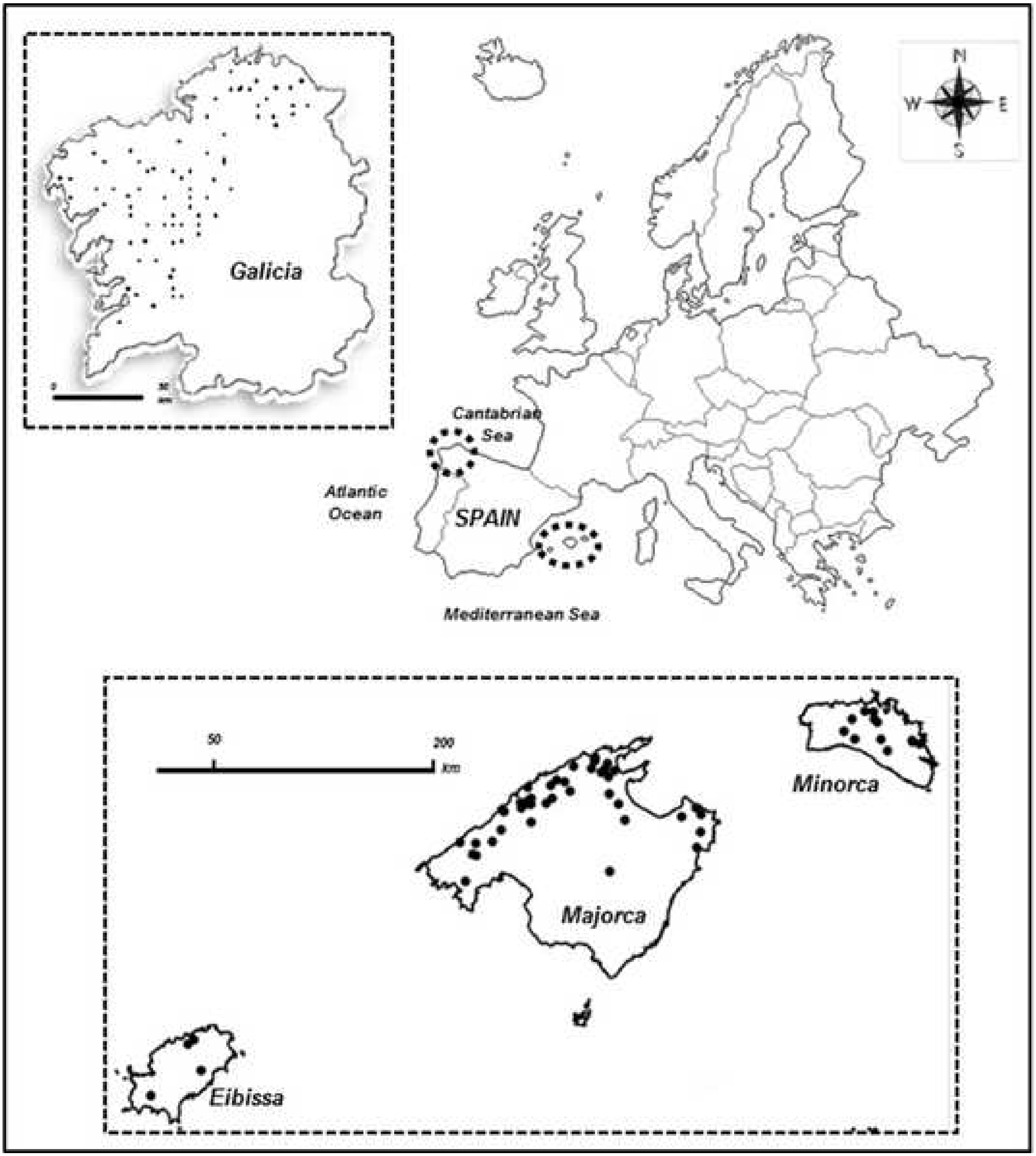

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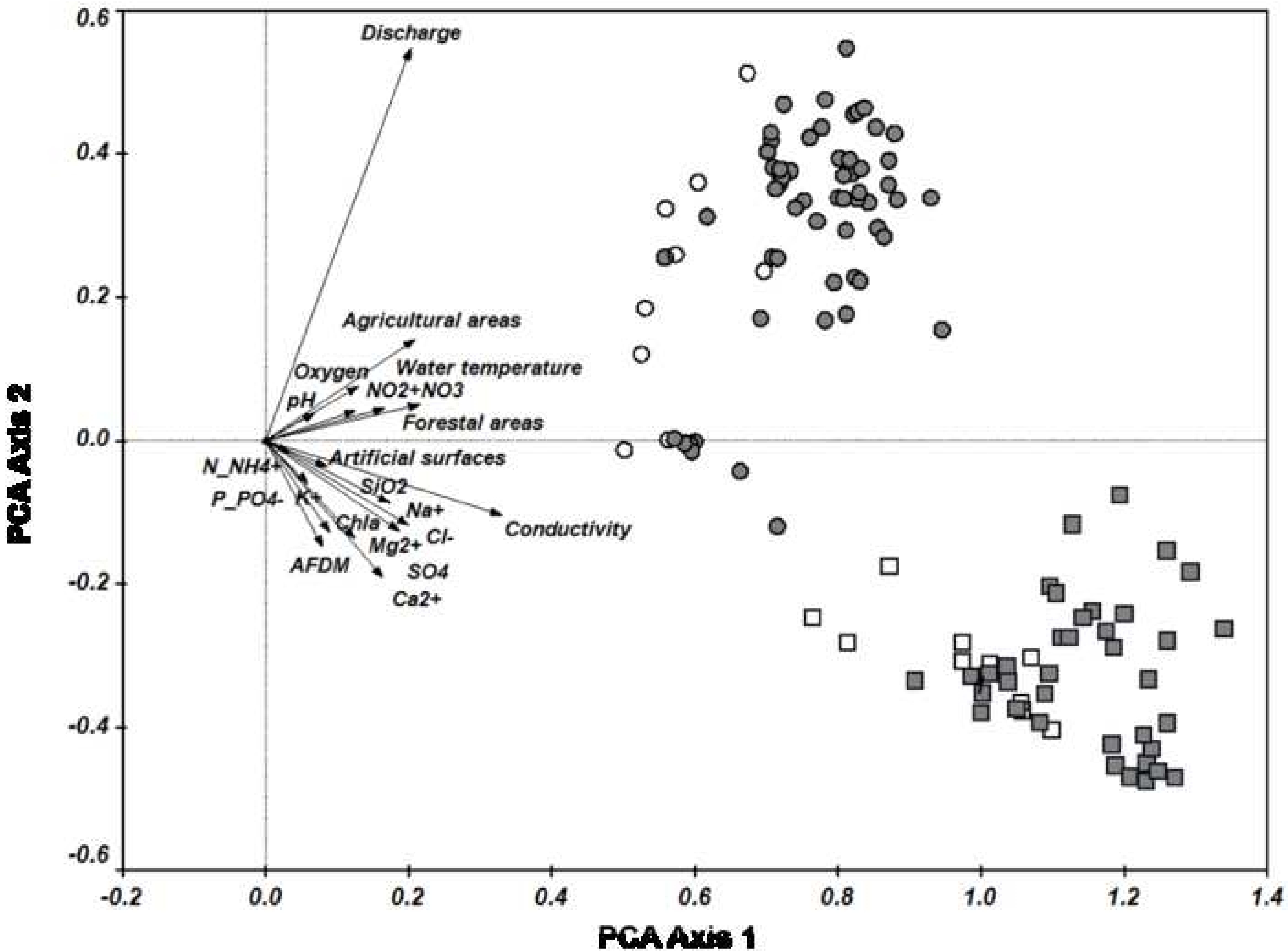

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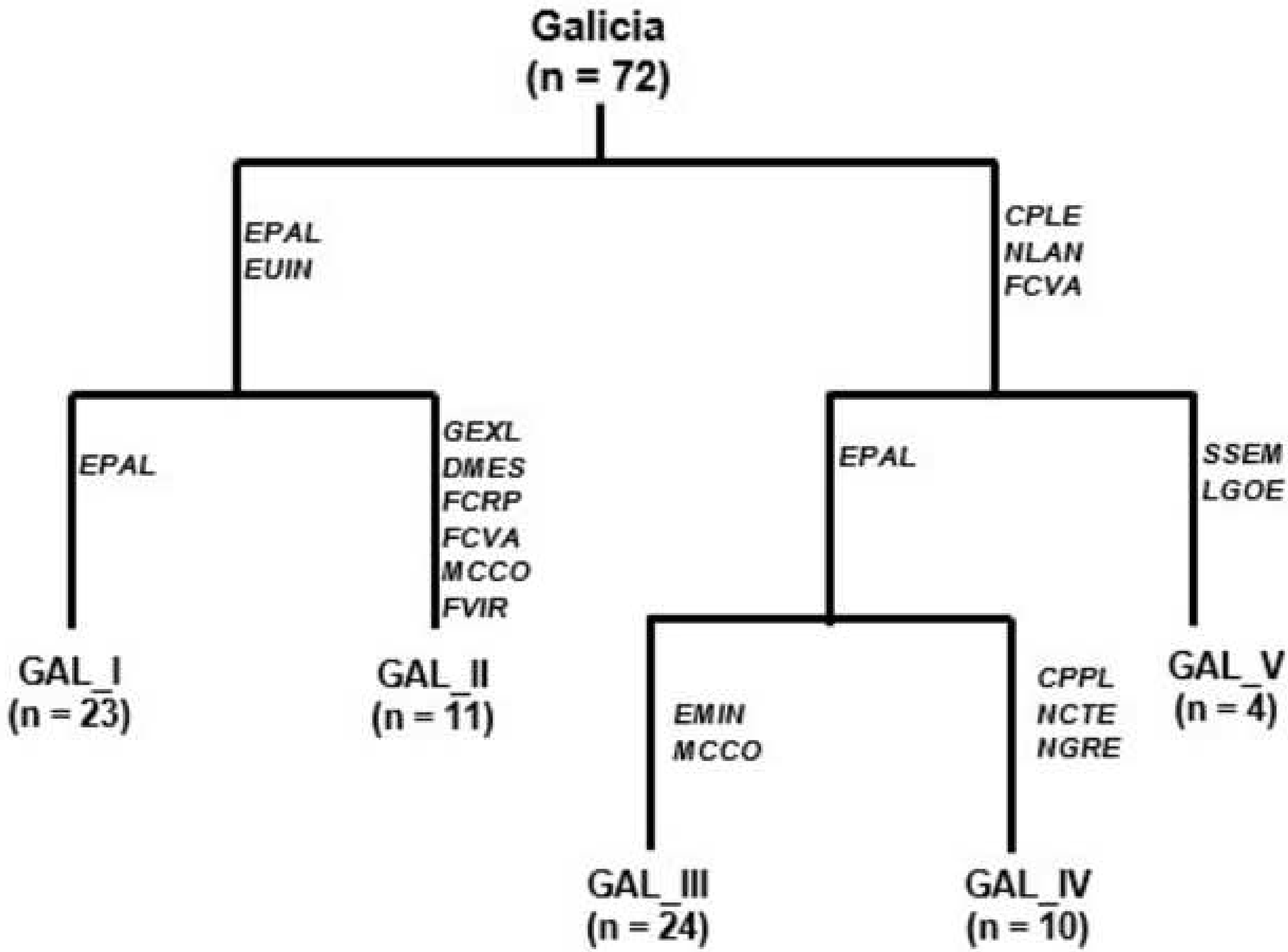

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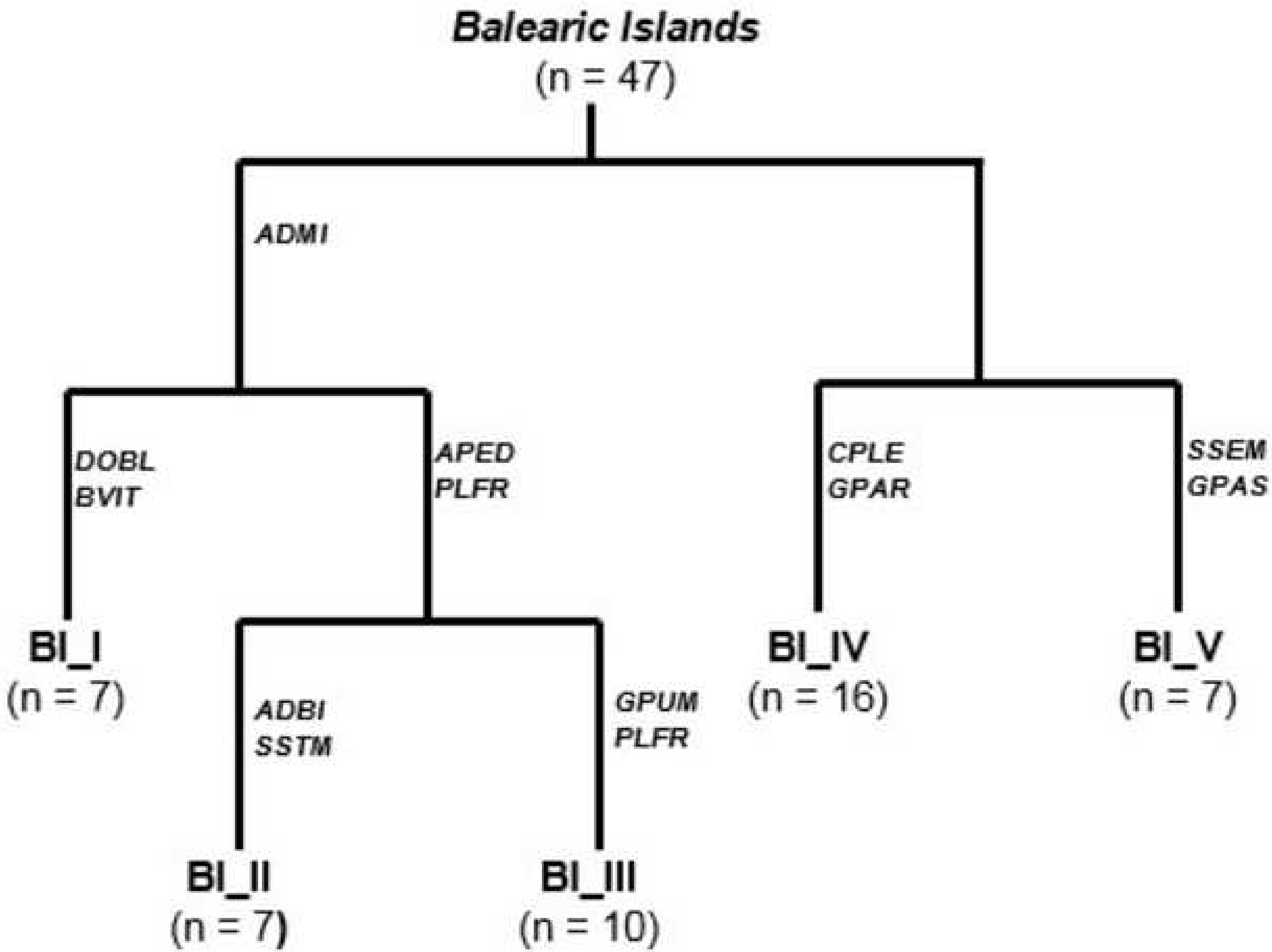

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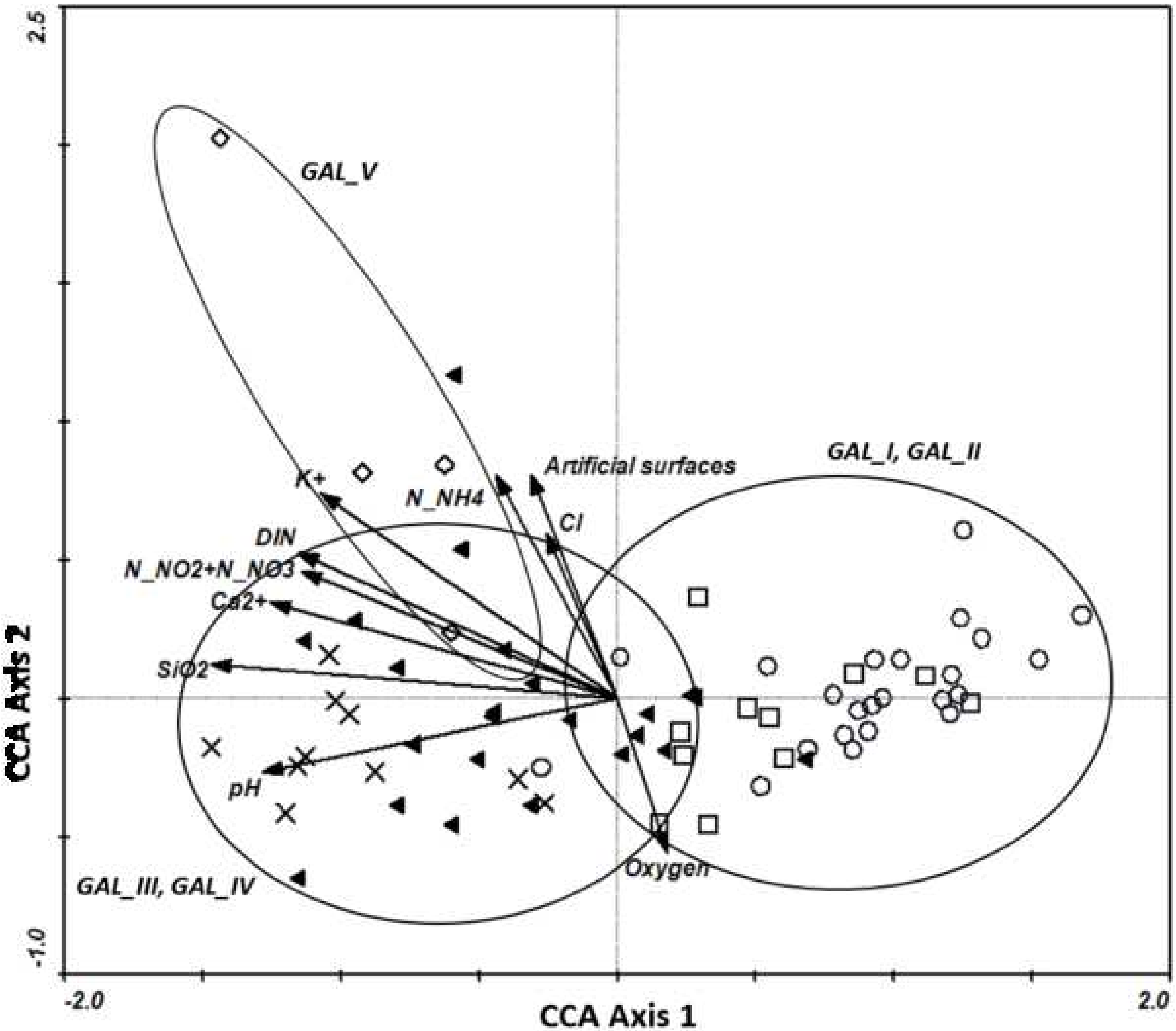

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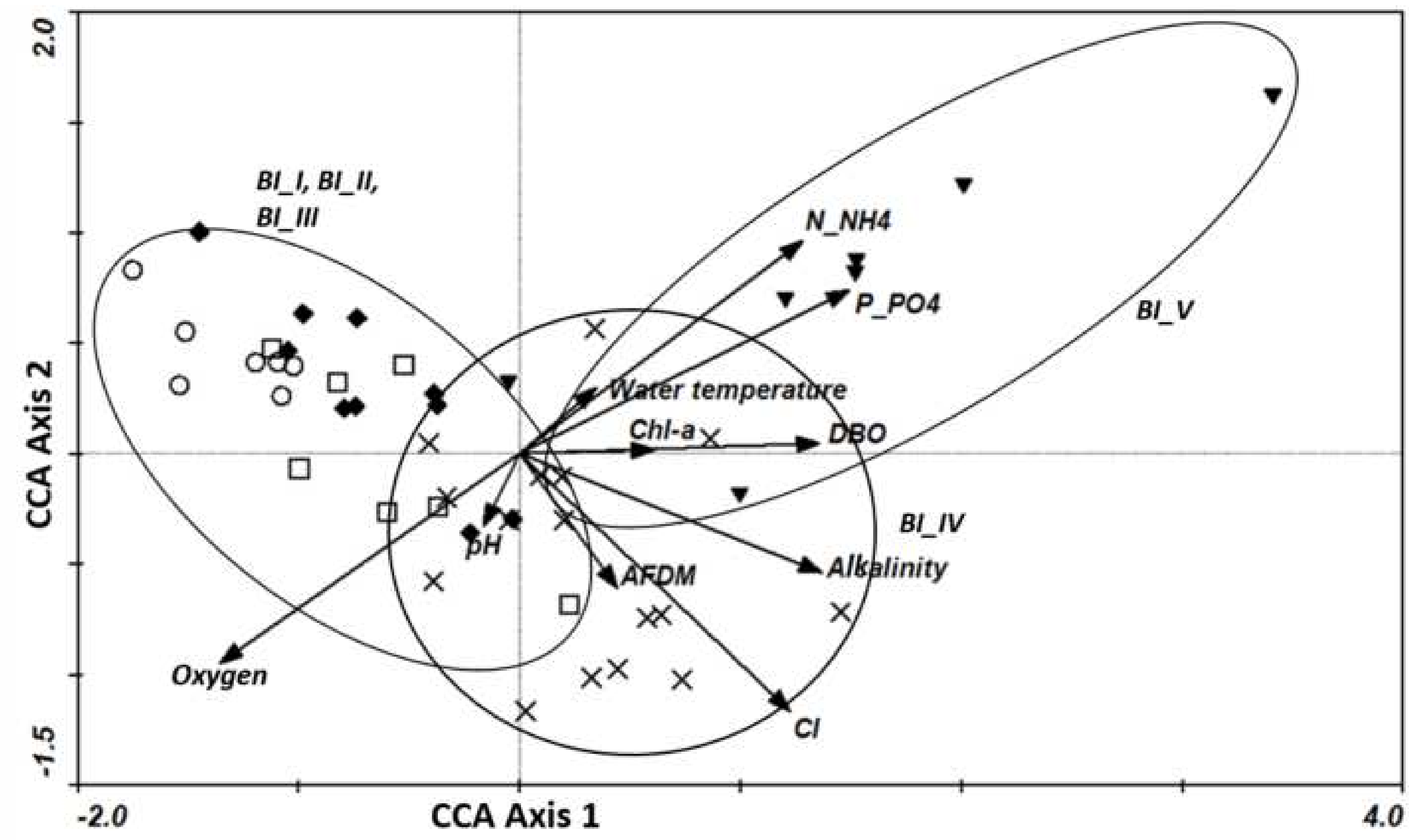

Tables

Study area	Catchment area	Altitude (m)	Nº of reference sites	Total nº of sites	Localization
Galicia	Small	<200		16	Coastal Galicia
	Small	200-800	5	31	
	Small	>800	4	4	
	Medium	200-800		11	
	Medium	<200		10	
Balearic Islands	Small (canyons)	200-800	4	5	Majorca
