Relative importance of climate and spatial processes in shaping species composition, functional structure and beta diversity of phytoplankton in a large river

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Abstract

Although metacommunity dynamics of lentic phytoplankton are relatively well-documented, studies on the role of environmental and spatial processes in shaping phytoplankton communities of large rivers are still scarce. Here, we examined six phytoplankton data sets, which were collected in 1978-2017 from large river-scale segments (mean spatial extent 1,117 km) in the Danube River. Our aim was to elucidate role of climatic, spatial and temporal predictors in variation of phytoplankton beta diversity using variance partitioning for compositions of species and functional groups sensu Reynolds. We hypothesised that phytoplankton beta diversity (measured as average distance to group centroid) would be positively related to both climatic heterogeneity and spatial extent used as a proxy for dispersal limitation. Additionally, we tested alternative dispersal models to evaluate different spatial processes structuring phytoplankton community. Our results revealed that spatial variables were more important than climatic factors in controlling both species and functional group composition. Climatic heterogeneity showed significant positive relationship with beta diversity. In contrast, there was no significant relationship between beta diversity and spatial extent, suggesting that spatial effect on beta-diversity was attenuated by anthropogenic disturbance. The better performance of non-directional model compared to model of water directionality suggested that spatial dynamics of phytoplankton metacommunity was in large part regulated by differences in the regional species pools. Spatial and temporal variables outperformed environmental (including climatic) factors in explaining phytoplankton metacommunity structure, indicating that phytoplankton exhibited strong biogeographical patterns. Thus, dispersal limitation interfered with species-sorting processes in determining phytoplankton community structure. In conclusion, our findings revealed that the development of a more reliable bioassessment program of the Danube River should be based on separation into basin regions.

Key words: Riverine phytoplankton, Metacommunity structure, Dispersal limitation, Biogeographical pattern, Climatic heterogeneity, Danube River

1. Introduction

Understanding the mechanisms that determine the structure of natural communities is a prime goal in ecology and biogeography (Cottenie, 2005; Ricklefs, Jenkins, 2011). Traditionally, ecological studies have been primarily focused on abiotic and species interactions to explain coexistence of species in local areas, while biogeography aims to consider evolutionary, historical and climatic drivers of regional biotas (Jenkins, Ricklefs, 2011). During recent years, a shift in the emphasis of ecological studies towards spatial scales beyond local habitats serves as a bridge between ecology and biogeography (Ricklefs, Jenkins, 2011). For example, one of the main objectives of the modern ecology studying the relationship between local and regional diversity patterns is the role of regional processes (i.e., historical dispersal) in shaping composition of contemporary local communities (Ricklefs, 1987). The integration of local and regional perspectives is a widespread and growing focus of contemporary multi-scale ecological studies (Logue et al., 2011), yet our ability to predict the relative importance of local and regional factors in spatio-temporal structuring of natural communities is still limited (Brown et al., 2017).

Metacommunity concept that has emerged recently in ecology explicitly recognizes that difference in community structure between neighbouring spatial sites is determined by interplay between local niche-based (e.g., environmental filtering) and spatial (e.g., dispersal) processes (Leibold et al., 2004). Currently, there are four metacommunity paradigms (neutral, species-sorting, patch-dynamics and mass-effects), which differ in the relative importance of local and regional processes and dispersal rate among local communities (Leibold et al., 2004; Logue et al., 2011). Neutral model assumes that species are identical in their response to environment and regional processes such as dispersal are the dominant force structuring local communities

(Hubbell, 2001). In contrast, species-sorting paradigm accentuates individual responses of species to varying environmental conditions, provided that there is a sufficient dispersal of species from regional pool so that regional spatial processes are not important under this paradigm (Leibold et al., 2004). In patch-dynamic and mass-effect paradigms, which can be considered as special cases of species-sorting (Winegardner et al., 2012), both local and regional processes play a role in structuring communities (Leibold et al., 2004). For patch-dynamics it is assumed that species differ in their colonization and/or competitive abilities and limited dispersal is a driving mechanism of community dissimilarity even in homogeneous environment. In mass-effect model, although all species use different niches, surplus dispersal allows some species to exist in suboptimal conditions, thus resulting in homogenization of community composition along environmental gradient. Several metacommunity models can be related simultaneously to spatio-temporal dynamics of natural metacommunities (Cottenie, 2005; Gravel et al., 2006). Furthermore, the relative role of local environmental vs. regional processes can vary with determinants such as spatial scale, habitat type and dispersal capabilities of organisms (Cottenie, 2005; Heino et al., 2015b). Therefore, the primary concern of metacommunity studies is not to fit natural metacommunities to one of the four perspectives, but rather determine how changes in relative contribution of environmental factors and dispersal rate affect metacommunity dynamics (Brown et al., 2017).

Beta diversity, generally defined as turnover of community composition in space and time (Soininen, 2010), is widely used to measure regional biodiversity and has important implications for biodiversity conservation and ecosystem management (Legendre et al., 2005). Empirical studies in freshwater systems have typically found that beta diversity is driven by interplay of environmental and spatial factors (Soininen et al., 2011; Bini et al., 2014; Santos et al., 2016; Zorzal-Almeida et al., 2017; Lansac-Tôha et al., 2019; Leboucher et al., 2019). Statistical methods that allow to assess the relative importance of environmental and spatial variables in species turnover may be grouped in raw-data approach (variation partitioning based on constrained ordination) and distance-based approach (variation partitioning based on correlation of (dis)similarity matrices) (Legendre et al., 2005; Tuomisto, Ruokolainen, 2006; Anderson et al., 2011). According to species sorting theory, there should be a positive relationship between environmental heterogeneity and beta diversity because wider environmental gradients support more diverse communities (Leibold et al., 2004). In turn, dispersal limitation should result in a positive relationship between spatial extent of the study regions and beta diversity, because increasing geographical distances between sites reduces immigration rates of species into local communities, that, in turn, increases compositional dissimilarities (Hubbell, 2001). A few studies demonstrated positive relationship between environmental heterogeneity and beta diversity implying the dominance of species sorting (Zorzal-Almeida et al., 2017; Santos et al., 2016). Some studies did not find evidence that environmental heterogeneity drives beta diversity (Bini et al., 2014), suggesting decoupling impact of anthropogenic disturbance (nutrient enrichment) on the relationships between compositional dissimilarities and environmental distances. A broad-scale study in streams showed that the spatial distances were significantly positively correlated with beta diversity, indicating a substantial role of dispersal processes in species turnover across regional and continental scales (Leboucher et al., 2019). Furthermore, it was found that strength of spatial dependence of beta diversity decreased along anthropogenic gradient due to taxonomic homogenization caused by nutrient enrichment (Leboucher et al., 2019). In addition, beta diversity also changes over time due to temporally varying strengths of beta diversity drivers (Wojciechowski et al., 2017). Thus, the comprehensive understanding of how metacommunities are structured should take into account both spatial and temporal variability in environmental correlates of beta diversity.

According to the Baas-Becking (1934) hypothesis (“Everything is everywhere, but the environment selects”), microalgae are cosmopolitan and have no biogeographical distribution patterns resulted from regional differences in species pools and dispersal limitation (Finlay, 2002). Therefore, the spatial distribution of passively dispersing microalgae would be driven solely by environmental filtering. Despite this, recent studies have shown that across regional to continental scales benthic diatom communities exhibit strong geographical patterns (Potapova, Charles, 2002; Soininen, 2007; Heino et al., 2010; Tang et al., 2013; Bottin et al., 2014; Soininen et al., 2016), suggesting that spatial distribution of diatom communities is influenced by dispersal-related factors. Study focusing on spatial turnover in benthic and planktonic diatom communities showed that benthic diatoms (which possess lower dispersal abilities due to their association with solid substrates) exhibited higher rates of similarity decay with spatial distance than do planktonic diatoms (which possess higher dispersal abilities) (Wetzel et al., 2012). These findings indicate that planktonic algal communities are less strongly structured by dispersal limitation than benthic algal communities. Recent researches on riverine phytoplankton have revealed that this community is regulated by both environment and space, but the former has a stronger effect than the latter, indicating a predominant role of species sorting in shaping community structure (Qu et al., 2018; Wu et al., 2018; Bortolini et al., 2019; Graco-Roza et al., 2020). Other studies have shown that spatial variables could be as important as environmental ones (Bortolini et al., 2017; Lansac-Tôha et al., 2019), suggesting that unidirectional passive dispersal by water flow prevent phytoplankton from effective tracking environmental variation along the river channel. However, it is worth noting that all these studies were conducted at relatively small spatial scales (sub-basins), at which dispersal limitation played a negligible role, while mass effect was the most likely process responsible for phytoplankton spatial signals (Heino et al., 2015a). Dispersal limitation is a scale-dependent ecological process, as its importance in structuring communities increases with increasing spatial extent of a study (Soininen et al., 2011; Heino et al., 2015a). Given that the most of the studies of riverine phytoplankton metacommunities were conducted at relatively small spatial scales, the role of regional processes (including dispersal limitation) in structuring riverine phytoplankton, especially at a broad scale, still needs to be established.

Aggregation of species into functional groups can provide a complementary information to taxonomic composition on how metacommunities are structured (e.g., Heino et al., 2015b). Species within functional groups have similar functional traits that determine their similar positions on environmental gradients (Wilson, 1999). Since species within a functional group are ecologically equivalent, the functional group concept implies some degree of functional redundancy in the ecosystem (Blondel, 2003). Thus, in different regions with similar environmental conditions the composition of functional groups should be similar, regardless of their taxonomic compositions. A number of studies have demonstrated that phytoplankton functional group concept, which was originally developed for lakes (Reynolds et al., 2002), may provide useful information on the response of riverine phytoplankton to environmental constraints such as nutrient concentration, hydrologic disturbance and climate changes (e.g., Abonyi et al., 2020). However, spatial factors have only recently been included as potential important drivers of phytoplankton functional composition. Particularly for functional group concept sensu Reynolds, different responses to spatial processes were reported, without a clear pattern. Although some studies have found that functional composition was jointly driven by environment and space (Izaguirre et al., 2015; Bortolini et al., 2019), other have found no effect of spatial variables (Santos et al., 2016; Xiao et al., 2018). The differences between studies could be related to the different scales of the study area. For example, in a large-scale (continental) research, functional groups were mainly driven by environmental factors and were less dependent on spatial structuring (Soininen et al., 2016). One of the likely reasons for this is that functional groups are composed by sets of taxonomically distinct species that have similar ecological functions and, therefore, classification based on the functional group approach is highly sensitive to environmental changes, irrespective of taxonomic differences among geographic regions.

The Danube River phytoplankton investigations have a long history (reviewed by Dokulil, 2015). The most comprehensive reports on longitudinal phytoplankton changes were published among others by Wawrik (1962), Aponasenko et al. (1990), Kiss (1991), Kiss and Genkal (1996) and Dokulil (2014). Under the umbrella of the International Commission for the Protection of the Danube River (ICPDR) phytoplankton monitoring in the Danube River has been carried out for two decades (Literáthy et al., 2002; Dokulil, Kaiblinger, 2008; Dokulil, Donabaum, 2015; Stanković et al., 2020). Results of these longitudinal surveys combined with phytoplankton production data were summarised and brought into the context of existing river function concepts (Dokulil, 2014; Dokulil, 2015). Spatio-temporal patterns of phytoplankton distribution were better described by the riverine productivity model (RPM), which emphasises the role of light, temperature and discharge in controlling autochthonous production (Thorp, Delong, 1994). Therefore, high inter-annual variation in phytoplankton biomass reported in the Danube River is likely resulted from varying hydrological and meteorological conditions (Dokulil, 2014; Dokulil, 2015). Since climate is one of the major factors accounting for differences in rivers worldwide (Sabater et al., 2006), phytoplankton alterations along the course of the large rivers may be driven in large part by interregional differences in climatic parameters. Thus, evaluation of climatic factors is especially relevant for water quality assessment in complex hydrological situation of the large catchment of the Danube River (Dokulil, 2014; Dokulil, 2015). Moreover, spatial distribution patterns of phytoplankton resulted from dispersal-related mechanisms may represent noise in the relationships between algal assemblages and environmental variables (Bottin et al., 2014). Therefore, to improve the efficiency of biomonitoring we should know the response of the Danube River phytoplankton community not only to major environmental gradients but also to regional processes.

Here, we examined several phytoplankton data sets, which were collected in 1978-2017 from large river-scale segments in the Danube River. The spatial extent (i.e., the length of the river segments) of the study varied from 732 km to 1783 km. The large spatial scale of the study was particularly appropriate to test whether phytoplankton community exhibits biogeographical patterns, related to both local and regional processes. Therefore, in order to identify main spatial patterns of phytoplankton community of the Danube River we test for significant differences in species and functional group composition among the major basin regions (Upper, Middle and Upper Danube). We evaluated spatio-temporal variation in phytoplankton biomass and community composition as influenced by climatic variables. The role of climatic, spatial and temporal predictors in variation of compositions of phytoplankton species and functional groups sensu Reynolds was assessed using variance partitioning based on constrained ordination and correlation of (dis)similarity matrices. More specifically, we tested the following hypotheses: (1) Phytoplankton beta diversity would be positively related to both climatic heterogeneity and spatial extent, used as a proxy for dispersal limitation. We expected that positive relationship between beta diversity and climatic heterogeneity occurs because the high variability of environmental factors favours colonization of different species, increasing compositional dissimilarity (Leibold et al., 2004). In turn, beta diversity is positively related to spatial extent because the reduced rate of species exchange with increasing distance between sites results in dissimilar communities (Hubbell, 2001). (2) The influence of spatial factors on phytoplankton structure should increase with increasing spatial extent, because dispersal limitation tends to have a stronger impact on phytoplankton community at larger spatial scales (Soininen et al., 2011). (3) The effect of temporally varying climatic parameters should be more important if compared with purely temporal component of long-term variability of phytoplankton community, because changes in temperature, precipitation and hydrological regime are the key factors responsible for phytoplankton dynamics according to the riverine productivity model (Thorp, Delong, 1994). (4) Given that at large spatial gradient the functional group approach is highly sensitive to changes in environmental conditions (Soininen et al., 2016), we expected that distribution of phytoplankton functional groups would be more related to climatic parameters than to geographic location. (5) Additionally, we tested alternative (non-directional and unidirectional) dispersal models to evaluate different spatial processes structuring phytoplankton metacommunity. We expected that phytoplankton would be more shaped by unidirectional processes, since directional water flow produces a strong longitudinal gradient in the distribution of phytoplankton and limits its ability to effectively track local-scale environmental variation along the river channel (Lansac-Tôha et al., 2019).

