Hydrological intermittency drives diversity decline and functional homogenization in benthic diatom communities

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Abstract: Over the last decades, the combined effects of global climate changes and severe land use modifications have been exacerbating river hydrological alterations and habitat fragmentation in many Mediterranean rivers. This trend is predicted to intensify, with expected significant impacts on taxonomic and functional diversity of benthic communities in the next future.
The present research aims at investigating the long-term combined effects of flow intermittency, climate and land use changes on benthic diatom communities, by analysing data collected over 11 years in Mediterranean streams of the NW-Italy.
We demonstrated that the ongoing global changes and local environmental pressures determined a significant decline in diatom species diversity at both local and regional scales. More in detail, flow intermittency affected both diatom diversity and life history traits, with communities of intermittent reaches taxonomically and functionally different and less heterogeneous than assemblages characterizing perennial ones. Communities inhabiting intermittent sections showed high percentages of small, mainly stalked and pioneer taxa belonging to the low profile guild, highlighting the strong environmental pressure exerted by the hydrological alterations. Conversely taxa colonizing permanent reaches were bigger, belonging to the high profile guild and able to produce colonies, denoting environmental stability.
The results we obtained could be ascribed to the long-term effects of drying in Mediterranean streams and, as first in the literature, we highlight that diatoms are able to provide long-terms responses to environmental changes caused by water stress, when hydrological disturbance is persistent.

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Cover Letter

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To the attention of
the editor of Science of the Total Environment
July 20th, 2020

Dear Editor,
we would be grateful if you would consider the possibility of including our contribution entitled **“Hydrological intermittency drives diversity decline and functional homogenization in benthic diatom communities”** in a forthcoming issue of Science of the Total Environment.

We are confident that our manuscript fulfills the aims and scope of the journal, being an interdisciplinary study that investigates the environmental impacts of climate and land use changes on freshwater ecosystems in the Mediterranean basin and, thus, potentially having a high international relevance.

Over the last decades, the combined effects of global climate changes and the local land use changes have been exacerbating the natural flow intermittency and habitat fragmentation in many Mediterranean rivers worldwide. And this trend is predicted to intensify with expected significant biodiversity and functional losses in a next future. In order to highlight the impacts of these phenomena on lotic ecosystems, here we investigated the taxonomic and functional response of the benthic diatom communities experiencing climate- and land use-driven exacerbation of flow intermittency over the last 11 years in a Mediterranean region of the NW-Italy.

By comparing the environmental parameters and diatom communities in permanent and intermittent reaches, we demonstrated that diatom communities are highly impacted by flow intermittency and that hydrological differences are due to the combined effects of the ongoing climate change and local environmental pressures. We highlighted a significant decline of diatom species at both local and regional scales due to the extinction of rare species and the increase of few dominant species in intermittent reaches. When considering community composition, we demonstrated a taxonomic change and a functional homogenization of the diatom communities inhabiting intermittent reaches, which were dominated by low profile, pioneer taxa, typical of disturbed conditions. Although flow intermittency is part of the natural hydrological cycle of Mediterranean rivers, its climate- and land use-driven exacerbation represents a threat to diatom benthic communities. These results are extremely alarming considering that Mediterranean rivers represent a biodiversity hotspot and provide fundamental ecosystem services worldwide.

This manuscript describes original work and is not under consideration by any other journal. All authors approved the manuscript and this submission.

Thank you for receiving our manuscript and considering it for review. We appreciate your time and look forward to your response.

Sincerely Yours,

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Hydrological intermittency drives diversity decline and functional homogenization in benthic diatom communities

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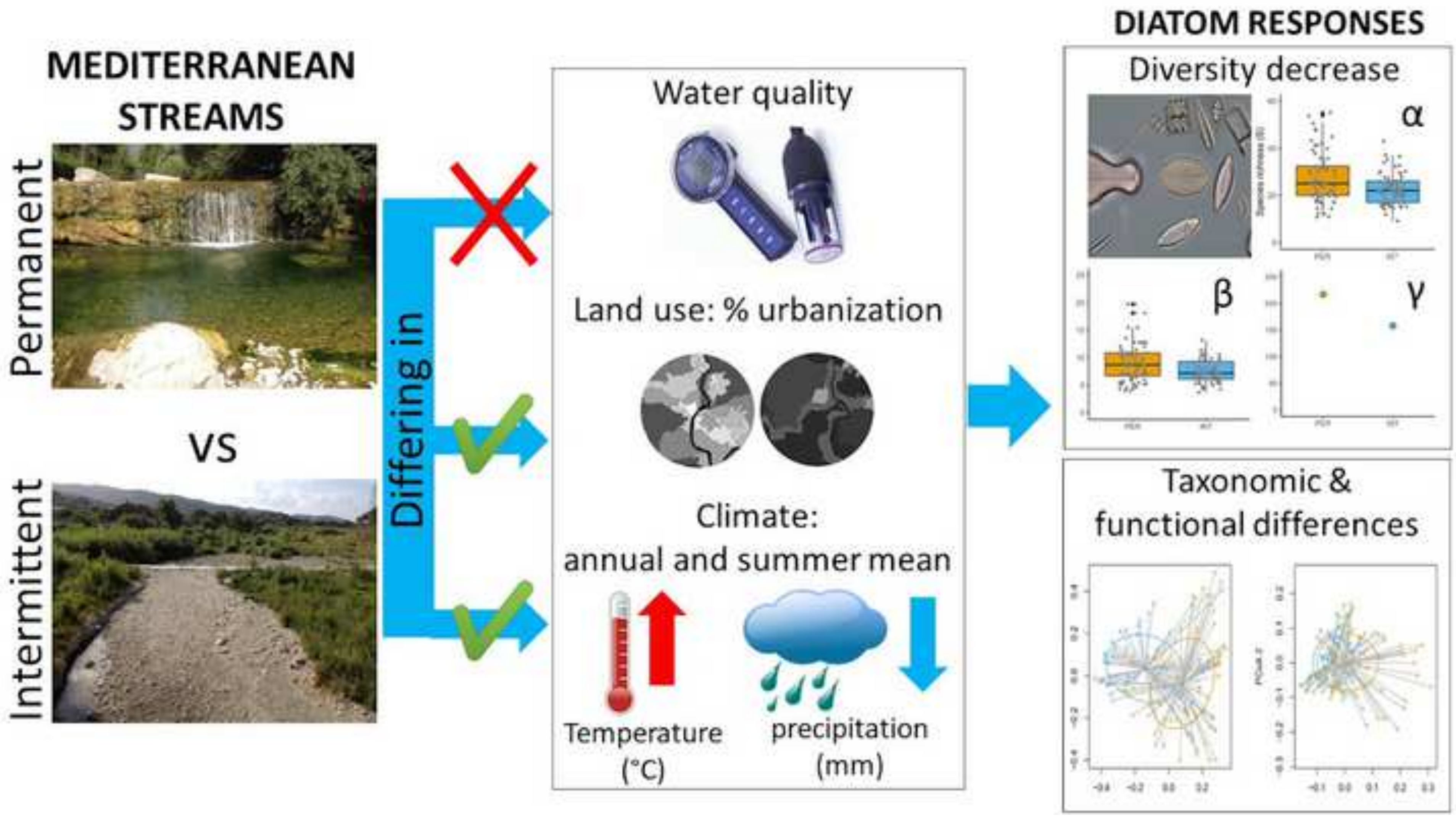
Running Title: Flow intermittency affects diatom communities

Keywords: Bacillariophyceae, climate change, diversity partitioning, non-flow, functional traits, Mediterranean rivers

***Graphical Abstract**



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

Highlights

- Climate and land use changes exacerbate flow intermittency in Mediterranean rivers
- We studied long-term effects of flow intermittency on diatom communities
- Diatom diversity is lower in intermittent than perennial reaches at several scales
- Flow intermittency changed diatom assemblages and reduced functional heterogeneity

Hydrological intermittency drives diversity decline and functional homogenization in benthic diatom communities

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ABSTRACT

Over the last decades, the combined effects of global climate changes and severe land use modifications have been exacerbating river hydrological alterations and habitat fragmentation in many Mediterranean rivers. This trend is predicted to intensify, with expected significant impacts on taxonomic and functional diversity of benthic communities in the next future.

The present research aims at investigating the long-term combined effects of flow intermittency, climate and land use changes on benthic diatom communities, by analysing data collected over 11 years in Mediterranean streams of the NW-Italy.

We demonstrated that the ongoing global changes and local environmental pressures determined a significant decline in diatom species diversity at both local and regional scales. More in detail, flow intermittency affected both diatom diversity and life history traits, with communities of intermittent reaches taxonomically and functionally different and less heterogeneous than assemblages characterizing perennial ones. Communities inhabiting intermittent sections showed high percentages of small, mainly stalked and pioneer taxa belonging to the *low profile* guild, highlighting the strong environmental pressure exerted by the hydrological alterations. Conversely taxa colonizing permanent reaches were bigger, belonging to the *high profile* guild and able to produce colonies, denoting environmental stability.

The results we obtained could be ascribed to the long-term effects of drying in Mediterranean streams and, as first in the literature, we highlight that diatoms are able to provide long-terms responses to environmental changes caused by water stress, when hydrological disturbance is persistent.

1. INTRODUCTION

Over the last decades, the combined effects of global climate changes and the growing human demand for water and other ecosystem services have been exacerbating flow intermittency in many areas (IPCC Core Writing Team, 2014). This trend is predicted to intensify, causing a global increase of drought frequency and the spatial and temporal extent of intermittent reaches in rivers worldwide (Datry et al., 2017a; b). Indeed, climate change-runoff models forecast future decreases in runoff patterns in mid-latitude regions coupled with an increase of air temperatures (Kundzewicz et al., 2008; Larned et al., 2010). This is particularly evident in the Mediterranean region, where an annual precipitation reduction up to 20% and a contemporary intensification of the extreme events have been observed during the last century (García-Ruiz et al., 2011). Although intermittent rivers are widely distributed in the Mediterranean region (Bonada & Resh, 2013), their number and extension is predicted to rapidly increase (IPCC Core Writing Team, 2014) and changes in air temperature and precipitation patterns can affect water temperature and the duration of the wet and dry phases that naturally characterizes their hydrological cycle during summer (Giorgi & Lionello, 2008). In addition, the local land use changes have been recognized to cause habitat fragmentation and to reduce habitat heterogeneity worldwide (Sala et al, 2000). More in detail, the conversion of natural land uses into agricultural and urban areas led to changes in hydrology, water chemistry and morphological conditions of rivers (Newall & Walsh, 2005; Medeiros et al., 2020; Song et al., 2020), likely exacerbating the negative effects of increasing flow intermittency on the lotic biota.

Under this scenario, Mediterranean freshwater ecosystems are expected to face a huge species loss (Chiu et al., 2017), due to the harsh environmental conditions induced by both hydrological alterations and habitat fragmentation, which act as a filter and select for species displaying specific traits that enhance their survival in these extreme environments (Datry et al., 2017c). In particular, drying up of the streambed causes longitudinal, lateral and vertical habitat fragmentation, limiting the recruitment of new individuals, which drift from upstream or colonizing the riverbed from lateral refugia, such as pools, or from the hyporheic zone, with consequences on species distribution and abundances (Tolonen et al., 2019). Focusing on diatom communities, it has been recently demonstrated that droughts in Mediterranean streams can led to a significant decrease of endangered species (Falasco et al., 2016a), thus reducing species diversity at both local and regional scale, and to changes in community functional traits (Elias et al., 2014; B-Béres, et al., 2019; Novais et al., 2020). For instance, the genera *Cymbella* and *Gomphonema* are able to face desiccation by embedding cells within a mucilage-protective layer (Sabater et al., 2017), while motile taxa, such as *Navicula*, *Nitzschia* and *Surirella*, can actively move on the substrate towards refugia or simply penetrate the biofilm toward deeper layers (Falasco et al., 2016b; 2018a). Flow intermittency also changes diatom community composition throughout the year, with shifts from lotic- to lentic-adapted species up to aerophilous ones during the lentification and the drying up of the riverbed respectively (Datry et al., 2017a). Thus, species surviving during the lentification phase can often represent both a taxonomic and functional subset of those typical of the wet phase (Tolonen et al., 2019). In addition, modifications in river physical elements (such as river banks, channel morphology, substrate composition and riparian canopy) induced by land use changes can play an important role as drivers of diatom composition (Kutka & Richards, 1996; Hill et al., 2000; Hlúbiková et al., 2014) and can furtherly lead to a decrease in species richness (Bona et al., 2008).

Beside species richness, another important biological response to environmental changes is the degree of compositional variation in communities across space or in time, namely β-diversity (Soininen, 2010). Larned et al. (2010) hypothesises a conceptual model aimed at exploring diversity patterns at different scale in temporary rivers, by predicting maximum β-diversity when the effect of additional aquatic patches is balanced by the homogenising effect of patch connection. In other words, β-diversity reaches its maximum when the connectivity and dispersal among the patches, created by hydrological variations, is intermediate. Thus, β-diversity is mainly driven by habitat heterogeneity and can be used to assess the loss of environmental quality, through a measure of community composition (Piano et al., 2017a). However, results on the existing relationship between β-diversity and habitat heterogeneity are often controversial and, concerning diatom communities, not well documented (Jyrkänkallio- Mikkola et al., 2016).

The present research aims at investigating the long-term effects of the combined pressure of both flow intermittency and land use changes in a Mediterranean region of NW-Italy (namely Liguria), by analysing benthic diatom communities collected over a period of 11 years. In particular, we analysed data obtained during the routine biomonitoring programs carried out by the Environmental Protection Agency (ARPAL) in Liguria (NW-Italy) in the framework of the WFD (2000/60/CE) and covering a temporal range of 11 years, from 2008 to 2019. In this region, summer precipitations follow a downward trend, suggesting that this region is becoming drier (Deitch et al., 2017). In addition, the land use is profoundly modified by anthropogenic activities, with high urban coverage especially along the coast, where most of the intermittent reaches are located. We compared communities inhabiting permanent and intermittent reaches during baseflow, in order to highlight differences in terms of diatom taxonomic diversity at multiple scales and in terms of taxonomical and functional community composition. In fact, a deep understanding of the existing relationships between hydrological alterations and benthic communities at different levels still represent an important scientific gap that urgently need to be filled (Wu et al., 2019).

We hypothesised that cumulative effects of non-flow events, determined by the ongoing climate change coupled with strong changes in river hydromorphology related to urbanization of the sampling sites, would result in diatom (i) species loss at both local and regional scales and (ii) taxonomic and functional homogenization. We also hypothesised that (iii) diatoms colonizing permanent and intermittent reaches would show different functional traits, measured in terms of class size, growth forms and ecological guilds. In particular, we expected bigger sized diatoms, which are known to be adapted to stable flow conditions (i.e. high profile diatoms, characterized by stalked growth forms and possibly producing colonies, Rimet & Bouchez, 2012), in the permanent reaches, while pioneer communities, composed of smaller sized taxa and thus likely adapted to unstable environments (i.e. low profile diatoms, characterized by adnate growth form, Rimet & Bouchez, 2012), in the intermittent ones.

2. MATERIALS AND METHODS

2.1 Study area

In total, we selected 55 sampling sites characterized by different flow regimes in Liguria, a NW-Italian region with a surface area of about 5400 km^2 (Figure 1). More in detail, we chose 25 sites with permanent flow (hereafter PER), classified as M4 according to the WFD (i.e. small and medium Mediterranenan rivers flowing in the mountains), and 30 sites characterized by intermittent flow and experiencing drying during summer (hereafter INT), classified as M5 according to the WFD (i.e. Mediterranean temporary rivers). To mitigate potential confounding factors related to water quality, we excluded a priori all the sites with an ecological status “poor” or “bad” according to the historical data provided by ARPAL and assuring a comparable ecological status between the two groups of sites. The PER sampling sites were located between 1 and 649 m a.s.l, with a distance from the source ranging from 22.4 and 62.3 Km and catchment surface ranging from 150 to 675 Km^2. Streambed in the permanent reaches was generally natural and heterogeneous among the stations, mainly composed of rocks, boulders and cobbles with some gravel and sand. The INT sampling sites were located between 1 and 446 m a.s.l, with a distance from the source ranging from 6 and 41.5 Km and catchment surface ranging from 8 to 285 Km^2. Streambed in the intermittent reaches was mostly natural, but a significant number of sampling sites resulted somehow artificial, characterized by streambed and banks modifications. However, in most of the reaches, the streambed composition was heterogeneous and composed mainly of boulders, cobbles and gravel but also sand and silt (see Table SM1 for more details on the environmental characterization of PER and INT sites).

2.2 Diatom samples

Epilithic diatoms were collected following the monitoring program of the ARPAL (see Table SM2), for a total of 144 samples, among which 72 samples were collected in PER sites and 72 samples were collected in intermittent sites. According to the standard procedure, in each reach we chose five cobbles from the main flow and we collected periphyton by scraping their upper surface by means of a toothbrush (European Committee for Standardization, 2003). Samples were fixed with ethanol (70%) and transported to the laboratory, where they were treated with H_2O_2 (30%) and HCl (European Committee for Standardization, 2003). Permanent slides for the light microscope analysis were mounted by means of Naphrax®. Diatom identification was based on several diatom floras and monographies, as well as recent taxonomic papers (Krammer and Lange-Bertalot, 1986–1991a,b; Lange-Bertalot and Metzeltin, 1996; Krammer, 1997a,b; 2002; 2003; Reichardt, 1999; Lange-Bertalot, 2001; Werum and Lange-Bertalot, 2004; Blanco et al., 2010; Hofmann et al., 2011; Bey and Ector, 2013; Falasco et al., 2013; Ector et al., 2015). As required by the standard procedure, we identified at least 400 valves in each sample. For each species, we compiled a list of functional traits focusing on class size, ecological guilds and growth forms, defined according to Rimet and Bouchez (2012).

2.3 Environmental data

<u>Water quality data.</u> Water samples for the physical and chemical analyses were collected together with diatom samplings. In total, over the whole sampling period (2008-2019), we collected 144 water samples. In both PER and INT reaches we measured the chemical and physical parameters required by the WFD, namely ammoniacal nitrogen ($N\text{-}NH_3$), nitrate nitrogen ($N\text{-}NO_3$), total phosphorous (Ptot), conductivity (COND), dissolved oxygen (DO, both in mg L^{-1} and %), pH and water temperature (TEMP), by using standard method procedures

<u>Land use data.</u> We defined the land use of each sampling site on digital maps in QGis (Quantum Gis Development 2020) in a buffer defined as a circle of 500 m radius with the sampling site as the centre. Land use was obtained from regional data (http://www.cartografia.regione.liguria.it/) and we calculated the % of the following five land use categories: urban areas, agricultural areas, woods, open areas and water bodies.

<u>Climatic data.</u> Climatic data were obtained from the WorldClim website (www.worldclim.org). We analysed data on monthly precipitations (mm) and monthly minimum and maximum air temperature (°C) from 2008 to 2018, with a spatial resolution of 2.5 minutes. Data from 2019 were not included as they were not available on the website yet. More in detail, by using the function “extract” from the package *raster* in the R software (R Core Team, 2020), for each sampling site, we extracted the value of precipitations, maximum and minimum air temperatures for each month in the

considered timeframe. We then averaged the obtained values for (i) all months (hereafter annual climatic data) and (ii) for only June, July and August months (hereafter summer climatic data), which represent the drought period for the study area, to obtain a mean value of all climatic data for each sampling site.

2.4 Statistical analyses

All statistical analyses were performed with the R statistical software (R Core Team, 2020).

Environmental data. We first performed data exploration following Zuur et al. (2010) by visually checking the distribution of environmental data and the presence of outliers with dotplots. Nutrients (were then log-transformed to achieve a homogeneous distribution). In order to explore possible differences between PER and INT sampling sites in terms of environmental and physical-chemical conditions we performed three Principal Component Analyses (PCA), one for each environmental database: i) physical and chemical data (Temp = temperature; %DO = dissolved oxygen saturation; Cond = conductivity; log_Ptot = log-transformed total phosphorous concentration; log_N-NO_2 = log-transformed nitrate concentration; log_N-NH_3 = log-transformed ammonia concentration; ii) land use data; and iii) climatic data (annual climatic data: year_prec = year precipitations, year_tmin = year minimum temperature, year_tmax = year maximum temperature; summer climatic data: summer_prec = summer precipitations, summer_tmin = summer minimum temperature and summer_tmax = summer maximum temperature). We separately analysed these datasets because we were interested in highlighting which environmental components, namely physical-chemical parameters, land use or climatic data, could better explain differences among PER and INT sites. A Permutational Multivariate Analysis of Variance (PERMANOVA, Anderson, 2001) was then applied to the three environmental dissimilarity matrices based on Euclidean distances to test for differences between PER and INT sites with the function “adonis” from the *vegan* package (Oksanen et al. 2019). Statistical significance was tested via 999 random permutations. We then performed a two-sample t-test to check for differences between PER an INT sites for each environmental variable.

Diversity partitioning. To investigate for differences among PER and INT sites in terms of taxonomic diversity, we partitioned the total diversity at the regional scale (γ) into its local diversity components, namely the average local taxa richness of each site (α), and the variation among sites (β) for both site categories. Variation in taxa composition among local communities (β-diversity) was calculated with the Whittaker’s multiplicative formula as the ratio between γ- and α-diversity (Jost, 2007; Anderson et al., 2011), which expresses the number of times by which the richness at regional level increases compared to the richness at local level. Differences in taxa richness among PER and INT sites were tested with a randomization procedure, whereby we permuted samples over the two hydrological categories 999 times (McGlinn et al., 2018), using the function “get_mob_stats” in the package *mobr* (Xiao et al., 2018). We estimated significant differences between treatments by comparing the observed value of the taxa richness to the null expectation of randomly distributed taxa across sites (Legendre & Legendre, 1998). Diversity partitioning was performed on observed taxa richness (S) and other two diversity metrics, namely rarefied richness (S_n), and effective number of species (S_{PIE}). In particular, S_n controls species richness for the number of individuals among treatments, while S_{PIE} represents evenness (McGlinn et al., 2018). Rarefied species richness (S_n) and evenness (S_{PIE}) are obtained from individual-based rarefaction curves that were either calculated at the level of individual samples (α-level) or by pooling all individuals across samples (γ-level). Evenness was calculated as the probability of intraspecific encounter (*PIE*) and transformed into an expected number of equally abundant species (S_{PIE}) to obtain an easier interpretation (McGlinn et al. 2018). In a second step, we decomposed total beta diversity, here intended as the dissimilarity between communities in PER and INT sites (β_{total}) into its turnover (β_{repl}) and nestedness (β_{rich}) components with the function beta in the *BAT* package (Cardoso et al., 2020). We then tested for differences between PER and INT sites in terms of all β-diversity components with a t-test.

Taxonomic and functional composition. We performed a Principal Coordinate Analysis (PCoA) to visually inspect possible differences in terms of both taxonomical (Bray-Curtis distance) and functional (Gower distance) composition among diatom samples collected in sites with different water regime and land-use (i.e. PER vs INT). Possible dissimilarity in taxonomical and functional composition of diatom communities collected in INT and PER sites was tested through a PERMANOVA (Anderson, 2001) applied on distance matrices, by using the function "adonis" in the package *vegan* (Oksanen et al., 2019). To investigate taxonomic differences, the taxonomic matrix, with the relative abundance of each recorded taxon in each sample, was converted into a site-by-site distance matrix using the Bray-Curtis distance with the function "vegdist" of the *vegan* package (Oksanen et al., 2019). The distance of each site to its associated group multidimensional median was calculated and differences among such site distances were tested by means of multivariate analogue of the Levene's test for homogeneity of variance with 9999 permutations to determine whether the dispersions between the two groups were different. The same procedure was repeated on the functional matrix, containing trait abundances for each sampled site. To generate the functional matrix, we first created a species-by-trait matrix that was multiplied by the species-by-site matrix to obtain the site-by-trait matrix with the function "functcomp" in the package *FD* (Laliberte et al., 2014), in which each entry corresponds to the sum of the relative abundances of all the species present in a site that have a particular trait state. Functional traits considered for generating the functional matrix were class size, life-forms and ecological guilds (Rimet & Bouchez, 2012). To test whether flow regime and land use changes cause biotic homogenization in both taxonomic and/or functional structure of diatom communities we performed the test of homogeneity for multivariate dispersion (Anderson et al., 2006) following the procedure proposed by Brice et al. (2017) and with the PERMANOVA, with the package *vegan* (Oksanen et al., 2019) by using the functions“betadisper and “adonis” respectively. Finally, to check whether functional differences between PER and INT reaches were due to selective environmental filtering of some functional traits, we compared the two categories by performing a t-test on each functional trait.

3 RESULTS

3.1 Environmental data

When considering the water quality parameters, all the investigated sites were characterized by a good or elevated water quality status going from “moderate” to “high” (Table 1). According to the PCA performed on water quality parameters, PER and INT sites broadly overlap (Figure 2a). The first axis explained 26.2% of the total variance and was negatively correlated with log_Ptot (-0.537), while the second axis explained 19.1% of the total variance and was positively correlated with pH (0.570) and log_N-NH_3 (0.509). The similarity among PER and INT sites was confirmed by the results of the PERMANOVA, which detected no significant differences in terms of physical-chemical parameters ($F_{1,143}$ = 3.17, P = 0.064). Although the PERMANOVA did not highlight significant differences among the two hydrological categories, the results of the two-sample t-tests showed significant differences in terms of nitrate and total phosphorous concentrations (Table 1). In particular, the former was higher in INT sites, whereas the latter was higher in PER sites. However, as shown in Table 1, observed values were always included in the first or second water quality class following the Italian water quality standards (D. Lgs 152/2006).

Conversely, from the visual inspection of the PCA performed on land use (Figure 2b), we could clearly distinguish two groups of sites, corresponding to PER and INT reaches. The observed pattern was confirmed by the PERMANOVA, which showed significant differences between the two hydrological categories ($F_{1,143}$ = 32.2, P = 0.001). PC1 explained 35.7% of the total variance and was positively correlated with urban areas (0.697), while PC2 explained 25.1% of the total variance and was negatively correlated with agricultural land use (-0.806). The results of the two-sample t-tests showed significant differences among the two site categories, in terms of wood areas (with higher percentages in PER sites) and urban areas (with higher percentages in INT sites), while no differences were detected in terms of agricultural areas, open areas and water bodies (Table 1).