	Small (mountains)	200-800	6	17	Majorca
	Small (lowlands)	<200	2	25	Majorca, Minorca and Ibiza

Tables

	Galicia				Balearic Islands			
Environmental variables	Mean	S.D.	Min	Max	Mean	S.D.	Min	Max
Water temp (ºC)	11.86	2.98	4.25	20.85	18.55	2.51	14.85	26.65
Dissolved oxygen (mg L^{-1})	8.27	1.14	4.55	10.91	7.61	2.75	0.69	12.49
pH	6.62	0.52	4.97	7.76	7.95	0.31	7.14	8.50
EC (μS cm^{-1})	50.41	33.04	12.90	219.00	1298.83	708.66	245.00	3084.00
Discharge (L s^{-1})	770.30	946.86	0.00	5551.00	9.80	30.56	0.00	197.01
Alcalinity (meq L^{-1})	0.81	1.62	0.00	11.14	6.03	2.29	1.91	12.00
BOD_5 (mg L^{-1})	0.85	2.47	0.00	20.00	4.98	4.44	0.00	28.00
P_PO_4 (mg L^{-1})	0.14	0.64	0.00	5.14	0.42	0.90	0.02	4.00
N_NO_2+ N_NO3 (mg L^{-1})	2.71	2.31	0.08	11.90	2.82	5.34	0.23	36.00