2. Materials and methods

2.1. Datasets

The Danube River flows 2783 km on its way to the Black Sea, passing through Europe from west to east. It transfers water through nine countries - Germany, Austria, Slovakia, Hungary, Croatia, Serbia and Montenegro, Bulgaria, Romania and Ukraine. The Danube basin drains an area of 817,000 km^2. Based on the catchment geology the river is divided into Upper, Middle and Lower Danube (Lászlóffy, 1965). The Upper Danube (2783-1791 km) extends from the confluence of the source rivers Breg and Brigach in Donaueschingen to Devín Gate below Vienna, the Middle Danube (1791-931 km) from Devín Gate to Iron Gate and finally the Lower Danube (931-0 km) from Iron Gate to Sulina. The Upper Danube is characterized by a steep gradient (average slop 101-40 cm km^{-1}), the Middle and Lower Danube by a low gradient (average slop 6 and 3.9 cm km^{-1}, respectively), except for the cataracts of Iron Gate (average slop 28 cm km^{-1}) (Lászlóffy 1965). Fifty hydroelectric power dams including a large dam system at Gabčikovo are situated in the Upper Danube. The largest hydropower dam and reservoir system Iron Gate Dam I and II represents the downstream border of the Middle Danube. On its entire course, the Danube River connects with 27 large and over 300 small tributaries. Almost all large tributaries are now impounded or connected to floodplain lakes. The Upper Danube has eight major tributaries, among which Lech, Isar, Inn, Iller, Traun, Enns join the Danube River on the right bank, and Morava and Regen join it on the left bank; the Middle Danube has nine major tributaries: the right-bank tributaries are Raba, Sió, Drava, Sava and Velika Morava, and the left-bank tributaries are Váh, Hron, Ipel and Tisa; and the Lower Danube has ten important tributaries: the southern tributaries are Timok, Iskar and Jantra, and the northern tributaries are

Jiu, Olt, Arges, Ialomita, Siret, Bistrița and Prut. On its way to the Black Sea the Danube River crosses four ecoregions from west to east: Central Highlands, Hungarian Lowlands, the Carpathians and Pontic Province ecoregions (Illies, 1978).

We included six phytoplankton datasets sampled in 1978, 1995, 2000, 2001, 2002 and 2017. Phytoplankton samples were collected in the Danube River (Fig. 1), from large river-scale sections ranged in the watercourse distance from 732 to 1783 km (mean 1117 km). River sections are described in detail in Table 1. Detailed information about the datasets of 1978 and 1995 can be found in Kiss (1991), Kiss and Genkal (1996). Phytoplankton samples were taken from the middle of the streamline and downstream of dams in impounded sections of the Danube River by the same person (Keve T. Kiss, one of the authors) over the entire period. The sampling strategy for phytoplankton investigations in the Danube River was based on the standardized sampling method (Kiss et al., 1996), that allowed a long-term comparison of phytoplankton datasets. The samples were fixed with acetic Lugol's solution. Microscopic counting of phytoplankton was carried out according to Utermöhl (1958). After sedimentation, 3-4 complete diameters (instead of randomized fields) of the Utermöhl counting chamber were counted with a magnification of 600x. Depending on phytoplankton density in samples, a 0.2 x 26 mm or 0.1 x 26 mm field was investigated (the diameter of the chamber is 26 mm) to count ~ 400 cells. This sampling protocol ensured a counting accuracy of 5% according to Lund et al. (1958). Phytoplankton species (except centric diatoms, see below) were measured during the count in the Utermöhl chamber. Among abundant species (more than 5 % in the sample) 10-20 individuals was measured, among the rare species only a few. Algal biovolume was calculated using characteristic geometric forms (Hillebrand et al., 1999) and expressed as fresh weight assuming a density of 1. Total phytoplankton biovolume (biomass) per sample was calculated as the sum of taxon-specific biovolumes.

All samples for centric diatom analysis were rinsed with distilled water to remove formaldehyde and subsequently treated with hot H_2O_2 (CEN 2014). The cleaned and washed sample was filtered through a stainless-steel syringe filter holder using 3 μm Isopore™ polycarbonate membrane filter (Merck Millipore, Germany). For scanning electron microscopy (SEM), the filter was then fixed onto a stub using double-sided carbon tape and coated with gold using a rotary-pumped spatter coater Quorum Q150R S (ZEISS). Fine structures of centric diatom frustules were observed and cell dimensions (diameter and height) were measured with Zeiss EVO MA 10 SEM (ZEISS) operated at 10 kV and 10 mm distance using SEM detectors. SEM diatom images (more than 100 micrographs from each sample; about 7,100 micrographs in total) were used to calculate average biovolumes for all centric diatom species.

Because water samples for chemical analyses were not collected during phytoplankton sampling, local environmental variables were inferred using indicator values of phytoplankton functional groups (see below). We also included seven climatic variables (annual temperature (°C), annual temperature SD, July, August and September temperatures (°C), annual precipitation (mm), and annual precipitation CV) drawn from the WorldClim database (Harris et al., 2014). Climate data covered the period 1901-2018 at high resolution spatial grid (0.5° × 0.5° latitude/longitude) and average monthly values were included in the analyses. Spatial variables were elevation (m), watercourse distance (km) and geographic coordinates (latitude and longitude) of sampling sites.

2.2. Phytoplankton functional groups

Phytoplankton taxa were aggregated into functional groups according to the functional group concept *sensu* Reynolds (Reynolds et al., 2002; Borics et al., 2007; Padisák et al., 2009). The relative abundances (percentages of total biomass) of functional groups were recorded for each sample. Based on the assignments of functional groups to trophic state, pollution and turbulence on a scale ranging from 0 to 5 (Borics et al., 2007), the indices (Q) of nutrient, pollution and turbulence levels for each sample were calculated using the following equation:

$$Q = \sum_{i=1}^{S} (pi \times F),$$

where $p_i = n_i/N$, n_i is the biomass of the i-th functional group, while N is the total biomass. The F is factor number allowing the Q index to range between 0 and 5. Calculated indices were used as environmental variables in the following data analysis. We considered here these variables as indicators for the degree of anthropogenic (nutrient level, pollution) and hydrologic (turbulence) stress.

2.3. Data analyses

We tested statistical differences ($p < 0.05$) in total phytoplankton biomass between years using one-way ANOVA. To test if there was a gradual change in total phytoplankton biomass over a time period of 1978-2017, we tested linear relationship between the dependent variable and years as an ordered factor. Relationships between total phytoplankton biomass, biomass of algal groups, climatic parameters and watercourse distance were assessed using linear correlation.

To assess beta diversity for phytoplankton taxa and functional groups, we performed a permutation test of multivariate homogeneity of groups dispersions (PERMDISP, Anderson et al., 2006). This test consists of measuring average distance from each observational unit to their group centroid and assessing variability in community structure. To measure average dissimilarity for sampling sites within each dataset (year), we used Bray-Curtis distances (D) on percent biomass of species and functional groups. PERMDISP method was performed using the function *betadisper* in 'vegan' package (Oksanen et al., 2019). To test for statistical differences ($p < 0.05$) in beta diversity between years, PERMDISP uses one-way ANOVA through 999 permutations and, where significant, tests for post hoc differences between individual years with Tukey's HSD test.

We assessed differences among years in climatic variables (mean annual temperature, temperature seasonality (SD), mean annual precipitation, and precipitation seasonality (CV)) using one-way ANOVA separately for each climatic variable. The gradual climate changes during the time period from 1978 to 2017 were tested using a linear model relating climatic variables to years as an ordered factor. The climatic heterogeneity in each year was assessed using all seven climatic variables in the PERMDISP analysis (Anderson et al., 2006), that was based on Euclidean distance. To test hypothesis 1, the relationships between beta diversity (for species and functional groups) and climatic heterogeneity and spatial extent (watercourse distance) were explored using linear regression.

Prior to the following analyses, relative abundance (percentage of total biomass) data for species and functional groups were Hellinger transformed according to Legendre and Gallagher (2001), using the function *decostand* in ‘vegan’. To diminish the effect of rare species, we selected phytoplankton species contributing > 1% of the total phytoplankton biomass and occurring in at least 3 years. Of the 331 taxa recorded in phytoplankton samples 132 met this criterion. Variables of environmental and climatic factors and site coordinates (latitude and longitude) were standardized (mean = 0, variance = 1) using the function *decostand* in ‘vegan’.

The significance of correlation coefficients between matrices of compositional similarity and matrices of climatic and spatial distances was tested using a Mantel test with 1000 permutations (Legendre et al., 2005). This (dis)similarity-based approach is an efficient method in analysing spatial variation in community similarity (Tuomisto, Ruokolainen, 2006; Anderson et al., 2011). We calculated similarity matrices for species and functional groups using Bray-Curtis similarity (1-D) on relative abundance data, separately for each data set (year). Spatial distance matrices for each data set were calculated using Euclidean distance. The watercourse distance was used as characteristic of site location instead of coordinates (latitude and longitude) in order to emphasise changes in community structure along the river course. Environmental distance matrices based on climatic variables were calculated using Euclidean distance, separately for each data set. To find subsets of climatic variables that produce the highest correlations with species and functional group composition in each data set, we run a forward selection procedure (Blanchet et al., 2008), using the function *ordiR2step* in ‘vegan’. Partial

Mantel tests, where either spatial or environmental matrix was considered as the explanatory matrix (Tuomisto, Ruokolainen, 2006), were run to test the relationship between compositional similarity and spatial distance while controlling for environmental distance, and vice versa. The Mantel and partial Mantel tests were run using the function *mantel* in package ‘ecodist’ (Goslee, Urban, 2007). To test hypothesis 2, we used linear correlation to test for significant ($p < 0.05$) relationships between the strength of spatial effect (measured using the partial Mantel correlation coefficient) and the spatial extent. In addition, the Mantel test performed on the basis of the entire data sets was used to test for correlation between similarity matrices of species composition and functional group composition.

We analysed changes in species and functional group compositions in spatio-temporal space using detrended correspondence analysis (DCA) followed by environmental factor fitting to examine the major structure of the data in unconstrained spacing. We run these analyses using the function *decorana* in ‘vegan’. To find which variables were significantly ($p < 0.05$) correlated with ordination, we fitted vectors of local environmental, climatic and spatial variables using the function *envfit* in ‘vegan’. The function *envfit* was also used to find species and functional groups that contributed significantly ($p < 0.05$) to spatio-temporal changes in phytoplankton composition. The significance of fitted vectors was assessed using permutation of the data. For the function *envfit* the goodness of fit statistic was squared correlation coefficient (R^2). The function *factorfit* was used to test for significant ($p < 0.05$) differences in species and functional group composition among Upper, Middle and Lower Danube. The function *factorfit* found averages of ordination scores for factor levels represented by different basin regions. The significance of fitted factor was tested with permutation procedure. The goodness of fit statistic was defined as $R^2 = 1 – ss_w / ss_t$, where ss_w and ss_t are within-group and total sums of squares, respectively (Oksanen et al., 2019).

To examine phytoplankton response to local environmental (Env), climatic (Cli), spatial (Spa) and temporal (Temp) variables and to determine the fractions explained by individual predictor sets and their covariance, we employed partial redundancy analysis (pRDA, Borcard et al., 1992), using the function *varpart* in ‘vegan’. Before pRDA, we selected the explanatory variables using a forward selection method developed by Blanchet et al. (2008). We used this method to prevent artificially inflated explanatory power of constrained ordination models. The forward selection was carried out using the function *ordiR2step* in ‘vegan’ and was done separately for each response data matrix (species and functional groups). We analysed the adjusted R^2 values and the components were tested at a $p < 0.05$ significance. Local environmental variables (trophic state, pollution and turbulence) were inferred from the composition of phytoplankton functional groups (see above). Therefore, fraction of the environmental factors can be overestimated. Climatic variables were annual temperature, annual temperature SD, July, August and September temperatures, annual precipitation, and annual precipitation CV. Spatial variables were derived from Moran’s eigenvector maps (MEM) approach (Dray et al., 2006; Dray, 2020). The whole set of generated MEM eigenvectors (positive and negative) was used in the forward selection procedure (see below). Time variable was presented by a dummy variable differentiating each phytoplankton data set (year). To test hypothesis 3, we compared the fraction of variation explained jointly by climatic and temporal variables with the proportion of variation explained only by temporal variable for both species and functional group composition. We compared the fractions of variation purely explained by climatic factors and spatial variables in composition of functional groups to test hypothesis 4.

Finally, spatial aspects of the Danube River phytoplankton data were assessed through different spatial eigenfunction models accounting for the effects of different scenarios of spatial dynamics of phytoplankton community. First, we applied Moran’s eigenvector maps (MEM) framework, that is used to model non-directed processes occurring in spatial proximity (Dray et al., 2006; Dray, 2020). Non-directed spatial patterns in phytoplankton dynamics can be generated, e.g., by local dispersal due to lateral hydrological connectivity or specific patches of a river channel (i.e., due to nutrient distributions or influence of dams and impoundments). For

MEM models, the connectivity matrix represented by transect of sampling sites was created for each year using the function *cell2nb* from 'spdep' package (Bivand et al., 2019). We measured the watercourse distances (d; length of the river between connected sites), calculated weights using the weighting function: weight = $1 - d/d_{\max}$, where d is the distance between connected sites, $d_{\max}$ is the maximum value of d in the dataset. The weight represents the ease of transfer of matter between sites. The spatial weighting matrix was then created for each year using the function *nb2listw* in 'spdep'. At the final step, the resulting spatial weighting matrix was used to compute spatial MEM eigenvectors by the function *scores.listw* in package 'adespatial' (Dray et al., 2020). Second, we used asymmetric eigenvector maps (AEM) framework, which was specifically designed to model directional spatial patterns, such as structures produced by directional flow in rivers (Blanchet et al., 2011). A site-by-edge binary matrix was constructed based on the information, which combines connection between sampling sites and direction of water flow, using the function *aem.build.binary* in 'adespatial'. Then, the same weighting function was applied as the one used for MEM models and the function *aem* in 'adespatial' was used to compute spatial eigenvectors (AEM). Third, we tested whether damming has higher impact on spatial dynamics of phytoplankton than watercourse distance. For this, we constructed MEM models using number of dams (DAM) instead of watercourse distance as the weighting factor. Therefore, the following weighting function was applied: weight = $1 - (N/N_{\text{total}})$, where N is number of dams between two consecutive sites, N_{total} is the total number of dams located in the studied river stretch. For each of the three spatial models, at first, RDA was run using the whole set of spatial eigenvectors. Then, in order to reduce the number of spatial variables and produce more parsimonious models, we performed a forward selection procedure (Blanchet et al., 2008), using the function *ordiR2step*. To test hypothesis 5, the relative performance of spatial models was assessed with pRDA (Borcard et al., 1992), using the function *varpart* in 'vegan'.

All statistical analyses were carried out in R ver. 3.6.3 (https://cran.r-project.org/).

3. Results

3.1. Response of phytoplankton biomass and beta diversity to climatic and spatial variables

The total phytoplankton biomass decreased significantly ($p < 0.001$) over the study period with the lowest value in 2017 ($F = 7.196$, $p < 0.001$; Fig. S1). Centric diatoms were the most important contributors to the total phytoplankton biomass (on the average 49.9%), followed by green algae (21.1%), pennate diatoms (11.4%), cryptophytes (9.4%) and cyanobacteria (3.4%). Correlation analysis showed that the total phytoplankton biomass was related negatively to mean annual precipitation ($r = -0.413$; $p < 0.001$) and positively to precipitation CV ($r = 0.489$; $p < 0.001$). Biomass of centric diatoms was positively related to temperature SD ($r = 0.581$; $p < 0.001$), while biomass of green algae showed negative relationship with temperature SD ($r = -0.553$; $p < 0.001$). There were significant relationships between biomass of some algal taxonomic groups and watercourse distance: centric diatoms were related positively ($r = 0.428$; $p < 0.001$) to watercourse distance, whereas green algae were related negatively ($r = -0.360$; $p < 0.01$).

Taxonomic beta diversity varied over the years with the lowest value in 1995 and the highest value in 2002 (Fig. 2a), yet the only significant difference was in 1995 ($F = 18.371$, $p < 0.001$). The Tukey's HSD pairwise test showed that taxonomic beta diversity in 1995 was significantly lower compared with all the years (Table S1). Similarly, functional group beta diversity showed significant variability over the years with the lowest value in 1995 and the highest value in 2002 ($F = 7.254$, $p < 0.001$; Fig. 2b). The Tukey's HSD pairwise test showed that functional group beta diversity in 1995 was significantly lower than in 1978, 2001, 2002 and 2017, while in 2002 it was significantly higher compared with 1978, 1995, 2000 and 2017 (Table S2).

Climatic parameters varied significantly over the years (Fig. S2). Mean annual temperature was maximal in 2000 and minimal in 1995 ($F = 4.560$, $p < 0.01$; Fig. S2a); temperature seasonality (SD) was lowest in 2002 and highest in 2001 and 2017 ($F = 7.355$, $p <$

0.001; Fig. S2b). Mean annual precipitation showed a significant ($p < 0.001$) upward trend over time with the lowest value in 1978 and the highest value in 2002 ($F = 9.896$, $p < 0.001$; Fig. S2c), while precipitation seasonality (CV) exhibited a significant ($p < 0.001$) downward trend with the highest value in 1978 and the lowest value in 2017 ($F = 13.914$, $p < 0.001$; Fig. S2d). Climatic heterogeneity varied among the years with the lowest value in 1995 and the highest value in 2001 (Fig. 2c), but there was the only significant difference between 1995 and 2001 ($F = 2.507$, $p < 0.05$; Table S3).

There was no significant linear relationship between climatic heterogeneity and spatial extent ($r = 0.359$; $p = 0.451$). Thus, the absence of collinearity between climatic heterogeneity and spatial extent allowed us to test independent effects of predictor variables on beta diversity. As correlation analysis showed, there were significant positive relationships between climatic heterogeneity and taxonomic beta diversity ($R^2 = 0.679$, $p = 0.027$) and functional group beta diversity ($R^2 = 0.692$, $p = 0.025$ (Fig. 3a). The relationship between spatial extent and beta diversity was not statistically significant both for species ($R^2 = 0.126$, $p = 0.260$) and functional groups ($R^2 = 0.016$, $p = 0.391$). On a graph, the relationship was represented by a curve with initially increasing values of beta diversity across a range of spatial extents from 732 to 1,153 km, then slowing down at maximum spatial extent 1,783 km (Fig. 3b).

3.2. Mantel test

Partial Mantel test showed pure effect of spatial distances on turnover in species and functional group composition after controlling for the effect of climatic distances, and vice versa. When considering phytoplankton species similarity, in 1995 and 2000, when the effects of climatic distances were removed, the main structuring factor was spatial distances alone (Table 2). If the effect of spatial distances was removed, the effect of climatic distances was not statistically significant. This suggested that sites that were closer together were also more similar in species composition, independently of climatic parameters. Conversely, in 2001 and 2002, when effect of spatial distances was removed, climatic distances were significant alone, while effect of spatial distances was not significant, after controlling for climatic conditions (Table 2). This suggested that sites sharing similar climatic conditions could support similar phytoplankton compositions, regardless of the spatial distances. In 1978 and 2017, species composition showed significant relationships with both climatic and spatial distances suggesting their joint effect (Table 2). Regarding the similarity matrices of phytoplankton functional groups, the turnover patterns in their composition were similar to that of the species composition (Table 2). The exception was 1995 and 2000, when neither spatial distance no climatic distance showed significant relationship with the community similarities. There were no significant linear relationships between the strength of spatial effect (measured as the partial Mantel correlation coefficient) and spatial extent of the study both for species ($p = 0.516$) and functional groups ($p = 0.208$). Nevertheless, the maximum value of the spatial effect was at the largest spatial extent in 1978 (Table 2). Mantel correlation calculated on the basis of the entire data sets revealed that similarity matrices of species composition and functional group composition were significantly positively related ($R = 0.764$; $p < 0.001$).

3.3. Ordination

In the DCA for species composition, local environmental, climatic and spatial variables showed significant (<0.05) relationships with phytoplankton composition (Fig. 4a; Table S4). Based on R^2-values, the spatial variables had the strongest relationship with species composition (Table S4). Among the spatial variables, latitude had the strongest effect followed by elevation on axis 1, while longitude had the strongest relationships followed by watercourse distance with axis 2. From the climatic variables, precipitation CV and mean annual precipitation had significant relationships with the first two axes (Table S4). Among the local variables, pollution also had strong relationship with the first two axes (Table S4). Along DCA axis 1, variability in species composition was mainly related to the long-term changes in anthropogenic pollution and, to a lesser extent, climate changes over the years. A quite different species composition in 1978 displayed by distinct location of samples on the positive side of DCA axis 1 suggested the impact of anthropogenic pollution (Fig. 4a). Indeed, pollution-tolerant and eutrophic cyanobacteria (*Oscillatoria limosa* C.Agardh ex Gomont, *Planktolyngbya limnetica* (Lemmermann) Komárková-Legnerová & Cronberg) (Borics et al., 2007; Padisák et al., 2009), pennate diatoms (*Asterionella formosa* Hassall, *Nitzschia fruticosa* Hustedt, *Ulnaria ulna* (Nitzsch) Compère) (Van Dam et al., 1994) and green algae (*Actinastrum hantzschii* Lagerheim, *Closteriopsis acicularis* (Chodat) J.H.Belcher & Swale, *Crucigenia quadrata* Morren, *C. tetrapedia* (Kirchner) Kuntze, *Desmodesmus communis* (E.Hegewald) E.Hegewald, and *Pediastrum duplex* Meyen) (Borics et al., 2007; Padisák et al., 2009) had positive scores on axis 1, whereas pollution-sensitive and mesotrophic centric diatoms (*Cyclostephanos delicatus* (Genkal) S.J.Casper & W.Scheffler, *Pantocsekiella delicatula* (Hustedt) K.T.Kiss & E.Ács, *P. ocellata* (Pantocsek) K.T.Kiss & E.Ács, *Stephanodiscus minutulus* (Kützing) Cleve & Möller, and *Thalassiosira pseudonana* Hasle & Heimdal) (Padisák et al., 2009) had negative scores on this axis (Table S5). Motile flagellates such as synurophytes (*Mallomonas akrokomos* Ruttner), chrysophytes (*Chrysococcus rufescens* Klebs), cryptomonads (*Komma caudata* (L.Geitler) D.R.A.Hill), green algae (*Chlamydomonas intermedia* Chodat) and centric diatoms (*Skeletonema potamos* (C.I.Weber) Hasle) also had negative scores on this axis (Table S5), reflecting response to climate changes. DCA axis 2 represented downriver gradient in climatic parameters with higher annual precipitation in Upper Danube and larger variability of precipitation and temperature in Middle Danube. This axis separated communities of Upper Danube with higher abundance of benthic diatoms (*Melosira varians* C.Agardh, *Amphora ovalis* (Kützing) Kützing, *Cocconeis placentula* Ehrenberg), cryptophytes (*Chroomonas coerulea* (Geitler) Skuja, *Cryptomonas curvata* Ehrenberg) and green algae (*Desmodesmus denticulatus* (Lagerheim) S.S.An, T.Friedl & E.Hegewald, *D. spinosus* (Chodat) E.Hegewald, *Pandorina morum* (O.F.Müller) Bory, *Pseudopediastrum boryanum* (Turpin) E.Hegewald, and *Sphaerocystis* *schroeteri* Chodat) from communities of Middle and Lower Danube that were dominated by centric diatoms (*Cyclostephanos invisitatus* (M.H.Hohn & Hellermann) E.C.Theriot, Stoermer & Håkasson, *Cyclotella atomus* Hustedt, *C. meduanae* H.Germain, and *Stephanodiscus tenuis* Hustedt) (Table S5).

In the DCA for composition of functional groups, local environmental, climatic and spatial variables showed significant ($p < 0.05$) relationships with groups (Table S6). However, these relationships generally were weaker compared with the respective relationships for species composition (Table S4, S6). In ordination space, group composition showed a relatively larger degree of overlap between years than species composition (Fig. 4b). Along DCA axis 1, group composition was related mostly to downriver longitudinal changes in temperature variability (SD), precipitation, pollution and nutrient enrichment (Table S6). DCA axis 2 mainly reflected the long-term changes in nutrient enrichment and precipitation CV (Table S6). Along axis 1, functional groups **X3**, **Y**, **J**, **W1**, **W2** and **Tb** were separated from groups **A**, **B** and **D** (Table S7), representing changes in group composition between Upper and Middle Danube. Along axis 2, groups **F**, **Lo**, **H1**, **Ws** and **Td** were separated from groups **P**, **G** and **X2** (Table S7), reflecting changes in group composition over the years.

Species composition differed significantly ($R^2 = 0.402$, $p < 0.001$) between the three basin regions (Upper, Middle and Lower Danube). In contrast, the basin regions showed no significant differences ($R^2 = 0.050$, $p = 0.103$) in composition of functional groups.

3.4. Variance partitioning by pRDA

Results of the pRDA performed for phytoplankton metacommunity represented by species indicated the greater importance of spatial and temporal predictors in the variation of species composition (Fig. 5a, Table S8). For the climatic predictor set, the variables selected in the model were temperature SD, July temperature and precipitation CV. The spatial predictor set included MEM eigenvectors of a broad and fine scale (MEM2, MEM6, MEM7, MEM8, MEM20). The pure climatic component (5%) was slightly lower than the pure spatial (6%). The pure temporal component (13%) was substantially higher than joint fraction of temporal and climatic variables (3%). In contrast, the pRDA performed for phytoplankton metacommunity represented by functional groups indicated that the temporal predictor was the main factor to explain the variation in composition of functional groups (Fig. 5b, Table S9). The climatic predictor set included temperature SD and precipitation CV. The spatial predictor set had variables of a broad and fine scale (MEM2, MEM6, MEM7, MEM8, MEM9, MEM22, MEM50). The pure climatic component (4%) was significantly lower than the pure spatial (10%). The shared fraction between temporal and climatic variables (1%) was negligible compared to the pure temporal component (13%).

3.5. Comparison of dispersal models

Variation partitioning showed that MEM and AEM models shared a large amount of the explained variation (19% on average) (Fig. 6a), indicating high degree of collinearity between the models. In some years (1995, 2000 and 2017), only MEM model had significant unique contributions, while the pure fractions of AEM were close to zero. Although the pure fractions of AEM were quite high in 1978 and 2002, they were equal or less than those of MEM. As a result, fractions explained exclusively by MEM were higher (on average 6% of the variation) than those explained by AEM (on average 2% of the variation). This indicates that non-directional MEM model, which reflects the effects of local hydrological connectivity, represented phytoplankton spatial patterns better than AEM model based on directional processes. Variation partitioning contrasting MEM model based on watercourse distances and MEM model based on dam numbers (DAM) showed that MEM outperformed DAM in explaining phytoplankton composition because fractions explained exclusively by MEM were higher (on average 4% of the variation) than those explained by DAM (on average 1% of the variation). These models also shared a substantial proportion of explained variance (20% on average) (Fig. 6b). Together, these results suggested that the spatial patterns of phytoplankton composition were better explained by the model accounting for spatial proximity among sites, i.e., local influence of tributaries, dams and impounded river sections.

4. Discussion

4.1. Environmental and spatial constraints of phytoplankton beta diversity

In this study, we evaluated the relative importance of climatic and spatial factors in the variation of taxonomic and functional structure of the Danube River phytoplankton, using dataset sampled over a long time-period at different spatial extents. We hypothesised that phytoplankton beta diversity would be positively related to both climatic heterogeneity and spatial extent due to sub-continental scale of the study. This hypothesis was only partially supported. Our results revealed significant and positive relationship between beta diversity and climatic heterogeneity, indicating significant effect of spatial variation in climatic parameters on compositional heterogeneity of phytoplankton community. In contrast, the relationship between beta diversity and spatial extent was not significant, although there was an increasing trend of beta diversity with an increase in watercourse distance. We also hypothesised that the influence of spatial factors on phytoplankton structure should increase with increasing spatial extent. This is because dispersal-driven assembly mechanisms are scale-dependent processes (e.g., Soininen et al., 2011). However, we found only limited support for this hypothesis. The partial Mantel test showed that the maximum strength of spatial effect was at the largest spatial scale of our study, but unlike we assumed, the strength of relationship between compositional similarities and spatial distances was unrelated to spatial extent. Taken together these results may lead to inaccurate conclusion that dispersal-related factors played a role in structuring of phytoplankton community, but to a lesser extent than climatic conditions. However, the result of the pRDA indicated that spatial factors played an equal (for species) or even larger (for functional groups) role vs. the role of climatic parameters. The simplest explanation for this discrepancy in our results may be that the high temporal variation of determinants of community structure preclude adequate evaluation of spatial patterns in microalgal metacommunities when the explanation is based on snapshot sampling (Heino et al., 2015b; Wojciechowski et al., 2017). Moreover, anthropogenic disturbance (nutrient enrichment), that varied considerably over the entire time span of our study (Dokulil, 2006; Istvánovics, Honti, 2012; Abonyi et al., 2018), could be a disassembly mechanism decoupling the relationship between beta diversity and spatial extent (Bini et al., 2014). We next discuss our main findings and causes of the discrepancy between the results in detail.

4.2. Factors responsible for longitudinal changes in phytoplankton

In our study, the length of the studied river segments varied from 732 to 1,783 km (Table 1). The largest dataset of 1978 was represented by samples collected from Upper, Middle and Lower Danube, while other datasets were composed by samples from Upper and Middle Danube. According to the results of partial Mantel test, the pure effect of climate factors was not statistically significant at relatively small spatial extents in 1995 and 2000. This indicates that at smaller spatial scale the variability of climate parameters along the watercourse might be too low to produce spatial turnover of community composition. On the other hand, the statistically significant relationship between community similarity and spatial distance suggested that some unmeasured environmental variables were responsible for spatial patterns in phytoplankton metacommunity (Soininen, 2007). Consequently, the temporal variation in the strength of the spatial effect may be explained by the temporally varying contribution of spatially associated factors such as hydrology and nutrients that are known to affect phytoplankton composition (Dokulil, 2014; Dokulil, 2015; Abonyi et al., 2018), but were not included directly in our analysis. Considering pure spatial aspect, the small-scale spatial patterns of phytoplankton composition can be driven by mass effect (Heino et al., 2015a). Due to surplus dispersal of species into suboptimal conditions, this process reduces variation in species composition and leads to a decoupling between community structure and environmental variables (Leibold et al., 2004). The low beta diversity coupled with a lack of a relationship between community similarity and climatic distance in 1995 and 2000 suggested that mass effect was likely to be a driving mechanism of phytoplankton metacommunity at small spatial extents. Besides mass effect, dispersal limitation also may be evident at very small spatial scales for microalgal communities (Bottin et al., 2014; Dong et al., 2016; Lansac-Tôha et al., 2019). In 1995 and 2000, phytoplankton was mainly sampled in Upper Danube, the river section with multiple dams and in-stream reservoirs. Thus, because dams act as dispersal barriers for passive dispersers, we suggest that limited dispersal of phytoplankton imposed by dams can also prevent species sorting from occurring, leading to the lack of the relationships between phytoplankton composition and climatic variables.

At broader spatial extents, the relative importance of spatial and climatic variables is directly related to the magnitude and frequency of precipitation. In 2001 and 2002 under conditions of heavy precipitation and high precipitation seasonality, climate variables alone contributed to the spatial variations in phytoplankton composition. A possible reason for the lack of the relationships between phytoplankton similarity and spatial distance is that during high discharge events induced by rainfall the phytoplankton composition is strongly dependent on spatial rainfall patterns such as spatially-varied flow in the main channel (Reynolds, Descy, 1996), enhanced lateral connectivity (Tockner et al., 2000; Chaparro et al., 2019) and inflow from tributaries (Kiss, Genkal, 1996; Dokulil, 2014). Thus, under high river flow conditions phytoplankton species are sorted mainly along rainfall gradient, whereas dispersal limitation becomes unimportant in shaping community structure. In 1978 and 2017 under conditions of low precipitation and more regular flow regime, compositional similarity showed a significant relationship with both climatic and spatial distances. Moreover, spatial effects outperformed effects of climatic variables, indicating that environmental filtering along climate gradient was likely constrained by limited dispersal of phytoplankton among sampling sites. Therefore, at large spatial scale of our study the role of dispersal processes in structuring phytoplankton metacommunity cannot be discarded. However, because other important environmental factors (i.e., nutrients, hydrology) were not included in the partial Mantel test, these results should be interpreted with caution.

Results of the pRDA gave additional insight into relative importance of drivers shaping phytoplankton metacommunity, indicating that spatial variables (including broad- and fine-scale variables) outperformed large-scale climatic factors. Furthermore, a small shared fraction of variation between climatic factors and spatial variables indicates that the major part of climatic variability driving changes in community structure was not spatially structured. Although we cannot rule out the possibility that other unmeasured factors can account for some part of spatial variation, our results suggested that biogeographic patterns of phytoplankton community are not only determined by environmental and climatic factors, but dispersal limitation also an important driver of community variation. The DCA ordination showed that species composition of phytoplankton community was significantly different among the three basin regions (Upper, Middle and Lower Danube). Therefore, the pronounced longitudinal patterns in phytoplankton distribution were caused by distinct regional species pools, which determined differences in species occurrences in particular geographic areas as shown for centric diatoms (Kiss et al., 2012). Our findings are consistent with few studies reporting that effects of spatial factors can overcome effects of environmental factors in explaining biogeographic patterns of freshwater algal communities (Heino et al., 2010; Tang et al., 2013; Xiao et al., 2018). However, as far as we know, this is the first study that reports the higher importance of limited dispersal vs. species sorting in shaping riverine phytoplankton metacommunities.

Concerning environmental drivers of phytoplankton biogeography, the DCA ordination revealed spatial patterns in phytoplankton composition in response to the west-east climatic gradient of decreasing precipitation and increasing seasonality of precipitation (precipitation CV) and temperature (temperature SD). This gradient represents climatic changes across different ecoregions of the Danube River basin. In our study, the most of the studied river stretches cross two ecoregions from west to east: Central Highlands and Hungarian Lowlands. The shift from mountainous and hilly areas to lowland relief co-occurs with climatic changes from humid alpine to a moderately dry continental (ICPDR, 2013). Besides climate regional differences, the phytoplankton longitudinal patterns also reflect differences in hydromorphology between Upper and Middle Danube as evidenced by notable longitudinal changes in biomass of dominant algal groups (centric diatoms and green algae). Centric diatoms increase downward and attain the highest biomass values in the Middle Danube, whereas green algae exhibit upward trend. Significant relationships between temperature seasonality and biomass of centric diatoms and green algae (positive and negative, respectively) reflect opposite positions of these algal groups along geographic gradient associated with changes in climate conditions in the Danube River basin. According to previous reports, higher biomass of green algae reflects impact of an extensive dam construction in the Upper Danube (Dokulil, Kaiblinger, 2008; Dokulil, 2015). Phytoplankton functional composition also responds to differences in hydrological conditions between the Upper and the Middle Danube. Small chlorococcalean algae (**J**) and cryptophytes (**Y**) are associated with the Upper Danube that corresponds with the previous reports (Stanković et al., 2020). Co-occurrence of these two groups with euglenoids and diniflagellates (**W1**, **W2**) and *Chrysococcus, Koliella* and *Chromulina* (**X3**) indicates a specific set of environmental conditions of dammed river sections which are characterised by shallowness, increased light availability and moderate turbulence (Abonyi et al., 2020). In contrast, the occurrence of benthic diatoms (**Tb**) reflects free-flowing river segments similar to highly turbid headwater systems. Further downstream, deep, well-mixed, and turbid environments favor centric diatoms, and therefore a co-dominance of functional groups **A**, **B**, and **D** is a regular characteristic of the Middle Danube (Stanković et al., 2020). The clear response of taxonomic and functional group compositions of the Danube River phytoplankton to changes in climatic and hydrologic parameters corresponds to other large-scale studies on algae distribution in rivers (Potapova, Charles, 2002; Bottin et al., 2014).

We applied different dispersal models accounting for the non-directional, directional and dam-impacted spatial dynamics of phytoplankton community. If two models are substantially confounded, but both contribute significantly to explaining community variation, this means that metacommunity is regulated by two types of processes simultaneously (Blanchet et al., 2011). Pairwise comparisons of eigenvector models showed that the models were largely confounded, but across the whole dataset the non-directional model (MEM) had better performance than the directional model (AEM). This indicates that spatial dynamics of phytoplankton community was more shaped by local influence of tributaries, dams and impounded river sections than by the prevailing water flow. To our knowledge, there are only few studies comparing various dispersal processes in shaping lotic algal communities, which demonstrate strong effect of directional flow on community structure of benthic diatoms in lowland and mountain rivers (Liu et al., 2013; Dong et al., 2016). Also, the directional downstream water flow was an important agent structuring riverine phytoplankton communities (Bortolini et al., 2017; Lansac-Tôha et al., 2019). Thus, our result was unexpected in comparison with earlier researches reporting high signature of directional dispersion in lotic algal communities. Differences between our and previous researches may be due to the strong biogeographic patterns exhibited by the Danube River phytoplankton that were possible to detect due to large geographic scale of the study (Heino, 2011). Furthermore, our results suggested that directional and local processes likely exhibited different degree of importance depending on flow conditions. For instance, this is evidenced by the AEM model components in 2001 and 2002, when increased rainfall amount and variability was recorded. Thus, the relative strength of the directional water flow processes increases during rainfall-induced high-flow events. Mechanistically, the processes responsible for directional changes in phytoplankton composition are linked to algal transport and gradual changes in nutrient and underwater light availability along the downriver flow path. It should be noted that during elevated discharge the MEM or DAM model components gain importance simultaneously with AEM. This indicates that gradual longitudinal changes in phytoplankton composition were maintained by active hydraulic transport and local sources of inoculum (i.e., impounded reaches and tributaries). In comparison, under conditions of low precipitation and steady flow in 2000 and 2017 MEM model explained alone a significant amount of longitudinal variation in phytoplankton composition. Therefore, in the lack of pronounced hydrological gradient the local dispersal due to lateral connectivity and influence of dams and impoundments become predominant mechanisms driving longitudinal distribution of phytoplankton metacommunity. Moreover, a reduction of hydrologic connectivity in the main channel due to an extensive damming and discharge regulation in the Upper Danube leads to a patchy nutrient distribution (i.e., large range in nutrient concentrations). Consequently, changes in nutrient concentration along the river watercourse can also induce a spatially-structured species-sorting effect that locally influences phytoplankton community. In fact, species-sorting related to local variation in water chemistry can contribute to spatial community turnover that were detected under low discharge (see above). This result is consistent with other research, which found an increased importance of local environmental filtering in stream ecosystems during decreased discharge (Liu et al., 2013). It should also be borne in mind that phytoplankton was sampled downstream of dams in impounded reaches of the Upper Danube. This may have introduced some bias to the observed differences in the relative performance of alternative spatial models at small scales of our study. We do not, however, expect this factor to affect the contribution of different models when they were applied to large spatial extents.

4.3. Factors regulating phytoplankton changes at long-term scale

In the River Danube, long-term changes in phytoplankton community structure are thought to be related to the temporal variability of temperature and precipitation-dependent hydrology, which regarded as key factors of phytoplankton temporal dynamics according to the riverine productivity model (Thorp, Delong, 1994). Increasing trends in precipitation (mainly in the Upper Danube) and temperature seasonality are in a good agreement with climate change scenario in the River Danube basin (ICPDR, 2013). Our study revealed the decreasing trend of the total phytoplankton biomass over the entire period of the study that corresponds to the results of long-term phytoplankton studies in Middle Danube (Verasztó et al., 2010; Abonyi et al., 2018). According to our findings, the total phytoplankton biomass was negatively related to mean annual precipitation, indicating that meteorological conditions had indirect influences on phytoplankton through changes in hydrology.

Our expectation that temporally varying climatic parameters should be important predictors of long-term variability of phytoplankton community gains only partial support from the pRDA. Indeed, the shared fraction between climatic and temporal factors was considerable in comparison with the pure climate fraction indicating that climatic variables were temporally structured. However, the shared fraction between climatic and temporal predictors was negligible if compared to the purely temporal component of the explained variation in taxonomic and functional group compositions. This suggests that large part of temporal variation of phytoplankton community can be attributed to unmeasured environmental factors and stochastic processes relaying on random dispersal such as priority effect (Fukami, 2015). Overall, our results showed that temporal variables were even more important than environmental and spatial variables in structuring the Danube River phytoplankton community, as also found in studies of subtropical phytoplankton (Bortolini et al., 2019; Lansac-Tôha et al., 2019).

In agreement with the pRDA results, the DCA ordination showed that the contribution of climatic variables to the long-term changes in taxonomic and functional group compositions was statistically significant. Under condition of the global warming, due to changed thermal structure of water column in stratified systems algal species with small-sized cells and those that are able to regulate their buoyancy will be favoured (Winder, Sommer, 2012). In agreement with this, an increasing trend over time showed flagellate algae from different orders such as synurophytes

(*Mallomonas akrokomos*), chrysophytes (*Chrysococcus rufescens*), cryptophytes (*Komma caudata*) and green algae (*Chlamydomonas intermedia*). Among functional groups, the relative abundance of small chlorococcalean algae (**F**: *Kirchneriella lunaris* (Kirchner) Möbius, *K. obesa* (West) West & G.S.West, *Dictyosphaerium* spp., *Micractinium pusillum* Fresenius, *Oocystis lacustris* Chodat), large flagellates (**Ws**: *Synura petersenii* Korshikov; **Lo**: *Ceratium hirundinella* (O.F.Müller) Dujardin, *Peridinium* spp., *Gimnodinium* spp.) and cyanobacteria with gas vesicles (**Lo**: *Coelosphaerium kuetzingianum* Nägeli, *Merismopedia glauca* (Ehrenberg) Kützing, *M. tenuissima* Lemmermann; **H1**: *Anabaena* spp., *Aphanizomenon flosaquae* Ralfs ex Bornet & Flahault) increased also over time. The increasing presence of stratification-tolerant algae in the main river channel can be explained by their supply from floodplain waterbodies during flood events when high lateral connectivity is established in the upper stretches of the Danube River (Chaparro et al., 2019). Additionally, in the Upper and Middle Danube limnophilic phytoplankton taxa are supplied by impounded tributaries. Increase in relative abundance of epiphytic diatoms (*Cocconeis placentula*) and filamentous green algae (*Mougeotia*), belonging to a group **Td,** most likely occurred in response to higher precipitation and more frequent high-flood events that resulted in their detachment from substrates. Contrarily, under conditions of reduced precipitation and low water discharge colonial diatoms (**P**: *Fragilaria crotonensis* Kitton, *Aulacoseira granulata* (Ehrenberg) Simonsen) and mixotrophic flagellates (**X2**: *Cryptomonas* spp.) tolerant to water column mixing and moderate turbulence increased in abundance. In general, these findings agree with previous study, showing increased abundances of benthic diatoms and limnophilic elements of phytoplankton in response to higher amount of precipitation and flooding frequency in the middle reach of the Danube River (Abonyi et al., 2018). Water temperature rise also promoted changes in species composition, for instance, a gradual increase in abundance of stenothermic species *Skeletonema potamos* was recorded over the last few decades (Kiss et al., 2012). Hence, our results indicate that the riverine productivity model (RPM), which emphasises the driving forces of light, temperature and discharge (Thorp, Delong, 1994; Dokulil, 2014; Dokulil, 2015), is a reliable model to describe spatio-temporal distribution of the River Danube phytoplankton at the regional scale.

In addition, results of the DCA ordination revealed that in 1978 the species composition was distinctly different from those of consequent years that can be attributed to the effect of temporal change in trophic level. The shift in composition of functional groups from eutrophic (**P**, **G**) to mesotrophic taxa (**Lo**, **Ws, Td**) over time reflects changes in community composition in response to re-oligotrophication trend in the Danube River due to nutrient load reduction in the 1990s and 2000s (Dokulil, 2006; Istvánovics, Honti, 2012, Abonyi et al., 2018). Anthropogenic nutrient enrichment is associated with taxonomic homogenization and overall decline in beta diversity (Donohue et al., 2009; Vilar et al., 2014; Zorzal-Almeida et al., 2017). Therefore, we speculate that in 1978 high nutrient level decreased phytoplankton compositional heterogeneity, that resulted in a non-significant relationship between beta diversity and spatial extent (see above). Our result is consistent in certain extent with prior research, the study of benthic diatoms also found that increase in beta-diversity across multiple spatial scales was attenuated by increasing nutrient enrichment (Leboucher et al., 2019).

4.4. Drivers of species and functional group compositions

We expected that functional group composition would be better explained by climatic parameters than by spatial variables, based on previous findings at large geographic scale (Soininen et al., 2016). However, we found no support for this hypothesis, since the pRDA demonstrated that functional groups were more dependent on spatial factors. Our findings are in agreement with previous study of subtropical reservoirs, demonstrating that environmental factors (including hydrological and climatic) were less important than spatial predictors in explaining functional composition of phytoplankton community (Bortolini et al., 2019). Despite a non-significant difference in composition of functional groups between the three basin regions of the Danube River, the spatial predictors (MEMs) of broader scales for functional composition were the same as for taxonomic composition, indicating strong biogeographic pattern in longitudinal distribution of functional groups. Moreover, fine-scale spatial predictors were even more significant for functional groups than for species, suggesting that different dispersal abilities of the groups according to their functional traits (i.e., motility, buoyancy, cell shape and size) were responsible for fine-scale spatial patterns. These findings have important implication for bioassessment in that the development of reliable taxonomic and functional indicators of water quality in the Danube River should be based on separation into limited geographical areas with uniform environmental characteristics and floristic composition (Potapova, Charles, 2002; Bottin et al., 2014).

According to the result of Mantel test, species and functional groups showed significant concordance in the spatio-temporal dynamics, suggesting that distributions of species and functional groups were mainly influenced by similar underlying mechanisms. The results of the pRDA indicated that taxonomic and functional composition were nearly equally sensitive to longitudinal variation in climatic parameters. Using climatic variables as predictors of metacommunity structure in large-scale studies allows to identify whether geographical patterns in species distribution are climate-driven. Otherwise, they might be spuriously interpreted as pure spatial structures (Rocha et al., 2017). Including climatic variables in our study, we were able to conclude that the observed spatial patterns in phytoplankton metacommunity represent effect of limiting dispersal. Overall, the high congruence in the response of taxonomic and functional compositions to environmental constraints in our study suggests a low level of functional redundancy of phytoplankton community. This highlights the importance of measures directed towards protection and conservation of current state of biodiversity in riverine landscapes of the Danube River basin.

5. Conclusions

In this study, the relative role of climatic factors, spatial and temporal variables in spatio-temporal variation of taxonomic and functional structure of the Danube River phytoplankton were analysed using raw-data and distance-based approaches. The main outcomes are:

(1) At large spatial scales of our study, we found that spatial effect outperformed effect of climatic variables, indicating that dispersal constraints likely preclude efficient species sorting along climate gradient. However, we did not find an increase in the strength of spatial affect with increasing spatial extent due to temporally varying hydrological conditions which are important in determining the role of dispersal-related factors in phytoplankton communities.

(2) Phytoplankton beta diversity showed a significant and positive relationship with climatic heterogeneity. Although we found a rising trend of beta diversity with increasing spatial extent, the beta diversity-scale relationship was not significant. Decrease in phytoplankton compositional heterogeneity due to high nutrient level in 1978 resulted in the non-significant relationship between beta diversity and spatial scale of the study.

(3) Temporally varying climatic parameters were important drivers of long-term dynamics of phytoplankton community. Yet the largest part of temporal phytoplankton dynamics was unrelated to local environmental and climatic factors, indicating a high level of stochasticity in temporal variability of phytoplankton communities.

(4) Spatial distribution of phytoplankton functional groups showed strong dependence on geographical location. A high degree of regional specificity in taxonomic and functional composition of the Danube River phytoplankton implies considerable differences in the regional species pools. Therefore, the development of more reliable water quality indicators should be based on separation into the major basin regions, namely the Upper, Middle and Upper Danube.

(5) Spatial dynamics of phytoplankton community was more strongly dependent on local influence of tributaries, dams and impounded river sections than on flow directionality. At the same time, the longitudinal variation in community composition was driven by a climate gradient spanning the entire Danube basin. Given that high discharge events lead to an enrichment of riverine phytoplankton with benthic and limnophilic elements, bioassessment program should account for regional and inter-annual variation in meteorological parameters.

Data availability statement

The biological data used in this study are not available in any database.

Declaration of competing interests

Authors declare no conflict of interests.

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Table 1

Description of the River Danube's sections where phytoplankton samples were collected during the study.

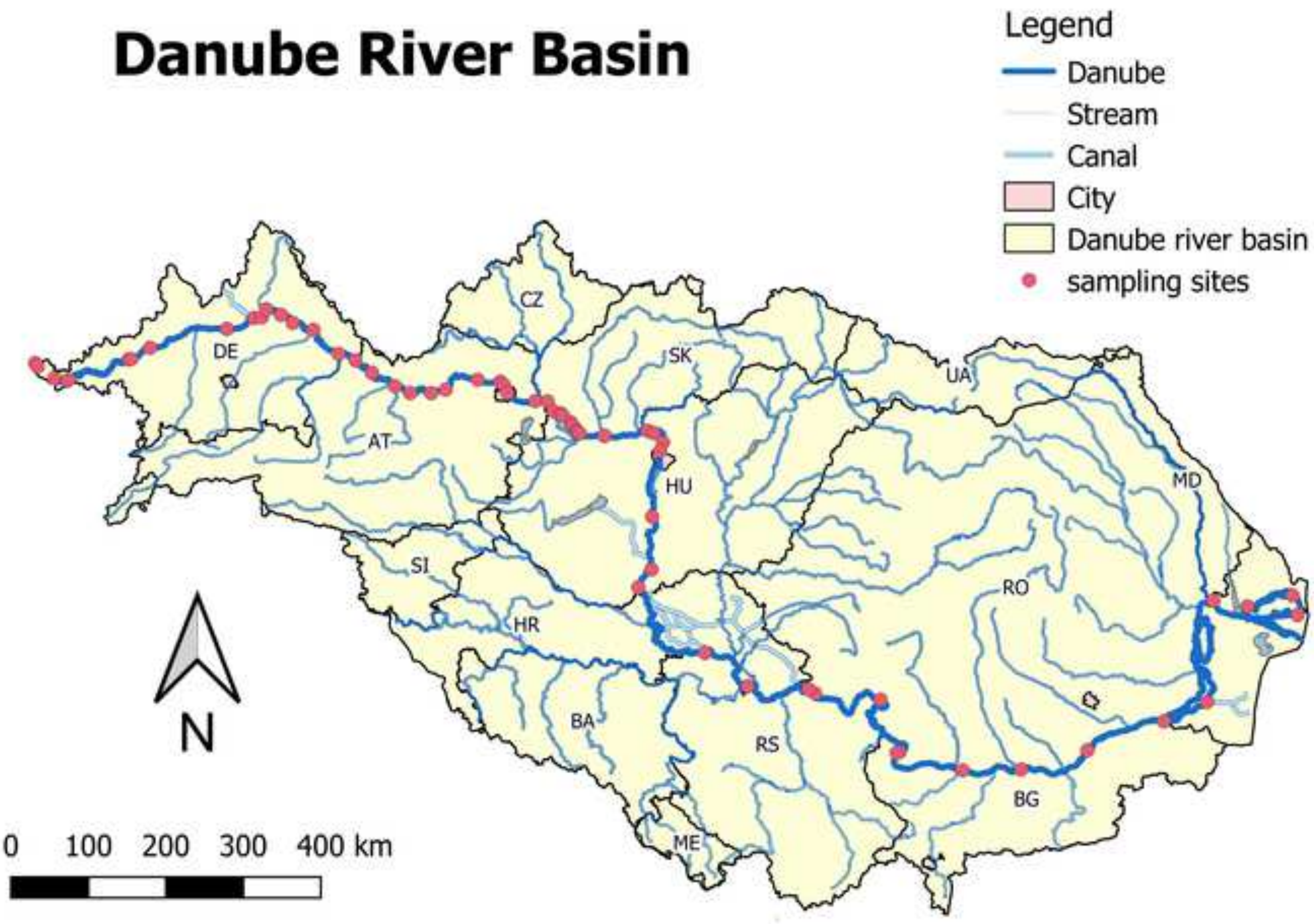
Month and year of sampling	No. of sites	Watercourse distance (10^3 km)	No. of dams and barrages	River sections
August-September 1978	14	1.783	2	Upper Danube (Bratislava – Gabcikovo); Middle Danube (Komarom – Calafat); Lower Danube (Bechet – Izmajil).
September 1995	15	0.732	16	Upper Danube (Bad Abbach - Gabcikovo); Middle Danube (Göd).
August 2000	9	0.789	18	Upper Danube (Ingolstadt – Bad Deutsch); Middle Danube (Nagybajcs – Göd).
August 2001	14	1.128	36	Upper Danube (Nasgenstadt – Bad Deutsch); Middle Danube (Komarom – Baja).
August 2002	9	1.153	44	Upper Danube (Breg-Furtwangen – Melk); Middle Danube (Komarom – Göd).
September 2017	10	1.114	47	Upper Danube (Donaueschingen – Bad Deutsch); Middle Danube (Komarom – Göd).

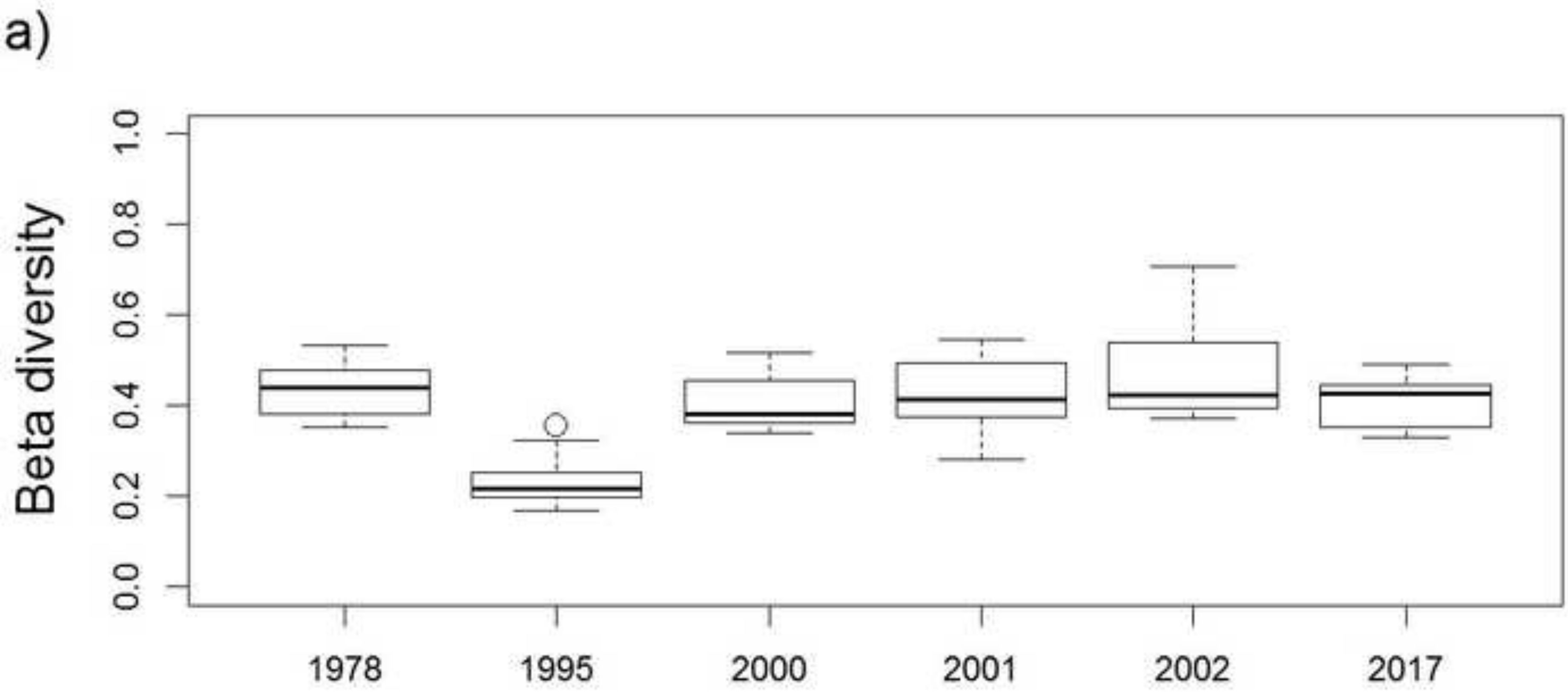
Table 2

Results of Mantel and partial Mantel tests for the correlation (*r*) of taxonomic and functional group similarity (Bray-Curtis index) with geographic and climatic distances (Euclidean distance). The two-tailed *p*-values (null hypothesis: *r* = 0) were obtained by the bootstrapping procedure with 1000 resampling (significant *r* values are indicated in bold).

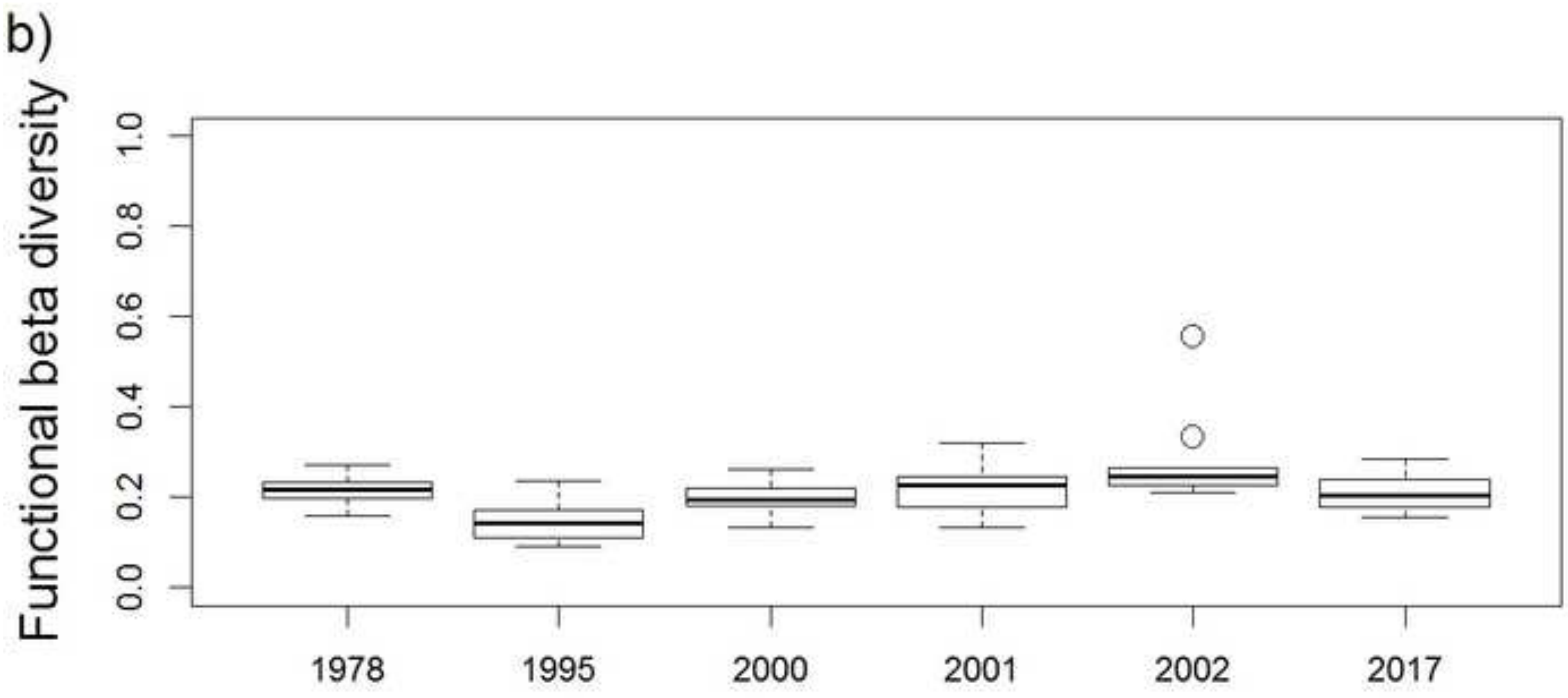
Year	Geographical		Climatic		Geographical - Climatic		Climatic - Geographical	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Taxonomic similarity								
1978	– **0.781**	0.001	– **0.533**	0.002	– **0.675**	0.001	**– 0.430**	0.004
1995	– **0.341**	0.005	– 0.157	0.214	– **0.308**	0.022	– 0.042	0.767
2000	– **0.470**	0.006	– 0.141	0.378	– **0.579**	0.003	0.045	0.804
2001	– **0.749**	0.001	– **0.801**	0.001	– 0.155	0.258	– **0.448**	0.001
2002	– **0.348**	0.043	– **0.557**	0.004	0.245	0.816	– **0.430**	0.022
2017	– **0.592**	0.001	– **0.450**	0.009	– **0.530**	0.003	– **0.342**	0.046
Functional similarity								
1978	– **0.619**	0.001	– **0.585**	0.001	– **0.448**	0.002	**– 0.399**	0.004
1995	– 0.131	0.396	0.001	0.999	– 0.140	0.392	0.051	0.762
2000	– 0.049	0.766	– 0.052	0.822	– 0.046	0.791	– 0.050	0.809
2001	– **0.674**	0.001	– **0.736**	0.001	– 0.081	0.519	– **0.406**	0.004
2002	– 0.298	0.072	– **0.516**	0.004	– 0.042	0.390	– **0.386**	0.032
2017	– **0.496**	0.005	– **0.379**	0.022	– **0.421**	0.015	– **0.337**	0.040

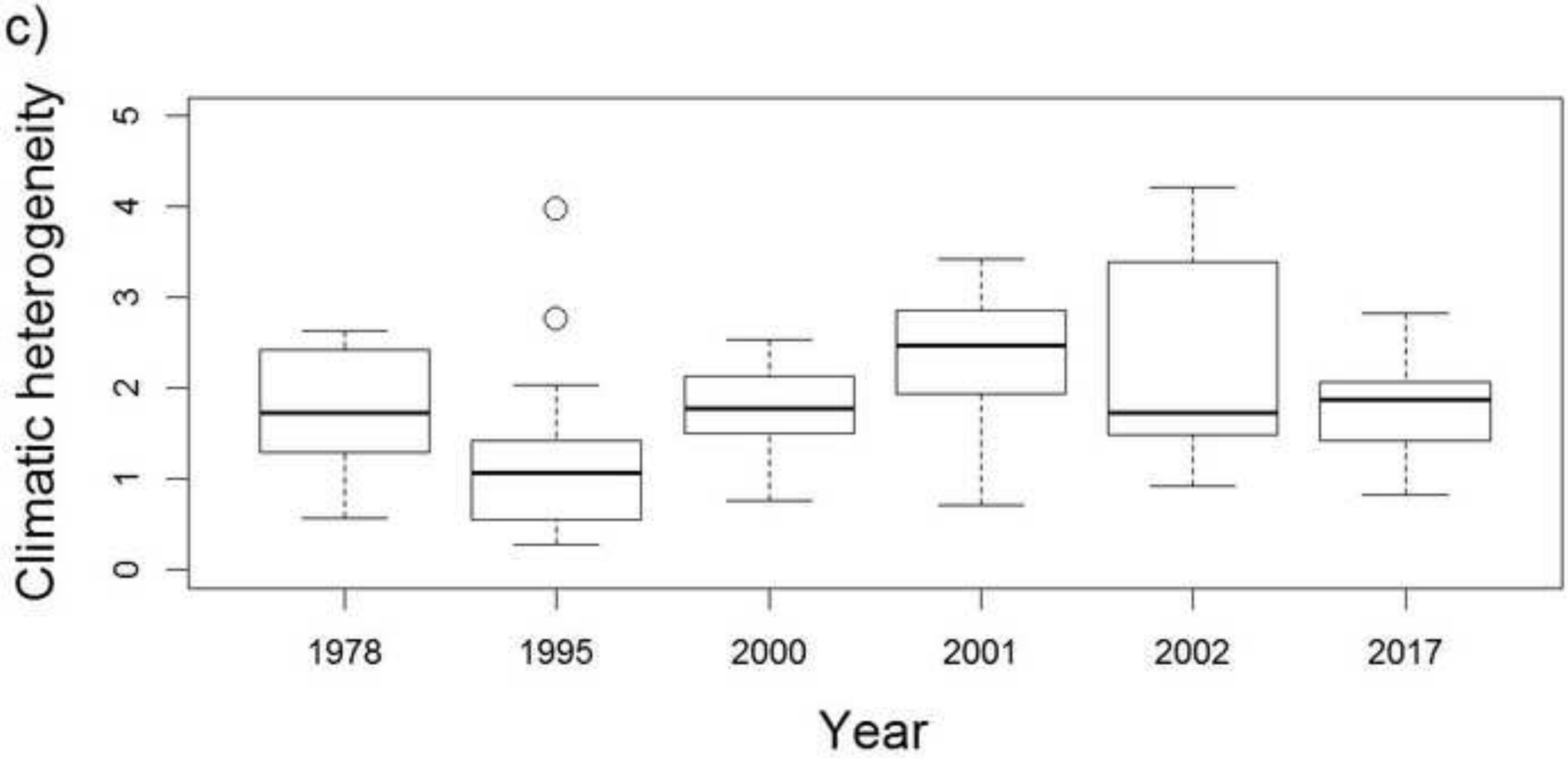
Figure 1

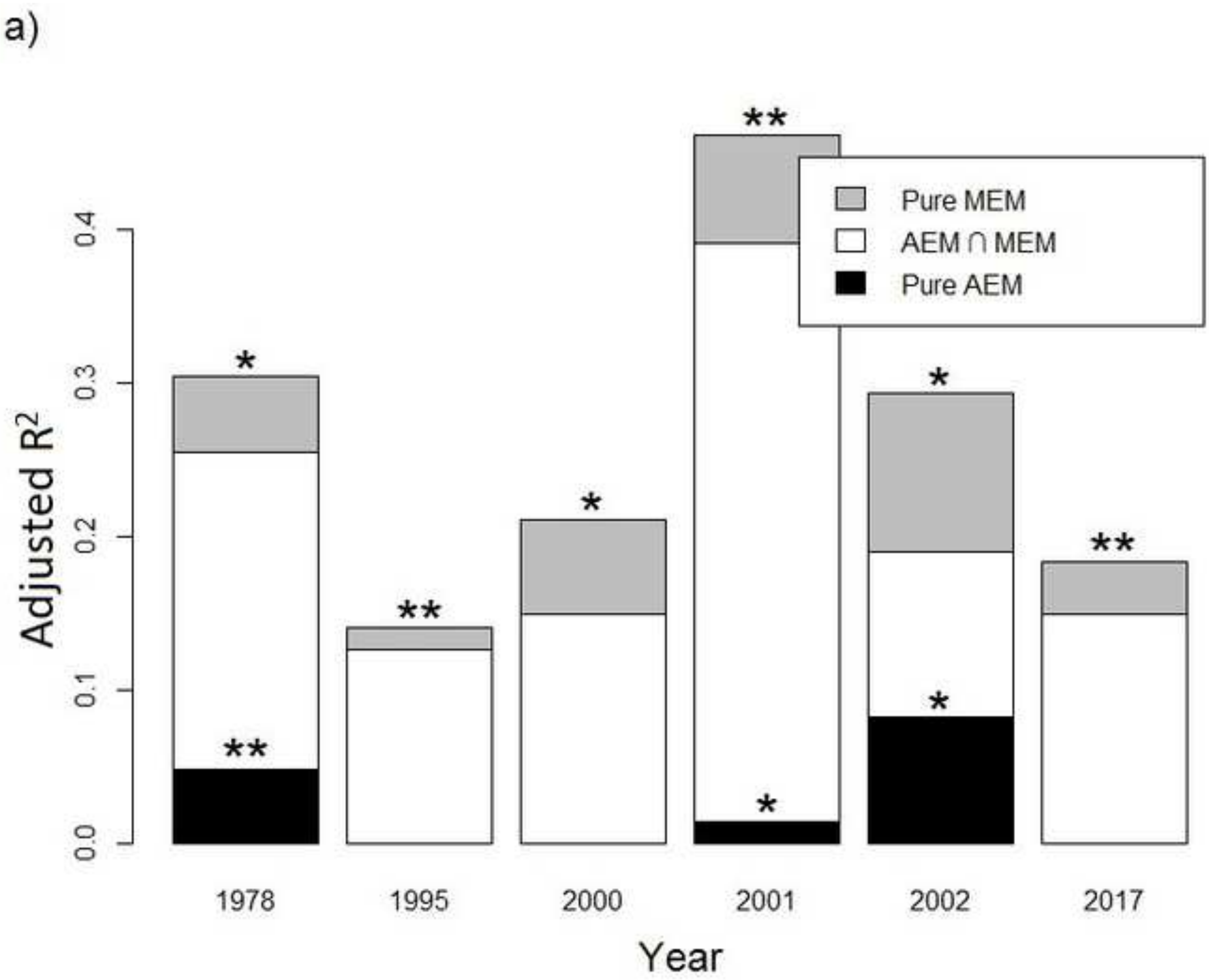

Danube River Basin
Legend
Danube
Stream
Canal
City
Danube river basin
● sampling sites
DE
CZ
SK
UA
AT
HU
MD
SI
RO
HR
BA
RS
BG
ME
N
0 100 200 300 400 km


a)
Beta diversity
0.0
0.2
0.4
0.6
0.8
1.0
1978
1995
2000
2001
2002
2017

Click here to access/download;Figure;F2b.jpg




c)
Climatic heterogeneity
0
1
2
3
4
5
1978
1995
2000
2001
2002
2017
Year


a)
Adjusted R^2
0.0
0.1
0.2
0.3
0.4
Pure MEM
AEM ∩ MEM
Pure AEM
*
**
**
*
**
*
*
*
**
1978
1995
2000
2001
2002
2017
Year

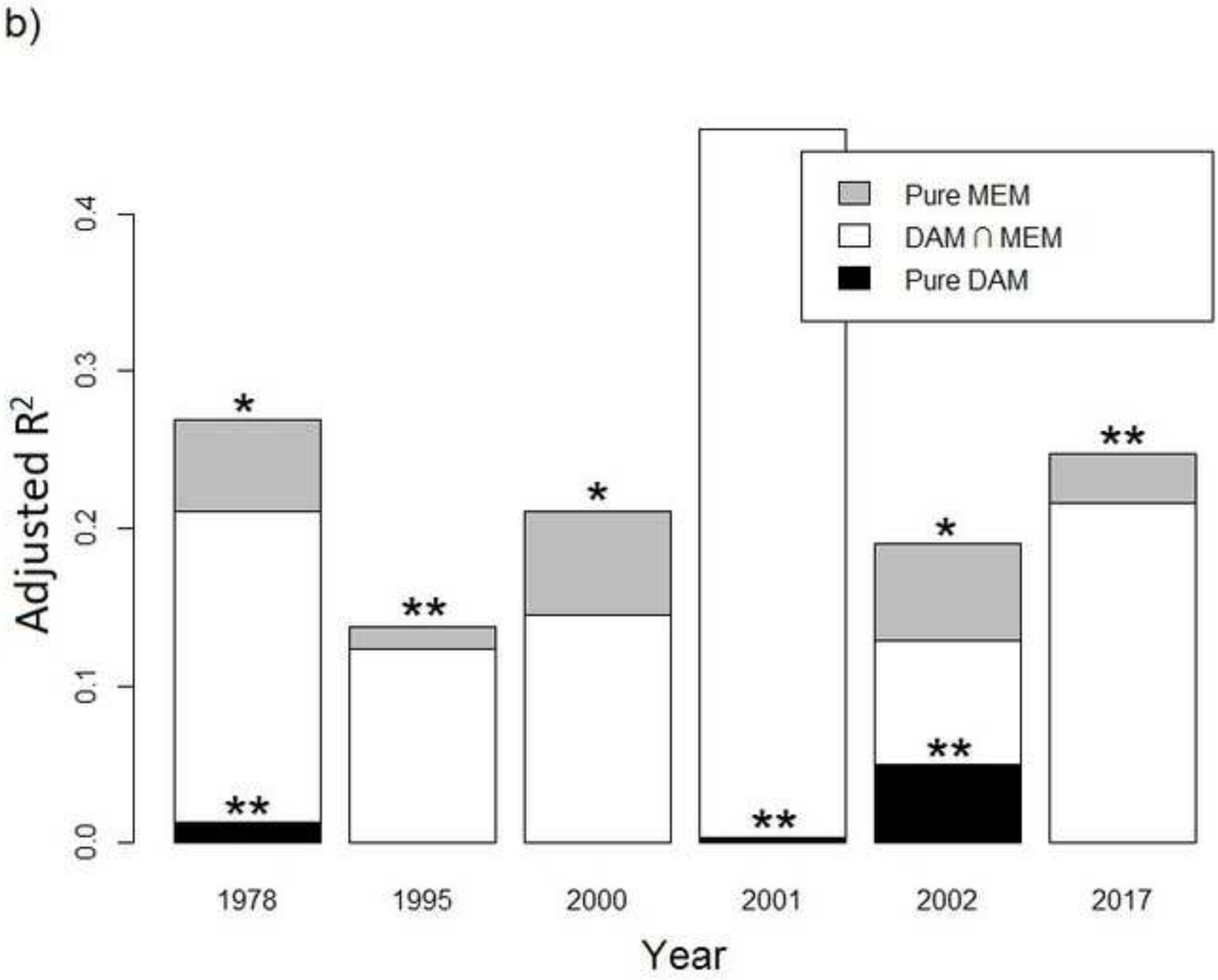

b)
Adjusted R^2
0.0
0.1
0.2
0.3
0.4
Pure MEM
DAM ∩ MEM
Pure DAM
*
**
**
*
**
*
**
**
1978
1995
2000
2001
2002
2017
Year

Figure legends

Fig. 1. Map of the Danube River basin showing the distribution of phytoplankton sampling sites along Danube River.

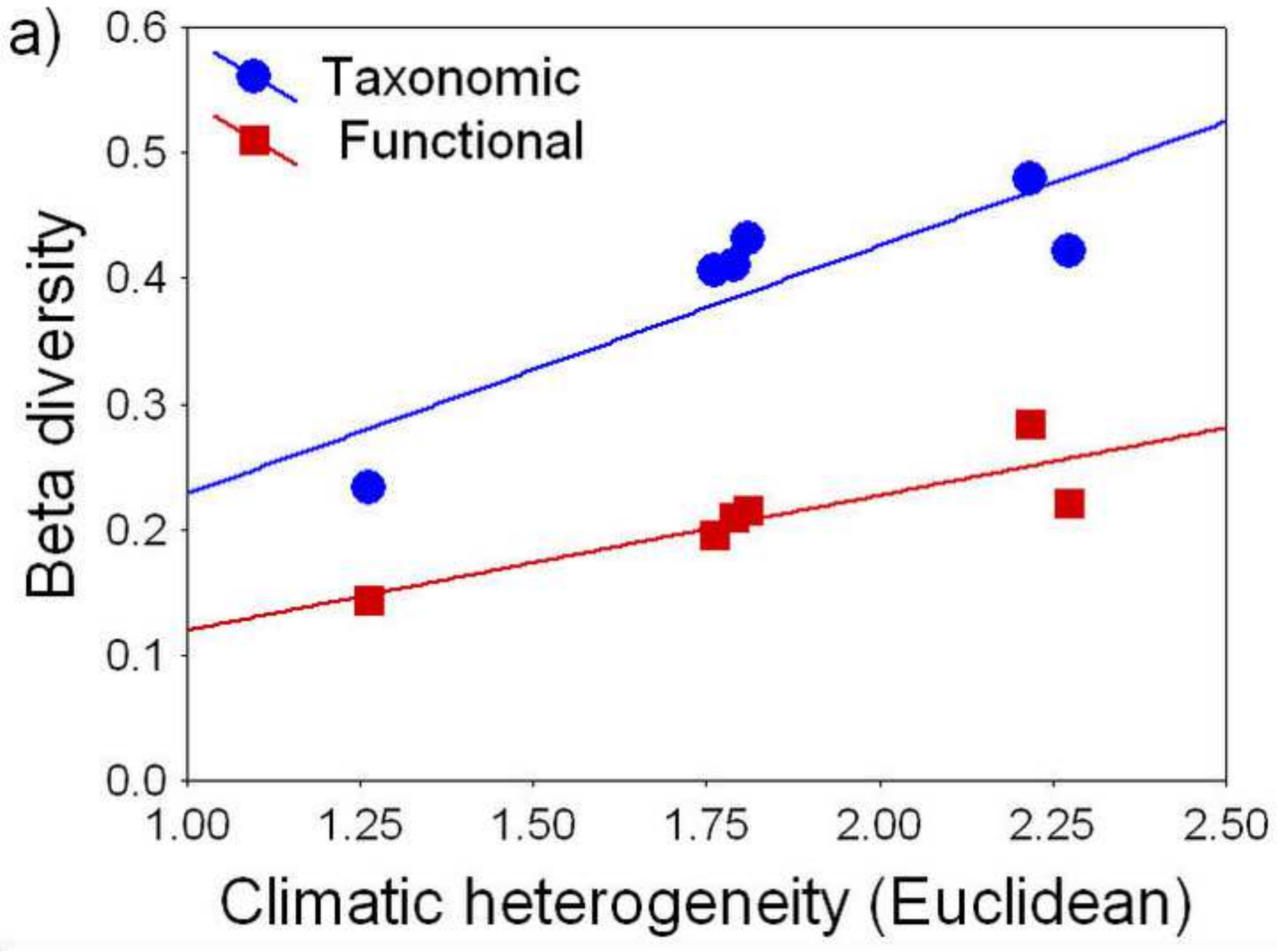
Fig. 2. Dynamics of beta diversity of phytoplankton species (a) and functional groups (b) and climatic heterogeneity (c) over the years.

Fig. 3. The relationships between phytoplankton species and functional group beta diversity and climatic heterogeneity (a) and spatial extent (b). Line graphs present significant ($p < 0.05$) linear relationships (a) and curves fitted by eye (b).

Fig. 4. DCA plots for axes 1 and 2 using phytoplankton species (a) and functional groups (b). Local environmental, climatic and spatial variables significantly ($p < 0.001$) related to community structure are shown with arrows. Key: Temp.SD, temperature SD; Temp.July, temperature in July; Temp.Sept, temperature in September; Precip.m.annual, mean annual precipitation; Precip.CV, precipitation CV; Lat, latitude; Long, longitude; Distance, watercourse distance.

Fig. 5. Variation partitioning-based Venn diagrams showing the relative contribution of local environmental (Env), climatic (Cli), spatial MEM eigenvectors (Spa) and temporal dummy variable separating years (Temp) for composition of phytoplankton species (a) and functional groups (b). Values represent the adjusted R^2-values. Negative fraction values are not presented. Statistical significance: ** $p \leq 0.01$.

Fig. 6. Result of variation partitioning performed on spatial eigenfunction models for phytoplankton communities in different years: (a) non-directional Moran's eigenvector map (MEM) models versus directional asymmetric eigenvector map (AEM) models and (b) MEM models versus spatial models based on dam numbers (DAM). Statistical significance: * $p \leq 0.05$, ** $p \leq 0.01$. Negative fraction values are not shown.


a)
Taxonomic
Functional
Beta diversity
0.0
0.1
0.2
0.3
0.4
0.5
0.6
1.00
1.25
1.50
1.75
2.00
2.25
2.50
Climatic heterogeneity (Euclidean)

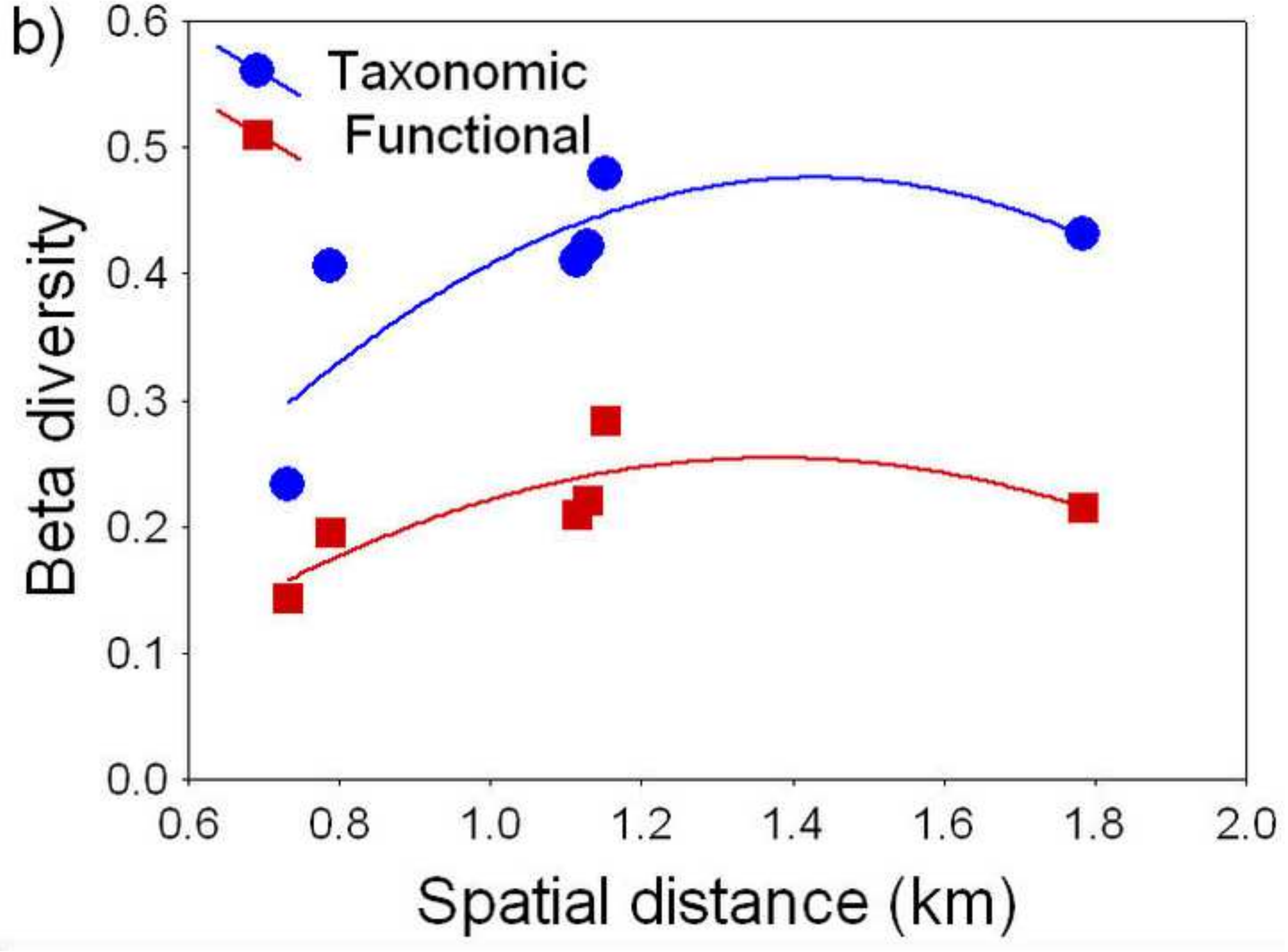
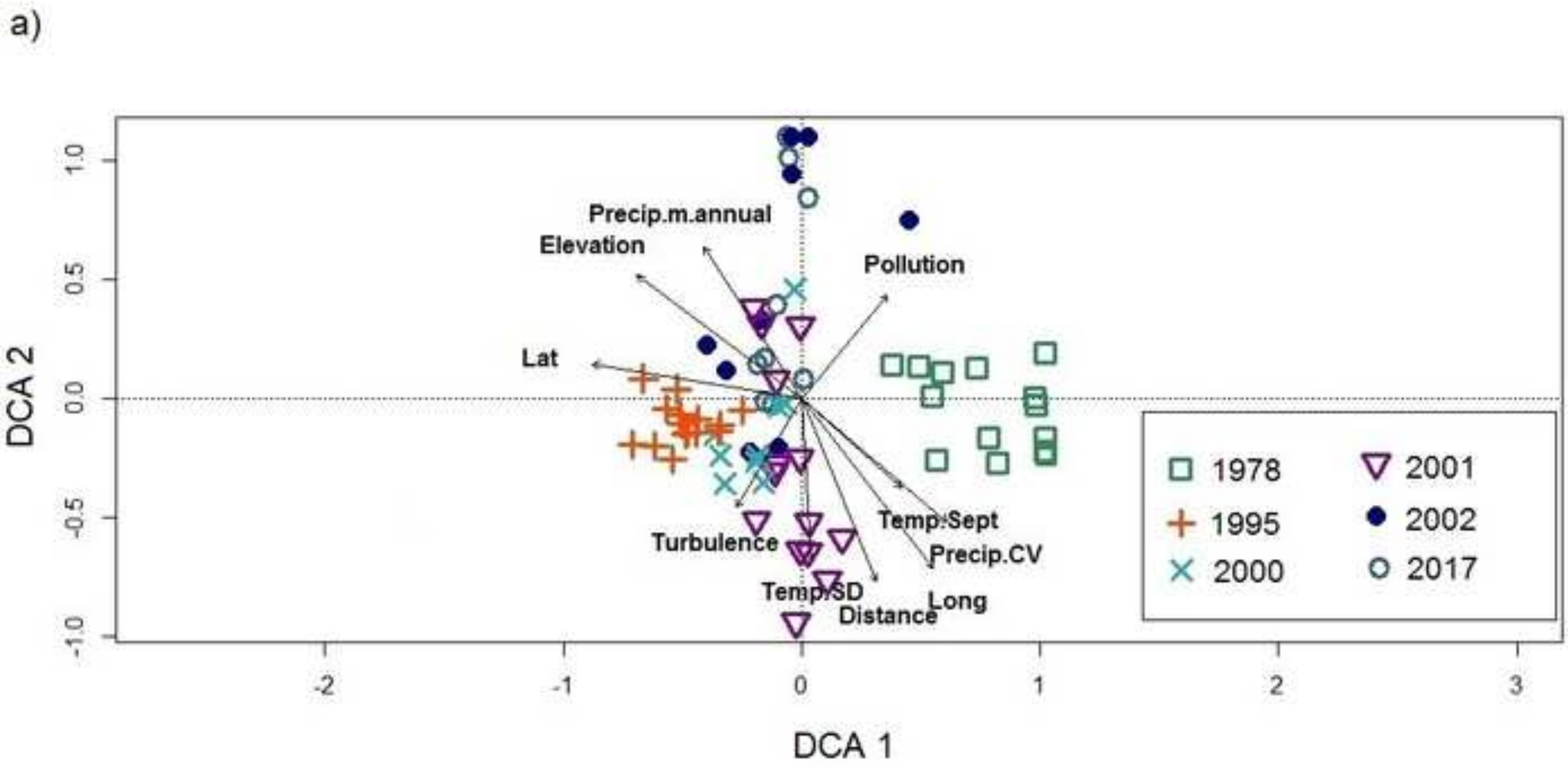
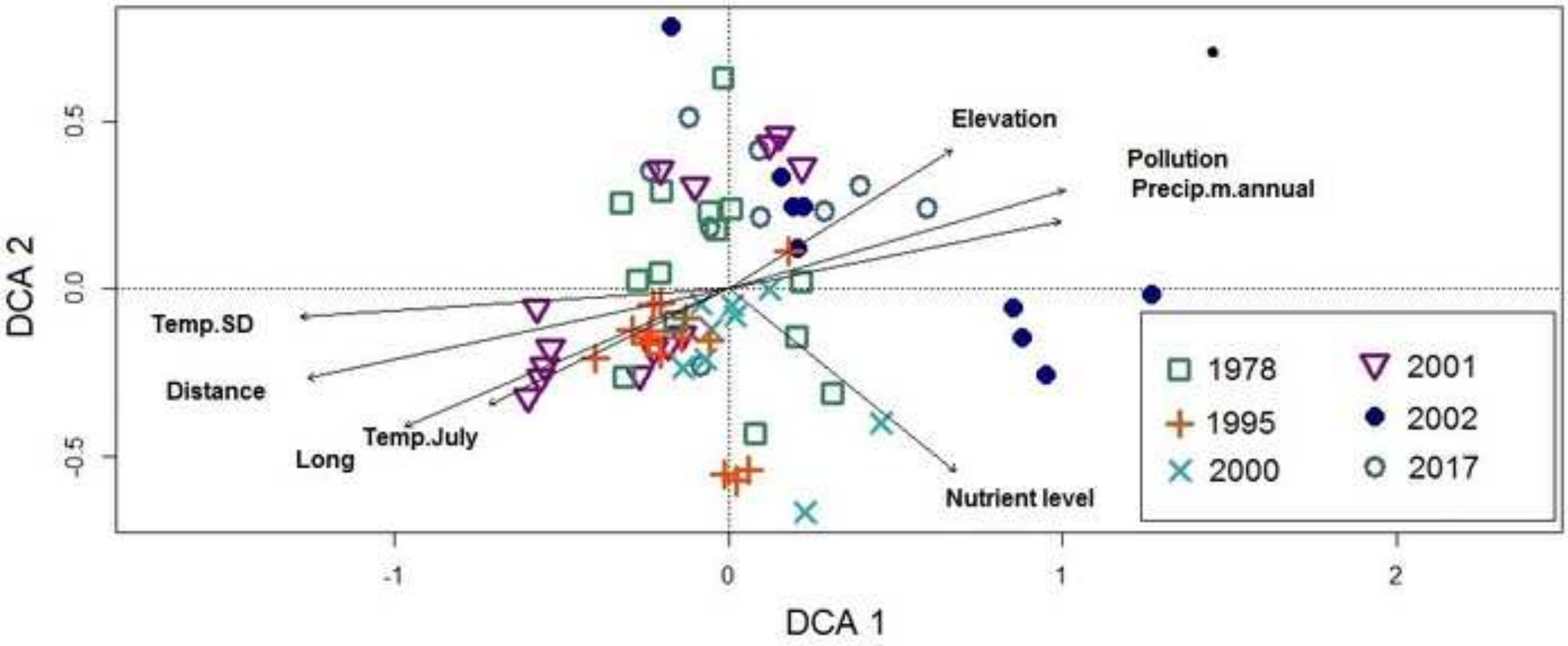
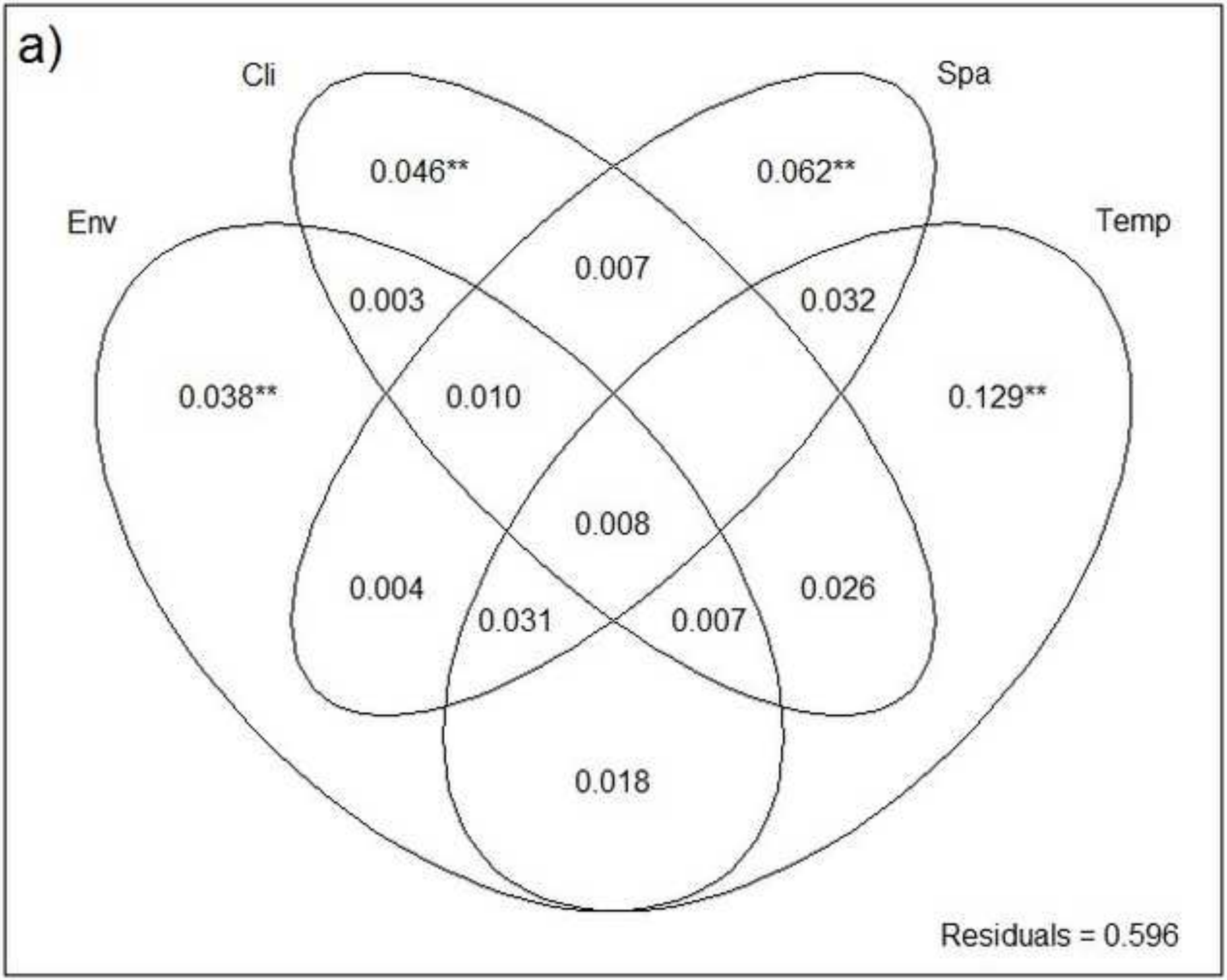

b)
Taxonomic
Functional
Beta diversity
0.0
0.1
0.2
0.3
0.4
0.5
0.6
Spatial distance (km)
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0

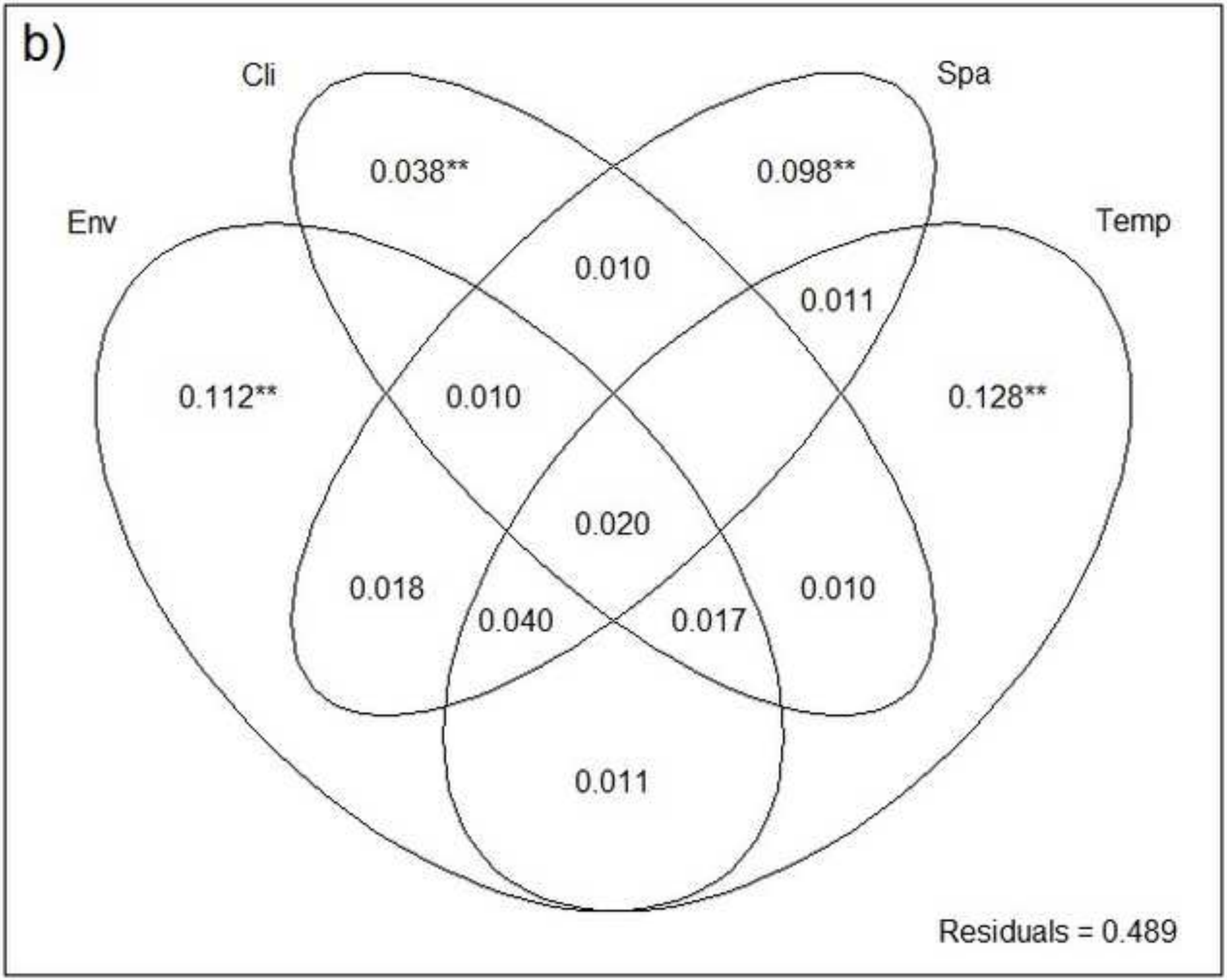
figure 4a


a)
Precip.m.annual
Elevation
Pollution
Lat
Turbulence
Temp.Sept
Precip.CV
Temp.SD
Distance
Long
DCA 2
1.0
0.5
0.0
-0.5
-1.0
-2
-1
0
1
2
3
DCA 1
□ 1978
+ 1995
× 2000
▽ 2001
● 2002
○ 2017

b)


DCA 2
0.5
0.0
-0.5
-1
0
1
2
DCA 1
Elevation
Pollution
Precip.m.annual
Temp.SD
Distance
Temp.July
Long
Nutrient level
1978
1995
2000
2001
2002
2017


a)
Cli
Spa
Env
Temp
0.046**
0.062**
0.007
0.003
0.032
0.038**
0.010
0.129**
0.008
0.004
0.026
0.031
0.007
0.018
Residuals = 0.596


b)
Cli
Spa
Env
Temp
0.038**
0.098**
0.010
0.011
0.112**
0.010
0.128**
0.020
0.018
0.040
0.017
0.010
0.011
Residuals = 0.489

Credit Author Statement

Alexander G. Rusanov: conceptualisation, data analysis and visualisation, drafting and writing the manuscript;
Tibor Bíró: reviewing, supervising;
Keve T. Kiss: conceptualisation, sampling, species identification, revising the original draft and editing;
Krisztina Buczkó: species identification;
István Grigorszky: sampling, species identification, reviewing and providing suggestions;
András Hidas: species identification, data preparation;
Mónika Duleba: species identification;

Zsuzsa Trábert: species identification;

Angéla Földi: species identification;

Éva Ács: conceptualisation, species identification, reviewing, supervising.

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: