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Geological and hydrochemical prerequisites of unexpectedly high biodiversity in spring ecosystems at the landscape level

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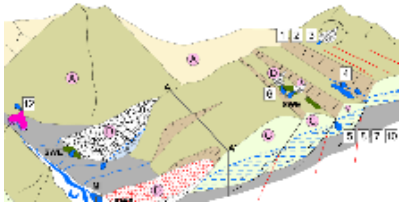
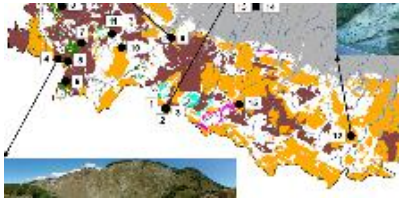
*Graphical Abstract

Pools of representative spring habitats as regional freshwater biodiversity hotspots



Hydrogeological characterization



Geodiversity

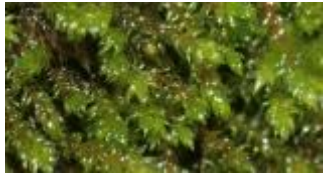
Ecomorphological diversity

Extensive physical and chemical gradients

13 µS cm^{-1}	Conductivity	11560 µS cm^{-1}
1	Shading	5
0.2 mg L^{-1}	Nitrates	48 mg L^{-1}
0 µg L^{-1}	Chromium	705 µg L^{-1}

Impacts:

Water diversion, Intensive agriculture and industry in the plains.



High γ biodiversity

***Highlights (for review : 3 to 5 bullet points (maximum 85 characters including spaces per bullet point)**

- ► Study region: High geodiversity with diverse lithotypes and aquifer structures.
- ► Consequence: Wide variation in ecomorphological types and hydrochemistry.
- ► Effect: High landscape-level (γ) biodiversity.
- ► Conservation: Protection of representative and proportional regional groups of springs.
- ► Springs: Ideal systems in which to investigate geo-biodiversity relationships.

*Manuscript (double-spaced and continuously LINE and PAGE numbered)-for final publication
Click here to view linked References

Geological and hydrochemical prerequisites of unexpectedly high biodiversity in spring ecosystems at the landscape level

Marco Cantonati [a,*], Stefano Segadelli [b], Daniel Spitale [a], Jacopo Gabrieli [c], Reinhard Gerecke [d], Nicola Angeli [a], Maria Teresa De Nardo [b], Kei Ogata [e], John D. Wehr [f]

[a] *MUSE – Museo delle Scienze, Limnology & Phycology Section, Corso del Lavoro e della Scienza 3, I-38123 Trento, Italy*

[b] *Emilia-Romagna Region, Geological, Seismic & Soil Survey, Viale della Fiera, 8, 40127 Bologna, Italy*

[c] *University Ca' Foscari of Venice, IDPA-CNR, Calle Larga Santa Marta 2137, 30123 Venice, Italy*

[d] *University of Tübingen, Department of Evolution and Ecology, Auf der Morgenstelle 28E, D-72076 Tübingen, Germany*

[e] *Università degli Studi di Napoli Federico II, Dipartimento di Scienze della Terra, dell'Ambiente e delle Risorse (DiSTAR), via vicinale cupa Cintia 21, Complesso Universitario di Monte S.Angelo, Edificio L 80126 Napoli*

[f] *Fordham University, Louis Calder Center - Biological Field Station, 31 Whippoorwill Road, PO Box 887, Armonk, NY 10504 USA*

[*] Corresponding author. *E-mail address:* marco.cantonati@muse.it (M. Cantonati).

ABSTRACT

This study explores the factors affecting the biodiversity of diatoms, vegetation with focus on bryophytes, and invertebrates with focus on water mites, in a series of 16 spring-habitats. The springs are located primarily from the mountainous part of the Emilia-Romagna Region (Northern Apennines, Italy), and two pool-springs from agricultural and industrial lowland locations. Overall, data indicate that biological diversity (Shannon-Wiener, α-diversity) within individual springs was relatively low, e.g.: $S_{diatoms}$ = 0-46, $S_{water\text{-}mites}$ = 0-11. However, when examined at the regional scale, they hosted a very high total number of taxa (γ-diversity; $S_{diatoms}$ = 285, $S_{water\text{-}mites}$ = 40), including several new or putatively-new species, and many Red-List taxa. This pattern suggested there is high species turnover among springs, as well as high distinctiveness of individual spring systems. A key goal was to assess the hydrogeological and hydrochemical conditions associated with this high regional-pool species richness, and to provide a guide to future conservation strategies. There was a striking variety of geological conditions (geodiversity, captured mainly with lithotype and aquifer structure) across the study region, which led to wide variation in the hydrosphere, especially in conductivity and pH. Agriculture and industrial activities (anthroposphere) in the lowlands resulted in nutrient enrichment and other forms of pollution. Across all 16 spring-systems, several hydrogeological conditions most strongly influenced the presence or absence of particular biota and were determinants of species importance: spring-head morphology, hydroperiod, discharge, current velocity, and elemental concentration. These findings have important practical consequences for conservation strategies. Our data show that it is imperative to protect entire regional groups of springs, including representatives of the different ecomorphological spring types, lithologies, and degrees of human influence. These findings suggest that springs, when studied from an ecohydrogeological perspective, are excellent systems in which to further investigate and understand geo-biodiversity relationships.

Keywords: Springs, Diatoms, Bryophytes, Zoobenthos, Water mites, Geodiversity

1. Introduction

Spring habitats possess remarkably distinctive ecological features and hold great importance for biodiversity conservation; however, they are fragile ecosystems affected by many human impacts, particularly water diversion and habitat destruction (e.g., Glazier, 2014). Springs in natural or near-natural conditions have often been described as biodiversity hotspots (e.g., Cantonati et al., 2012a), but the type of diversity and key factors have rarely been specified (but see e.g. Nascimbene et al., 2011; Pascual et al., 2020). Among the reasons often mentioned for spring habitat uniqueness and biodiversity are the marked heterogeneity of water characteristics, their complex microhabitat mosaic structure, and their characteristic ecotonal environment. Springs typically transition from groundwater to surface water, and intergrade between aquatic and terrestrial habitats, leading to localized spatial heterogeneity (Cantonati et al., 2012a).

The heterogeneous nature of spring habitats has been classified in a variety of ways (e.g., Glazier, 2014; Cantonati et al., 2020). Seepages are known as the spring type richest in diatom and meiofauna species (Cantonati et al., 2012a). Low-conductivity springs on siliceous (e.g., granitic) substratum, when not affected by acidification or other types of contamination, host very species-rich and distinct diatom assemblages (e.g., Cantonati et al., 2009). Flowing springs are those most similar to streams, and indeed often the transition from springhead to spring-fed streams is seamless, but nevertheless there are diatom species with a marked preference for the uppermost section of the running-water system, but not in lower reaches (e.g., Cantonati et al., 2012b). Limestone Precipitating Springs (LPS) or petrifying springs are one of the few spring types indicated as priority habitat by the European Union Habitat Directive (EU-HD, 1992; Cantonati et al., 2016), and shelter biota highly-adapted to this this special, limestone-precipitating hydrochemical microhabitat. Biota in these systems include specific algae (in particular the bio-calcifying desmid alga *Oocardium stratum*; Linhart and Schagerl, 2015). Inland-saline (mineral) springs are potentially of high evolutionary significance as putative stepping-stone habits that facilitated the radiation in inland waters of algal taxa of marine origin (e.g., Cantonati et al., 2019b).

Species composition and spatial distribution in springs is mainly determined by ion content, particle-sizes of major substrata (determined by current velocity), shading, nutrients, and dissolved metals (Bonettini and Cantonati, 1996; Cantonati et al., 2012b). Disturbances markedly alter spring-habitats and can result in clearly-detectable changes in diatom assemblages, in particular by alterations to the physical structure of springs and contamination with nitrates (e.g., Angeli et al., 2010). Diatom Red List species (percentage of species in threat categories) were shown to react sensitively to habitat alteration and destruction (e.g., water capturing), as well as to nitrate pollution (Cantonati et al., 2012a). The recent (Hofmann et al., 2018) publication of an updated and expanded version of the diatom Red List for Central Europe (first edition: Lange-Bertalot, 1996) allows for an even more effective use of this tool for spring-habitat integrity evaluation in this geographic region.

Spring-ecosystem hydrochemistry is influenced by a variety of pre- and post-emergence factors affecting the aquifer, including geology (e.g., Cantonati et al., 2020), climate, vegetation, and land-use (Merk et al., 2020). The close relationship between spring-dwelling communities and geological formations of the aquifers has been clearly demonstrated for diatom assemblages (Werum and Lange-Bertalot 2004). In some cases, the occurrence of individual diatom species appears to be correlated with the dominant lithotype in the drainage basin, such as dolomite or granite. The occurrence of some diatom species in spring ecosystems has been studied in detail and their ecological characterization often shows that their distribution is driven by a complex combination of hydrogeological and chemical factors. For instance, *Achnanthidium dolomiticum* (Cantonati and Lange-Bertalot, 2006), as the specific epithet suggests, is found on dolomite [$CaMg(CO_3)_2$] substrata. However, Cantonati et al. (2012b) found that this species is characteristic of karstic springs with strongly fluctuating discharge. Recently, *A. dolomiticum* was also found in springs emerging on other rock types, but were nonetheless influenced by groundwater enriched in magnesium (Cantonati et al., 2017).

Bryophytes in spring habitats, like diatoms and other photoautotrophs, are strongly influenced by the geological characteristics of the substratum (e.g., Nascimbene et al., 2011). Among invertebrates, water mites are apparently a group with among the greatest number of spring-dependent species; this points to a

particularly strong relationship between this group and conditions in the spring environment (e.g., Gerecke et al., 2018).

Despite these patterns in individual studies, few papers have addressed the broader relationship between biota and spring hydrogeology (e.g., Van der Kamp, 1995; Cantonati et al., 2020). The link between geological diversity, or "geodiversity" (e.g., Gray et al., 2013) and biodiversity is increasingly gaining interest, but far more studies are needed to provide empirical evidence for this connection (Alahuhta et al., 2020). The role of geodiversity in positively influencing biodiversity has been recognized for terrestrial ecosystems (e.g., Bailey et al., 2017), but studies on freshwater habitats are still rare (e.g., Kärnä et al., 2019, who worked on streams). The Northern Apennines (Italy) are renowned as a highly complex geoscape and area of high geological diversity (e.g., Castellarin, 2001; Boccaletti et al., 2004; Marroni et al., 2010). Therefore, we focused this study specifically in the Emilia-Romagna Region for a deeper understanding of spring-habitat biodiversity and a better understanding of their ecological and geological drivers.

We examined the hydrogeological and hydrochemical conditions associated with high regional-pool species richness and landscape-level (ɣ) biodiversity with the goal to show how high lithological and aquifer-structure diversity generate a multiplicity of ecomorphological and hydrochemical environmental settings, and how this translates into high ɣ diversity. We focused our studies primarily on two groups of organisms, diatoms and water mites, which have contrasting ecological roles within the spring ecosystem. The consequences of our findings for spring-habitat conservation strategies are highlighted.

2. Methods

We examined sixteen springs: Names and codes (with explanations) are listed in Table 1, and will be used throughout the paper. Data from these springs are listed by increasing mineral content, from lowest to highest conductivity.