From the PCA performed on climatic data (Figure 2c), we could again clearly distinguish two groups of sites, corresponding to PER and INT reaches. The PC1 explained most of the variance (76.2%) and was negatively correlated with year_tmin (-0.453) and summer_tmin (-0.451), while PC2 explained 18.7% of the total variance and was positively correlated with summer_tmax (0.727). PERMANOVA highlighted significant differences between the two categories in terms of climatic features ($F_{1,143} = 16.1$, $P = 0.001$). According to the results of the two-sample t-tests performed on climatic data, permanent reaches were characterized by higher annual and summer precipitations than INT sites, while INT sites showed higher minimum and maximum air temperatures, all over the year and during summer, in comparison to PER sites (Table 1).

3.2 Taxonomic responses to different water regimes

In total we recorded 240 species of diatoms (see Table SM3 for a complete list of the recorded species). The results of the diversity partitioning showed that species richness (S) was significantly different between PER and INT sites in terms of all the diversity components (α- β- and γ-diversity) (Figure 3a-c). PER sites were characterized by a higher number of taxa at both local ($\alpha = 27.6 \pm 10.6$) and regional ($\gamma = 217$) scale than INT reaches ($\alpha = 22.6 \pm 6.44$; $\gamma = 158$). Moreover, the variation of species richness among sites (i.e. β- diversity) was significantly higher in PER ($\beta = 9.11 \pm 3.66$) than INT sites ($\beta = 7.57 \pm 2.31$), highlighting a higher heterogeneity in terms of species richness in PER reaches. When considering rarefied richness (S_n) (Figure 3d-f), we observed significant higher values of α- and γ-diversity in PER ($\alpha = 23.0 \pm 8.87$; $\gamma = 198$) than INT sites ($\alpha = 19.1 \pm 5.60$; $\gamma = 146.3$), but not of β-diversity (PER = 2.87 ± 1.15; INT = 2.56 ± 0.814). Regarding evenness (S_{PIE}) (Figure 3g-i), we denoted a higher dominance of few taxa in INT than PER reaches at both local (PER: $\alpha = 6.32 \pm 3.96$; INT: $\alpha = 4.39 \pm 2.37$) and regional scale (PER: $\gamma = 15.2$; INT: $\gamma = 6.99$), as well as in terms of variation of dominant species (PER: $\beta = 3.48 \pm 2.32$; INT: $\beta = 2.04 \pm 1.03$). When analysing the community dissimilarity, we observed that total β-diversity and its turnover and nestedness components were significantly higher in PER than in INT sites (Table 2). In both groups, patterns of diatom community composition were mainly explained by the turnover component of dissimilarity, while nestedness played a much minor role (Figure 4).

We then analysed whether the observed differences between the two hydrological categories could also be mirrored in the taxonomic and functional composition of the diatom communities. Visual inspection of the PCoA ordination performed on the taxonomic matrix (Figure 5a) depicted a clear difference in terms of taxonomic composition between PER and INT reaches, which was confirmed by the PERMANOVA ($F_{1,143} = 4.67$, $P = 0.001$). Results of the test of homogeneity for multivariate dispersion highlighted a significant homogenization of diatom communities in the INT reaches in comparison to PER ones, in terms of species composition ($F_{1,143} = 15.5$, $P = 0.001$). When considering the diatom functional matrix, the PCoA showed that communities characterizing the INT reaches were not only functionally different from those found in PER sites but they could be considered a subgroup of those colonizing the PER sites (Figure 5b). Results of the PERMANOVA and of the test of multivariate dispersion demonstrated that diatom communities inhabiting INT sites were functionally different (PERMANOVA: $F_{1,143}$ = 7.94, P=0.001) and more homogeneous ($F_{1,143}$ = 9.70, P = 0.002) than those in PER reaches.

When analysing the response of diatom functional metrics, we detected significant differences in terms of class size, life-forms, ecological guilds and relative abundance of pioneer species among the two site categories (Table 2; Figure 6). When considering class size, we observed significant higher values in PER than INT sites, dropping from an average value of 2.35 μm^3 in PER sites to 2.16 μm^3 in INT sites. Regarding life-forms, we observed significant higher relative abundances of pad attached diatoms in PER than INT sites, as well as of colonial diatoms, mainly due to the higher abundance of zig-zag and ribbon forming colonies in PER than INT sites. Contrarily, stalked taxa were significantly more abundant in INT than PER sites. Focusing on ecological guilds, we found significant higher abundances of the high profile guild in PER than INT sites, whereas the low profile guild was more abundant in INT than PER sites. Finally, pioneer species were significantly more abundant in INT than PER sites.

4. DISCUSSION

Although hydrological intermittency is part of the natural hydrological cycle in many Mediterranean streams, the indiscriminate use of the water resources coupled with global climate changes has been exacerbating this phenomenon (Datry et al., 2017a). In addition, over the last decades, land use alterations and increased urbanization strongly contributed to the physical disruption of the river habitat (Romano et al., 2017). Since from our analysis the two groups of sites broadly overlapped in terms of water quality (see Figure 2), here we could highlight that the combined effect of the flow intermittency and urbanization negatively affects diatom communities in Mediterranean streams, both in terms of diversity and composition.

Indeed, when considering diatom community diversity, we observed a significant species loss (S) in intermittent reaches, both at local and regional scales, in accordance with other studies (B-Béres et al. 2019; Stubbington et al., 2017). In addition, by partitioning the effective species richness (S_n) and the evenness (S_{PIE}), we could demonstrate that this phenomenon is due to both the reduction of species in the regional pool and by the increase in the relative abundance of few dominant species. This may be due to the strong environmental filter generated by flow intermittency that favours the dominance of few opportunistic taxa to the detriment of less competitive organisms (Richardson &

Sato, 2015; Várbíró et al., 2020). This hypothesis is furtherly corroborated by the lower β-diversity observed in intermittent than permanent reaches for both total richness (*S*) and evenness (S_{PIE}), which suggests that diatom communities experiencing flow intermittency are more homogeneous than those found in perennial streams. In addition, when analysing β-diversity as dissimilarity among communities we could show that diatom assemblages are more similar among each other in intermittent than in permanent sites, due to a lower contribution of both turnover and nestedness components.

Similar results were observed when analysing the community structure, as we observed significant differences between permanent and intermittent reaches from both the taxonomic and functional point of view. In particular, we observed a taxonomic and functional homogenization of diatom communities inhabiting the intermittent reaches, which appeared simpler and less heterogeneous than those characterizing stations with permanent flow, confirming the results obtained by B-Béres et al. (2019). In addition, diatom communities of intermittent reaches represented a subset of those located upstream, similarly to what observed by Larned et al. (2010). Other biological groups, such as macroinvertebrates and fish, showed similar responses as communities inhabiting sites experiencing prolonged fragmentation (e.g. intermittent rivers) often represented a highly nested subsets of communities living in connected habitats (Datry et al., 2014; 2017c; Miyazono and Taylor, 2015). This is in agreement with the *habitat templet theory* (Southwood 1977, 1988; Townsend and Hildrew 1994), which states that strong environmental filters, such as flow intermittency, are expected to differentially affect species from the regional pool by either favouring species that can tolerate drying conditions and/or disfavouring species lacking resistance or resilience mechanisms to the disturbance (Wu et al., 2019). This selection process is thus expected to favour particular traits (Webb et al. 2010; Luck et al. 2012) conferring resistance (i.e. set of traits that enable organisms to survive during non-flow periods) and/or resilience (set of traits that enable organisms to recolonize and recruit after non-flow events).