N_NH_4 (mg L^{-1})	0.12	0.68	0.00	5.60	0.51	1.60	0.00	6.16
DIN (mg L^{-1})	2.83	2.53	0.09	12.10	3.33	5.48	0.23	36.00
SiO_2 (mg L^{-1})	2.61	3.67	0.03	17.18	6.46	4.18	0.42	25.22
SO_4^{2-} (mg L^{-1})	4.75	1.29	1.14	10.50	103.93	59.03	3.33	285.65
Cl^- (mg L^{-1})	7.27	4.69	0.59	21.39	160.47	178.68	18.40	740.39
Mg^{2+} (mg L^{-1})	1.50	1.26	0.00	5.39	30.36	21.52	3.84	95.22
Ca^{2+} (mg L^{-1})	2.23	2.38	0.01	13.89	84.46	32.95	29.77	181.70
Na^+ (mg L^{-1})	5.99	4.11	0.04	23.10	72.49	73.08	6.86	264.90
K^+ (mg L^{-1})	0.67	0.81	0.04	5.97	5.59	8.73	0.57	50.33
Chl *a* (mg m^{-2})	0.57	0.74	0.01	4.08	15.41	11.78	1.23	49.37
AFDW (g m^{-2})	0.17	0.16	0.00	1.07	13.74	10.27	1.88	50.00
Artificial surfaces (%)	1.04	3.29	0.00	22.48	1.57	2.03	0.00	8.88
Agricultural areas (%)	52.15	28.46	0.00	100.00	34.67	26.07	0.00	96.83