Springs were selected on the basis of the following criteria: (1) location in different types of nature reserves; (2) representativeness of the diverse ecomorphological and hydrochemical spring types, and of the main lithotypes occurring in the study area; (3) availability of medium-term data series; (4) location within the main northern Apennine aquifer types; (5) location in the different altitudinal belts; (6) presence of a permanent hydraulic regime; and (7) in natural or near-natural conditions. Seventy separate morphological, physical, and chemical variables (Supplementary Material Table 1) and variables were determined for each of 16 selected springs.

2.1. Sampling surveys

Field work was carried out in the summers of 2011 and 2012 (hydrochemistry and biota). Hydrogeological observations and monitoring of selected springs were performed continuously within a 6-year timeframe, from 2007-2013. The position of the springs was recorded by measuring the geographic coordinates with a GPS (Garmin eTrex 10; Garmin Ltd., Olathe, KS, USA). Geomorphology (configuration) was assessed recording the main characteristics on sampling forms and drawing sketches. Current-velocity was assessed using the five-point scale reported in Cantonati et al. (2012b). Canopy cover (as a measure of shading) was assessed with a hemispherical densiometer.

Depending on local conditions, discharge measurements were performed either using the volumetric method (with low flow rates) or using flumes and weirs to convey all the water inside a graduated container. Measures were repeated at least three times at each site. Temperature, specific conductance at 25 °C, and pH were measured on-site by means of handheld Eutech temperature-conductivity meter (Cole-Parmer, Vernon Hills, IL, USA) along with each discharge measurement. Finally, at the Poia_MiSp-SC and Laba_LPS-le springs, discharge values were determined with an STS digital pressure transducer (model DL/N-64, STS - Sensor Technik Sirnach, Sirnach, Switzerland), whereas temperature and conductivity were

measured with a digital thermometer-conductimeter (Hanna Instruments; Woonsocket, RI, USA). To calculate variability indices, discharge, temperature, and electrical conductivity data collected in the field during the EBERs project have been integrated with own (SS) datasets or available from the literature (Chiesi and Forti, 2009; Filippini, 2009-2010; Gherardi, 2011-2012).

2.2. Geology and Hydrogeology

Geological data used for this work was derived from the database of the geological map of the Emilia-Romagna Region, scale 1:10,000 (Geological cartography of Appennines, scale 1:10,000, 2012).

A variable termed 'lithology' was defined to express the main geological subdivisions in the Northern Apennines and their influence on the mineral content of the studied spring waters. Lithological classes were ordered on the basis of the degree of solubility of the rock and therefore on the basis of their capacity to condition the chemistry of the waters during flow inside the aquifer. 'Lithology' expressed the range of conditions from poorly-soluble rocks (e.g. siliciclastic arenites and basalts) to karst rocks composed of evaporatic Triassic - Messinian rocks, and classified from 1 to 8, as follows: (1) siliciclastic arenites, (2) ophiolites (basalts), (3) ophiolites (peridotites), (4) ophiolites (serpentinized peridotites), (5) lithic arenites, (6) limestone-marly flyschs and calcarenites, (7) alluvial deposits, and (8) Triassic and Messinian evaporites (details in Table 1).

The springs were classified on the basis of variation in discharge through time, and quantified using the variability index (Rv) introduced by Meinzer (1923). This index is a function of the maximum (QM), minimum (Qm), and mean (Qmed) discharge values within the hydrological year: Rv = ((QM – Qm)/Qmed)*100). Values of Rv<25% indicate springs with constant discharge, whereas Rv between 25% and 100% are defined as sub-variable. Higher values (Rv > 100%) identify springs with variable discharge. This index was also implemented for temperature and specific conductance data. For several springs (Helo_LCSe, CaLS_LCFS, GeRi_MiSp-Su, FoVR_PS-Sh, and FoVR_PS-Su), the Rv values had to be estimated.

2.3. Hydrochemistry

175 Sampling was conducted using polyethylene (PE) bottles previously cleaned with ultra-pure HNO_3
176 (Ultrapure grade, Romil, Cambridge, UK), and then rinsed several times with ultra-pure water (Purelab Ultra
177 Analytic, Elga Lab Water, High Wycombe, UK) to minimize the risk of external contaminations. Water
178 samples for major ions and nutrients were kept chilled (ca. 4 °C) in fridges and fridge bags until analysis.
179 Detailed hydrochemical analyses were carried out following standard methodology (APHA, 2000). Ca^{2+},
180 Mg^{2+}, Na^{+}, K^{+}, NH_4^{+}, Cl^{-}, NO_3^{-}, SO_4^{2-}, Br^{-}, F^{-} were analysed by ion chromatography (ICS 1500 Dionex Corp.,
181 Sunnyvale, CA, USA), and dissolved nutrients (N-NO_2^{-}, N-NH_4^{+}, P-PO_4^{3-}, TP, TN, Si) by standard absorption
182 spectrometry (details in Cantonati et al., 2012b).

183 The bottles of water samples for trace elements were capped after sampling, packed in double LDPE
184 bags, and transported to the IDPA-CNR Laboratories (Milan, Italy), where samples remained frozen until
185 they were analysed. The samples were thawed at room temperature under a class 100 laminar flow clean
186 bench, and 10 mL aliquots were transferred to 12 mL ultra-clean LDPE vials and acidified with ultra-pure
187 HNO_3 to obtain 2% solutions (v/v). Concentrations of 29 elements (Li, Be, Na, Mg, Al, Ti, V, Cr, Mn, Fe, Co,
188 Ni, Cu, Zn, Ga, As, Rb, Sr, Mo, Ag, Cd, Sn, Cs, Sb, Ba, Tl, Pb, Bi, U) were determined by Inductively Coupled
189 Plasma Sector Field Mass Spectrometry (ICP-SFMS; Element2, Thermo-Fisher, Bremen, Germany) equipped
190 with a desolvation system (APEX IR, Elemental Scientific, Omaha, US). The system was calibrated by
191 external calibration using a certified multi-standard solution containing the 29 trace elements (IMS-102,
192 UltraScientific, US). Concentrations in the standard solutions ranged from 0.005 to 50 ng/g for all the
193 elements except Na, Mg, Al, Fe, Mn and Ba which were calibrated at higher concentration values (from 1.0
194 to 1000 ng/g). The accuracy was evaluated (ION 96.2, National Water Research Institute, Canada) and
195 ranged from 76% (Al) to 105% (Sn). Average precision was always better than 12%.

196 In order to evaluate the relative contribution from natural (e.g., rock and soil dust) versus anthropogenic
197 sources, trace element concentrations were expressed in the form of crustal enrichment factors (EF_{Ba}). EF_{Ba}
198 is defined as the concentration ratio of a given element to that of Ba (or any other conservative element
199 which derives mainly from rock and soil dust), normalized to the same concentration ratio characteristic of
200 the upper continental crust (after Wedepohl, 1995). However, given the large variations in the composition

201 of rock and soil, enrichment factors within ± 10 times the mean crustal abundance (i.e. EF_{Ba} values ranging
202 from ~ 0.1 to 10) do not likely demonstrate the input from sources other than rock and soil dust.
203 Conversely, any EF_{Ba} value significantly greater than 10 strongly suggests contributions from other natural
204 sources and/or anthropogenic sources. Elements with EF_{Ba} values between 10 and 100 are considered
205 moderately enriched, indicating one or several other sources in addition to the crustal material. Finally,
206 elements with EF_{Ba} values greater than 100 are considered highly enriched, suggesting a severe
207 anthropogenic contribution (after Veysseyre et al., 2001).
208

209 *2.4. Diatom sampling, identification, and quantification*

210 Diatom assemblages were sampled and treated following the methods described previously for spring
211 habitats by Cantonati et al. (2012b), with specific designation of the spring-head area (= eucrenal, defined
212 here as the submerged portion at the time of sampling and the inner bank clearly influenced by the water
213 during the year), choice of substrata, and sample treatment. Epilithic diatoms were collected by brushing
214 ten stones. For diatoms epiphytic on the dominant bryophyte species, the epibryon were collected from
215 the most frequent and abundant bryophytes in each sample location (see Supplementary Material Table 4).
216 The collected materials, including the bryophytes, were cut into small pieces and were digested using
217 hydrogen peroxide (Cantonati et al., 2012b). The cleaned material was mounted in Naphrax (refractive
218 index of 1.74). For each sample, three cover-slips were prepared on one permanent slide, and a pooled
219 total of about 450 valves were counted. All samples (original samples, suspensions of prepared material,
220 and permanent mounts) have been catalogued and deposited in the collections of the MUSE – Museo delle
221 Scienze (Trento) (access codes: cLIM007 DIAT 1956-2002) along with information about the abundance of
222 the species found and the main environmental variables.

223 Counting was conducted with a Zeiss Axioskop 2 at 1000x magnification (Zeiss, Oberkochen, Germany).
224 The most updated taxonomy and nomenclature available at the time when counts were performed was
225 applied (in particular, Levkov, 2009; Hofmann et al., 2011; Lowe et al., 2014), and other identification
226 reference works followed were as in Cantonati et al. (2012b). Nomenclature and some taxonomic concepts

227 were updated using Cantonati et al. (2017), AlgaeBase (Guiry and Guiry, 2020), DiatomBase (Kociolek et al.,
228 2020), Diatoms of North America (Spaulding et al., 2019), the Freshwater Diatom Flora of Britain and
229 Ireland (Jüttner et al., 2020). To confirm identifications and document taxa with poorly-observed
230 ultrastructure, several taxa were examined with SEM (Zeiss-EVO40XVP, Carl Zeiss SMT Ltd., Cambridge, UK)
231 at the MUSE - Museo delle Scienze (Trento) or at the University of Frankfurt using a Hitachi S-4500 (Hitachi
232 Ltd., Tokyo, Japan) at high vacuum on gold-coated stubs.

233

234 *2.5. Bryophytes and vascular plants*

235 Within the eucrenal, all the bryophytes and vascular plants were identified and assigned a score
236 according to their visually estimated percent cover: 1 = < 5%, 2 = 5-25%; 3= 25-50%; 4 = 50-75%; 5 = 75-100
237 (e.g., Braun-Blanquet, 1964; Tomaselli et al. 2011). Species nomenclature follows Aeschlimann et al. (2004)
238 for vascular plants, Hill et al. (2006) for mosses, and Ros et al. (2007) for liverworts. Specimens of vascular
239 plants were collected as needed to confirm field identifications at a later stage. Bryophytes were
240 systematically collected and will be deposited in the Herbarium of the MUSE – Museo delle Scienze, Trento,
241 Italy (Code = TR).

242

243 *2.6. Zoobenthos*

244 Cumulative sediment samples from all significant substrata were preserved in 70% denaturated ethanol
245 or in 4% formaldehyde; animals from the latter was later transferred to 70% denaturated ethanol. During
246 sorting, water mites were then preserved in Koenike's fluid (glycerol:acidic acid:distilled water 10:3:6).
247 Before sorting, samples were washed and divided into two fractions (>1.000 µm and <1.000 µm). The large-
248 scale fraction was investigated at lower magnification, the fine fraction at high magnification under a Zeiss
249 SV6 stereo microscope. All samples were examined completely; individuals encountered were counted
250 from the whole sample, although not all were directly enumerated. In cases of high densities, a quarter of
251 each dish was counted and the resulting number multiplied by 4. Resulting specimen numbers were
252 rounded and were used for calculating categories of relative density for all taxonomic groups recognized.