In this framework, the analysis of functional traits further corroborates this assumption, as we could demonstrate that intermittent sites are characterized by higher percentages of small sized pioneer species, both features reflecting high reproductive rates and conferring great resilience (Lange et al., 2016), confirming previous observations (B-Béres et al., 2014, 2016; Stenger-Kovács et al., 2013). Conversely, permanent sites are inhabited mainly by higher percentage of high profile taxa, characterized by bigger size and pad attached growth forms, reflecting a good environmental stability, confirming the results of Várbíró and colleagues (2020). In our research, permanent reaches favoured also the establishment of colonial species, and in particular those forming zig-zag and ribbon colonies. This was indirectly in accordance with Novais et al. (2020), who observed that most of the species characterizing Portuguese intermittent rivers were solitary. Moreover, our result confirmed the conclusions reached by other researches stating that colonies need a particularly stable environment to settle down and develop (Elias et al., 2014; Várbíró et al., 2020). Although we observed higher abundances of stalked diatoms in intermittent sites, this life form has been usually classified as sensitive to the hydrological variations (Elias et al., 2014, but see Sabater at al., 2017, who defined species belonging to the genera *Cymbella* and *Gomphonema* as particularly resistant to drought). However, it should be noticed that the group of the stalked diatoms is extremely heterogeneous and includes several different genera, such as those with simple (i.e. *Achnanthidium* spp.) or arbuscular (i.e. *Gomphonema* spp. Or *Cymbella* spp.) stalks, characterized by different sensitivity to physical disturbance. For instance, *Achnanthidium* is known to be one of the most resistant genus to physical disturbance, due to its morphological features and the position occupied within the biofilm (i.e. the inner layers) (Passy 2007). On the contrary, the arbuscular diatoms are secondary colonisers, which develop upon the basal layers taking advantage of light but, at the same time, being more exposed to shear stress and hydrological variations (Rimet and Bouchez, 2012). The great heterogeneity of this group likely explains the contrasting results observed in literature when analysing the pattern of stalked diatoms in response of the hydrological variations. Another important driver of stalked diatom pattern is the strong control that grazers exert on this growth form. It is already well known that herbivory is an important factor regulating the biomass and community structure of benthic algae in streams (Stevenson et al., 1996). It is also known that macroinvertebrate scrapers are generally favoured by rheophilous microhabitats with coarse mineral substrates (Piano et al. 2019) and, at the same time, stalked diatoms are more subject to scrapers' pressure than other growth forms (Holomuzuli et al, 2010). We can hypothesize that the adverse environmental conditions found in intermittent reaches disfavoured the presence of macroinvertebrate scrapers (see Piano et al., 2019b for an example in intermittent Alpine streams) and altered their grazing efficiency, indirectly favouring the development of stalked diatoms. Therefore, although the trait-based approach, which reflects both dispersal capability and environmental adaptability of the species, has been often invoked as a suitable and reliable tool for the hydrological disturbance assessment (B-Béres et al., 2019; Wu et al., 2019; Novais et al., 2020; Várbíró et al., 2020), the response of stalked diatoms to flow intermittency should be interpreted with caution in future work.

When comparing the response of diatom communities between Mediterranean and Alpine intermittent streams, results are surprisingly different. In particular, in a previous study (Piano et al., 2019a) we observed that diatoms inhabiting recently intermittent reaches were taxonomically and functionally comparable to the permanent sections located upstream. Therefore, our results seem to contradict the *natural flow regime paradigm*, which states that the structure and function of a lotic ecosystem, and the adaptation of its constituent aquatic species, are determined by the pattern of temporal variation in river flows (Poff et al. 1997; Lytle and Poff 2004). This apparent contradictory result could be explained in light of three main factors: i) the intensity of the flow intermittency; ii) the diatom community composition; and iii) the combined effect of the land use.

From one hand, based on our experience (Falasco et al., 2016a,b; 2018a), water retreat is faster in the Mediterranean area than in the Alpine regions, due to the higher air temperatures characterizing the Mediterranean summers and the consequent higher evaporation rates. A slow and gradual water retreat allows biological communities to activate resistance mechanisms to face the drought, such as producing resting forms (Souffreau et al., 2013) or simply moving toward more suitable conditions (Falasco et al., 2016a; 2018a). In addition, a higher amount of humidity can be retained by the biofilm, and organisms colonizing the lowest layers can survive (Sabater et al. 2016). Moreover, a slow water retreat allows the creation of refugia (i.e. residual pools or hyporheic zone) where individuals can survive during harsh conditions (Falasco et al., 2016b) and favouring the recolonization processes after water returns. Conversely, the fast water retreat occurring in Mediterranean streams hampers the activation of resistance and resilience mechanisms. In addition, drought lasts longer and the extension of dry reaches is higher in the Mediterranean than in the Alpine areas.

On the other hand, resilience mechanisms are likely influenced by both species composition during the pre-drought event and the composition characterizing assemblages in the upstream sections which serve as source during the recolonization process (Falasco et al., 2018b). When comparing the composition of diatom assemblages in Alpine and Mediterranean rivers, we observed how low profile taxa dominates in the fast-flowing Alpine streams (around 75% in the permanent sections sampled in Piano et al., 2019), while this same guild represents on average only 48% of the communities in the permanent sites of the Mediterranean area. As it is already well known that species belonging to the low profile guild possess a higher resilience than the others, we can suggest that communities dominated by low profile species can easily recover compared to more heterogeneous other communities.

Third, it should also be noted that in the present study intermittent reaches are also highly urbanized compared to those examined in the Alpine area. In fact, most of the intermittent reaches included in this research were located in the core of urban contexts, particularly overexploited by tourists during summer, and they showed widespread habitat modifications, with artificial banks and streambeds, a significant presence of weirds, no canopy coverage nor riparian vegetation. All these features led to a further increase of river habitat fragmentation and connectivity loss in the intermittent sections of the Mediterranean area, which likely exacerbate the effects of flow intermittency on diatom communities.

5. CONCLUSIONS

By analysing multiple aspects of benthic diatom communities in permanent and intermittent reaches in Mediterranean streams, here we demonstrated that the combined effects of the ongoing climate change and local environmental pressures have been significantly altering diatom biodiversity in the last 11 years. The results we obtained could be ascribed to the long-term effects of drying in Mediterranean streams and, as first in the literature, we highlight that diatoms are able to provide long-terms responses to environmental changes caused by water stress, when disturbance is persistent. Indeed, diatoms are usually considered useful indicators to short-time stresses (i.e. a couple of weeks) probably due to their short life cycles (Karaouzas et al., 2018). Although Mediterranean benthic communities are naturally exposed to intermittency and showed a certain degree of adaptation to drying, our results highlighted that these communities should be still considered fragile and threatened, especially in a global climate change scenario exacerbated by local anthropogenic disturbance (Smeti et al., 2019). In addition, when considering the trophic food web, the alteration of diatom communities in intermittent reaches could affect the river autotrophic processes, leading to a decrease of primary production and diatom chlorophyll a (Piano et al., 2017b). Hydrological intermittency could promote river heterotrophy and alter the fatty acid composition of the biofilm thus reducing grazing efficiency of the macroinvertebrate scrapers, hence their ecological niche (Piano et al., 2019). In this framework, it is mandatory to deepen our knowledge on the potential effects of droughts on river biological communities and, at the same time, to improve and adapt biological indices to assess the water quality of intermittent rivers.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

Data sharing and data accessibility

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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CAPTIONS

Table 1 – Average values (± SD) of water quality, land use and climatic data in PER and INT sites and results of the two-sample t-tests. Significant results are reported in bold.

Table 2 - Average values (± SD) of the β-diversity components and functional traits in PER and INT sites and results of the two-sample t-tests. Significant results are reported in bold.

Figure 1- Map of the sampling sites located in Liguria (NW-Italy). PER = orange circles; INT = light blue squares.

Figure 2 - results of the PCA performed on: (a.) water quality parameters; (b.) land use coverage measured at each sampling site; and (c.) climatic variables —annual and summer average precipitation amount, minimum and maximum air temperatures. Points represent each single sample while arrows represent the loadings of each environmental variable included in the analysis. Ellipses represent standard deviations around the centroids of the two groups (PER = orange circles; INT = light blue squares).

Figure 3 - Partitioning of species richness (S, first row), rarefied species richness (S_n, second row) and evenness (S_{PIE}, third row) into local diversity (α-diversity, left panel), variation among local communities (β-diversity, central panel) and total diversity at the regional scale (γ-diversity, right panel). P-values above each graph depict the significance of differences between PER (orange) and INT (light blue) reaches.

Figure 4 - Partitioning of total β-diversity into its nestedness (dark grey) and turnover (light grey) components in PER and INT reaches.

Figure 5 - Ordination of the taxonomic (left panel) and functional (right panel) dissimilarity matrices according to the first two PCoA axes (Orange circles = PER sites; light blue squares = INT sites). Ellipses represent standard deviations around the centroids of the two groups.

Figure 6 - Boxplots representing differences in diatom functional traits between PER (orange) and INT (light blue) sites.

Table 1

	Variables	*PER*	*INT*	Two-sample t-test
Water quality data	**N-NH$_3$ (mg L^{-1})**	0.050 (±0.078)	0.037 (±0.054)	*t* = 1.05; *P* = 0.295
	N-NO$_3$ (mg L^{-1})	0.460 (±0.502)	0.737 (±0.384)	***t* = -3.81; *P* < 0.001**
	Ptot (mg L^{-1})	0.074 (±0.092)	0.042 (±0.043)	***t* = 2.68; *P* = 0.008**
	Cond (µS cm^{-1})	322 (±119)	345 (±107)	*t* = -1.24; *P* = 0.215
	DO (%)	102 (±12.0)	105 (±12.1)	*t* = -1.43; *P* = 0.154
	pH	8.26 (±0.310)	8.27 (±0.396)	***t* = -2.55; *P* = 0.012**
	Temp (°C)	15.4 (±3.16)	17.1 (±4.48)	*t* = -0.145; *P* = 0.885
Surrounding land use (%)	**Urban areas**	21.2 (±18.0)	48.3 (±25.3)	***t* = -7.41; *P* < 0.001**
	Agricultural areas	27.1 (±15.3)	23.7 (±22.8)	*t* = 1.047; *P* = 0.287
	Woods	35.4 (±25.3)	11.8 (±14.4)	***t* = 6.89; *P* < 0.001**
	Open areas	4.81 (±7.91)	3.18 (±5.87)	*t* = 1.42; *P* = 0.159
	Water bodies	11.5 (±7.08)	13.0 (±7.69)	*t* = -1.25; *P* = 0.212
Climatic variables	**year_prec (mm)**	78.8 (±24.5)	71.0 (±13.4)	***t* = 2.39; *P* = 0.019**
	year_tmax (°C)	17.7 (±1.27)	19.4 (±0.55)	***t* = -10.2; *P* < 0.001**
	year_tmin (°C)	9.93 (±1.99)	12.6 (±1.34)	***t* = -9.66; *P* < 0.001**
	summer_prec (mm)	41.7 (±15.4)	33.0 (±9.58)	***t* = 4.11; *P* < 0.001**
	summer_tmax (°C)	26.8 (±1.10)	27.7 (±0.46)	***t* = -5.69; *P* < 0.001**
	summer_tmin (°C)	17.7 (±2.08)	20.0 (±1.29)	***t* = -8.19; *P* < 0.001**