Forest and semi-natural areas (%)	40.97	30.07	0.00	100.00	60.79	24.50	0.73	100.00

Tables

	GAL_I		GAL_II		GAL_III		GAL_IV		GAL_V	
Environmental variables	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Water temp (ºC)	11.24	3.01	9.83	1.97	12.24	2.64	13.94	3.45	13.48	2.26
Dissolved oxygen (mg L^{-1})	8.17	1.11	8.98	1.09	8.19	1.07	8.37	0.84	7.03	1.62
pH	6.26	0.40	6.26	0.58	6.85	0.31	7.15	0.37	6.90	0.37
EC (μS cm^{-1})	35.03	21.29	35.12	13.90	53.49	24.71	62.08	21.54	133.20	58.74
Discharge (L s^{-1})	505.05	550.63	280.17	330.02	1084.56	908.77	1478.44	1691.98	289.90	158.62
Alcalinity (meq L^{-1})	0.19	0.26	0.13	0.27	0.82	1.02	1.57	1.33	4.22	5.07
BOD_5 (mg L^{-1})	0.35	0.78	0.27	0.47	1.33	4.03	0.80	1.03	2.50	1.73
P_PO_4 (mg L^{-1})	0.01	0.01	0.04	0.08	0.28	1.04	0.06	0.06	0.60	0.82
N_NO_2+ N_NO3 (mg L^{-1})	1.22	0.86	1.97	1.42	3.09	2.02	4.15	2.25	7.53	3.12
N_NH_4 (mg L^{-1})	0.00	0.01	0.00	0.00	0.10	0.28	0.01	0.01	1.50	2.73
DIN (mg L^{-1})	1.22	0.87	1.97	1.42	3.19	2.10	4.16	2.25	9.03	2.96
DIN/P_PO_4	310.97	236.35	215.80	227.21	216.56	294.49	178.80	235.81	36.05	22.18
SiO_2 (mg L^{-1})	0.46	1.22	0.26	0.16	2.95	2.98	7.63	4.86	6.82	2.03
SO_4^{2-} (mg L^{-1})	4.55	0.79	4.59	0.76	4.67	1.70	4.75	0.33	6.73	2.12
Cl^- (mg L^{-1})	7.33	4.96	6.12	4.64	6.72	4.09	6.51	2.74	15.36	4.45
Mg^{2+} (mg L^{-1})	0.75	0.58	0.80	0.39	1.71	1.18	2.57	1.49	3.73	0.62
Ca^{2+} (mg L^{-1})	0.88	0.88	1.26	0.77	2.74	2.16	3.12	2.17	7.39	4.40
Na^+ (mg L^{-1})	4.68	3.78	4.24	2.44	6.29	2.98	6.00	1.88	16.50	5.02
K^+ (mg L^{-1})	0.28	0.21	0.42	0.26	0.77	0.54	0.71	0.30	2.77	2.23