253 The material was sorted at the taxonomic level of orders, or, in the case of Diptera, families. As a general
254 rule, for taxa in low densities, all specimens were sorted and enumerated, while for more frequent taxa,
255 only a representative number of specimens was preserved. During treatment, attention was paid to
256 obvious differences in morphology and size classes, trying to sort out different morphotaxa in relation to
257 their relative abundance. In order to get insight into the whole diversity spectrum (often reflected by
258 differences in body weight and consequently in sedimentation rate), specimens of all taxa were selected
259 during the whole sorting process, from the first to the last Petri dish. For each spring, and each
260 order/family, a tube with two labels (taxon name and site abbreviation; information on collecting site and
261 date) was deposited in the collection. A part of the material has been sent to specialists, the rest was
262 deposited in the collections of the MUSE– Museo delle Scienze (Trento) Limnology Section (access codes:
263 cLIM007).

264

265 *2.7. Data processing and statistical analyses*

266 For all diatom species collected in this study, a threat status (a measure of rarity) was assigned,
267 according to current (Hofmann et al., 2018) and previous Red List data (Lange-Bertalot, 1996). For the
268 species present in both lists, a check was made if the conservation status is improving or declining.
269 Hofmann et al. (2018) provide further ecological attributes (trophic and mineralization preferences, aerial
270 species) used in this study (Supplementary Material Table 3).

271 Shannon-Wiener diversity (Shannon, 1948) was calculated using a base-2 logarithm. Canonical
272 Correspondence Analysis (CCA) was calculated with the package *vegan* (Oksanen et al., 2019) in the R
273 statistical environment (R CORE TEAM, 2018). CCA was selected over other ordination techniques, such as
274 redundancy analysis (RDA), after evaluation of the length of the gradient. Model selection was performed
275 with the *orddistep* function of vegan, with an automatic, stepwise model with constrained ordination. Only
276 the environmental variables significant in the last step of selection was then used for the final CCA model.
277 Significance of the CCA model, terms, and constrained axes was tested with an ANOVA like permutation
278 test (999 permutations).

A Mantel test was used to assess correlation between epibryon and epilithon diatoms and vegetation. The significance of the statistic is evaluated by permuting rows and columns of the first dissimilarity matrix. The function to perform the Mantel test is available in the vegan package. To study α, β, and ɣ diversity for diatoms, we used an additive diversity partitioning, where the mean values of α diversity at lower levels of a sampling hierarchy are compared to the total diversity in the entire data set (ɣ diversity, Crist et al., 2003); this function is available in the vegan package.

3. Results

3.1. Ecomorphology, Geology and Hydrogeology

The ecomorphological classification of the springs studied here led to the recognition of seven spring-types, listed in order of increasing mineral content of the spring water (Table 1, Fig. 3). The spring codes are explained in Table 1, and include, after the underscore, an abbreviation of the spring type, and, after the hyphen, an abbreviation of the possible sub-category. Within shaded, flowing springs, a subgroup was characterized by the occurrence of the macroscopic crustose red alga *Hildenbrandia rivularis*.

The geological (lithological) and hydrogeological classification of the studied springs is provided in Table 1. Our study also included two lowland pool springs in alluvial deposits, locally called *fontanili*. Hard Rock Aquifers (HRA) is the best represented group because the ophiolites, and in particular the turbiditic units, both silico-clastic, and calcareous and calcareous-marly, cover very vast and seamless territories (Fig. 1).

3.2. Hydrochemistry

The study revealed several factors that ranged widely among spring-types (Fig. 4, Supplementary Material Table 1, Table 2). The conductivity gradient of the studied springs (13-11560 µS cm^{-1}) spanned more than three orders of magnitude. In one system, Prin_ShFS-pH, very high pH (11.2) was due to enrichment with sodium and chloride. The two mineral springs (Poia_MiSp-SC, GeRi_MiSp-Su) were characterized by high sodium, chloride, sulphate, and total phosphorus (TP) values. TP enrichment is

304 otherwise rare in most systems, even in the agricultural lowland limnocrenes (*fontanili*) contaminated by
305 nitrate and chloride (Table 2). The MtNe_ShFS-Hi (aquifer: deep-seated gravitational deformations in slopes
306 - DSGSD) and CiLi_HygS springs, both coming to daylight on ophiolithic rocks, were enriched with sodium,
307 magnesium, chromium, nickel, arsenic, uranium, molybdenum, cadmium, antimony, titanium
308 (Supplementary Material Table 2, Table 2).

309

310 *3.3. Diatoms*

311 In the spring GeRi_MiSp-Su, bryophytes were not found (see below), and diatoms were absent also on
312 lithic material. Diatom analyses of samples from the remaining 15 springs revealed a total species richness
313 of 285 taxa (272 identified to the species -or intraspecific- level) belonging to 63 genera (Supplementary
314 Material Table 3). Red-List threat-category data were available for nearly all the taxa identified to species
315 level: only nine taxa were not listed (Hofmann et al., 2018). A high proportion (60%) of the species for
316 which Red List information was available were found to belong to one of the key threat categories (1, 2, 3,
317 G, R, V, D oligotraphentic) (Supplementary Material Table 3). A selection of frequent and abundant,
318 characteristic, rare, and Red-List species is summarized in Fig. 5.

319 Two of the species identified were listed in the highest threat category, i.e. threat category 1
320 ("threatened with extinction"): *Achnanthidium trinode* (Fig. 5 11b) and *Eunotia cisalpina* (Fig. 5 2b). Several
321 more species in the studied springs are members of threat category 2 ("strongly threatened"), including
322 *Achnanthidium dolomiticum*, *A. rosenstockii*, *Brachysira calcicola*, *B. vitrea*, *Cymbella tridentina* (Fig. 5 12a),
323 *C. tumidula* (Fig. 5 12b), *Cymbopleura austriaca*, *Encyonema hebridicum*, *Encyonopsis lange-bertalotii*,
324 *Eucocconeis flexella*, *Eunotia kruegeri*, *E. nymanniana*, *E. palatina*, *E. sudetica*, *E. tetraodon*, *Kolbesia*
325 *carissima* (Fig. 5 2d), *Navicula angusta* (Fig. 5 1a), *N. dealpina*, *Psammothidium altaicum*, *Rossithidium*
326 *anastasiae*, *R. pusillum*, *Sellaphora stroemii*.

327 On the basis of in-depth studies on LM morphology, plastid shape and arrangement, SEM ultrastructure,
328 ecology and distribution, and extensive literature research, eight species in the genera *Achnanthidium*,

329 *Amphora*, *Delicata*, *Eunotia*, *Fragilaria*, *Halamphora*, *Navicula*, and *Planothidium* were recognized as new
330 to science (Cantonati et al., 2019a;b; MC, Horst Lange-Bertalot, unpublished data).

331 Ecological preferences were assigned (based on Hofmann et al. 2018) for 252 of the 272 taxa identified
332 at least to the species level (Supplementary Material Table 3). Of these only 24 species (= 9.5%) were
333 classified as aerial, and included *Cymbopleura austriaca*, *Delicata minuta*, *Diploneis minuta*, *Encyonema*
334 *alpinum*, *E. bipartitum* (Fig. 5 9f), *Halamphora montana*, *H. normanii*, *Hantzschia amphioxys*, almost all
335 *Humidophila* spp. found (excepting *H. schmassmannii*), *Hygropetra balfouriana*, *Nitzschia harderi*, *Nupela*
336 *lapidosa*, *Pinnularia obscura*, *Simonsenia delognei*, *Surirella terricola*, *Tetracyclus rupestris*, *Tryblionella*
337 *debilis*. Most species were encountered in only one or two springs. Table 3 shows richness (*S*) and Shannon-
338 Wiener (*H'*) ranges. Species richness in the epilithon ranged from 7 (Poia_MiSp-SC) to 46 (CaLS_LCFS);
339 richness in the epibryon ranged from 5 (Poia_MiSp-SC) to 65 (Helo_LCSe). The variable 'lithology' was
340 correlated significantly with both Shannon-Wiener diversity of epibryon diatoms (r = - 0.74; p = 0.003) and
341 vegetation (r = - 0.78; p < 0.001, Fig. 6).

342 A CCA (Table 4, Fig. 7) indicated that the distribution of the sites and characteristic species was mainly
343 determined by differences in chromium, nitrates, elevation (for epilithon), and by nitrates, magnesium,
344 bicarbonate alkalinity, and elevation (for epibryon). This is confirmed by the placement of typical mountain
345 species (*Psammothidium daonense*, *Achnanthidium trinode* (Fig. 5 11b), *Nupela lapidosa*, *Gomphonema*
346 *tenoccultum* (Fig. 5 11c), *Odontidium neolongissimum*) close to the apex of the elevation vector. The
347 eutraphentic *Fragilaria famelica* is positioned close to the head of the nitrate vector, and indeed this
348 species was abundant in FoVR_PS-Su, where the highest nitrate values were measured (Table 2).

349 The total species richness (ɣ-diversity) recorded across all springs was partitioned into the average
350 number of species that occur within a sample (α) and the average number of species absent from a sample
351 (β), Table 5. The partition showed that α diversity was on average 23.5 in the epilithon and 28.4 on average
352 in the epibryon, each of which is relatively low, compared to total ɣ-diversity (199 and 214 species).

353

354 *3.4. Bryophytes and vascular plants*

A total of 93 macrophytic plant species were observed across all sites (43 bryophytes, and 49 vascular plants; Supplementary Material Tables 4-5), with an average richness of 9.6 per site. In the spring GeRi_MiSp-Su both bryophytes and vascular plants were absent, whereas the richest spring was Helo_LCSe, with 13 and 15 species respectively for bryophytes and vascular plants. Of note were the bryophytes *Hygrohypnum eugyrium* (the second record in Italy), *Fissidens viridulus*, *Racomitrium aciculare*, and *Southbya tophacea*; taxa that were not recorded after 1950 in the Emilia-Romagna Region. Most species were encountered only once or twice. Species observed more than 5 times are common in many aquatic habitats, such as *Bryum pseudotriquestrum*, *Palustriella commutata*, and *Brachythecium rivulare*. Other less frequent species occurred in particular spring types, including *Didymodon tophaceus* and *Eucladium verticillatum* in LPS springs, and *Sphagnum* spp. in seepages. The vegetation composition, designated here as plant and bryophyte assemblages together, was highly correlated with the epibryon diatom assemblages (Mantel statistic $r = 0.95$; $p < 0.001$), and correlated, but less so, with the epilithic diatoms (Mantel statistic $r = 0.30$; $p < 0.011$).

3.5. Zoobenthos

3.5.1. Overview on invertebrates

The invertebrate groups encountered and the numbers of specimens counted, are given in Supplementary Material Table 6. The most abundant groups were chironomids, followed by ostracods, mites, and stoneflies. The total specimen number in samples collected during the investigation exceeded ten thousand. A considerable abundance and frequency of groundwater taxa (= stygophilous) were observed. Among these, particularly common were amphipods in the genus *Niphargus*, stygobiont hydrobiid snails, and the mites genera *Stygothrombium* and *Frontipodopsis*, the latter two taxa previously not published from spring sites in Italy. An interesting finding was also a larva of the psephenid beetle genus *Eubria*, a taxon with a very scattered distribution in Europe. The relationship between selected groups of invertebrates and lithology is shown in Fig. 8. Of these, only chironomid species composition was significantly correlated to lithology ($r = -0.65$; $p = 0.006$).

