Environmental and spatial factors drive diatom species distribution in Alpine streams: implications for biomonitoring

Elisa Falasco[1], Francesca Bona[1], Catia Monauni[2], Agnese Zeni[2], Elena Piano[1]*

[1] DBIOS, University of Turin, Via Accademia Albertina 13, I-10123, Turin, Italy

[2]APPA – Settore tecnico per la tutela dell'ambiente – U.O. Acqua, Via Lidorno 1, I-38123, Trento, Italy

*Corresponding author: elena.piano@unito.it

ABSTRACT

Given their documented capacity to track changes in environmental conditions and human alterations, benthic diatom communities are at present widely used in biomonitoring programs to evaluate stream water quality. However, it is becoming more and more evident that species ecological preferences are not the only drivers of diatom community composition, since dispersal-related processes also play a role. This is particularly compelling in Alpine streams, where orographic conformation and human-related impacts limit dispersal of organisms. In addition, several environmental variables may influence diatom community in pristine or impacted sites. We here investigate the differential role of environmental and spatial factors in driving the community assemblages of diatoms in streams of the Eastern Italian Alps, focusing on both taxonomic and functional composition. We analysed data from 110 samples collected on two different geological substrates, i.e. calcareous and siliceous, during the last eight years of biomonitoring programs, among which 64 collected in reference sites and 46 in impacted sites. We first evaluated whether diatom communities in reference and impacted sites are differentially shaped by environmental and spatial factors, highlighting the major role of spatial constraints in both of them. In particular, anthropogenic disruption of longitudinal connectivity in streams likely shaped impacted communities, as demonstrated by the increasing abundance of motile taxa, which are associated with physical disturbance. Conversely, reference communities were mostly affected by spatially structured environmental variables, especially those related to streambed lithology. We then compared the taxonomic and functional composition of diatom communities between the two geological substrates in both reference and impacted sites to better highlight the differential role of this factor. Our results demonstrate that lithology strongly drives diatom community composition in reference but not in impacted sites, confirming our previous observations. The analysis of functional traits, however, highlighted how differences were due not only to the geological substrates, but also to other environmental variables, like flow velocity. Overall, the effect of the spatial component on the structure of diatom assemblages can represent a background noise in the framework of the river quality assessment, and this should be taken into account especially in those countries, like Italy, covering a broad range of mountain areas.

Key-words: reference sites, ecological guilds, functional groups, dispersal limitation, variation partitioning

Highlights

- Diatoms track changes in environmental conditions and human alterations
- Dispersal is expected to affect diatom distribution in Alpine streams
- Substrate lithology affects diatom community in reference sites
- Chemical variables and spatial processes affect diatom community in impacted sites
- Biomonitoring programs should account for the combined effects of spatial and environmental processes

1. INTRODUCTION

Given their documented capacity to track changes in environmental conditions and human alterations, diatoms have been gradually included in all the European monitoring programs as biological indicators for the quality assessment of lotic freshwater ecosystems, after the publication of the Waterframe Directive (2000/60/CE). Diatom responses to environmental variables acting at multiple scales of analysis have been widely documented in literature (Rimet et al., 2004; 2007; 2009; Tison et al., 2005; Grenier et al., 2006; Bona et al., 2007; Soininen, 2007). For instance, Soininen (2007) identified flow velocity and substratum composition as key determinants of diatom composition at local scale, whereas ion concentrations and trophic conditions mainly drive changes in river diatom communities at a regional scale (Bona et al., 2007; Soininen, 2007). However, it has been recently proved that species ecological preferences alone are not sufficient to explain diatom community composition, since dispersal processes also play an important role in shaping communities (Heino, 2013).

Owing to their physical, topographic and climatic heterogeneity, Alpine streams are extremely heterogeneous, encompassing large variation in environmental variables, like flow velocity, temperature and ion concentrations, over short distances (Ward, 1994). This environmental heterogeneity likely exerts a selective pressure on organisms inhabiting Alpine lotic freshwater ecosystems, which track their optimal environmental conditions according to their ecological niche. In Alpine streams, where ion and nutrient concentrations are usually very low, geology and, as a consequence, conductivity and hardness resulted among the most important factors in shaping diatom communities (Rimet et al., 2004; 2007; 2009; Tison et al., 2005; Grenier et al., 2006), but also altitude, dissolved oxygen, nitrate, calcium, turbidity and pH are among the main environmental factors shaping diatom assemblages in such oligotrophic ecosystems (see Falasco et al., 2012 for an example in Western Alps and Cantonati, 1998; Cantonati et al., 2001; 2006; 2007; 2009; Beltrami et al. 2012 for examples in the Eastern Alps). However, recent works have highlighted that the expected responses of diatoms to environmental parameters are not completely fulfilled, especially in mountain streams, due to the effect of spatial processes (Bottin et al., 2014; Dong et al., 2016).

Although composition of diatom communities is clearly shaped by environmental variables and habitat heterogeneity at local scale (i.e. species sorting), the role of spatial factors should be kept into account at regional/global scale (Soininen, 2007; Verleyen et al., 2009). The importance of diatom dispersal-related processes in the framework of the biological monitoring has been recently proved in lacustrine ecosystems (Vilmi et al., 2016). Although lake communities are more spatially-structured than stream ones, due to the higher dispersal limitation characterizing the lentic species (Soininen, 2007; Marquardt et al., 2017), Bottin et al. (2014) remarked the importance of the spatial component also in mountain streams, where steep slopes surrounding streams might limit organism dispersion (Dong et al., 2016). In addition, also human alterations such as stream channelization, flow regulations and the construction of reservoirs, interrupt habitat connectivity and limit dispersal of organisms. For instance, water scarcity, caused by both local impacts and global changes, has recently become the most critical threat for diatom lotic assemblages in mountain streams, altering the taxonomical and functional composition of benthic communities ((Bona et al., 2008; Falasco et al., 2018; Piano et al., 2019). Thus, anthropogenic impacts in Alpine streams are expected to alter not only chemical variables but also the spatial processes underlying the composition of diatom communities, overriding the role of natural environmental filters or physical barriers (such as lithological substrates and orographic barriers). A better understanding of the role of environmental and dispersal-related processes in shaping diatom communities is then compelling to enhance the implementation of diatom-based biomonitoring programs in Alpine streams.

One of the main outcomes of anthropogenic disturbance on the composition of biotic communities is biotic homogenization, which is defined as an increase in the similarity of species composition (Olden et al., 2004). Environmental filters and dispersal barriers caused by the human activities likely cause the loss of rare and specialised species, and the gain of widespread tolerant ones, with consequent biotic homogenization. In addition, according to the "habitat templet theory" (Southwood, 1977, 1988; Townsend and Hildrew, 1994), the above mentioned selection processes may have major effects on particular functional traits, like morphological or physiological attributes, or they could affect the realized niche of a certain species (Webb et al., 2010), causing also functional homogenization (Olden et al., 2004). Thus, the investigation of these processes may provide information on the effects of anthropogenic pressures on biotic communities.

According to Leibold and Chase (2017), the relationship between taxonomic and functional homogenization allows us to infer which processes are acting: while taxonomic homogenization underlies dispersal limitation, functional homogenization is a signal of environmental filtering. In particular, anthropogenic environmental filters and spatial barriers are expected to differentially affect species within the original communities based on their functional traits. Therefore, exploring shifts in the functional profile of a community could shed light into mechanisms underlying community assemblages (Leibold and Chase, 2017).

We here analysed the community composition of diatoms in streams of the Eastern Italian Alps (Trento province), by collating a dataset of data collected during eight years of biomonitoring programs (2008-2016). In particular, we aimed at: i) investigating the differential role of environmental variables and spatial factors in driving the community composition in pristine and impacted sites within Alpine streams; and ii) unravelling whether taxonomic and/or functional homogenization occur in pristine and impacted sites and whether the lithological substrate may drive these processes. We hypothesized that: i) diatom composition in pristine sites would be mainly shaped by spatial factors, because the variation in chemical parameters within these oligotrophic ecosystems is expected to be low; conversely, chemical parameters would have a determinant role in impacted sites; ii) functional homogenization would occur in impacted sites as a consequence of environmental filters; and iii) lithological substrate would cause taxonomic differentiation in pristine sites, where species can track their ecological preferences without constraints of chemical variables (Rimet et al., 2004; 2007; 2009; Tison et al., 2005; Grenier et al., 2006), but not in impacted sites, where chemical parameters are the main drivers of diatom community composition.

MATERIALS AND METHODS

1.1. Study area

The Trento province is located in the North-East of Italy and covers an area of nearly 6000 km^2. Within this area, two hydroecoregions (hereafter HERs), i.e. homogeneous areas in terms of geology, altitude and climate (Wasson et al., 2002), were defined consistently with WFD requirements. Both HERs are Alpine and, within each HER, reference sites with potentially homogeneous, pristine environmental conditions and unaltered diatom communities were identified (Rimet et al., 2004). The first HER (hereafter calcareous HER), is the Southern Pre-Alps and Dolomites hydroecoregion, characterized by mountain streams flowing on calcareous substrate (massive and carbonated rocks). The second HER (hereafter siliceous HER) is the Inner Alps hydroecoregion, with high mountains and streams flowing on siliceous substrates (crystalline rocks). Diatom biomonitoring in streams has been carried out by the Environment Protection Agency of Trento province (APPA TN) since 2004. The monitoring network, including 166 sites in the entire Trento province, was designed to provide a coherent and comprehensive overview of ecological and chemical status within each river. In accordance with the monitoring program, every site is sampled twice in a year every six years (every three years for reference sites), considering biological, hydromorphological and physico-chemical quality elements, and pollutants discharged into the river basin or sub-basin.

In this study area, we analysed diatom communities collected from both HERs, in 17 reference and 16 impacted sites (Table 1), during the last eight years of biomonitoring programs (2008-2016). 11 reference sites were selected following the official national criteria (DM 56, 14/04/2009), i.e. sites characterized by null or slight human impacts, unaltered hydrological conditions, intact three-dimensional river connections, null or slight hydromorphological modifications, high water quality, and biological communities showing taxonomical composition and densities corresponding to the unaltered conditions. Beside these official stations, 6 further sites with minor hydromorphological alterations were considered similar to reference sites because of their high water quality and were added to reference category for this study. Impacted sites are located in water bodies characterized by agricultural and urban pressures and they have high nutrient concentration and pesticide contamination (Fig. 1).

1.2. Physical and chemical analyses

Water samples for the physical and chemical analyses were collected along with diatom sampling. In both reference and impacted sites we analysed the following 15 parameters using standard methods (see Table 2): Biological Oxygen Demand (BOD_5), carbonate hardness (HARD), Chemical Oxygen Demand (COD), Chlorides (Cl^-), conductivity (COND), dissolved oxygen (DO), nitrates ($N\text{-}NO_3$), orthophosphates ($P\text{-}PO_4$), sulphates (SO_4), temperature (TEMP), pH, total nitrogen (N_{tot}), total phosphorous (P_{tot}), total suspended sediments (TSS), turbidity (TURB).

1.3. Diatom collection and treatment

In total, we collected 64 diatom samples from 17 reference sites and 46 diatom samples from 16 impacted sites (see Tab. 1 for details).

Samplings were performed between 2008 and 2016. Following the WFD requirements, each site belonging to surveillance monitoring was sampled at least twice during the 8 years monitoring program (mainly in spring and autumn). Some sites (Table A1), belonging to core network monitoring, were sampled in three different years (2 samples per year X 3 years = 6 samples). Epilithic diatoms were collected following the standard procedure (European Committee for Standardization, 2003). In each site, we chose 5 cobbles from the main flow and we collected periphyton by scraping their upper surface by means of a toothbrush. Samples were than preserved in ethanol and moved in laboratory for the treatment with H_2O_2 (30%) and HCl (European Committee for Standardization, 2003). Slides for the observation at the light microscope 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, 1986-1991 a, b; Lange-Bertalot and Metzeltin, 1996; Krammer, 1997 a, 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). We identified at least 400 valves in each sample. Diatom communities were analysed in terms of biodiversity, taxonomical composition, ecological guilds and growth forms (Rimet and Bouchez, 2012).

1.4. Statistical analyses

All statistical analyses were performed with the software R 3.4.2 (R Development Core Team, 2017).

1.4.1. Characterization of environmental features and diatom community

We performed the Mann-Whitney U-test to check for differences in environmental variables between references and impacted sites on the whole dataset and between the two HERs within the reference and impacted datasets. To highlight statistically significant species, representative of the two hydroecoregions for both reference and impacted sites, we performed an Indicator Species Analysis (Dufrêne and Legendre, 1997) with the function “multipatt” in the package *indicspecies* (Caceres and Legendre, 2009).

1.4.2. Environmental parameters vs spatial processes

To test whether diatom communities in pristine sites are more shaped by spatial factors than in disturbed sites (Hypothesis 1), we investigated the role of environmental vs spatial parameters in both reference and impacted sites. To achieve this aim, we performed a partial-RDA, following the approach suggested in Peres-Neto et al. (2006) and De Bie et al. (2012). Using redundancy analysis (RDA), we built up two explanatory matrices: a chemical matrix [C], including all chemical parameters, and a spatial matrix [S] with both the coordinates of the sampling sites and the spatial variables extracted by Moran’s Eigenvector Maps analysis (MEM, see Dray et al., 2006). The MEM analysis partition the spatial information into variables representing the potential autocorrelation between spatial points at different scales. With this procedure we generated a set of orthogonal, and thus non-collinear, spatial variables that are derived from geographical coordinates of the study sites, each of which corresponds to a specific spatial structure and scale. These variables can model coarse patterns in the community data and then progressively represent finer-scale patterns (Borcard et al., 2004). Both chemical and spatial variables were selected by means of a forward selection performed with the R package *packfor* (Dray et al., 2013) to obtain a parsimonious combination of variables, i.e. including only variables with a significant relationship with the community matrix. We separately tested the [C] and [S] matrices against the taxonomic matrix and we decomposed total community variation into pure components and their intersections. Significance was tested by means of a Monte Carlo test with 999 permutations.

1.4.3. Taxonomic vs functional composition

To test whether anthropogenic pressure causes biotic homogenization in the taxonomic and/or the functional structure of diatom communities (Hypothesis 2), we analysed the taxonomic and functional response of diatom communities. First, we evaluated whether reference and impacted sites differed in their composition of diatom communities. Changes in taxonomic and functional composition (homogenization or differentiation) among the reference and impacted sites were analysed using the test of homogeneity for multivariate dispersion (Anderson et al., 2006) following the procedure proposed by Brice et al. (2017). This test represents a method to evaluate the homogenization within communities belonging to the same category and to compare these values among categories, e.g. among reference and impacted sites or among the calcareous and siliceous HERs. The test of homogeneity for multivariate dispersion was performed with the function “betadisper” in the package *vegan* (Oksanen et al., 2018). 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, to which the test was applied. 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 9,999 permutations to determine whether the dispersions between the two groups were different. We then repeated the test of homogeneity for multivariate dispersion on a 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* (Laliberté 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 life-forms, ecological guilds, size classes and biovolume (Rimet and Bouchez, 2012). We also tested shifts in taxonomic and functional composition between reference and impacted sites with a PERMANOVA (Anderson, 2001) applied on distance matrices, using the function "adonis" in the package *vegan* (Oksanen et al., 2017). PERMANOVA is a non parametric multivariate analysis of variance that measures location differences in centroids of the different categories. Thus, we applied this test for investigating possible taxonomic and/or functional turnover among categories. Statistical significance was tested via 9999 random permutations.

The same procedure was finally adopted to detect differences between the two HERs, which are proxies of lithological substrates, to test whether geology causes differentiation in diatom composition in pristine but not in impacted sites (Hypothesis 3).

1.4.4. Analysis of functional traits

The set of functional metrics extrapolated from the functional matrix were subjected to a non-parametric Mann-Withtney U-test to test for differences between reference and impacted sites and between the calcareous and siliceous HERs within both reference and impacted sites.

2. RESULTS

2.1. Physical and chemical analyses

Observed values of physical and chemical parameters of reference sites showed low nutrient contents (Tab. 2) thus classifying them as oligotrophic in accordance with the Italian Water Legislation (D. Leg. 152/2006 and successive ones). BOD_5 concentrations never exceeded 2.5mg/L, being included in the range of the first water quality class of the national classification, like COD that on average never exceeded 3mg/L. DO was generally high, and pH ranged from 6.70 to 8.50, according to the riverbed lithology. Impacted sites generally showed lower quality, since, on average, $N\text{-}NO_3$ values were included in the range of the third water quality class (D. Leg. 152/2006 and successive ones), with mean values of 1.90 to 2.02 mg/L on calcareous and siliceous substrates respectively. Despite slightly higher than in reference sites, BOD_5 and COD levels corresponded to a moderate organic level (BOD_5 highest value = 5.1 mg/L; COD highest value = 13.8 mg/L) so as P_{tot} whose maximum value reached 0.17 mg/L.

Reference and impacted sites showed significant differences in terms of all chemical parameters, except for temperature and TSS and all variables were higher in impacted than reference sites (Tab. 2; Fig. B1). Among reference sites, results of the Mann-Whitney U-test showed that $N\text{-}NO_3$, N_{tot}, $P\text{-}PO_4$, conductivity, hardness and pH were significantly higher in the calcareous than siliceous hydroecoregion, while opposite results were observed for BOD_5 and turbidity (Tab. 2; Fig. B2). Among impacted sites, we observed no significant differences between the two geological substrates (Tab. 2; Fig. B3).

2.2. Diatom community composition

In total, we analysed 64 diatom samples from reference sites and identified 120 taxa. In these samples, the most abundant and frequent species was *Achnanthidium minutissimum,* which was detected in all samples and represented the most abundant and frequent species, with a relative abundance on average of 35% per sample. *Achnanthidium pyrenaicum* (about 16% mean relative abundance), *Achnanthidium lineare* (9%). *Gomphonema pumilum* (8%), *Fragilaria arcus* (6%), *Encyonema minutum* (2%), *Fragilaria vaucheriae* (<2%), *Cocconeis lineata* (<2%), *Diatoma mesodon* (<2%)

were the most abundant taxa detected in at least 50% of the reference sites. In impacted sites, we analysed 46 diatom samples and identified 128 taxa. Again, *A. minutissimum* was the most abundant (mean relative abundance 12%) and frequent (detected in 98% of the samples) species. Beside *A. minutissimum*, 16 species were observed in at least 50% of the samples; among them, *Fistulifera saprophila* (about 11% of mean relative abundance), *Cocconeis euglypta* (6%), *Amphora pediculus* (6%), *Mayamaea permitis* (5%), *Nitzschia fonticola* (5%), *A. pyrenaicum* (3%), *Nitzschia inconspicua* (3%), *E. minutum* (3%), *Navicula tripunctata* (3%) and *Reimeria sinuata* (2%) were those exceeding 2% of relative abundance. According to the Indicator Species Analysis, several species are exclusive of the two HERs in pristine sites, with 10 species representative of the calcareous HER and 8 species representative of the siliceous HER (Tab. 3). Conversely, only few species are exclusive of the two HERs in impacted sites, with 2 species representative of the calcareous substrates and 4 species representative of the siliceous substrates (Tab. 3). *Fragilaria vaucheriae* is shared between reference and impacted sites as an indicator species for the siliceous HER, whereas *Gomponema olivaceum* is representative of both siliceous reference sites and calcareous impacted sites.

2.3. Environmental vs spatial components

We fist analysed whether diatom communities in pristine sites are more shaped by spatial factors than in disturbed sites (Hypothesis 1). The partial RDA performed on samples collected in reference sites (Fig. 2a) pointed out the minor role of chemical parameters, which together just explained 4% of the total explained variation. Only four chemical parameters (SO_4, pH, N-NO_3 and DO) were included in the final environmental matrix after forward selection. On the other hand, the spatial matrix, which included seven PCNM vectors referring to both coarse and fine scale spatial autocorrelation after forward selection, and the spatially structured environmental parameters represented the key components in explaining diatom variability between the two HERs (8% and 12% respectively).

The partial-RDA performed on samples collected in impacted sites (Fig. 2b), highlighted again the dominant role of the spatial component, which alone explains 15% of the total variance. After forward selection, BOD, COD, Cl, HARD, N-NO_3 and SO_4 were included in the environmental matrix, whereas the spatial matrix included six PCNM vectors at large scale. Chemical parameters resulted important in shaping the communities of the impacted sites, explaining 10% of the total variance. The spatially structured environmental parameters explained 6% of the total variance.

2.4. Taxonomic vs functional composition

Second, we analysed whether anthropogenic pressure causes taxonomic and/or functional homogenization of diatom communities (Hypothesis 2). Visual inspection of the PCoA ordination depicts a clear difference in both taxonomic and functional composition between references and impacted sites (Fig. 3). The results of the test of homogeneity for multivariate dispersion and PERMANOVA (Tab. 4) highlighted how reference and impacted sites show significant different taxonomic composition, with reference sites more homogeneous than impacted sites. However, even if the functional composition was significantly different between reference and impacted sites, we could not detect any homogenization effect. The analysis of the community functional profile (Fig. 4) revealed significant higher abundances of *low* and *high profile* species in reference sites, whereas *motile* species were more abundant in impacted sites. Regarding life-forms, adnate species dominated in impacted sites, while pad-attached and stalked species showed significant higher abundances in reference sites.

Finally, we analysed whether lithological substrate causes biotic differentiation of diatom communities in pristine but not in impacted sites (Hypothesis 3). When considering reference sites, visual inspection of the PCoA performed on the taxonomic matrix clearly highlighted different species composition in siliceous and calcareous hydroecoregions (Fig. 5). This pattern was further confirmed by the results of the PERMANOVA (Table 4), which revealed significant differences in species composition between the two groups. Results of the test of homogeneity for multivariate dispersion (Fig. 5; Tab. 4) highlighted that taxonomic and functional composition of diatom communities were homogeneous within the two groups, even if a nearly significant difference in terms of heterogeneity between diatom communities collected on siliceous (more heterogeneous) and calcareous (more homogeneous) hydroecoregions were observed in terms of ecological guilds. In particular, with the analysis of functional groups, we could detect how the *high profile* guild was significantly more abundant in the calcareous than siliceous area, while the *low profile* guild showed the opposite trend (Fig. 6). In terms of growth forms, the adnate, pad-attached and mucous-colonial taxa resulted significantly more abundant in the siliceous than calcareous hydroecoregion, whereas the opposite trend was observed for stalked-attached taxa (Fig. 6).

Concerning impacted sites, PCoA ordinations performed on the basis of both taxonomical and functional composition displayed an overlap between the two groups (Fig. 5). The test of homogeneity for multivariate dispersion underlined no significant differences between the two HERs, even if results of the PERMANOVA highlighted a slightly significant difference between the two groups in terms of taxonomic composition (Tab. 4). These results were furtherly corroborated by the Mann-Whitney U-test performed on total biovolume and relative abundance of ecological guilds and growth forms, which did not highlight differences between the two HERs (Fig. 6).

DISCUSSION

Traditionally, diatoms have been described as cosmopolitan organisms, whose communities are shaped by local environmental features (“everything is everywhere but environment selects”; Baas-Becking, 1934). For several years, this theory has been strengthened by the use of the *wide species concept* adopted in the European floras (Krammer and Large-Bertalot, 1986-1991), which led to the “force-fitting” of diatom taxa to “European” names and, as a consequence, to confusion concerning species distribution and diversity, which resulted strongly underestimated (Vanormelingen et al., 2008). However, in recent years, the role of spatial control, strictly associated to the dispersal capacity, has been recognized as an important key factor for the determination of diatom community structure (Soininen, 2007), especially in Alpine streams (Bottin et al., 2014). We here investigated the differential role of environmental variables and spatial factors in driving the community composition in pristine and impacted sites within Alpine streams in NE-Italy. Our results confirmed that, in Alpine streams, differences in the composition of the communities are in part due to dispersal limitation. Previous studies in literature highlighted the importance of spatial variables, even at regional scale. For instance, in 2002, Potapova et Charles, observed that at national-scale (USA rivers), almost one-third of the variation in diatom species composition was explained by spatial factors, about 14% by spatially structured environmental parameters and more than 50% by local environmental features. Soininen (2007) highlighted that spatial component accounted 20-31% of the total variance explaining diatom community variations. However, the same author confirmed that at intermediate scale (i.e. 10-3000 km, corresponding to the scale of the present study), there is a joint control of both historical effects and contemporary ecological features. Dong et al. (2016) found that the spatial component played a significant role in shaping diatom communities (about 12%) even at small spatial extent (maximum distance between sites less than 30 km). In 2017, Marquardt and colleagues highlighted that the variation in compositional dissimilarity of some planktonic diatom communities collected in tropical reservoirs was better explained by geographic distance than local environmental features.

In accordance with Bottin et al. (2014), we found a stronger effect of the spatial component in impacted (15%) than in reference (8%) sites. This is in contrast with our initial hypothesis (Hypothesis 1) for which the importance of spatial component should be less evident in those sites affected by anthropogenic disturbance. In particular, we highlighted how environmental conditions in impacted sites likely cause biotic homogenization of diatom communities (Hypothesis 2), causing taxonomic but not functional homogenization. Therefore, the main functions in the community are maintained, while spatial processes likely eliminate some functional redundant species (Leibold and Chase, 2017). Spatial processes determining diatom community composition are controlled by several factors, such as geographical barriers (i.e. spatial distance between sites), topographical and geomorphological features (i.e. high mountains), ecological traits of the species (i.e. their motility) but also by hydrological constraints, like artificial impoundments, which interrupt the longitudinal river connection (Dong et al., 2016). Even if the pressure of chemical parameters is evident in these streams, artificial alterations of hydrological connectivity likely play a major role in determining the structure of diatom community. Although the community spatial structure may be determined by multiple underlying mechanisms, like dispersal limitation, colonization stochasticity (i.e. priority effects) or individual spillover from upstream populations (i.e. mass effect), the analysis of the functional profile of diatom communities supports the role of dispersal limitation because the motile guild is dominant in impacted sites, which has been observed to be associated with both hydrological (Elias et al. 2015; Falasco et al. 2016) and morphological (Smucker and Vis, 2010; Bona et al. 2016) alterations. Our results therefore suggest that considering diatom response not only to trophic conditions but also to physical alterations is crucial in biomonitoring programs.

In reference sites, spatially structured environmental variables explain a great part of the total variance. This result suggests how chemical parameters strictly linked to peculiar areas (i.e. to HERs) are the main drivers of diatom community composition, further strengthening the role of geology. In fact, the different lithology characterizing the two hydroecoregions led to significant differences in some important geochemical variables, such as conductivity and hardness, which were higher on calcareous than on siliceous substrates (Rimet et al., 2004), with repercussions on diatom species composition. As already observed in previous studies, hydroecoregion environmental characteristics, like geology, resulted among the most important factors in shaping diatom reference communities of high altitude streams (Tison et al., 2005; Rimet et al., 2004; 2007). This is confirmed by our results, since in reference sites taxonomic composition of diatom communities significantly differed between the two HERs but this is not evident in impacted sites. This result confirms our third hypothesis, underlying that the influence of geology is much more important in reference than impacted sites, where the effect of geology is overridden by chemical parameters. The Indicator Species Analysis confirmed such differences in terms of diatom communities in according with previous findings, highlighting some species as significantly representative of reference conditions on calcareous substrates, i.e. *Gomphonema pumilum* (Cantonati, 1998; Cantonati et al., 2012; Tison et al., 2005; Rimet et al., 2004; 2007; Beltrami et al., 2012), *Gomphonema tergestinum* (Rimet et al., 2005; 2007; Tison et al., 2005) and *Cymbella affinis* (Tison et al., 2003; 2005), and on siliceous substrates, i.e. *Encyonema minutum* (Tison et al., 2005; Cantonati et al., 2012; Beltrami et al., 2012), *Encyonema silesiacum* (Rimet et al., 2004; Bona et al., 2007; Beltrami et al., 2012), *Fragilaria vaucheriae* (Rimet et al., 2004; Tison et al., 2005; Beltrami et al., 2012) and *Reimeria sinuata* (Rimet et al., 2004; Tison et al., 2005; Beltrami et al., 2012).

We found contrasting results concerning *Gomphonema olivaceum*, which resulted indicator species of siliceous substrates in our reference sites, while typical of the calcareous typologies in other studies (see Rimet et al., 2004; Tison et al. 2005; Beltrami et al., 2012). However, in the present study *G. olivaceum* also resulted as indicator species of calcareous substrates in the impacted typologies, suggesting that probably geology is not the main parameter controlling the distribution and abundance of this taxon. Even though *Achnanthidium lineare* did not result among the statistically significant indicator species, we noticed it was mainly abundant on the calcareous hydroecoregion, confirming previous findings in the study area (Beltrami et al., 2012).

Diatom communities in the two hydroecoregions within reference sites also differed in terms of functional composition, confirming the significant functional shift obtained with the PERMANOVA. In the siliceous sites, the *low profile* guild dominates the community, suggesting that the hydrological regime differs between the two hydroecoregions. We can hypothesize that rivers flowing on the siliceous substrates are possibly characterized by turbulent flows and unstable substrates, which did not allow the development of a mature three-dimensional periphyton. Conversely, the calcareous hydroecoregion presented more mature and stable communities, dominated by the *high profile* guild, i.e. taxa which tolerate higher nutrient load, but sensitive to physical disturbance, suggesting more stable flow conditions compared to siliceous streams (Rimet and Bouchez, 2012). Our hypothesis is supported by Soininen (2007), who pointed out the role of flow velocity among the key environmental factors shaping diatom composition at local scale. In light of these results, the recent hydrological alteration that is affecting Alpine streams (Falasco et al. 2018; Piano et al. 2019) might represent a serious threat to diatom communities.

Conversely, differences in functional composition between HERs were less evident in impacted sites, confirming the absence of both functional homogenization and functional shift detected with the test of homogeneity for multivariate dispersion and PERMANOVA respectively.

Overall, our results are in accordance with the observations of Heino (2013) and highlight the possibility that water quality bioassessment based on diatoms should be considered more reliable at small scale. In fact, at drainage basin scale, the species sorting should be more effective than at larger scale, where the importance of the spatial component and dispersal limitation is typically high. As highlighted by Bottin et al. (2014), the effect of the spatial component on the structure of diatom assemblages can represent a background noise in the framework of the river quality assessment, and this should be taken into account especially in those countries, like Italy, covering a broad range of mountain areas. Given that physical barriers limiting dispersal in Alpine streams are mainly represented by orographic borders, the identification of reference sites within each drainage network could represent a solution to this constraint.

In addition, although we observed taxonomic homogenization in impacted sites, the turnover of species resulted to be more important than biotic homogenization in shaping diatom communities within Alpine streams. These results suggest that the variance between reference and impacted sites is comparable and that they are characterized by different species, thus strengthening the role of diatoms as bioindicators. In light of these patterns, the implementation of metrics that measure this component (e.g. Hillebrand et al., 2017) should be encouraged in biomonitoring programs.

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Tables

Table 1. Characterization of sampling sites (CAL = calcareous HER; SIL = siliceous HER).

SITES	HER	origin	distance from the source/morphology	influence of the upstream basin	n samples	n total
REFERENCE SITES	**CAL**	intermittent	meandering	null	2	64
		spring	< 10 km		2	
		pluvial/nival	< 5 km		12	
			5-25 km		4	
			25-75 km	slight	4	
	SIL	glacial	< 10 km	not detectable	6	
		pluvial/nival	< 5 km		6	
			5-25 km		28	
IMPACTED SITES	**CAL**	pluvial/nival	< 5 km	null	4	46
			5-25 km		16	
				strong	8	
			25-75 km	slight	2	
		subterranean	< 10 km	null	2	
	SIL	pluvial/nival	1.24	not detectable	6	
			0.9		8	

Table 2. average (mean), standard deviation (sd), minimum (min) and maximum (max) values of chemical parameters observed in the calcareous HER (CAL) and in the siliceous HER (SIL) in both reference and impacted sites (N-NO$_3$ = nitrates; N_TOT = total nitrogen; P-PO$_4$ = orthophosphates; P_TOT = total phosphorous; SO$_4$ = sulphates; Cl$^-$ = chlorides; BOD$_5$ = biochemical oxygen demand; COD = chemical oxygen demand; HARD = hardness; COND = conductivity; DO = dissolved oxygen; TEMP = temperature; TSS = total suspended solids; TURB = turbidity).

Sites	**HER**	**value**	**N-NO$_3$ (mg/l)**	**N_TOT (mg/l)**	**P-PO$_4$ (mg/l)**	**P_TOT (mg/l)**	**SO$_4$ (mg/l)**	**Cl$^-$ (mg/l)**	**BOD$_5$ (mg/l)**	**COD (mg/l)**	**HARD (mg/l)**	**COND (µS/cm)**	**DO (mg/l)**	**pH**	**TEMP (°C)**	**TSS (mg/l)**	**TURB (NTU)**
REFERENCE SITES	CAL	mean	0.700	0.680	0.008	0.010	5.60	0.670	0.521	2.09	132	220	10.9	8.24	8.00	5.00	1.12
		sd	0.200	0.260	0.008	0.012	4.30	0.470	0.102	1.10	36.7	47	0.720	0.190	2.80	0.00	0.954
		min	0.400	0.250	0.005	0.005	1.40	0.200	0.500	2.50	17.0	128	9.40	7.70	4.30	5.00	0.300
		max	1.12	1.28	0.040	0.040	16.4	2.00	1.00	6.20	168	277	12.7	8.40	15.0	5.00	4.20
	SIL	mean	0.330	0.390	0.005	0.012	17.8	0.940	0.80	2.70	51.0	100	10.7	7.60	7.50	12.0	7.12
		sd	0.160	0.230	0.001	0.016	17.6	1.12	0.500	0.800	37.7	66	0.940	0.390	2.80	24.0	17.7
		min	0.100	0.250	0.005	0.005	0.800	0.100	0.500	2.50	5.00	14	9.00	6.70	2.80	5.00	0.200
		max	0.900	1.10	0.010	0.060	58.0	6.20	2.30	5.90	139	250	12.6	8.50	14.0	129	98.0
IMPACTED SITES	CAL	mean	2.02	2.29	0.059	0.045	13.4	7.25	1.30	4.30	190	339	11.4	8.39	9.10	6.00	4.24
		sd	1.21	1.38	0.038	0.035	9.90	4.19	1.00	2.80	63.3	99.0	1.25	0.220	4.00	4.00	4.25
		min	0.500	0.600	0.010	0.005	2.60	0.900	0.500	2.50	42.0	92.0	9.40	7.80	1.00	5.00	0.600
		max	7.50	8.40	0.070	0.160	43.0	19.4	5.10	13.8	291	531	15.2	8.80	17.3	25.0	22.3
	SIL	mean	1.90	2.13	0.056	0.070	42.7	10.5	1.10	5.00	133	267	11.4	8.07	7.80	5.00	3.30
		sd	1.26	1.24	0.031	0.037	70.2	3.19	0.700	2.40	97.3	150	0.960	0.160	3.40	0.00	2.88
		min	0.80	0.900	0.020	0.020	5.30	6.80	0.500	2.50	74.0	157	10.1	7.90	3.00	5.00	1.00
		max	4.00	4.20	0.110	0.130	238	16.5	2.90	9.20	398	975	12.9	8.30	13.6	5.00	11.2

Table 3. Species resulting indicators of calcareous (CAL) and siliceous (SIL) HER in reference and impacted sites from the Indicator Species Analysis

SITES	**CAL**			**SIL**		
	species	**IndVal**	***P***	**species**	**IndVal**	***P***
REFERENCE SITES	*Gomphonema pumilum*	0.817	0.001	*Encyonema minutum*	0.844	0.001
	Denticula tenuis	0.678	0.001	*Fragilaria vaucheriae*	0.831	0.001
	Diatoma erhenbergii	0.640	0.006	*Encyonema silesiacum*	0.716	0.002
	Cymbella excisa	0.589	0.001	*Gomphonema olivaceum*	0.699	0.002
	Gomphonema tergestinum	0.520	0.020	*Reimeria sinuata*	0.686	0.038
	Nitzschia fonticola	0.519	0.039	*Psammothidium bioretii*	0.539	0.020
	Gomphonema elegantissimum	0.497	0.006	*Gomphonema pumilum* var. *rigidum*	0.474	0.026
	Cocconeis placentula var. *placentula*	0.462	0.018	*Achananthidium atomoides*	0.447	0.034
	Cymbella affinis	0.424	0.040			
	Cymbella excisiformis	0.405	0.023			
IMPACTED SITES	*Gomphonema olivaceum*	0.712	0.010	*Nitzschia inconspicua*	0.814	0.002
	Encyonema ventricosum	0.707	0.004	*Fragilaria vaucheriae*	0.679	0.046
				Rhoicosphenia abbreviata	0.600	0.020
				Diatoma mesodon	0.513	0.029

Table 4. Results of the test for differences in species composition between reference and impacted sites and between the calcareous (CAL) and the siliceous (SIL) HER within both reference and impacted sites as inferred from the test of homogeneity for multivariate dispersion and PERMANOVA analysis. Significant values are highlighted in bold.

	Taxonomic composition		**Functional composition**	
Reference vs impacted sites				
Homogeneity test	$F_{1,108} = 16.2$	**$P < 0.001$**	$F_{1,108} = 0.140$	$P = 0.709$
PERMANOVA	$F_{1,108} = 24.1$	**$P = 0.001$**	$F_{1,108} = 49.9$	**$P = 0.001$**
CAL vs SIL in reference sites				
Homogeneity test	$F_{1,62} = 0.315$	$P = 0.577$	$F_{1,62} = 3.93$	$P = 0.052$
PERMANOVA	$F_{1,62} = 7.32$	**$P = 0.001$**	$F_{1,62} = 4.81$	**$P = 0.002$**
CAL vs SIL in impacted sites				
Homogeneity test	$F_{1,44} = 0.194$	$P = 0.662$	$F_{1,44} = 0.598$	$P = 0.444$
PERMANOVA	$F_{1,44} = 1.79$	**$P = 0.047$**	$F_{1,44} = 1.92$	$P = 0.110$

Figures

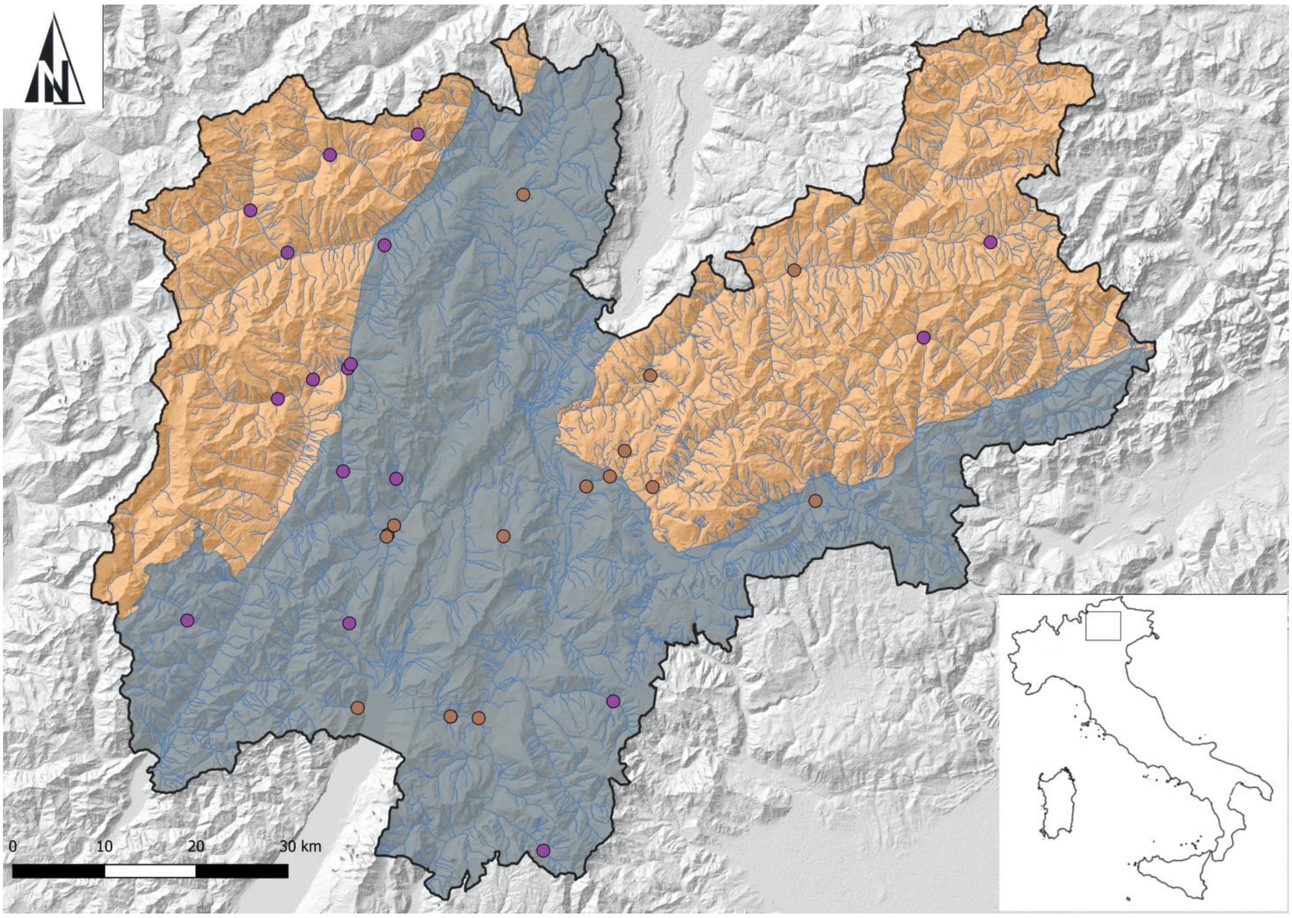


Figure 1. Map of the sampling sites (purple circles = reference sites; brown circles = impacted sites) in the study area (light blue = calacareous HER; orange = siliceous HER).

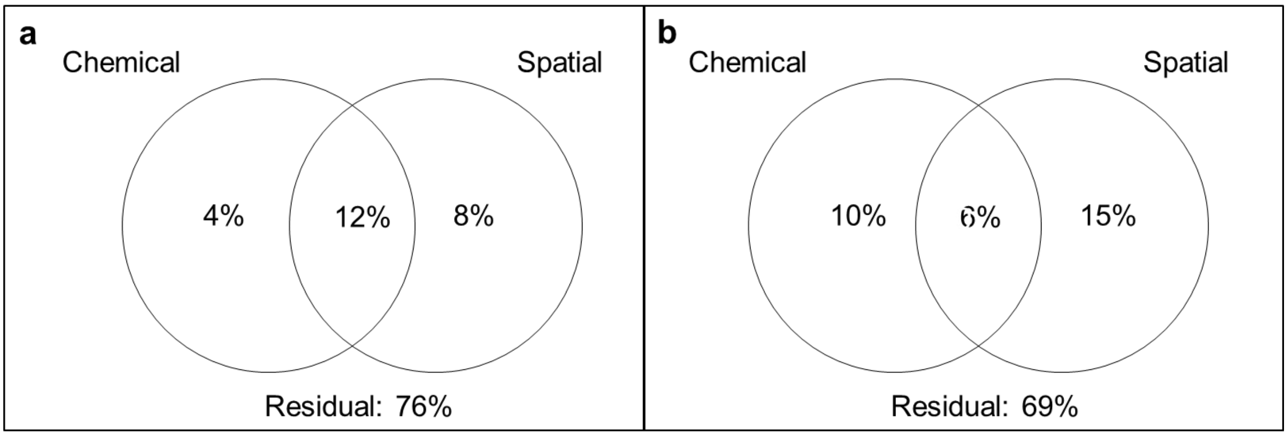


Figure 2. Results from variation partitioning (partial redundancy analysis). The relative contributions (% of explanation) of chemical and spatial variables, as well as the shared components explaining variation in diatom communities in reference (a) and impacted (b) sites.

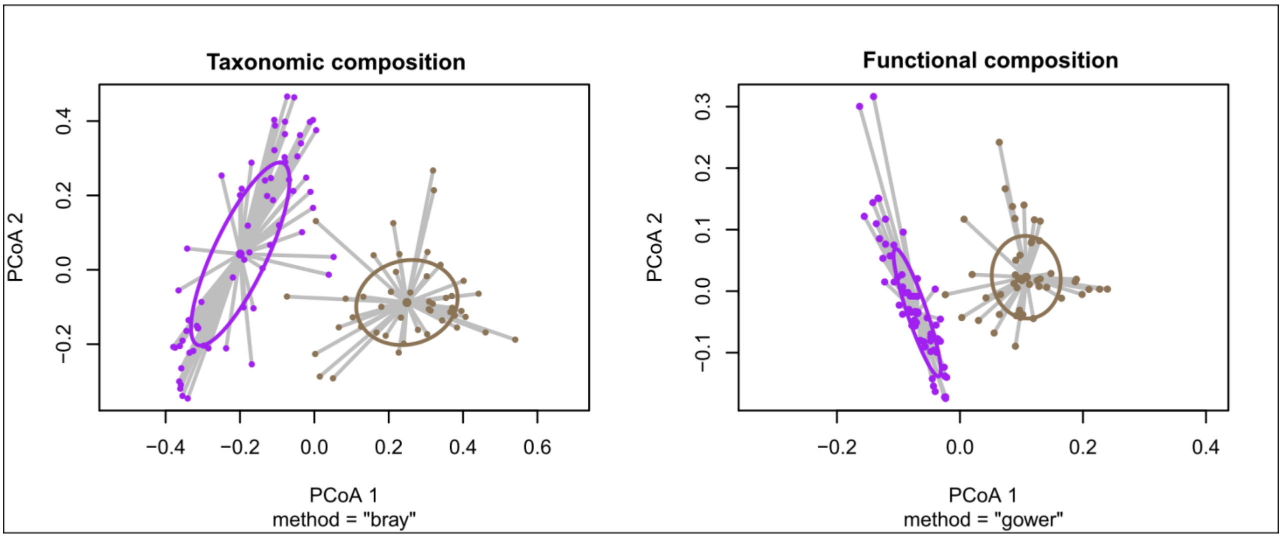


Figure 3. PCoA ordinations of samples based on taxonomic (left panel) and functional (right panel) composition of diatom communities in reference (purple) and impacted (brown) sites.

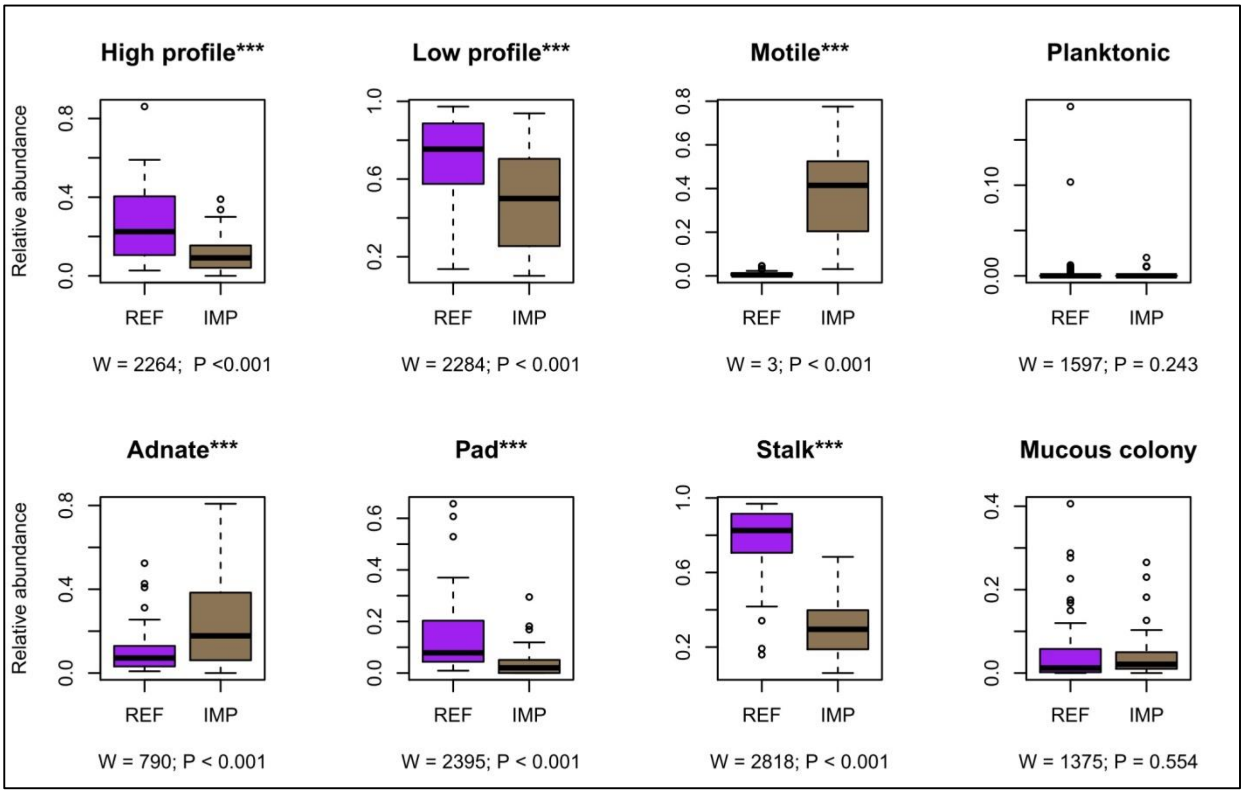
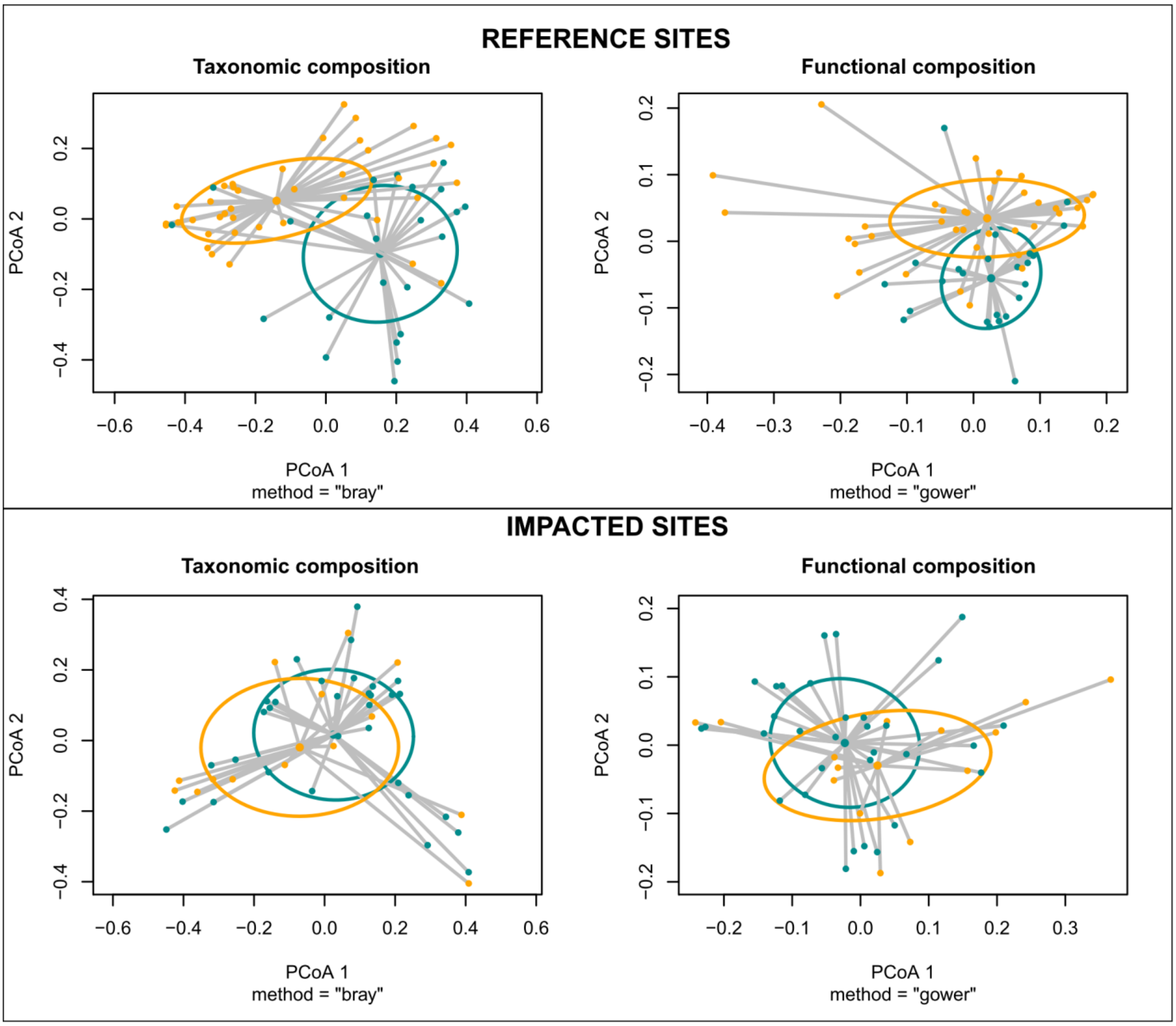


Figure 4. Boxplots depicting the relative abundances of ecological guilds and life-forms in reference (purple) and impacted (brown) sites. Results of the Mann-Whitney U-test are reported below each graph. Asterisks refer to significant differences between the two categories (* < 0.05; ** < 0.01; *** <0.001).



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665 Figure 5. PCoA ordinations of samples based on taxonomic (left panel) and functional (right panel) 666 composition of diatom communities in calcareous (light blue) and siliceous (orange) sites performed 667 on reference (upper panel) and impacted (lower panel) sites.

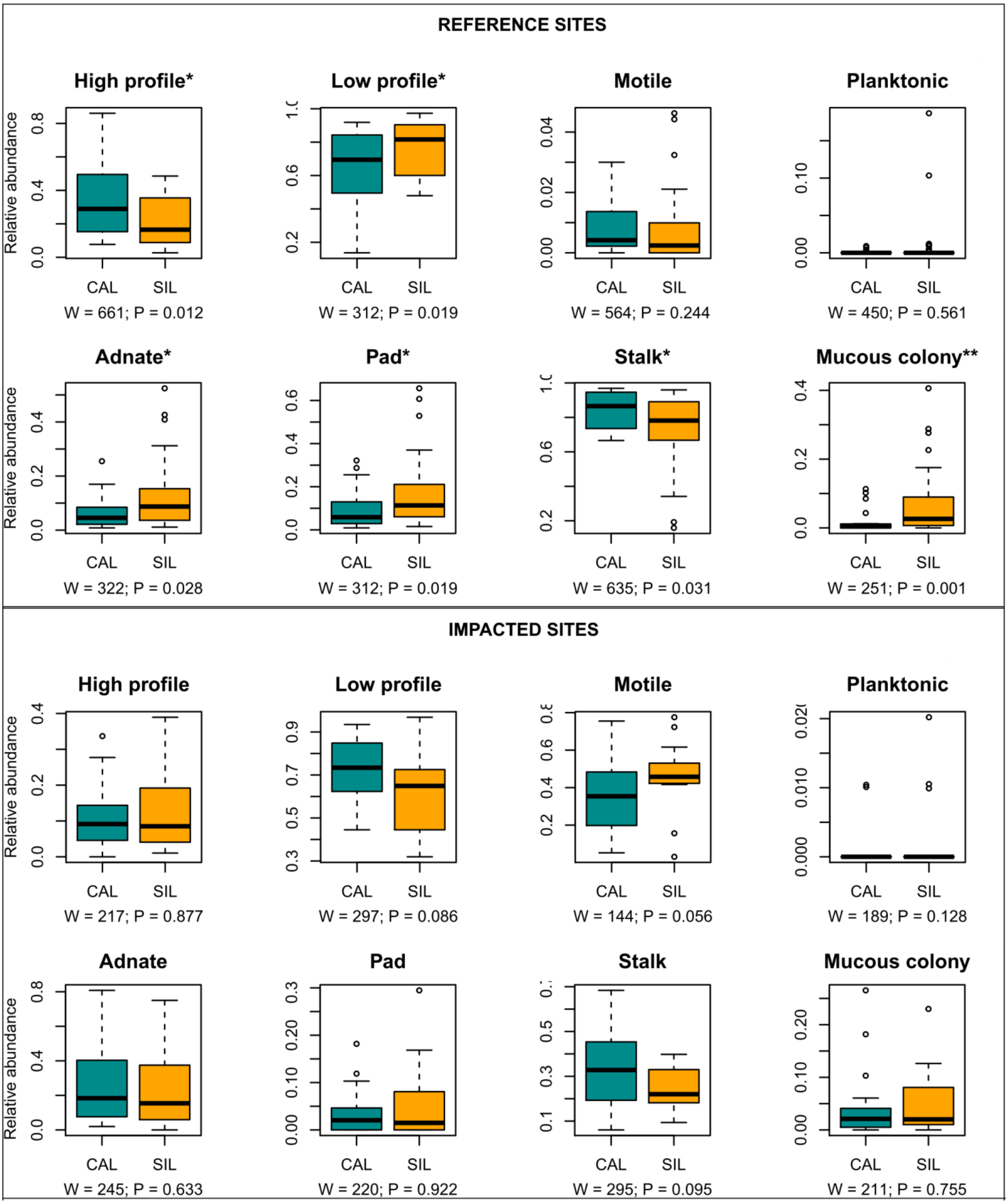

Figure 6. Boxplots depicting the relative abundances of ecological guilds and life-forms in calcareous (light blue) and siliceous (orange) substrates in reference (upper panel) and impacted (lower panel) sites. Asterisks refer to significant differences between the two categories (* < 0.05; ** < 0.01; *** <0.001).