Chl *a* (mg m^{-2})	0.31	0.23	0.37	0.42	0.71	0.83	0.78	0.83	1.33	1.62
AFDW (g m^{-2})	0.14	0.21	0.20	0.22	0.18	0.11	0.17	0.09	0.20	0.08
Artificial surfaces (%)	0.49	1.91	0.03	0.10	0.67	1.42	0.50	0.44	10.57	9.14
Agricultural areas (%)	33.40	29.59	39.30	21.64	65.61	22.97	66.15	13.69	79.43	13.44
Forest and semi-natural areas (%)	61.11	32.10	56.24	20.74	25.72	21.68	30.20	13.14	1.54	1.91

Tables

	BI_I		BI_II		BI_III		BI_IV		BI_V	
Environmental variables	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Water temp (ºC)	18.91	2.78	18.09	1.79	18.15	1.92	18.47	2.93	19.39	2.18
Dissolved oxygen (mg L^{-1})	8.23	1.78	9.19	1.18	8.39	1.96	8.31	1.74	2.73	2.08
pH	7.77	0.30	8.09	0.42	7.96	0.33	8.04	0.21	7.74	0.17
EC (μS cm^{-1})	966.21	497.21	746.61	410.40	1076.08	784.28	1707.25	629.51	1568.36	428.60
Discharge (L s^{-1})	1.77	2.97	0.57	1.23	24.46	58.54	10.94	17.77	3.53	3.21
Alcalinity (meq L^{-1})	5.76	0.79	4.13	1.18	4.27	1.19	7.17	2.28	8.08	1.86
BOD_5 (mg L^{-1})	3.57	1.18	1.86	1.46	3.70	1.27	5.56	2.57	10.00	8.30
P_PO_4 (mg L^{-1})	0.05	0.01	0.05	0.01	0.05	0.01	0.38	0.71	1.81	1.36
N_NO_2+ N_NO3 (mg L^{-1})	1.67	1.75	1.25	0.76	1.74	0.71	4.41	8.26	3.43	3.91
N_NH_4 (mg L^{-1})	0.04	0.05	0.01	0.01	0.04	0.05	0.02	0.03	3.31	2.78
DIN (mg L^{-1})	1.71	1.74	1.26	0.76	1.77	0.69	4.43	8.26	6.74	3.53
DIN/P_PO_4	25.08	15.89	30.76	25.45	39.68	21.61	57.82	129.62	6.91	5.28
SiO_2 (mg L^{-1})	6.43	2.64	4.38	2.10	4.39	3.68	6.37	2.37	11.69	5.83
SO_4^{2-} (mg L^{-1})	67.32	50.00	79.74	65.57	90.39	41.01	130.97	62.70	122.26	33.60
Cl^- (mg L^{-1})	111.46	147.36	38.05	26.61	96.62	122.14	278.69	216.49	152.88	61.73
Mg^{2+} (mg L^{-1})	25.89	12.67	17.35	9.72	18.66	6.58	44.14	25.39	33.04	20.97
Ca^{2+} (mg L^{-1})	95.50	20.70	62.79	26.29	80.70	26.87	86.78	31.15	95.14	46.09
Na^+ (mg L^{-1})	53.94	53.02	19.04	14.09	45.31	56.42	103.84	69.87	111.66	91.50
K^+ (mg L^{-1})	3.70	5.49	1.15	0.56	2.81	2.92	5.69	4.58	15.68	16.55
Chl *a* (mg m^{-2})	9.00	6.96	10.57	6.84	14.96	12.96	19.23	11.40	18.57	13.34
AFDW (g m^{-2})	11.99	5.74	13.92	16.08	8.14	5.40	17.83	11.27	13.93	4.49
Artificial surfaces (%)	0.63	0.75	0.88	1.58	0.71	0.78	2.48	2.55	2.35	1.91
Agricultural areas (%)	25.04	19.91	12.98	14.37	18.02	15.52	48.33	19.53	58.56	26.87
Forest and semi-natural areas (%)	67.29	18.96	82.76	14.77	77.71	15.40	47.25	15.22	39.09	26.55