3.5.2. Water mites

Distribution and frequency of mite species are given in Supplementary Material Table 7. A total of 40 water-mite taxa were observed, 36 of which are members of the true water mites. With regard to individual numbers, of the globally 827 specimens collected in the frame of this project, only 47 (5%), are representative of terrestrial groups. They represented (specimen numbers in brackets): Trombidiformes (3), Gamasida (4), and Oribatida (40, including 20 Phthiracaridae). True aquatic mites occurred in 14 of the springs studied, but in six of these occurred in low numbers. Terrestrial taxa were recorded only from sites GeRi_MiSp-Su (Gamasida, Oribatida, 10 specimens) and FoVR_PS-Sh (Oribatida, 1). Sites with very low water-mite density were Bari_ShFS-mc (*Partnunia aprutina*, *Protzia squamosa*, each one specimen), Poia_MiSp-SC (*Partnunia aprutina*, one specimen), Laba_LPS-le (*Aturus* cf. *natangensis*, one specimen, *Sperchonopsis verrucosa*, 2 specimens), FoVR_PS-Su (only one undetermined larva), FoVe_LCFS (*Feltria setigera*, *Lebertia schechteli*, each 2 specimens), and CaLS_LCFS (*Pseudofeltria aemiliana*, *Protzia eximia* gr., 1 specimen each). Furthermore, also CiLi_HygS (3 water mite taxa, 14 specimens) and Cara_LPS-sn (4 water mite taxa, 5 specimens) had a rather poor fauna. Higher population densities and species richness were observed at sites MtPe_ShFS-Hi, MaPa_ShFS-Hi, Laga_ShFS-Hi, MtNe_ShFS-Hi, Prin_ShFS-pH and Helo_LCSe.

Hygrobates psammocrenicus occurred in greatest abundance, mostly due to an extreme density of this species at one site: Laga_ShFS-Hi. *Sperchon thienemanni* was the next most common taxon, along with *Feltria setigera* and *Partnunia aprutina*, as frequently recorded species (4 sites). Other species found in higher numbers or more frequently (specimen numbers/frequency in parentheses) are *Lebertia schechteli* (36/3), *Atractides longisetus* (24/2), *Pseudofeltria appenninica* (14/2), *Hydrovolzia placophora* (13/2), *Atractides loricatus* (10/2), *Sperchonopsis verrucosa* (7/3), and *Panisus michaeli* (4/3). The remaining 25 species were found at one or two site(s) only and in low numbers, 14 of them as single specimens.

In this study, nearly 75% of the water mite species are classified to have a close relationship to spring habitats: crenobionts (= strictly bound to springs): 60%; and crenophiles (= with a preference for springs,

but also in other habitats): 14%. Typical crenobionts, some of them with a particular preference for special habitats, are *Panisus michaeli*, *Protzia squamosa paucipora*, *Sperchon resupinus*, *Bandakia concreta* (all with a known preference for helocrenes), *Hydrovolzia placophora*, *Lebertia cuneifera*, *Lebertia holsatica* (preferably in rheohelocrenes rich in macrophytes and fine detritus), and *Lebertia schechteli*, *Sperchon mutilus*, *Sperchon thienemanni* (in all types of springs). Important crenophiles in our study are *Lebertia maculosa*, *Sperchon squamosus* and *Atractides loricatus*.

The pattern revealed by ɣ-diversity partitioning (Table 3) parallels the results obtained for diatoms.

4. Discussion

The regional assemblages of spring organisms examined included a very high total number of species, including several new or putatively-new species, and many Red-List taxa. The ɣ-diversity partitioning for both diatoms and water mites demonstrated that α-diversity of the individual springs is relatively low, and that the main contribution to ɣ-diversity was due to β-diversity, that is, a high turnover of species among springs. This pattern and the percentages were very similar for diatoms and water mites.

These results are in good agreement with previous studies. Nascimbene et al. (2011) investigated algae, diatoms, lichens, and bryophytes in springs of the southeastern Alps and compared α-, β- and ɣ-diversity: In individual springs, these photoautotroph groups formed relatively species poor communities with a high species replacement among springs whilst regional species pools were important. Studying macrozoobenthos from 19 mountain springs of the River Sarca catchment in the Adamello-Brenta Nature Park, Bonettini and Cantonati (1996), observed low species numbers in individual biotopes, and a high total number of taxa. This condition demonstrates marked site to site (spatial) differences, which also emerged from the TWINSPAN ordination. In the present study, springs with the greatest number of species, and those that may be considered rare in northern Italy, were characterized by low flow variability. Pascual et al. (2020) underline the uniqueness of individual spring communities after studying α-, β- and ɣ-diversity of

macroalgae, diatoms, bryophytes, vascular plants, aquatic invertebrates and vertebrates in Mediterranean springs. Our findings also have important implications for conservation, suggesting that the protection of single sites will not be effective, because a biodiversity conservation plan for spring environments requires a plan developed at the regional level, and include a network of sites representative of the different ecomorphologies, lithologies, and other key ecological factors.

These results also reveal the need for a deeper understanding of the causes of high ɣ-diversity. Specifically, these data demonstrate that high geological diversity (lithotypes, aquifer hydrostructure, etc.), translates into high spring ecomorphological and hydrochemical diversity. Further, these patterns are reflected in high biodiversity. Other factors that may affect high ɣ-diversity include ecotonal character (aquatic-aerial) of spring habitats, the fact that spring habitats in the study region (Emilia-Romagna) are still relatively unexplored, and a general lack of high spatial resolution and in-depth taxonomic approaches of springs.

A large part of the geological framework of the Northern Apennines comprises Cretaceous to Miocene, marine neritic (shallow water) or turbiditic (deep water) deposits (siliciclastic arenites, marly-limestones or arenaceous-pelitic turbidites), as well as scattered outcrops of ophiolites, mostly composed of peridotites, serpentinites, gabbros, and basalts. The latter represent inducted remnants of the original oceanic crust of the Ligurian basin, developed in the Middle to Upper Jurassic, which separated the European from the Adriatic plate (Marroni et al., 2010). Each of these geological units are tectonically layered and deformed within the Apennine system, and constitute valuable groundwater reservoirs. In some circumstances, the sedimentary rocks exhibit heterogeneous and anisotropic hydraulic conductivity distributions, similar to those commonly observed for HRA (Gargini et al., 2006; 2008; Vincenzi et al., 2009; Piccinini et al., 2013; Vincenzi et al., 2014; Segadelli et al., 2017b). In particular, Gargini et al. (2014) and Piccinini et al. (2013) consider that the Northern Apennines groundwater reservoirs are all represented either by hard rock aquifers *sensu stricto* (like ophiolites) or by sedimentary units comprising hard rock aquifers as defined above (Fig. 1). These units can be classified as shallow/surficial, recharge-dominated, strongly heterogenous and anisotropic aquifers. Much less represented are the karstic aquifers, comprising Triassic -

458 Messinian evaporite sequences and Late Jurassic - Paleogene non-metamorphic carbonate successions (Fig.
459 1). The geological, structural and geomorphological survey conducted in part during the EBERs project and
460 in subsequent years (Gargini et al., 2014; Cantonati et al., 2016; Segadelli et al., 2017a;b; Cantonati et al.,
461 2020) highlighted in the study area a variability of hydrogeological situations as described and summarized
462 in the conceptual scheme proposed in Figs 1-2, with particular attention to the HRA group, because it
463 represents, from a geological standpoint, the backbone of the northern Apennines. In one system, very
464 high pH values were associated with low temperature reaction between meteoric water and ultramafic
465 rocks (Neal and Shand, 2002; Boschetti and Toscani, 2008; Boschetti et al., 2013).

466 The percentage of Red List diatom species identified in this study belonging to the most important
467 threat categories (60%) is remarkably high. As a comparison, ~ 50% Red List species belonging to threat
468 categories were previously found in high-integrity springs and high-mountain lakes of the Alps (Cantonati et
469 al., 2012a). It is also important to note that most species belonging to the highest threat categories
470 encountered were collected in low-conductivity and dystrophic mountain springs, habitats that were
471 singled out for their rich and peculiar diatom microflora by previous works (Cantonati et al., 2009; 2011;
472 2019), and, secondarily, in limestone-precipitating springs (LPS), which are as well known as special habitats
473 (Cantonati et al., 2016). The share of Red-List threat categories species was also high likely because in the
474 recently published Red List (Hofmann et al., 2018; summarized in Supplementary Material Table 3), the
475 status of most species changed as a result of the new Red List classifications (Lange-Bertalot, 1996), with
476 many placed in higher threat categories. The relatively low share of aerial diatom species in the present
477 study (less than 10%) is likely due to a focus on stable-discharge springs for this study, as compared to
478 previous investigations in the Alps. Most diatom, bryophyte and vascular-plant species were encountered
479 only once or twice because of the high diversity of spring typologies and the consequent distinctiveness of
480 the individual spring sites.

481 In the zoobenthos, a considerable abundance and frequency of stygophilous taxa were observed, which
482 suggests that the selected sites are probably of considerable biogeographical interest. As confirmed also by
483 this study, in Europe, but probably also on other continents, water mites (Hydrachnidia) are the group of

organisms (other than microbes and diatoms) with the greatest number and percentage of species, which in many different genera, independently evolved strong ecological links to spring habitats (Gerecke et al., 2018). Only in Hydrobiid snails, a similarly high share of spring-typical species is reported from western North America, due to a strong radiation of the genus *Pyrgulopsis* (Hershler et al., 2014). As with diatoms (Hofmann et al., 2018), these data offer information useful for a future Red List for European water mites. In the course of the EBERs study, *Pseudofeltria aemiliana* was detected and described as a species new to science (Gerecke, 2014). Unusual in water mites, and particularly surprising in a genus with strong sexual dimorphism, this species is more distinct in females than in males. As with all known species of the genus, it probably is a crenobiont with a preference for weakly seeping helocrenes. It can be considered an endemic species of the Northern Apennines. The material from the study area includes also further interesting records: the species found in highest abundance, *Hygrobates psammocrenicus*, has been described only recently from springs in the Apennines and on Corsica (Gerecke and Di Sabatino, 2013); *Stygothrombium chappuisi* is a very rare species reported from Italy only recently (Veneto, Emilia Romagna: Gerecke and Di Sabatino, 2013); and *Partnunia aprutina*, after its first description from Abruzzo (Gerecke, 1993), has been found again recently in several sites of the northern Appennines (Bottazzi et al., 2011). *Lebertia fontana* in Italy was previously known only from two sites in Trentino (Gerecke, 2009), so this is the first record South of the Alps, while *Lebertia mediterranea*, described from several sites in Italy, France (Corsica), Spain and Bulgaria, and later on detected also in the Austrian Alps (Fišer et al., 2012), is a species in need of revision. Specimens of the latter species found in this study differ from the original diagnosis in some morphological details. With reference to species closely linked to groundwater habitats, the most striking result was the finding of a large population of the typical interstitial-dwelling, laterally-compressed water mite *Frontipodopsis reticulatifrons*. This species is normally restricted to interstitial waters, and not found in springheads.

The topic of the relationship between biodiversity and geodiversity is now emerging with greater awareness in the international scientific community (Kärnä et al, 2019; Alahuhta et al., 2020). There is a clear need to develop new approaches through which to quantify and allow the comparison between

geodiversity and biodiversity in many types of ecosystems. In particular, as is already the case in other ecological questions, the adoption of suitable indices and metrics stimulates and facilitates the comparison between the biotic and abiotic diversity of a given area.

Our data suggest that only an integrated hydrogeological-ecological approach (= ecohydrogeological perspective; Cantonati et al., 2020) will permit researchers to accurately define the foundations for conservation actions and for the monitoring of springs. These systems should not be examined as simple points of aquifer-system discharge, but as ecotones with regional patterns that create complex GDEs (groundwater dependent ecosystem, Bertrand et al., 2012). Springs, studied in an ecohydrogeological perspective, are ideal systems in which to investigate and understand the geo-biodiversity relationship.

5. Conclusions

The main conclusions that can be drawn from our observations are as follows:

- We confirm for spring habitats that the study region (Emilia-Romagna) is highly complex geoscape and an area of high geodiversity. In relation to inland waters, and spring ecosystems in particular, this is mainly apparent in the occurrence of diverse lithotypes and aquifer structures.
- Our data demonstrate that the diversity of rock and aquifer types generates a wide variation in ecomorphological types and hydrochemistry.
- Considering two groups of organisms (diatoms, water mites) with contrasting ecological roles within the spring ecosystem, our study for a deeper understanding of spring-habitat biodiversity and uniqueness and of their determinants shows that the multiplicity of ecomorphological and hydrochemical types translates into high landscape-level (γ) biodiversity, with individual sites typically having relatively low diversity (α diversity) but differing markedly from one another (high individuality).

- The main consequence of our findings for spring-ecosystem conservation is that it is imperative to protect representative and proportional groups of springs at the landscape level.

We further note that springs, studied from an ecohydrogeological perspective, are ideal systems where to investigate geo-biodiversity relationships.

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Figure legends

Fig. 1. Geological map with the location of the springs studied. The small images exemplify the geodiversity that characterizes the landscape of the Emilia-Romagna Region. (4) The basalts of Mt. Ciapa Liscia, 1658 m a.s.l. (9) The serpentinized peridotites of Mt. Prinzera, 736 m a.s.l. (1) View to the East from the Fugicchia Pass: siliciclastic arenites, 1667 m a.s.l. (12) Parallel-stratified calcarenite deposits, 636 m a.s.l. See Table 1 for the meaning of the codes of the 16 springs.

Fig. 2. Hydrogeological block diagram of groundwater flow systems in the main hard rock aquifers outcropping in the study area. Spring numbers are as in Fig. 1.

Fig. 3. Representative images of springs of the different ecomorphological types and subcategories.

Fig. 4. Box plots of the main environmental biota determinants.

Fig. 5. LM and SEM (3a external view; 9e internal view) micrographs of selected frequent and abundant, characteristic, rare and Red List diatom species of the different ecomorphological types. The micrographs (sites) follow the usual order of increasing mineralization. **1a-d**, **Helo_LCSe**, 1a *Navicula angusta*, 1b *Gomphonema pseudobohemicum*, 1c *Eunotia soleirolii* "dwarf", 1d *Stauroneis smithii*; **2a-d**, **CaLS_LCFS**, 2a *Encyonema neomesianum*, 2b *Eunotia cisalpina*, 2c *Chamaepinnularia muscicola*, 2d *Kolbesia carissima*; **3a-d**, **FoVe_LCFS**, 3a *Geissleria acceptata*, 3b *Gomphonema sphenovertex*, 3c *Nitzschia dealpina*, 3d *Achnanthidium lineare*; **4a-c**, **CiLi_HygS**), 4a *Gomphonema minusculum*, 4b *Encyonema ventricosum*, 4c *Amphora micra*; **5a-c**, **MtNe_ShFS-Hi**, 5a *Encyonema vulgare*, 5b *Encyonema sublangebertalotii*, 5c *Encyonopsis moseri*; **6a**, **MtPe_ShFS-Hi**, 6a *Brachysira calcicola*; **7a-c**, **Laga_ShFS-Hi**, 7a *Sellaphora atomoides*, 7b *Sellaphora seminulum*, 7c *Gomphosphenia fontinalis* girdle view; **8a-b**, **MaPa_ShFS-Hi**), 8a

Navicula splendicula, 8b *Neidiomorpha binodiformis*. **9a-f**, **Prin_ShFS-pH**, 9a *Gomphonema auritum*, 9b *Navicula veronensis*, 9c-d *Humidophila irata*, 9e *Reimeria uniseriata*, 9f *Encyonema bipartitum*; **10a**, **Bari_ShFS-mc**, 10a *Gomphonema angustum*; **11a-c**, **Cara_LPS-sn**, 11a *Gomphonema lateripunctatum*, 11b *Achnanthidium trinode*, 11c *Gomphonema tenoccultum*; **12a-d**, **Laba_LPS-le**, 12a *Cymbella tridentina*, 12b *C. tumidula*, 12c-d *Denticula elegans*: 12c valve view, 12d girdle view; **13a**, **FoVR_PS-Su**, 13a *Fragilaria famelica* low-salinity morphotype; **14a-c**, **FoVR_PS-Sh**, 14a *Staurophora wislouchii*, 14b-c *Planothidium werumianum*: 14b RL valve, 14c raphe valve; **15a-b**, **Poia_MiSp-SC**, 15a *Nitzschia frustulum*, 15b *Fragilaria famelica*.

Fig. 6. Site-environmental variables, and species-environmental variables CCA biplots for the epilithon and the epibryon, respectively. See Table 1 for the meaning of the codes of the 16 springs. Species OMNIDIA acronyms: PDAO = *Psammothidium daonense*, ADOL = *Achnanthidium* sp. aff. *dolomiticum*, ADTR = *Achnanthidium trinode*, NULA = *Nupela lapidosa*, GTNO = *Gomphonema tenoccultum*, ONEO = *Odontidium neolongissimum*, GLAT = *Gomphonema lateripunctatum*, PMCR = *Psammothidium microscopicum*, GCUN = *Gomphonema cuneolus*, AINA = *Amphora inariensis*, NEXI = *Navicula exilis*, ADFO_mn = *Achnanthidium fontisalinae* sp. nov. mn, AIND = *Amphora indistincta*, CRUM = *Crenotia rumrichorum*, COPL = *Cocconeis pseudolineata*, FFAM = *Fragilaria famelica*, PTEN_mn = *Planothidium tenuilanceolatum* sp. nov. mn; ACAF = *Achnanthidium affine*, ENCM = *Encyonopsis microcephala*, DLMI = *Delicata minuta*, GPUM = *Gomphonema pumilum*, PGRI = *Psammothidium grischunum*, GACC = *Geissleria acceptata*, HPEP = *Humidophila perpusilla*, HUCO = *Humidophila contenta*, SENI = *Sellaphora nigri*, SSEM = *Sellaphora seminulum*.

Fig. 7. Scatterplots showing the relations between diatom-epibryon Shannon-Wiener diversity (*H'*) and vegetation Shannon-Wiener diversity (*H'*) and lithology. Explanations of the eight values of the variable 'lithology' are given in Table 1.

Fig. 8. Scatterplots showing the relations between selected zoobenthos groups (absolute abundances) and lithology. The meaning of the eight values of the variable 'lithology' are explained in Table 1.

Supplemental online materials

Supplementary material Table 1. The seventy morphological, physical, and chemical variables and parameters measured in the 16 springs.

Supplementary material Table 2. Enrichment factors for the trace elements and metals measured in the springs studied (2011).

Supplementary material Table 3. List of all diatom species identified in the samples. N. occurrences, min, max relative abundance %); Red-List status, aerial species, and ecology (according to Hofmann et al., 2018).

Supplementary material Table 4. Bryophytes.

Supplementary material Table 5. Vascular plants.

Supplementary material Table 6. Zoobenthos.

Supplementary material Table 7. Water mites.

Table
Click here to download Table: Table1_Ecomorph-&-hydrogeol-classification.docx

Table 1. Ecomorphological and hydrogeological classifications of the springs studied and related variables.

Spring code	**Helo_LCSe**	**CaLS_LCFS**	**FoVe_LCFS**	**CiLi_HygS**	**MtNe_ShFS-Hi**	**MtPe_ShFS-Hi**	**Laga_ShFS-Hi**	**MaPa_ShFS-Hi**	**Prin_ShFS-pH**	**Bari_ShFS-mc**	**Cara_LPS-sn**	**Laba_LPS -le**	**FoVR_PS-Su**	**FoVR_PS-Sh**	**Poia_MiSp-SC**	**GeRi_MiSp-Su**
Spring name	Helocrene at Lago Scuro (**Helo**)	Capanne Lago Scuro (**CaLS**)	Fontana del Vescovo (**FoVe**)	Ciapa Liscia (**CiLi**)	Monte Nero (**MtNe**)	Monte Penna (**MtPe**)	Lagacci (**Laga**)	Mangiapane (**MaPa**)	Prinzera (**Prin**)	Barigazzo (**Bari**)	Carameto (**Cara**)	Labante (**Laba**)	Fontanile Valle Re (**FoVR**) Sun	Fontanile Valle Re (**FoVR**) Shade	Poiano (**Poia**)	Gessi Riolo (**GeRi**)
Ecomorphology	Low Cond. (**LC**) Seepage (**Se**)	Low Conductivity (**LC**) Flowing Springs (**FS**)		Hygropetric (**Hyg**) spring (**S**)	Shaded (**Sh**) Flowing Springs (**FS**) with Hildenbrandia (**Hi**)				**ShFS** with high pH (**pH**)	**ShFS** with medium cond. (**mc**)	**LPS** small near-natural (**sn**)	**LPS** large exploited (**le**)	Pool Spring (**PS**) Sun (**Su**)	Pool Spring (**PS**) Shade (**Sh**)	Mineral Spr. (**MiSp**) Sodium Chloride (**SC**)	Mineral Spr. (**MiSp**) Sulphates (**Su**)
Cond. (µS cm^{-1})	13	27	57	62	56	62	111	265	255	305	462	451	788	815	11560	2170
Shading (%)	3	20	3	70	78	69	77	77	78	67	11	8	6	72	80	96
Discharge (L s^{-1})	0,01	0,8	1	1	1,2	3,5	3,5	0,9	0,03	0,4	0,07	15,5	0,5	0,5	50	2,5
pH	7,27	6,41	7,31	7,30	7,63	6,56	7,47	7,70	11,20	7,48	7,66	8,20	7,11	7,22	7,44	7,83
Na^+ (mg L^{-1})	1,5	1,3	1,6	1,5	0,39	1,8	1,0	2,8	33	6,7	4,4	4,8	21	22	2250	11,1
Cl^- (mg L^{-1})	1,7	1,2	1,1	1,4	0,7	1,2	1,0	1,5	24,0	2,7	2,6	7,5	41,0	41,0	4370,0	13,6
SO_4^{2-} (mg L^{-1})	1,7	1,5	1,8	2,8	0,81	2,5	2,2	10,7	9,7	27	45	27	69	68	2090	144
Lithology var. (1-8)	1	1	1	2	3	2	3	4	4	5	6	6	7	7	8	8
Lithology	siliciclastic arenites			basalts	peridotites	basalts	peridotites	serpentinized peridot.		lithic arenites	limest. flyschs; calcarenites		gravel and sand		gypsum	
Aquifer	turbidites			ophiolites						turbidites			alluvial deposits		evaporites	
Aquifer broad cat.	Hard Rock Aquifers (HRA)												Porous aquifer		Karst	

Table
Click here to download Table: Table2_Main-morphol-physical-&-chemical-vars.docx

Table 2. Main morphological, physical, and chemical characteristics as emerging from data processing and statistical analyses.

Spring code	**Helo_ LCSe**	**CaLS_ LCFS**	**FoVe_ LCFS**	**CiLi_ HygS**	**MtNe_ ShFS-Hi**	**MtPe_ ShFS-Hi**	**Laga_ ShFS-Hi**	**MaPa_ ShFS-Hi**	**Prin_ ShFS-pH**	**Bari_ ShFS-mc**	**Cara_ LPS-sn**	**Laba_ LPS-le**	**FoVR_ PS-Su**	**FoVR_ PS-Sh**	**Poia_ MiSp-SC**	**GeRi_ MiSp-Su**
Longitude E	10°2'56.534"	10°2'54.256"	10°2'26.790"	9°28'2.709"	9°31'13.674"	9°30'29.493"	9°35'41.766"	9°29'24.606"	10°4'45.008"	9°48'37.719"	9°45'35.165"	11°2'9.836"	10°31'22.143"	10°31'20.988"	10°26'21.274"	11°43'50.073"
Latitude N	44°22'41.151"	44°22'40.381"	44°22'44.767"	44°34'14.370"	44°33'38.595"	44°29'6.029"	44°37'53.091"	44°46'19.328"	44°38'45.603"	44°36'44.062"	44°39'50.840"	44°15'38.339"	44°45'53.362"	44°45'50.898"	44°23'20.168"	44°14'8.570"
Elevation (m a.s.l.)	1534	1547,5	1613	1381	1509	1324	926	736	492	880	758	603	31,3	32	430	167,5
Discharge ($L\ s^{-1}$)	0,01	0,8	1	1	1,2	3,5	3,5	0,9	0,03	0,4	0,07	15,5	0,5	0,5	50	2,5
Variab. Ind. Disch.	200	50	269	172	98	70	162	53	95	93	150	233	20	20	129	200
T (°C)	9,8	8,8	4,7	5,4	5,8	5,3	7,7	11,1	14	7,9	12,8	15,4	15	13,2	9,6	11,9
Conduct. ($\mu S\ cm^{-1}$)	13	27	57	62	56	62	111	265	255	305	462	451	788	815	11560	2170
pH	7,27	6,41	7,31	7,3	7,63	6,56	7,47	7,7	11,2	7,48	7,66	8,2	7,11	7,22	7,44	7,83
HCO_3^- ($mg\ L^{-1}$)	14	8,2	18	20	20	19	39	93	40	92	145	143	210	208	79	110
Ca^{2+} ($mg\ L^{-1}$)	6,9	4,2	10,7	11,5	0,77	10,3	2,4	23	10,3	43	88	69	143	142	880	651
Mg^{2+} ($mg\ L^{-1}$)	0,63	0,42	0,53	1,7	7,3	1,5	14,7	25	0,11	15	15,5	23	29	29	4,1	39
TP ($\mu g\ L^{-1}$)	<1	4	14	3	<1	7	6	<1	10	<1	6	4	43	3	33	11
NO_3^- ($mg\ L^{-1}$)	0,17	1,1	1,1	0,43	0,46	1,2	0,64	1,1	<0.05	1,1	0,12	1,9	41	48	0,56	14,6
Cr ($\mu g\ L^{-1}$)	0,28	0,24	0,5	1,1	40	0,88	16,8	15,4	0,18	0,85	0,12	0,38	1	0,29	3,4	1,06
Ni ($\mu g\ L^{-1}$)	0,54	0,15	0,19	0,31	2,6	0,24	4,4	10,8	0,14	0,74	0,74	1,33	3,3	1,35	3	5,4
Cu ($\mu g\ L^{-1}$)	1,11	0,24	0,13	0,11	0,17	0,17	0,14	0,93	0,54	0,18	0,19	0,17	1,11	0,25	7,1	0,33
Zn ($\mu g\ L^{-1}$)	1,21	0,36	0,2	0,13	1,15	0,62	0,32	0,71	0,28	0,26	0,36	0,2	3	0,44	0,32	0,38
Fe ($\mu g\ L^{-1}$)	15	9,3	18	28	7,2	14	13	82	8,4	59	58	38	391	93	274	643
Mn ($\mu g\ L^{-1}$)	11,1	0,15	0,061	0,079	0,26	0,079	0,18	1,9	0,26	1,6	0,33	0,078	10,2	0,43	0,52	0,23

Table
Click here to download Table: Table3_Diatom-S-&-H.docx

Table 3. Diatom species richness (*S*) and Shannon-Wiener diversity (*H'*).

S	epilithon	epibryon	debris	***H'***	epilithon	epibryon	debris
min	7	7	5	**min**	0,56	0,25	0,52
max	46	65	22	**max**	2,59	2,15	2,29
mean	25	28	16	**mean**	1,74	1,57	1,41
N	12	14	3	**N**	12	14	2

Table
Click here to download Table: Table4_Diatom-CCA-data.docx

Table 4. Diatom (epilithon and epibryon) Canonical Correspondence Analysis (CCA) data.

epilithon	***df***	***Chi2***	***F***	***P***	**epibryon**	***df***	***Chi2***	***F***	***P***
elevation	1	0,575	1,343	0,039	**elevation**	1	0,636	1,413	0,029
logNO3	1	0,523	1,222	0,132	**HCO3**	1	0,540	1,200	0,228
Cr	1	0,657	1,535	0,038	**Mg**	1	0,418	0,930	0,562
					logNO3	1	0,726	1,613	0,044
Residual	10	4,280			**Residual**	9	4,050		

Table
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Table 5. Landscape-level (ɣ) diversity partitioning for diatoms and water mites.

	DIATOMS								WATER MITES	
	Richness				Shannon-Wiener				Richness	
	epilithon		epibryon		epilithon		epibryon			
	statistic	%	statistic	%	statistic	%	statistic	%	statistic	%
alpha.1	23,5	0,12	28,4	0,13	1,69	0,51	1,57	0,50	5,07	0,13
gamma	199		214		3,35		3,13		39	
beta.1	175,5	0,88	185,6	0,87	1,65	0,49	1,56	0,50	33,9	0,87

Figure
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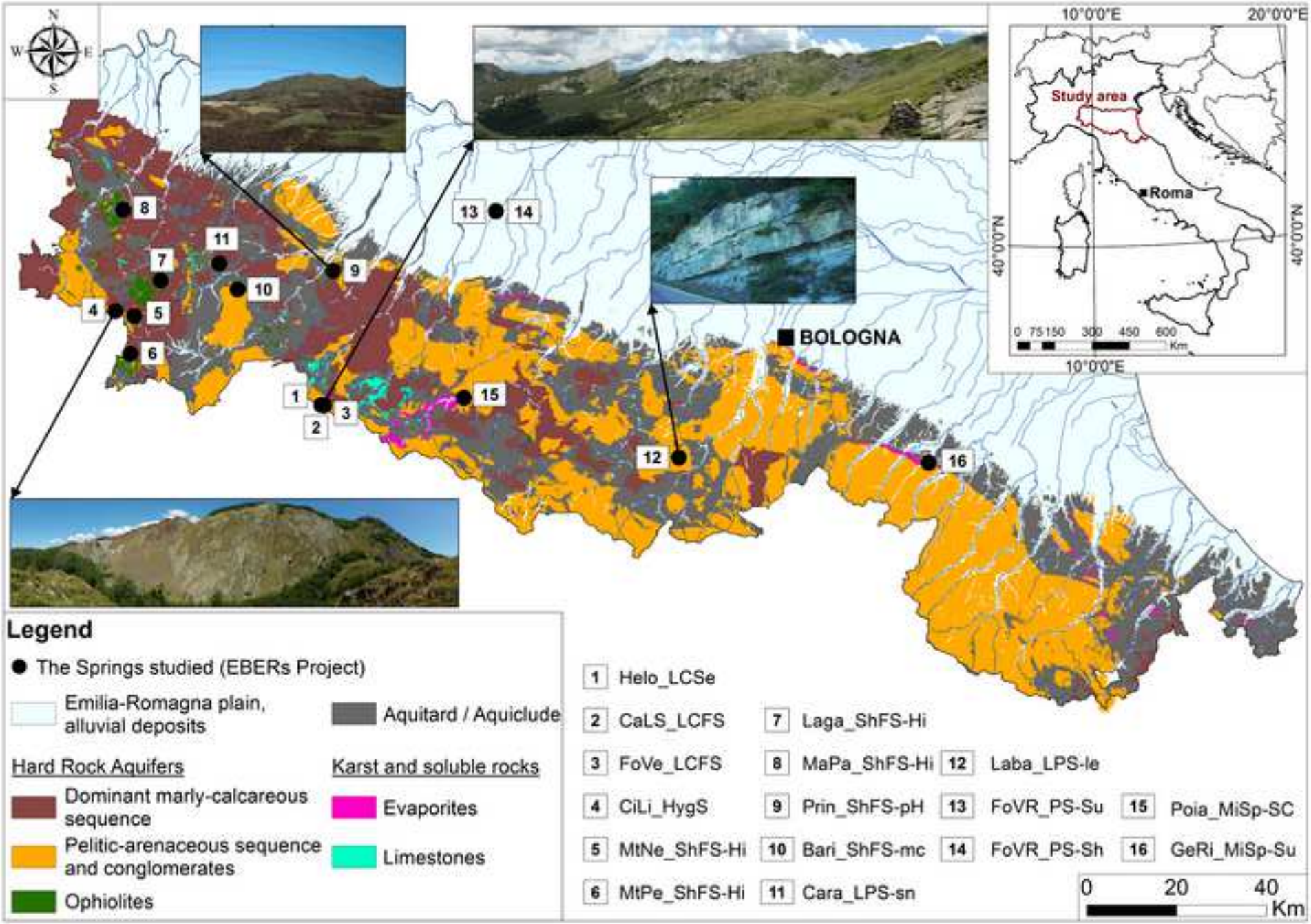

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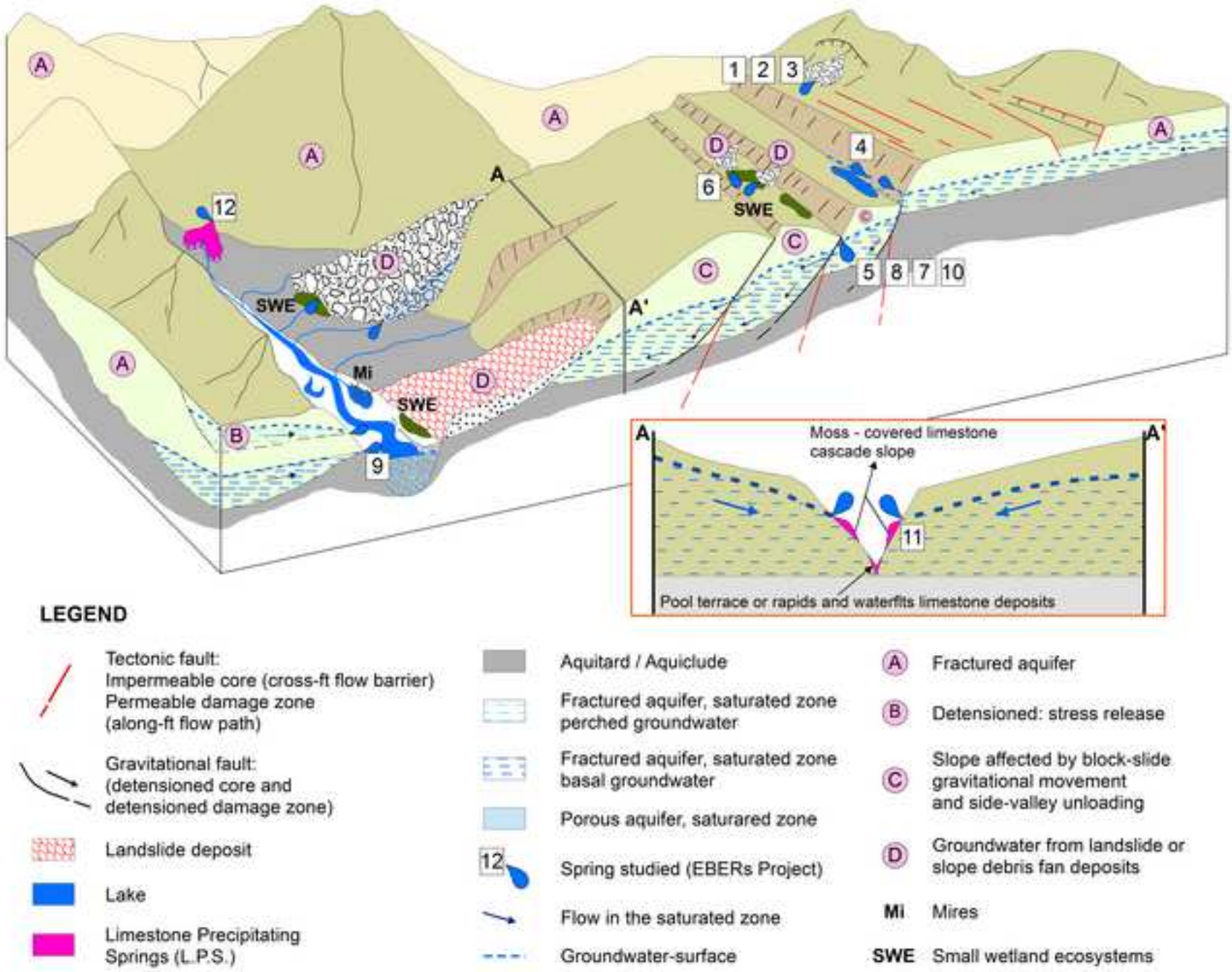

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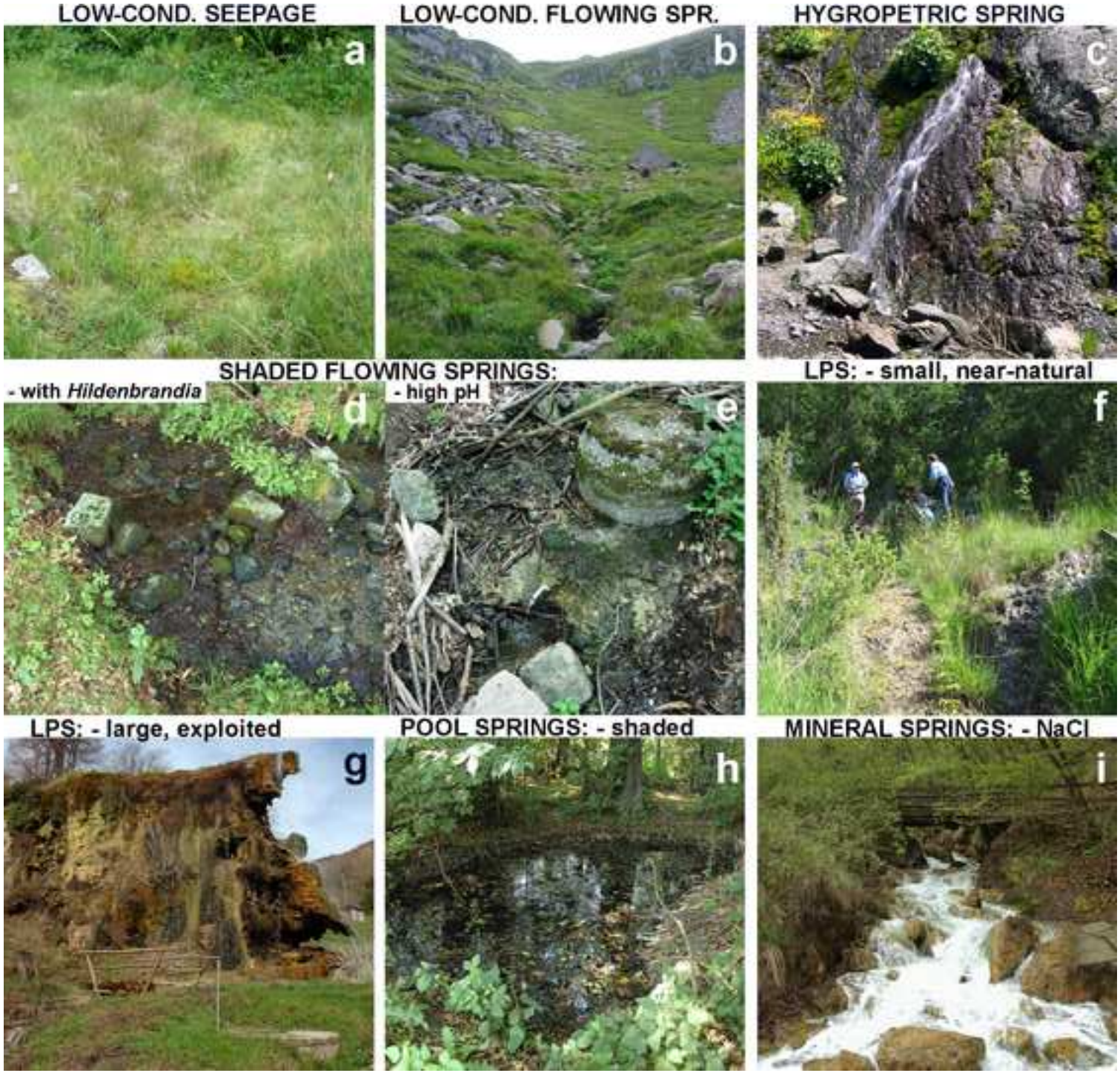

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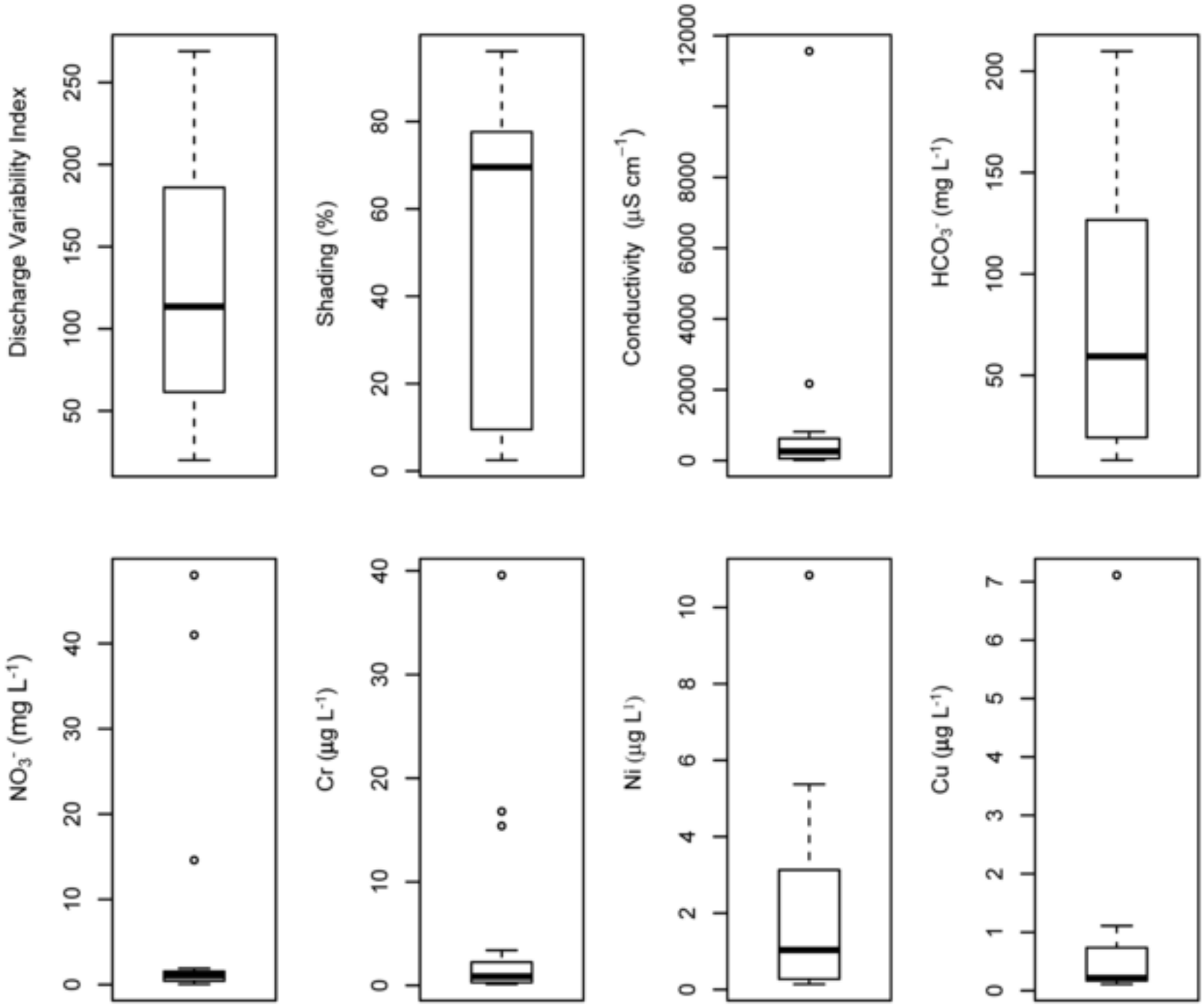

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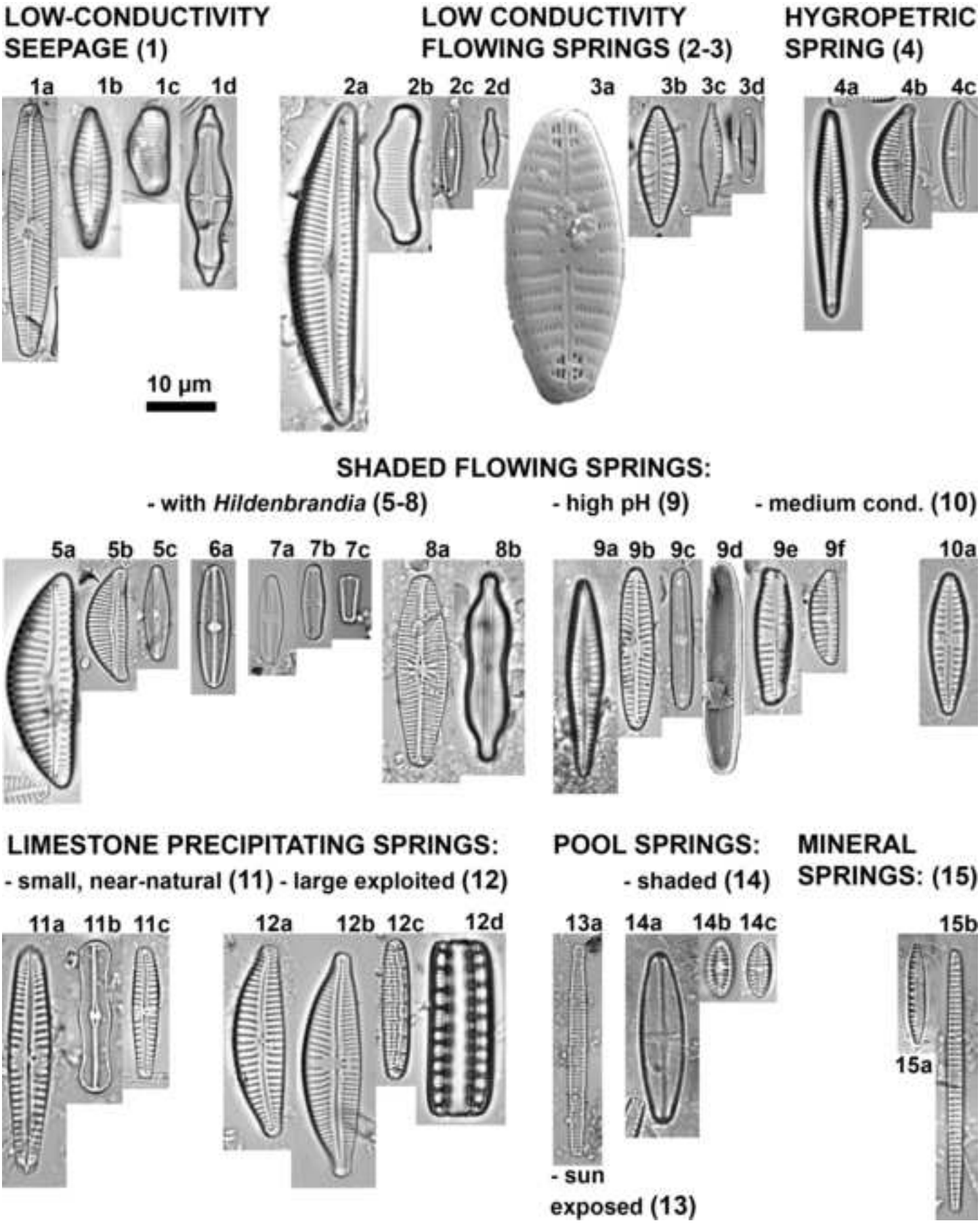

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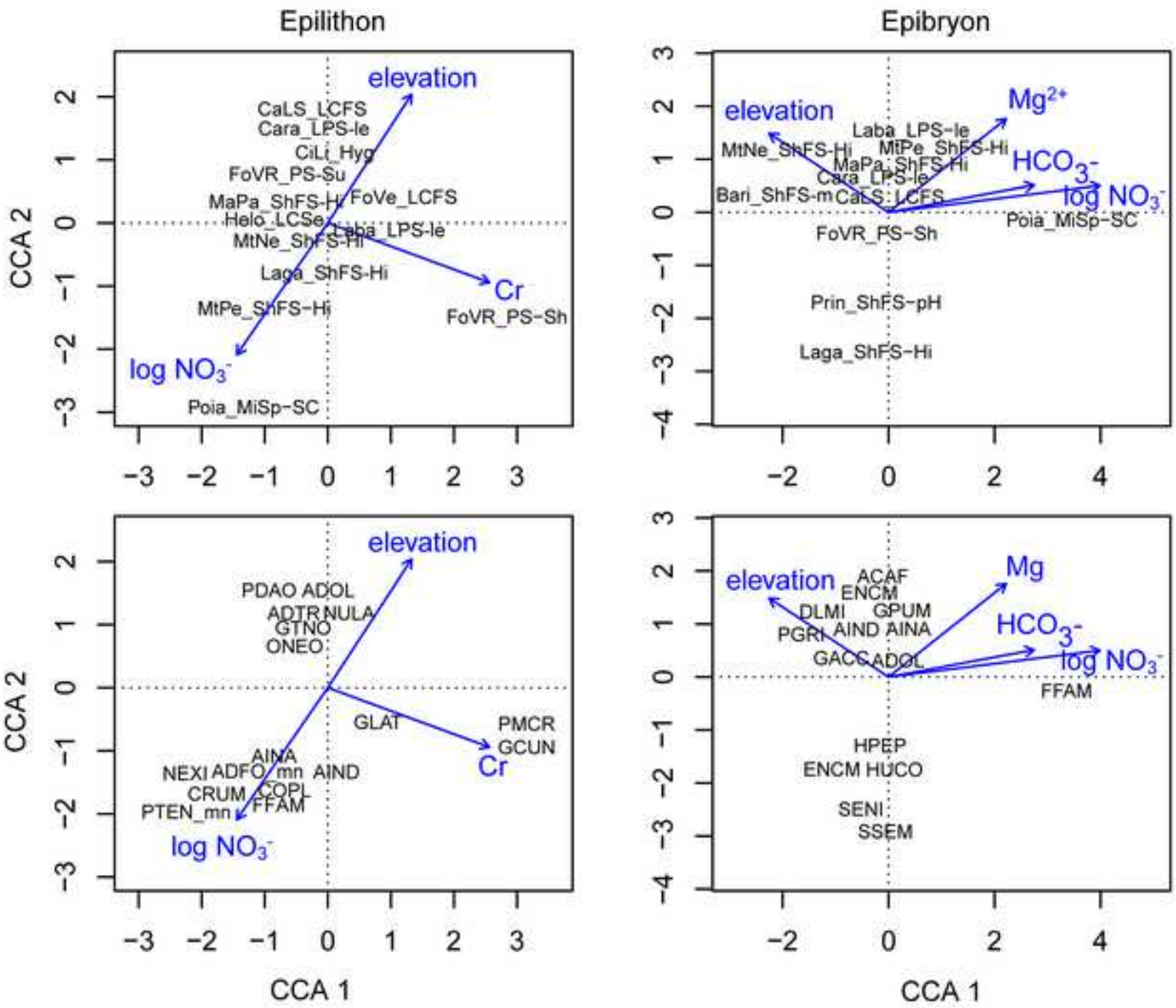

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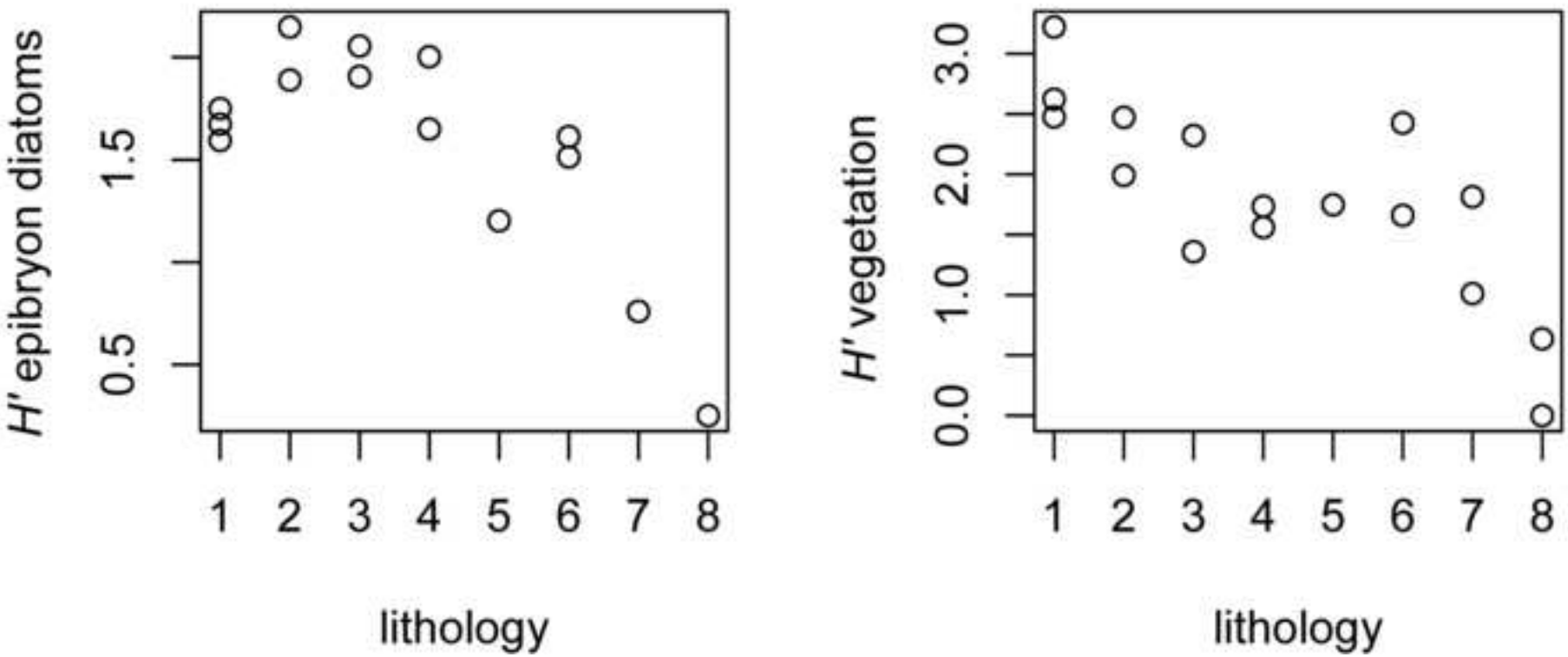
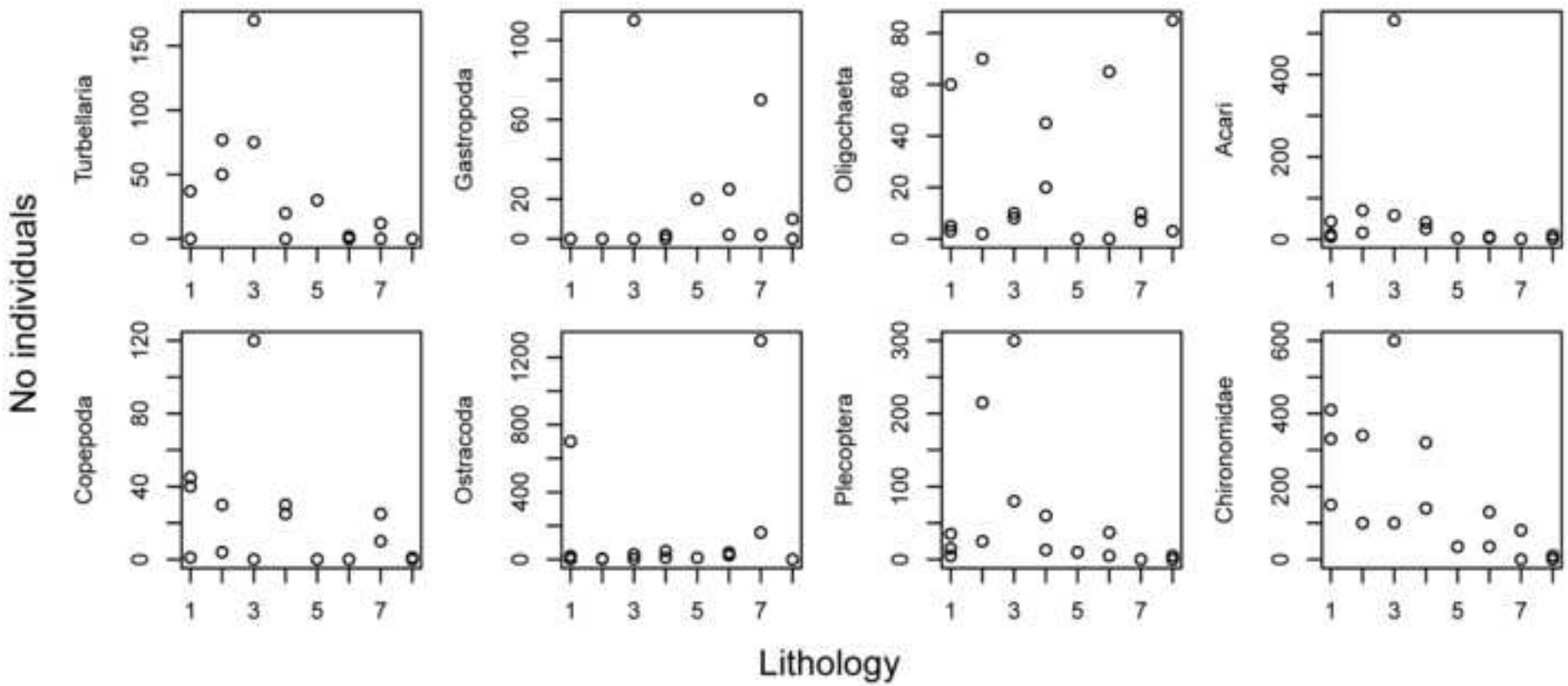
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