Table 2

Component	PER	INT
β_{tot}	0.77 (± 0.15)	0.66 (± 0.18)
β_{repl}	0.70 (± 0.17)	0.62 (± 0.19)
β_{rich}	0.07 (± 0.08)	0.04 (± 0.06)

	Trait	PER	INT
	Size class	2.35 (± 0.56)	2.16 (± 0.59)
	Pioneer	0.19 (± 0.17)	0.36 (± 0.22)
GROWTH FORMS	Adnate	0.09 (± 0.11)	0.12 (± 0.17)
	Pad	0.17 (± 0.18)	0.08 (± 0.10)
	Stalk	0.41 (± 0.27)	0.50 (± 0.23)
	Colonial	0.20 (± 0.17)	0.12 (± 0.11)
	Mucous	0.04 (± 0.07)	0.04 (± 0.06)
	Filament	0.004 (± 0.01)	0.002 (± 0.01)
	Zig-zag	0.06 (± 0.12)	0.03 (± 0.06)
	Rosette	0.006 (± 0.013)	0.006 (± 0.012)
	Ribon	0.08 (± 0.14)	0.03 (± 0.07)
	Arbuscular	0.006 (± 0.008)	0.008 (± 0.015)
ECOLOGICAL GUILDS	High profile	0.22 (± 0.18)	0.15 (± 0.14)
	Low profile	0.47 (± 0.28)	0.57 (±0.24)
	Motile	0.28 (± 0.25)	0.28 (± 0.23)
	Planktonic	0.02 (± 0.07)	0.01 (± 0.01)

Figure 1

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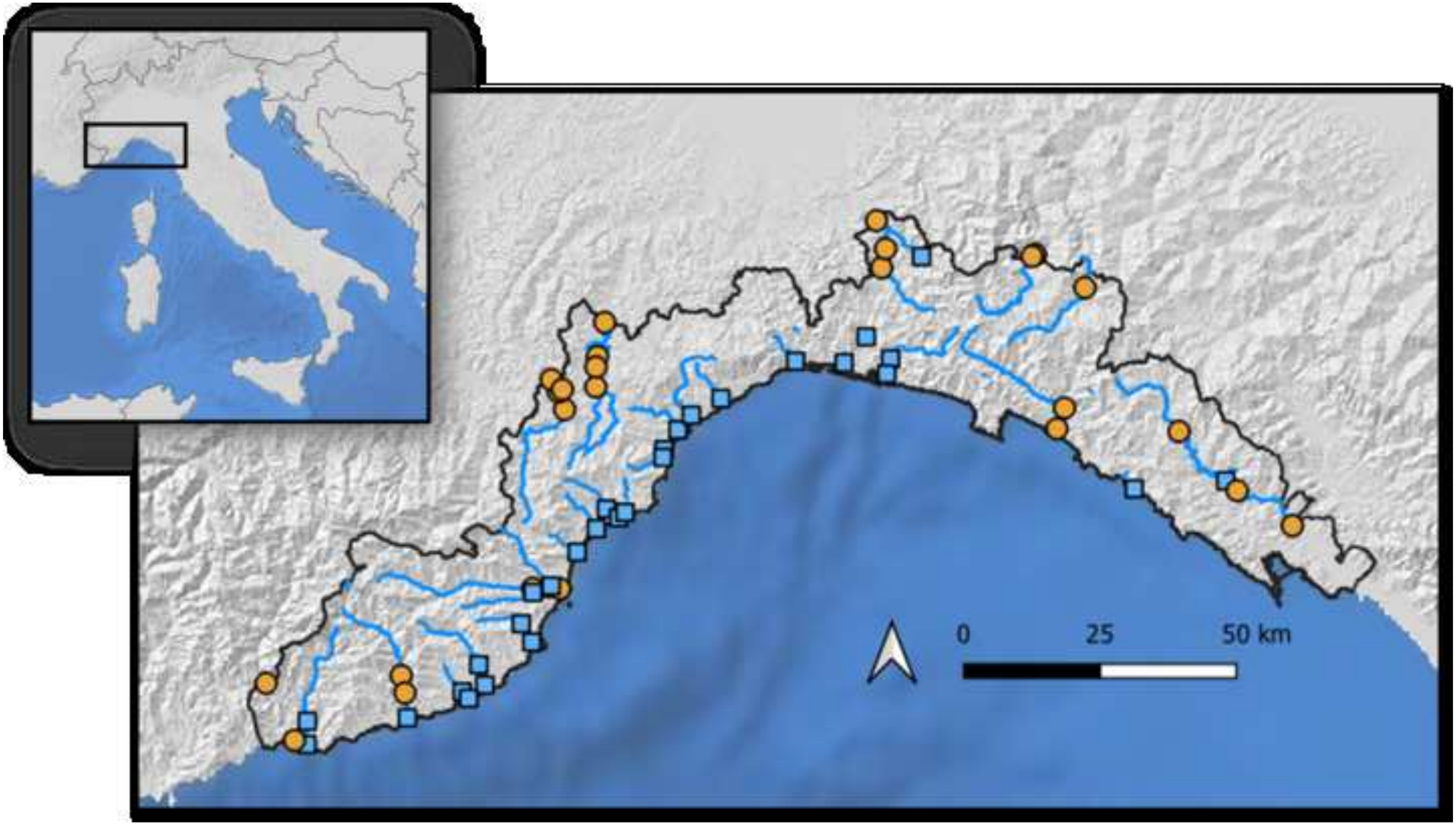

Figure 2

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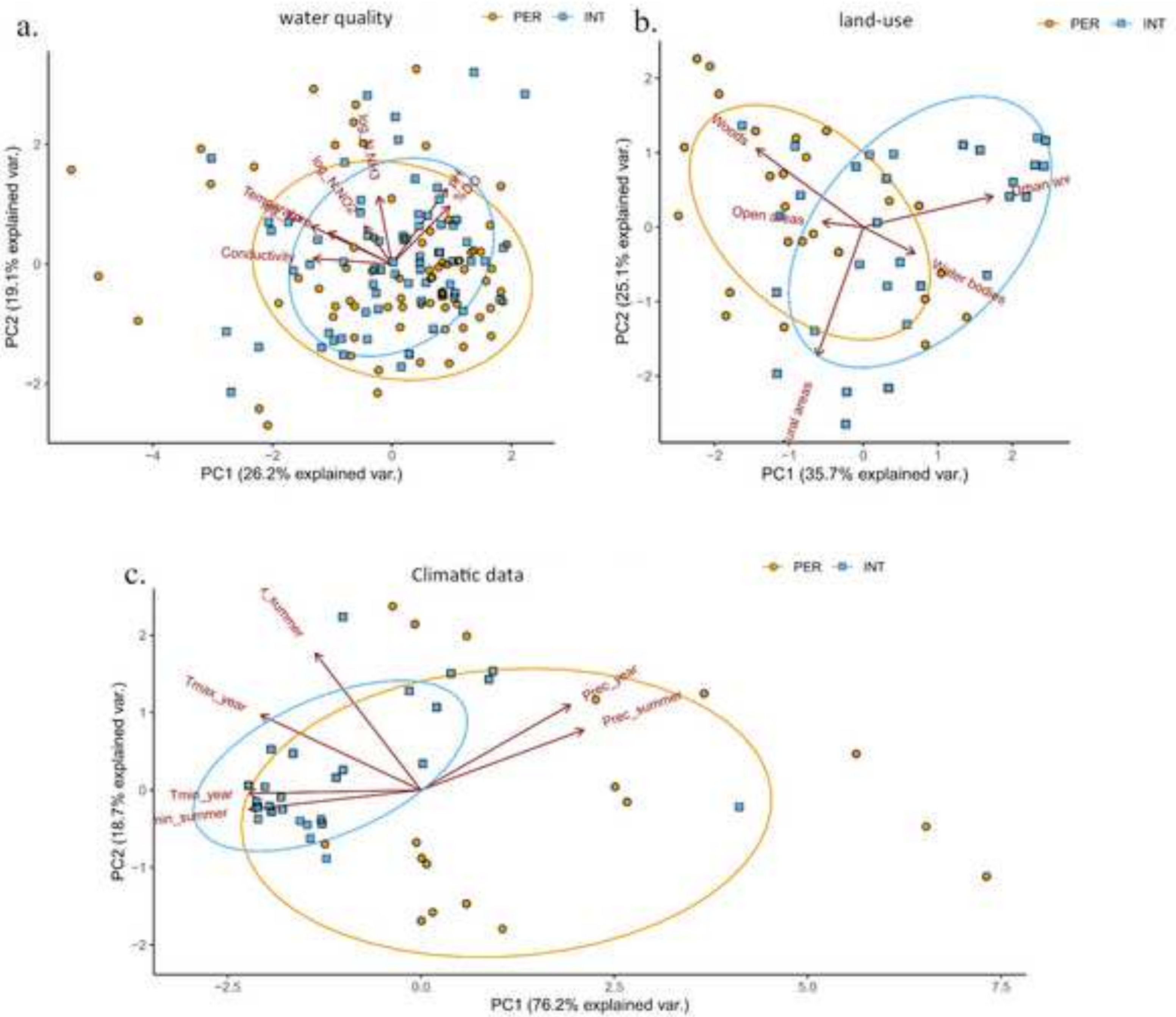

Figure 3

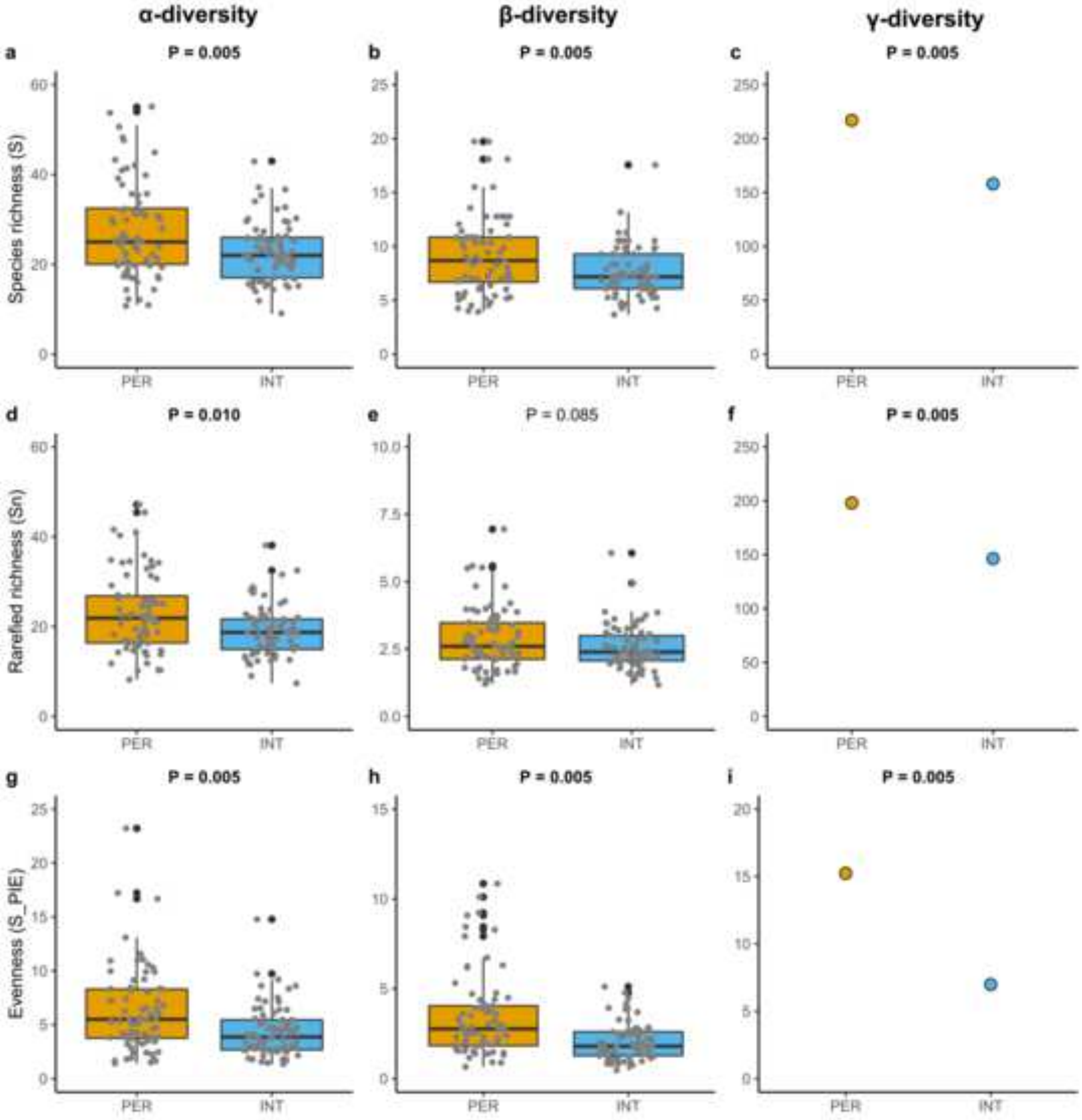
α-diversity
β-diversity
γ-diversity
a
P = 0.005
b
P = 0.005
c
P = 0.005
Species richness (S)
0
20
40
60
0
5
10
15
20
25
0
50
100
150
200
250
PER
INT
PER
INT
PER
INT
d
P = 0.010
e
P = 0.085
f
P = 0.005
Rarefied richness (Sn)
0
20
40
60
0.0
2.5
5.0
7.5
10.0
0
50
100
150
200
250
PER
INT
PER
INT
PER
INT
g
P = 0.005
h
P = 0.005
i
P = 0.005
Evenness (S_PIE)
0
5
10
15
20
25
0
5
10
15
0
5
10
15
20
PER
INT
PER
INT
PER
INT

Figure 4

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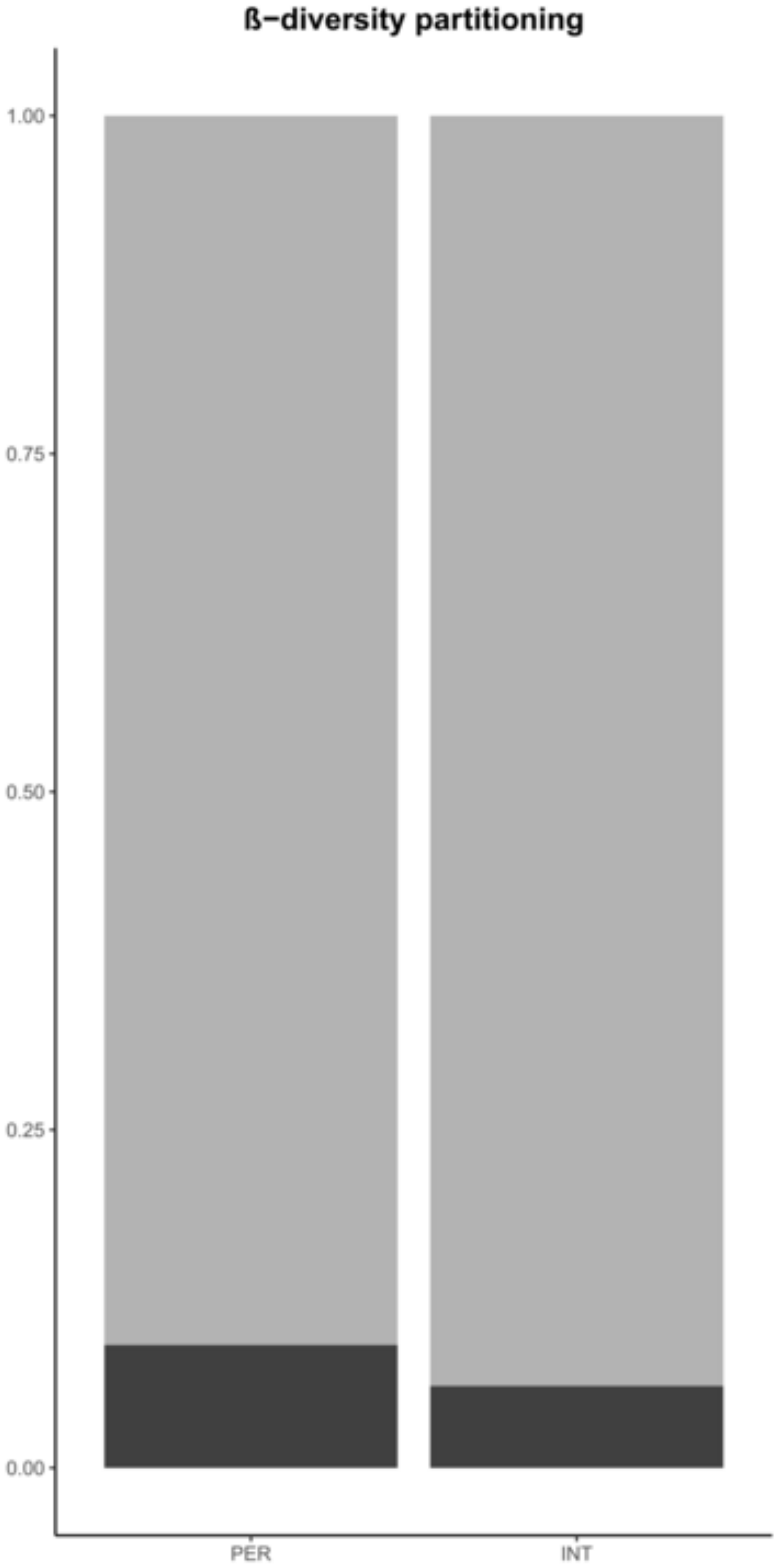

Figure 5

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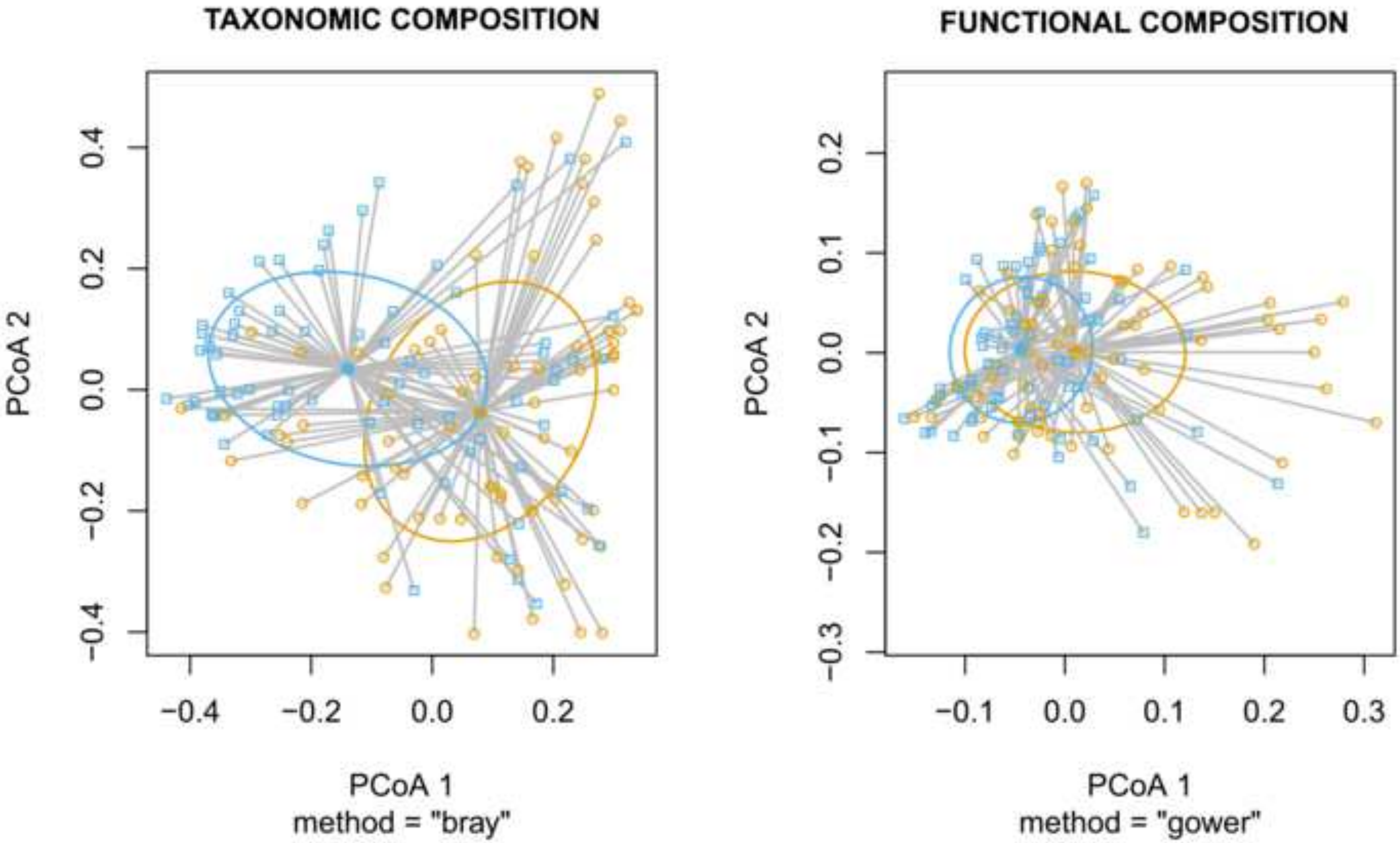
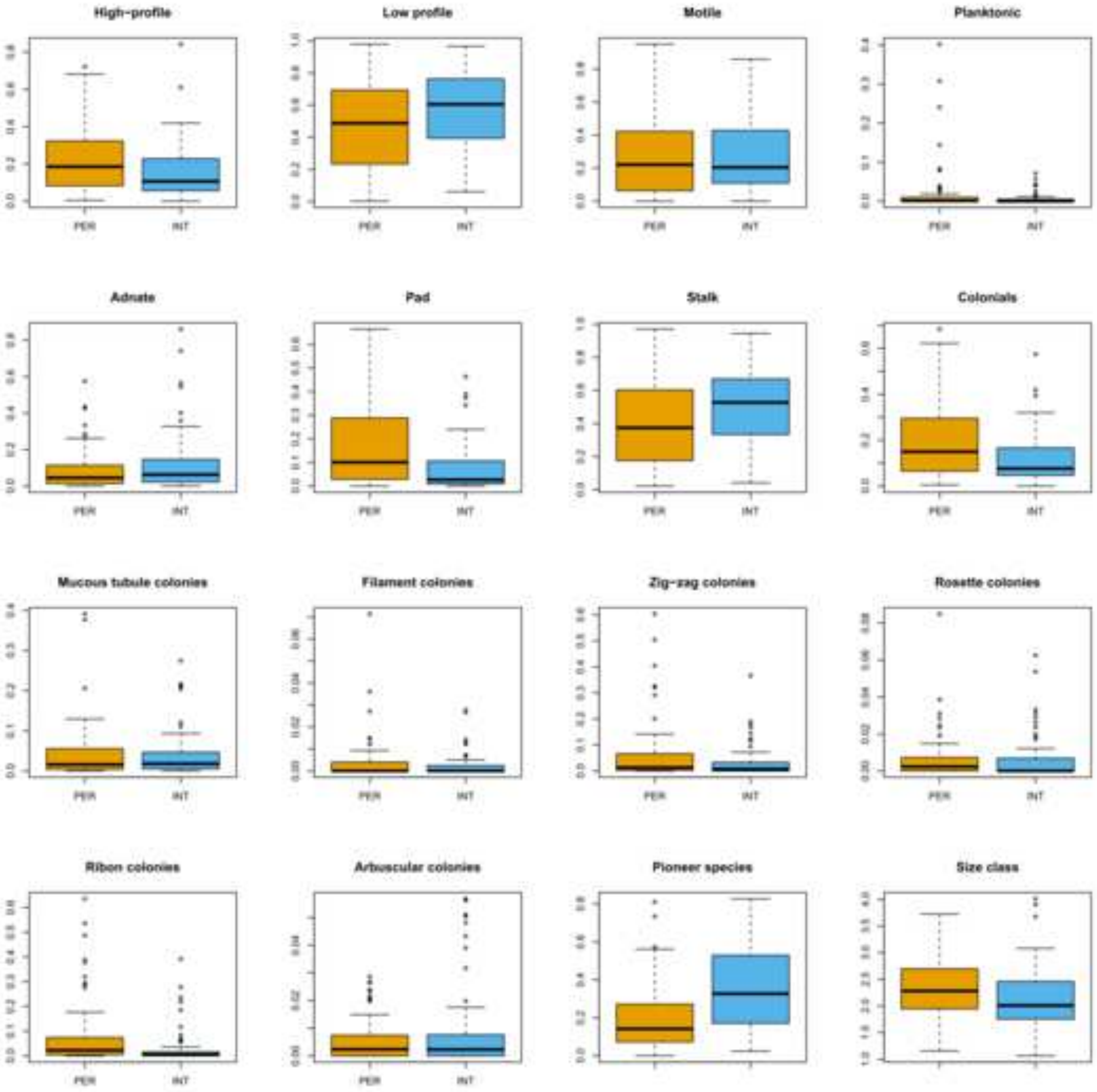


Figure 6


High-profile
Low profile
Motile
Planktonic
Adnate
Pad
Stalk
Colonials
Mucous tubule colonies
Filament colonies
Zig-zag colonies
Rosette colonies
Ribon colonies
Arbuscular colonies
Pioneer species
Size class
PER
INT

SM1

SM2

SM3

***conflict of Interest Statement**

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: