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Climbing the elevational gradient: Diatom diversity patterns across temporary ponds

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ABSTRACT

Temporary ponds are peculiar and vulnerable habitats widespread in different biogeographical regions, which ecological and social importance was historically neglected. Among all the life forms supported by ponds, diatoms represent a key tool for assessing the conservation status of temporary waters. In this perspective, this study investigates patterns of diatom α and β diversity across an elevation gradient in low-elevation (LP) and high-elevation (HP) temporary ponds, assessing the influence of environmental, local, and seasonal factors. Samplings were performed throughout the entire year in six temporary ponds located along an altitudinal gradient. Species richness did not differ significantly between LP and HP sites, but Shannon diversity was lower in LP, indicating fewer even communities. Species richness, as predicted by our models, was influenced by pH and electrical conductivity (EC), while elevation was the main driver of both Shannon and Simpson diversity. EC consistently had the strongest influence on α diversity indices. Community similarity declined with increasing elevation, halving every ~1059 m, demonstrating rapid species turnover along elevational gradients. Both elevation and environmental factors independently shaped community structure, whereas seasonal effects contributed little to community variation. Ecologically, these results suggest that diatom communities in temporary ponds are strongly structured by elevational and physicochemical gradients, making them sensitive to environmental changes such as climate shifts or pollution. Conservation efforts should prioritize protecting a range of habitats across elevations to preserve regional biodiversity, as loss of specific habitats could lead to significant declines in diversity.

1. Introduction

Temporary ponds are unique and peculiar ecosystems (Pinto-Cruz et al., 2022), representing a key tool for preserving and safeguarding aquatic biodiversity from Mediterranean (Coccia and Scalici, 2025) to alpine environments (Blanco et al., 2020). The term “pond” is the common name used to refer to small (<5 ha) water bodies which are shallow (<5 m), with <30 % emergent vegetation (Richardson et al., 2022). Their temporary nature is provided by the restricted hydroperiod: water scarcity and drought affect these ecosystems, especially during the summer season (Gòmez-Rodrìguez et al., 2010). Ponds are globally recognized as biodiversity hotspots (Oertli et al., 2005), supporting the provisioning of a broad variety of ecosystem services, e.g., water quality, aquatic organisms for food and medicines, recreation and tourism (Stamenković et al., 2019) and acting as ‘stepping stones’ to facilitate the movement of species through the landscape (Horváth et al., 2019). Over the past decades, ponds have been severely affected by a range of anthropogenic disturbances, resulting in significant negative effects on the structure and function of these ecosystems (Gallitelli et al., 2025; Hill et al., 2021). Climate change, driven by global warming, and the reduction of connectivity between ponds (isolation and disruption of migration corridors; Oertli et al., 2010) can lead to biodiversity loss as well as habitat loss and fragmentation (Szabo et al., 2024; Arntzen et al., 2017; Cushman, 2006). These effects can be observed across ponds in various regions, regardless of their location or elevation, highlighting the widespread vulnerability of these ecosystems to global change. Indeed, while lowland and coastal temporary ponds, which are more frequently studied, are already experiencing severe droughts and hydrological alterations (Coccia et al., 2024), recent evidence shows that high-altitude and alpine ponds (high-elevation habitats above the climatic treeline; Testolin et al., 2020) are equally affected. High-altitude ponds hydroperiod is doubly affected by snow during the winter

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season and dryness during the summer period (Taurozzi and Scalici, 2024). Moreover, the high-elevation small aquatic habitats are some of the most sensitive indicators of environmental change (Kumar et al., 2012). However, while a certain attention is given to low-elevation ponds, the ecological importance of mountainous small waterbodies is critically unrecognized (Wissinger et al., 2016), and there is still a lack of evidence on current trends in high-elevation biodiversity (Fahy et al., 2024).

Among all the life forms supported by temporary ponds, diatoms also represent a key tool for assessing the conservation status of temporary waters. Diatoms are unicellular golden brown algae (Bhardwaj et al., 2021) showing a global distribution, with a fundamental key role in aquatic ecosystems (Taurozzi et al., 2024). Their ubiquity and sensitivity to environmental alteration make them excellent bioindicators used long since (Salmaso et al., 2019). The response of diatoms to multiple stressors in temporary lentic waters has received much less attention, with studies focusing mainly on rivers and streams (Novais and Morais, 2024). Moreover, there is a lack of knowledge and conflicting evidence regarding diatom metacommunity dynamics and their responses to seasonal and environmental gradients (Jamoneau et al., 2018; Lindholm et al., 2018; Godhe and Rynearson, 2017). Elevational gradients, while crucial for identifying broad-scale species distribution patterns, can also enhance our understanding of how biological communities might respond to projected global climate change.

Elevational gradients are widely recognized as natural laboratories for investigating patterns and determinants of species diversity. Numerous studies have documented that species richness often follows a hump-shaped pattern along elevation, peaking at mid-elevations, although monotonic declines, particularly for woody plants, are also frequently reported (Herrera-Alsina et al., 2025; Di Musciano et al., 2024; Zhi-Yao and Jing-Yun, 2004). These patterns are shaped by an interplay of factors, including area availability, habitat heterogeneity, productivity, and climatic variables such as temperature and water availability (Herrera-Alsina et al., 2025; He et al., 2020). For example, lower elevations may support higher diversity due to greater productivity and broader ecological niches, while mid-elevations can host peak diversity where optimal conditions and habitat diversity converge. Several studies have also shown that plant species richness can display unimodal peaks and at mid-elevations, highlighting the effects of multiple factors (Kumar et al., 2024). However, responses can vary substantially among plant functional groups, indicating that different groups react uniquely to environmental gradients. Importantly, the shape and steepness of elevational diversity gradients can differ markedly among taxonomic groups, with plants, animals, and microbes each displaying unique responses to elevation (Bryant et al., 2008). Comparative analyses across taxa highlight both shared and distinct mechanisms, emphasizing the need for multi-taxon approaches to fully understand elevational biodiversity patterns.

For instance, several studies have supported the hypothesis of no richness–elevation relationships in diatom communities within streams (Teittinen et al., 2016), with environmental variables (water depth, pH, conductivity) exerting a stronger influence. Conversely, other research has shown that diatom distribution patterns can serve as reliable predictors of the altitudinal position of sampling sites (He et al., 2020; Gremmen et al., 2007; Ormerod et al., 1994), supporting the view that diatom richness generally show a monotonic increase with elevation. Moreover, diatom guild composition has been reported as highly sensitive to elevation gradients, while remaining relatively unaffected by seasonality (Wang et al., 2020).

Regarding temporary ponds, the current understanding of diatom metacommunities drivers and spatial and temporal (elevational and seasonal) turnover remains extremely limited. The findings from Heikkinen et al. (2022) suggest that, in subarctic mountain ponds, elevation was not sufficient to explain the variation in diatom species richness and community composition, but diatom biodiversity was shaped by a variety of local-scale environmental variables. Similarly, Della Bella and Mancini (2010) highlighted that diatoms tended more to reflect water chemistry through changes in community structure compared to physical habitat changes; Differently, Blanco et al. (2020) revealed that that diatom diversity is mainly controlled by elevation and hydroperiod, with highest richness in high-elevation ponds.

As shown, studies considering species richness and spatial and temporal turnover along elevation gradients of diatom communities are relatively scarce and, in some cases, contradictory in their findings. To reduce these knowledge gaps, we present a study on the seasonal, elevational and inter-annual variation of the taxonomic diversity of benthic diatom communities of temporary ponds. In this sense, we investigated the influence of seasonality, elevation and environmental local variables on diatom communities through a one-year sampling across temporary ponds along an elevation gradient, passing through Mediterranean and Alpine biogeographical regions. In particular, this study aims to (i) focus on temporal and seasonal effects as a proxy of drought on diversity; (ii) investigate the role of elevation and local environmental factors as drivers of diatom α diversity in temporary ponds; (iii) to disentangle the relative roles of local environment and elevation as drivers of diatom β diversity.

2. Materials and methods

2.1. Study area

The study focused on central Italy, including the two regions of Latium and Abruzzi. Six ponds were identified using Google Earth Engine ® (GEE) images and field surveys, following Taurozzi and Scalici (2024). The six ponds were chosen following an elevation gradient from the lowest (TP0, inside the Castelporziano Presidential Reserve) to the highest (TP2000, Gran Sasso e Monti Della Laga National Park), passing through intermediate elevations (0–400 – 800–1200 – 1600–2000 ± 75 m above sea level). The sampling sites were named following the acronymous made by two letters (TP = Temporary Pond) and the relative elevation, dividing them into low-elevation (TP0, TP400, TP800; hereafter, LP) and high-elevation (TP1200, TP1600, TP2000; hereafter, HP) ponds (Fig. 1a). The classification into LP and HP followed a geographical selection criterion, where the ponds located in the Campo Imperatore plain (>1000 m above sea level) were defined as HP and those below 1000 m above sea level as LP (Körner, 2007). The description of the sampling sites is shown in Table S1.

2.2. Sampling activities

The samplings were performed each month, one sampling per month, for one year, only when ponds were filled with water. For this reason, the total number of samplings was 41, as shown in Fig. 1b. In particular, 7 samplings were performed for TP0, 11 for TP400, 11 for TP800 and 4 for TP1200, TP 1600 and TP2000. For each sampling, three diatom samples were collected, resulting in a total of 123 samples. To standardize the sampling methodology, all samplings were performed by the same operator at approximately the same time of day (±30 min) (Fig. 1c). Diatom sampling activities were performed following Beltrami et al. (2007), Kelly et al. (1998) and Maitland et al. (2020): For each sample, at least 10 stones (dimensions between 64 mm and 256 mm) were scraped using a commercial toothbrush (taking care to use a new toothbrush for each sampling site); then, the toothbrush was immersed in a 50 ml Falcon containing 70 % diluted ethanol and distilled water. In this way the diatom component was also made suitable to be carried out in the laboratory for oxidation operations.

Before performing diatom sampling activities, 5 physicochemical parameters of ponds were recorded using a multiparametric probe Hannah® HI98194: Temperature (T °C), pH, Oxygen Reduction Potential (ORP, mV), Electrical conductivity (EC, μS/cm), Dissolved oxygen (DO, ppm). Temperature and pH directly affect metabolic processes and diatom species distributions (Azmuda et al., 2019); ORP provides

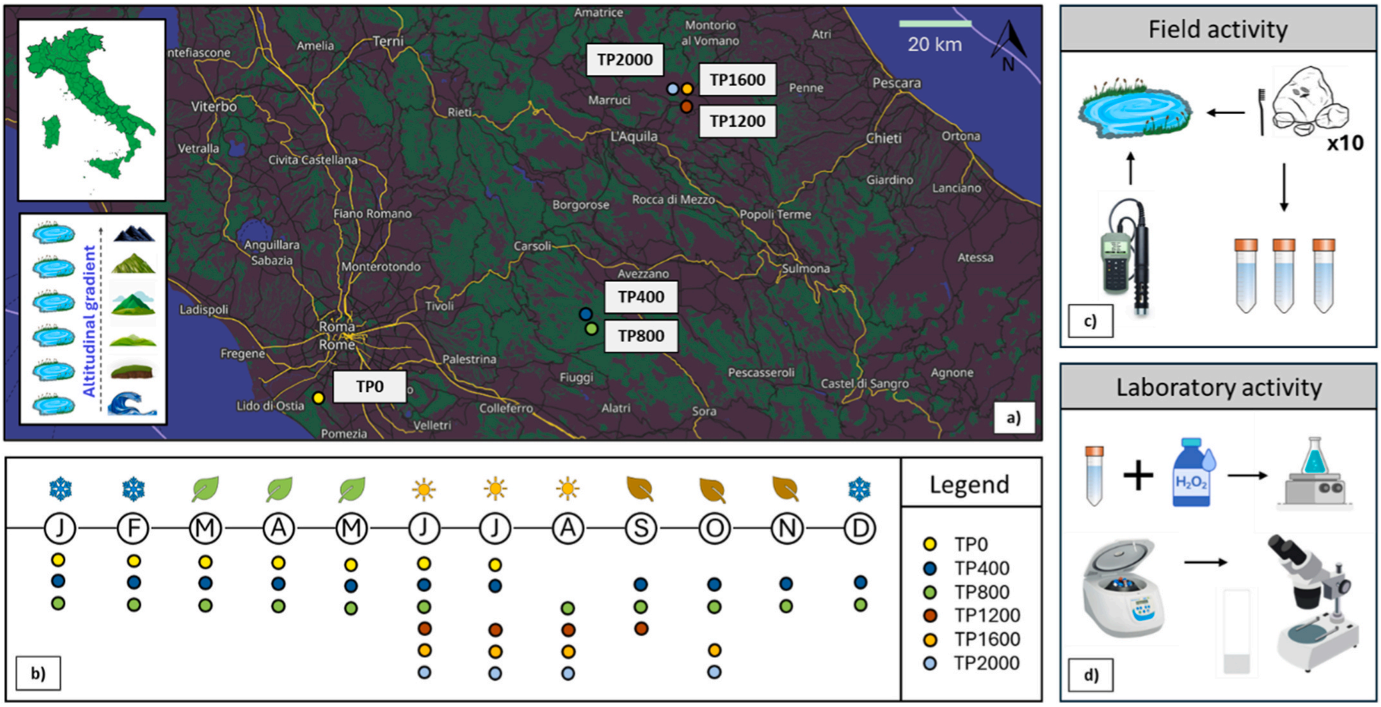

Fig. 1. a) Geographical distribution of the study sites considered: TP0, Castelporziano Presidential Estate; TP400, Simbruini National Park; TP800, Altipiani di Arcinazzo; TP1200, TP1600, TP2000, Gran Sasso and Monti della Laga National Park. b) Scheme of temporal distribution of sampling activities; dots represent sampling sites with water availability per month; empty spaces represent dry ponds. J–D: months of the year from January (left) to December (right). The icons at the top represent the season. Snowflake, winter; green leaf, spring; sun, summer; brown leaf, autumn. c) Field activities for diatom sampling and d) analysis and identification methods of diatoms, showing the laboratory activities. TP = Temporary pond. © OpenStreetMap. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

insights into the redox conditions and potential for different biogeochemical processes (Wang et al., 2009); EC reflects the ionic content and overall water chemistry, which can shape diatom assemblages (Leira and Sabater, 2005). DO is essential for aerobic life and can indicate the trophic and ecological status of the pond (Culberson and Piedrahita, 1996). Collectively, these parameters offer a comprehensive snapshot of the environmental conditions relevant to diatom ecology and are widely used in ecological studies to interpret community patterns and environmental gradients (Córdoba et al., 2022).

Diatom samples were processed and analysed in the laboratory by following the oxidation method explained in Beltrami et al. (2007) for inland waters and the valves identified using taxonomic guides (Table S2). For each slide, the analysis was considered completed when identification at the species level of 400 valves was achieved (ISPRA, 2014).

2.3. *Statistical analysis*

2.3.1. *Diatom α diversity*

Diatom α diversity was calculated using four α diversity indices: a) Species richness b) Shannon diversity index (H); c) Simpson index (D); d) Pielou's evenness (J). We selected these diversity indices to capture complementary aspects of community diversity and structure. Species richness simply counts the number of species present, providing a basic measure of biodiversity. The Shannon diversity index (H) accounts for both richness and the relative abundance of each species, reflecting the uncertainty in predicting the species identity of a randomly chosen individual (Chiarucci et al., 2011). The Simpson index (D) emphasizes dominance or evenness within the community, giving more weight to common species and thus highlighting the probability that two individuals randomly selected from a sample belong to the same species (Buzas and Hayek, 2005). Pielou's evenness (J) quantifies how evenly individuals are distributed among the species present, standardizing the Shannon index to a 0–1 scale (Kunakh et al., 2023). Diversity indices were calculated using the *diversity* function from the *vegan* package. To assess whether α diversity indices differed between low-elevation (LP) and high-elevation (HP) ponds, we performed non-parametric Mann–Whitney U tests on species richness, Shannon diversity, Simpson diversity, and evenness. This approach was chosen due to the limited sample size and the non-normal distribution of some indices.

2.3.2. *Influence of elevation on diatom α diversity*

For testing the first aim (i) and investigating temporal and seasonal effects as a proxy of drought on diatom diversity in LP and HP, Linear Mixed Models (LMMs), which are generally more robust against pseudoreplication (e.g., avoiding treating repeated samples as independent), were applied to the four α diversity indices calculated.

In each model, the elevation group was included as a fixed effect, while season was treated as a random effect to account for potential temporal variability. LMMs were applied using the function *lmer* in the R package *lme4* (Bates et al., 2015). To evaluate the contribution of the random effect (season) to model performance, Likelihood Ratio Tests (LRTs) were conducted by comparing models with and without the random effect. A non-significant result from the LRT indicated that the inclusion of season did not significantly improve the model fit.

2.3.3. *Influence of environmental variables on diatom α diversity*

For testing the second aim (ii), local environmental variables (except pH) were log10-transformed before statistical analyses due to their skewed distributions (Holmgren and Appelberg, 2000). Log-10 transformation helped ensure that statistical assumptions (Normality of residuals, homoscedasticity, independence) are met and enhanced the reliability of subsequent analyses (Jenkins, 2015). Statistical dependence between the explanatory variables (including elevation) was assessed using Spearman's rank correlation coefficients (r_s) using the *cor* function in R. As the variables were not strongly correlated ($r_s \leq |0.75|$), they were all retained for further analyses. Then, we compared Generalized Additive Models (GAM) and Linear Mixed Models (LMM) with Gaussian error distribution (Nelder and Wedderburn, 1972) to examine richness in relation to local environmental (T, pH, ORP, EC, DO) and spatial (elevation, season) factors in a multivariate setting. For these analyses, the previous categorization of ponds in LP and HP was not retained, because the main aim was to evaluate how continuous physicochemical gradients influence diversity metrics across all sampled ponds. By considering individual ponds rather than group-level comparisons, these models focused on detecting general nonlinear or linear relationships between environmental conditions and diatom diversity.

GAMs were specified as:

$$\mathrm{Diversity}_i = \beta_0 + s_1(T_i) + s_2(\mathrm{pH}_i) + s_3(\mathrm{ORP}_i) + s_4(\mathrm{EC}_i) + s_5(\mathrm{DO}_i) + s_6(\mathrm{Elevation}_i) + \beta_7 \cdot \mathrm{Season}_i + \varepsilon_i$$

where $s_k(\cdot)$ denotes a penalized spline smoother with basis dimension k = 6, and Season was included as a fixed factor;

LMMs were specified as:

$$\mathrm{Diversity}_{ij} = \beta_0 + \beta_1 T_{ij} + \beta_2 \mathrm{pH}_{ij} + \beta_3 \mathrm{ORP}_{ij} + \beta_4 \mathrm{EC}_{ij} + \beta_5 \mathrm{DO}_{ij} + \beta_6 \mathrm{Elevation}_{ij} + u_j + \varepsilon_{ij}$$

where $Diversity_{ij}$ is the response variable (e.g., species richness, Shannon index) for site i during season j, and $uj \sim N(0,\sigma_u^2)$ represents the random intercept for the season. The residual errors ε_{ij} were assumed to be normally distributed.

The best approximating model (GAM) was chosen using Akaike's information criterion (AIC; Akaike, 1974) and function *AIC* in R package *stats* (Sakamoto et al., 1986) and applied here using the *gam* function from the *mgcv* R package, with k = 6 degrees of freedom (Wood, 2017) (Appendix A). Smoothing functions were implemented using thin plate regression splines via the *s* function in the *mgcv* package. We specified a basis dimension of k = 6 for each smoother, following standard practice and to prevent overfitting, given the moderate sample size and number of predictors. This value provides sufficient flexibility to capture non-linear relationships, while avoiding excessive model complexity. The choice was further supported by visual inspection of fitted smooth terms and model diagnostics. The Relative Importance of Regressors in Linear Models, evaluating the contribution of each explanatory variable to the variation in diatom richness, was examined using the *relimp* function from the *relaimpo* package in R (Grömping, 2006). This function estimated how much each predictor variable contributed to the explained variance (R^2) in a linear model. We adopted the recommended metric *lmg*, which provides a decomposition of the model explained variance into non-negative contributions (Lindeman et al., 1980). This approach is particularly useful when multiple predictors are considered, as it quantifies their relative importance even in the presence of multicollinearity. While GAMs are flexible and effective in modeling non-linear relationships (Wood, 2017), *relaimpo* complements them by providing an interpretable breakdown of predictor influence in a linear framework, helping to identify the most influential explanatory variables.

2.3.4. *Testing the effects environmental and elevational variables on diatom β diversity*

For testing the third aim (iii), i.e. to analyze changes in community composition (β diversity) along environmental and elevational gradients, we used Mantel test (Mantel, 1967), a distance-based approach commonly employed to study diatom community (dis)similarities in relation to distance matrices (Wang et al., 2012). The Mantel statistic r (range −1 to 1) is a correlation between two dissimilarity or distance matrices. We first constructed a dissimilarity matrix for biological data, and distance matrices for environmental and elevation data (Teittinen et al., 2016). To calculate the pairwise dissimilarities, we used the Bray–Curtis dissimilarity index on diatom relative abundance data because this index considers differences in species abundances and emphasizes dominant species. Bray-Curtis dissimilarity was calculated using the *vegdist* function from the *vegan* package in R (Oksanen et al., 2022). Mantel test was run to assess the correlation between community dissimilarity and both elevation and environmental distances, using the *mantel* function in R package *vegan* (Oksanen et al., 2022). Then, to disentangle the individual effects of elevation and environment, we used partial Mantel tests for: (i) testing the relationship between community dissimilarity and elevation while controlling for environmental differences, and (ii) testing the relationship between community dissimilarity and environment while controlling for elevation. Partial Mantel test was run using the *mantel.partial* function from the *vegan* package in R (Oksanen et al., 2022). In all tests, the significance of the relationships was assessed using 9999 permutations.

We then calculated Initial Compositional Similarities and Halving Distances of the distance decay relationships (Soininen et al., 2007) to examine β diversity further. The initial similarity reflected β diversity at small spatial scales, with high similarity values indicating low β diversity. The Halving Distance measured how long is the distance (here elevational distance in meters above sea level) in which compositional similarity has decreased to half of the initial similarity (i.e., how quickly the community changes with distance). Long Halving Distances imply that the rate of compositional turnover does not change much with increasing spatial scale. To estimate these parameters, we performed a linear regression analysis of pairwise Bray-Curtis compositional similarity between ponds against their elevational distances. We calculated the initial similarity (at 1 m distance) as $\mathrm{Sim1} = b \times 1 + a$, and halving distance as $\mathrm{HD} = (b - a)/2\,b$, where 'b' and 'a' are regression parameters (Teittinen et al., 2016). The regression intercept (a) represents the expected community similarity at minimal elevational separation, i.e., when ponds are very close in elevation (approximated as 1 m distance since zero distance is undefined). This intercept provides an estimate of the baseline similarity between communities at very small spatial scales. The slope (b) quantifies the rate at which similarity decreases with increasing elevational distance, indicating the turnover rate of species composition across the elevational gradient. This linear modeling approach quantifies the decay rate of community similarity with spatial distance.

We used analysis of similarities (ANOSIM) to test if there were significant differences in species compositions between ponds (Clarke, 1993), using the *anosim* function from the *vegan* package in R (Oksanen et al., 2022). The ANOSIM statistic (R) is based on the difference of mean ranks between groups and within groups. R takes values between −1 and + 1; a value of 0 denotes random grouping. ANOSIM tells you if communities are different between specific categories (e.g. summer vs winter, low vs high elevation) (Teittinen et al., 2016).

Subsequently, we performed non-metric multidimensional scaling (NMDS) on the community abundance data using the *metaMDS* function from the *vegan* package (Oksanen et al., 2022) in R, to identify environmental variables significantly associated with diatom community composition. Then, we performed a PERMANOVA (Permutational Multivariate Analysis of Variance), using the *adonis2* function from the *vegan* package in R (Oksanen et al., 2022), to assess the influence of environmental variables on community composition. Unlike ANOSIM, which only tests for group separation, PERMANOVA quantifies the proportion of variation in community composition explained by continuous or categorical predictors. In this way, we determined which environmental gradients significantly structured the assemblages and to assess their relative influence on β diversity (Anderson and Walsh, 2013). We applied the *envfit* function from the same package to fit environmental vectors onto the NMDS ordination. This procedure tests the correlation between environmental variables (e.g., temperature, electrical conductivity, oxidation-reduction potential) and the ordination axes, assessing their significance through 999 permutations. Significant vectors indicate environmental gradients that are strongly related to patterns of community variation.

All statistical analyses were performed using R software (R Core

Team, 2024; version 4.4.2), and significance was assessed at $p < 0.05$.

3. Results

3.1. Season effect on diatom diversity

The α diversity did not significantly differ in species richness between low- and high-elevation ponds ($U = 154.5$, $p = 0.59$) (Appendix C). However, a clear trend emerged in diversity indices: both Shannon ($U = 108.0$, $p = 0.06$) and Simpson ($U = 114.0$, $p = 0.09$) values tended to be higher in HP sites (although not significant), suggesting more balanced communities at higher elevations despite comparable species counts. Evenness showed a similar, though non-significant, pattern ($U = 127.0$, $p = 0.18$), supporting the idea of more equitable species distributions in HP compared to LP ponds. These results align with patterns observed in the raw data, where LP ponds often hosted many species but were dominated by a few, while HP ponds exhibited more uniform taxa distributions (Fig. 2).

The results of the linear mixed models suggest that diatom alpha diversity showed distinct patterns across the elevational gradient. Species richness did not differ markedly between LP and HP, indicating a comparable number of taxa. However, the Shannon diversity index was consistently higher in HP sites, suggesting a more even distribution of species. The Simpson index followed a similar trend, though less pronounced. Evenness was also slightly greater at high elevations, pointing to more balanced communities (Table 1). Overall, these results indicate that while LP and HP ponds may host a similar number of species, HP sites support communities that are more structurally diverse and equitably composed.

3.2. Effect of environmental and local factors on diatom α diversity

The best GAM model explaining variation in diatom α-diversity included pH, EC, and elevation as the strongest predictors (Fig. 3a–d; Table 2; Appendix B). These results highlight the key role of these environmental factors in shaping α-diversity patterns across ponds.

The Relative Importance of Regressors in Linear Models (LMG method) revealed that the linear models explained approximately 17–21 % of the variance in the diversity metrics analysed (Fig. 3e). Among the environmental variables, EC consistently had the strongest influence, explaining 5.5 % of the variance in species richness, 5.5 % in Shannon diversity, and 7.1 % in Simpson diversity. Seasonal variation also played a notable role, particularly influencing evenness (6.2 %) and species richness (4.8 %). Elevation was more relevant for Simpson diversity (5.5 %) and, to a lesser extent, for Shannon diversity (3.5 %). Temperature showed a greater relative contribution to evenness (4.8 %) compared to species richness (0.4 %) and Shannon diversity (0.4 %). Other variables such as pH, ORP and DO contributed variably and moderately across the indices, with ORP being more important for species richness (1.1 %) and Simpson diversity (2.5 %).

Table 1
Results of linear mixed-effects models testing the effect of elevation (low-elevation ponds, LP vs high-elevation ponds, HP) on four α diversity indices: species richness, Shannon index, Simpson index, and evenness. Fixed effects represent the estimated difference between LP and HP. The random effect "season" was included in all models. P-values are provided both for the fixed effect of group and for the likelihood ratio test comparing each full model to a null model without the group term.

Diversity Index	Estimate (LP vs HP)	SE	*t*	*p* (fixed)	χ^2 (vs null)	*p* (LRT)
Species richness	−4.40	2.98	−1.48	0.152	1.05	0.305
Shannon index	−0.30	0.15	−2.03	**0.049**	4.11	**0.043**
Simpson index	−0.043	0.032	−1.35	0.185	1.87	0.171
Evenness	−0.061	0.036	−1.67	0.102	2.80	0.094

3.3. Effects of local environment and elevation on diatom β diversity

A strong positive correlation was found between community dissimilarity and elevation distance (Mantel $r = 0.54$, $p < 0.001$), indicating that communities become increasingly dissimilar with greater elevational separation. A weaker yest significant correlation was also observed between community dissimilarity and environmental distance based on physicochemical factors (Mantel $r = 0.16$, $p = 0.048$). Partial Mantel tests confirmed that the association between community dissimilarity and elevation remained strong and significant even when controlling for environmental differences ($r = 0.55$, $p < 0.001$). Conversely, the correlation between community dissimilarity and environment, though modest, remained significant when controlling for elevation ($r = 0.19$, $p = 0.027$). These results suggest that elevation is a primary driver of β diversity patterns in the studied communities, with environmental variation exerting a secondary but meaningful influence and both contribute independently.

Linear regression analysis (Initial Compositional Similarities) revealed a significant negative relationship between community similarity and elevational distance ($p < 0.001$). The intercept ($a \approx 0.41$) indicates that communities at minimal elevational separation exhibit approximately 41 % similarity. The slope ($b \approx -0.00019$) indicated that for each additional meter of elevational separation, community similarity decreases by approximately 0.00019. The calculated Halving Distance (~1059 m) indicates the elevational distance at which community similarity is reduced by half, highlighting a pronounced decay in similarity over relatively short elevational gradients (Fig. 4a). These results suggest that elevational distance acts as a significant spatial filter driving community turnover, likely reflecting environmental gradients and habitat heterogeneity associated with elevation.

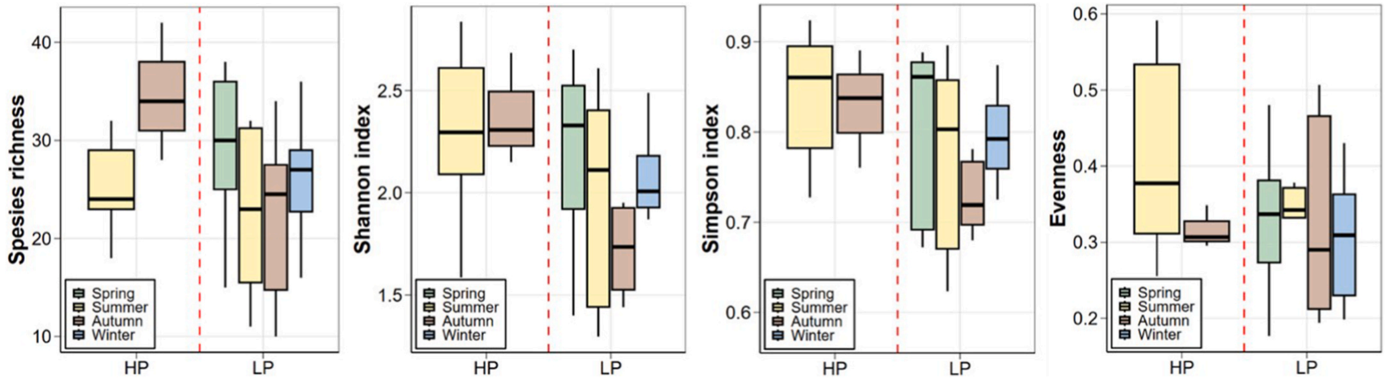

Fig. 2. Boxplot showing the species richness, Shannon index, Simpson index and Evenness across different groups (HP - LP) and seasons.

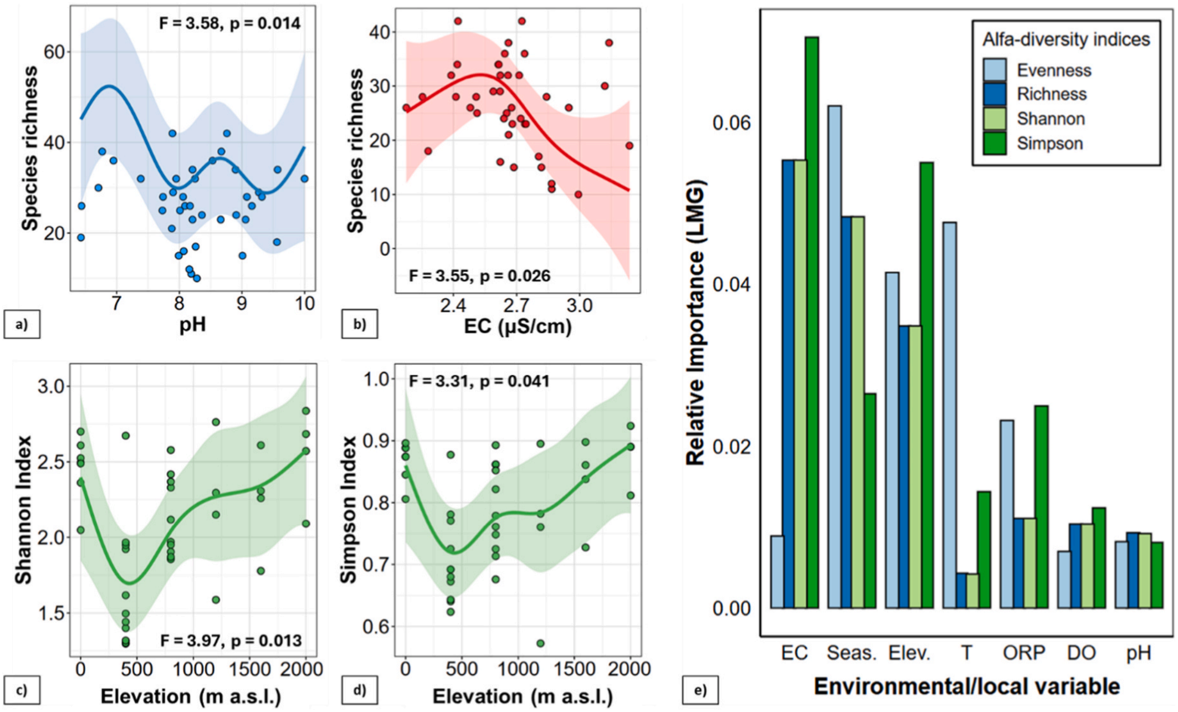

Fig. 3. Estimated relationship between a) pH and species richness, b) EC and species richness, Elevation and c) Shannon and d) Simpson diversity index, derived from GAM analysis. In all plots, observed data points are shown alongside GAM-fitted smooth curves representing the predicted relationships between each key environmental variable and diversity metrics. The shaded bands around the curves indicate 95 % confidence intervals, calculated by fixing other predictors at their mean values and setting season to a reference level ("Autumn"), thus isolating the effect of each variable. Points represent the observed values. e) Relative importance (LMG) of environmental and local variables in explaining variation in α-diversity indices, shown for multiple diversity metrics. Bars represent the contribution of each predictor variable to the explained variance of the diversity indices, with different colours indicating distinct α diversity indices. Seas. = seasonality; Elev. = elevation. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2

Summary of significant predictors from generalized additive models (GAMs) analysing relationships between environmental variables and diatom diversity indices. Only predictors with p-values below 0.05 are shown. For each model, the table reports the F-value, p-value, adjusted R-squared, generalized cross-validation (GCV) score, and Akaike Information Criterion (AIC).

Diversity Index	Significant Predictor(s)	F-value	p-value	Adjusted R^2	GCV	AIC
Species richness	pH	3.581	0.0146	0.360	71.951	283.96
	EC	3.559	0.0269			
Shannon index	Elevation	3.976	0.0130	0.327	0.196	46.33
Simpson index	Elevation	3.331	0.0416	0.219	0.010	−75.76
Evenness	– *(no significant terms)*	–	–	0.099	0.015	−58.34

ANOSIM results for seasonality revealed a low but statistically significant separation among groups ($R = 0.13$, $p = 0.016$), indicating slight seasonal variation in community structure (Fig. 4b). In contrast, the ANOSIM for elevation showed a much stronger and highly significant effect ($R = 0.86$, $p < 0.001$), demonstrating a pronounced differentiation of communities along the elevational gradient (Fig. 4c).

PERMANOVA analysis showed that combined chemical-physical parameters (T, pH, ORP, EC, DO) significantly explained variation in community composition ($R^2 = 0.198$, $F = 1.73$, $p = 0.006$). Complementarily, the *envfit* analysis indicated that temperature ($r^2 = 0.20$, $p = 0.023$), oxidation-reduction potential ($r^2 = 0.16$, $p = 0.038$), and electrical conductivity ($r^2 = 0.23$, $p = 0.009$) were the most influential environmental vectors correlating with the NMDS ordination of community data (Fig. 4d and e).

4. Discussion

This study represents the first research focused on the analysis of diatom communities in six different temporary ponds along an elevation gradient; To our knowledge, Mediterranean and Alpine diatom biodiversity has never been compared in one-year sampling including multiple Natural Reserves, Regional and Natural Parks. Moreover, for the first time, an elevation gradient ranging from 0 to 2000 m was examined to compare temporary ponds and diatom community responses to seasonal, elevation and environmental constraints.

4.1. *Influence of seasonality on diatom diversity (grouped for elevation)*

The obtained results indicated that the elevational groups of analysed ponds (LP and HP), did not provide significant outcomes for diatom α diversity, except for Shannon index. Shannon diversity is influenced by both species' richness (how many species there are) and evenness (how evenly distributed they are). The absence of a richness-elevation relationship (including Simpson and evenness indices), in this study may be explained by the fact that, according to Van Dam et al. (1994), diatoms are strongly influenced by local and environmental factors. Following Vyverman et al. (2007), the higher water temperatures characterizing LP could promote biofilm growth, accelerate metabolic cycles, and support greater primary productivity, creating favorable conditions for a broader range of diatom species. Passy (2007)

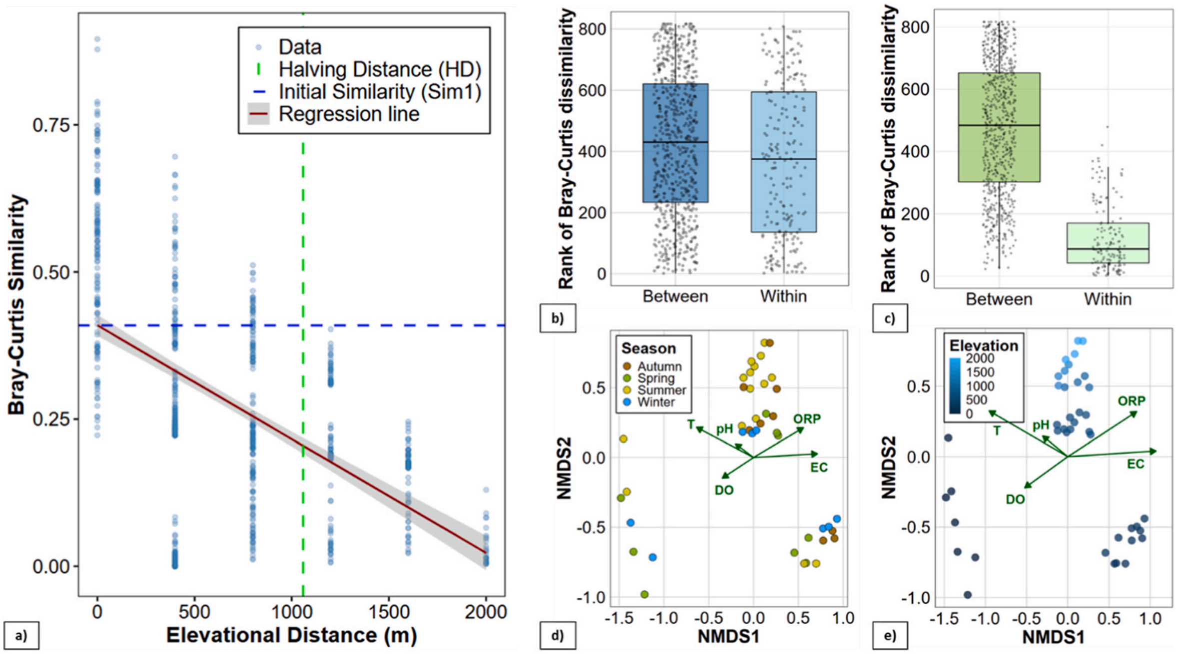

Fig. 4. a) Distance decay analysis of diatom community similarity as a function of elevational distance between ponds. Points represent Bray-Curtis similarity values for site pairs, while the red line shows the linear regression of similarity against elevational distance. The dashed blue line indicates the initial similarity (Sim1) at minimal elevational separation (1 m), and the dashed green line marks the halving distance (HD), the elevational distance at which community similarity is reduced by half, highlighting a pronounced decay of similarity over relatively short elevational gradients. b) Boxplot of ranked Bray-Curtis dissimilarities comparing within-season versus between-season diatom community differences. "Within" ranks represent pairwise dissimilarities between samples collected in the same season, while "Between" ranks correspond to dissimilarities between samples from different seasons. c) Boxplot of ranked Bray-Curtis dissimilarities comparing within-elevation versus between-elevation diatom community differences. Non-metric multidimensional scaling (NMDS) ordination of diatom community composition coloured by d) season and e) elevation. Environmental variables significantly correlated with community structure are represented as green arrows, indicating the direction and strength of their influence on the NMDS axes. Arrow lengths are proportional to the correlation strength, highlighting key environmental gradients shaping seasonal and elevational diatom assemblages. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

highlighted that the greater structural complexity (submerged vegetation, debris, variations in light and depth) typical of LP can increase environmental heterogeneity. However, our findings do not fully align with previous literature. Although the differences between the two groups are not statistically significant, the HP group appears to host a higher number of species, as shown graphically. Feret et al. (2017), compared littoral benthic diatoms of 63 natural lakes situated between 1350 and 2700 m a.s.l. (lower altitude lakes vs higher altitude lakes) and found that local factors were more important than regional factors. In contrast, our results may be interpreted in relation to the observed hydroperiods. We hypothesize that diatom communities reach a peak in species richness during the early stages following dry or frozen periods, when many pioneer species can rapidly colonize the environment as soon as water becomes available (Teittinen et al., 2016; Ledger et al., 2013). Conversely, as suggested by Celewicz and Gołdyn (2021), diatom communities in ponds with longer hydroperiods tend to stabilize, leading to a lower number of species as the pioneer taxa are gradually replaced (Dalu et al., 2022).

Our findings also indicated that no clear seasonal patterns in diatom diversity were observed within ponds. This result is consistent with Virta et al. (2020), who reported that diatom taxonomic α diversity remained stable at seasonal level despite observable changes in community composition in a coastal habitat. Differently, Wang et al., 2020 identified seasonals shifts in diatom community in an unregulated subtropical river in China, with environmental filtering having a strong effect during both spring and autumn. They also demonstrated that multiple processes play important roles in maintaining benthic community structure, with underlying mechanisms that vary seasonally. Similarly, Liu et al. (2025), reported substantial seasonal variations in the composition and abundance of diatom dominant species, as well as in the α and β diversity of the species-based community in a transitional zone if the Yellow River Delta. Interestingly, Underwood (1994) observed pronounced seasonal shifts in diatom communities of the Severn Estuary, which appeared to be related to the gradients of environmental stress and disturbance. The results from Reynolds (1973) supported the seasonal periodicity of diatom assemblages in a small eutrophic lake, indicating that diatom communities are typically governed more by physical than chemical factors.

Research focusing on diatom communities along elevational gradients is rare in literature and difficult to compare. In addition, our results contrast with existing studies on the effect of seasonality on diatom communities. However, given the limited amount of research even on this topic, providing a definitive explanation for the observed results is challenging. As suggested by Soininen (2007), it is reasonable to expect that diatom communities respond differently depending on the investigated environment. Thus, physical characteristics (lentic vs lotic sites), climatic (different biogeographic regions), and local and microhabitat differences can all influence diatom responses to seasonal variations (Cibic et al., 2012; Soininen et al., 2004). According to Rühland et al. (2015), diatom responses to seasonal variations are likely more pronounced in regions with strong seasonality, such as high-latitude, temperate, and many high-elevation regions. In the Mediterranean region, where this study was conducted, seasonality is generally less marked than in other temperate or continental climates. Nevertheless, to our knowledge, this study is the first attempt to investigate whether diatom communities undergo seasonal shifts in temporary pond environments along an elevation gradient. This topic, particularly under current climate change conditions, needs to be explored further and rapidly. Understanding the ecological responses of diatoms is essential for forecasting and mitigating the effects of climate change, especially regarding high-elevation habitats and their associated temporary freshwater ecosystems.

4.2. The role of elevation and local environmental factors as drivers of diatom α diversity

Based on GAM results, richness was significantly influenced by pH and EC. Virta and Soininen (2017) hypothesized that diatom species richness in Baltic Sea is related to water chemistry variables, including pH. Specifically, they observed a U-shaped relationship between species richness and pH (Fig. 3a), indicating that both low and high pH levels can affect diatom diversity. Our findings also align with Fernàndez-Moreno et al. (2024), who identified conductivity as the primary explanatory variable influencing diatom assemblages in wetlands in southern Spain, with pH also playing a significant role. Similar results were reported by Granqvist et al. (2024), where ponds with higher pH and conductivity levels supported more diverse diatom communities, whereas ponds with low pH and conductivity exhibited reduced species richness. The relative influence of EC (Fig. 3b) was also demonstrated in karst ponds by Novak and Zelnik (2021), who highlighted that diatom community composition varied among ponds with different EC levels, suggesting that conductivity shapes species distribution and abundance. We further suggest that, even in pond ecosystems, the relationship between electrical conductivity and richness is likely related to differences in the total concentration of dissolved ions. Low EC may limit essential nutrients or ions needed for growth, filtering out sensitive species, while high EC (often resulting from salinization, pollution, or evaporation) can be stressful for many freshwater diatoms, favoring only a few tolerant taxa and thus reducing richness. In contrast, moderate EC often supports higher species richness due to a balance of resources and environmental stability (Weilhoefer and Pan, 2006). As demonstrated by Potapova and Charles (2003), we hypothesize that these ions can affect nutrient availability, osmoregulation, and photosynthesis, which in turn determine which diatom species can thrive. Similar constraints may also apply to pH variations: pH can influence the solubility of nutrients (such as phosphorus and metals) as well as the toxicity of compounds like ammonia or heavy metals, thereby affecting diatom survival and growth. Moreover, diatoms require silica to build their frustules (cell walls) and in low pH (acidic) environments, silica availability can be reduced.

No apparent richness–elevation relationships were observed within ponds, consistent with the findings of Teittinen et al. (2016). However, elevation significantly influenced the GAM models for Shannon and Simpson diversity (Fig. 3c–d). Shannon and Simpson indices typically decrease with elevation, particularly in simplified communities or those dominated by generalist species. Similar patterns have been reported across various freshwater systems and organism groups. For example, Lotter et al. (1997) found that diatom diversity in Swiss mountain lakes declined with altitude, while Körner (2007) and Sundqvist et al. (2013) reported consistent decreases in plant and microbial diversity along elevational gradients. Xu et al. (2017) observed a unimodal pattern in plant diversity, with higher Shannon-Wiener and Simpson index values at mid-elevations. Comparable results emerged from Füreder et al. (2006), where the Shannon index of macroinvertebrate communities in alpine lakes peaked between 2001 and 2200 m a.s.l., declining at both lower and higher elevations. Similarly, García-Ríos et al. (2020) observed negative linear trends in Shannon diversity for macroinvertebrate communities with increasing elevation. Interestingly, in our study, higher Shannon and Simpson diversity indices were observed in HP. This may reflect reduced anthropogenic disturbance, lower nutrient enrichment, and higher water clarity in remote alpine environments, which can support more diverse and balanced diatom communities. Comparable patterns have been reported in other taxa; for example, Oommen and Shanker (2005) found higher plant and amphibian diversity at mid-to-high elevations in the Western Ghats, likely due to complex habitat heterogeneity and reduced human impact. Likewise, Jacobsen (2003) reported that some high-altitude streams in the Andes harbor surprisingly diverse macroinvertebrate assemblages, possibly due to unique ecological niches and reduced competition. Overall, these results may suggest that elevation, while often associated with environmental filtering can under certain conditions such as those typical of high-elevation temporary ponds, promote biodiversity through reduced anthropogenic disturbance, lower nutrient enrichment, and higher water clarity.

Our results highlight that even in small temporary alpine ponds, local environmental variables such as pH and conductivity can have a strong effect on diatom species richness. This suggests that diatom diversity in these habitats is not determined solely by dispersal limitation or geographic isolation but also responds directly to local physicochemical gradients. Importantly, the effect of elevation on Shannon and Simpson indices indicates that community structure and evenness, in addition to species richness, are shaped by broader climatic and topographic gradients. These patterns reflect underlying ecological processes such as environmental filtering, physiological stress and resource availability, which operate at multiple spatial scales. Understanding such mechanisms is particularly important in high-altitude ecosystems, which are highly sensitive to climate change and human disturbance.

Furthermore, according to the results from The Relative Importance of Regressors in Linear Model (Fig. 3e), EC was consistently the most influential variable across all diversity metrics, with temperature and seasonality also playing important roles, particularly in explaining variation in evenness and species richness. Generally, diatoms show increases in richness and abundances during late winter, spring and early summer (Nohe et al., 2020). In this study, the alternation of the wet phase between the two types of temporary ponds, occurring in winter for LP and during summer for HP, could generate a complementary pattern: when some ponds are full of water, others are frozen or dry and vice versa. This alternation may help maintain overall diatom richness at a relatively constant level. It is also plausible that, despite the shorter hydroperiod of HP compared to LP, and the similar number of shared diatom species, HP diatom communities are better adapted to reduced water availability and exhibit greater resistance and resilience to drought events (Falasco et al., 2020; Kuefner et al., 2020). Furthermore, the seasonal alternation of hydroperiods may complicate direct comparisons between LP and HP; we hypothesize that these two pond types could be considered two distinct environments, exhibiting significant differences in geographical, ecological and physicochemical characteristics. In this context, diatom communities can serve as valuable proxies for assessing the biological characteristics of ponds (Borrego-Ramos et al., 2021; Blanco et al., 2020) and their use as biological indicators was encouraged by the authors.

Similar to our findings on diatom diversity, Sharma et al. (2019) observed a unimodal pattern of plant species richness along an elevational gradient in the Eastern Himalaya, with peak diversity at mid-elevations. Their study highlighted the role of climatic and edaphic factors, such as temperature, soil properties, and precipitation, as key drivers of richness. This suggests that elevation-related patterns in biodiversity may reflect shared ecological mechanisms across taxonomic groups, including both terrestrial and aquatic communities. Similarly, Krömer et al. (2013) reported that several terrestrial plant taxa showed significant elevation-related changes, while Sanchez-Gonzalez and Lopez-Mata (2005) found that differences in plant species richness in Mexico were mainly influenced by elevation, particularly temperature and humidity. In contrast, Jacobsen (2008), observed a decrease in stream macroinvertebrate richness with increasing elevation, whereas He et al. (2020) reported a monotonic increase in diatom and macroinvertebrate richness with elevation. Our results highlight how different taxonomic groups and geographical contexts can influence species distributions along elevational gradients. These findings also underscore the importance of using multiple diversity metrics. While species richness responds primarily to local water chemistry (e.g., nutrient availability, ionic stress), abundance-weighted indices such as Shannon and Simpson capture broader gradients such as habitat stability and ecological stress, which are often reflected by elevation. It is important to note that other environmental factors not included in our analyses may also play signficantroles in shaping diatom α diversity. For instance, nutrient concentrations (e.g., forms of phosphorus and nitrogen) are strongly associated with primary producer richness and community composition (Groendahl and Fink, 2017; Elser et al., 2007). In general, light and nutrient availability increase diatom species richness and primary productivity (Liess et al., 2009). Similarly, substrate type (e.g., silt, sand, rock) can affect diatom attachment and growth, as different taxa shows specific microhabitat preferences (Passy, 2007). Although our study included natural ponds with varied conditions, substrate types were not categorized quantitatively. Finally, hydrological regime (including hydroperiod length and water level fluctuations) is known to structure communities in temporary freshwater systems (Vanschoenwinkel et al., 2009), with short hydroperiods often favoring stress-tolerant or rapidly colonizing species, as demonstrated for aquatic plants (Trémolières, 2004).

This supports the idea that different components of diversity respond to distinct ecological mechanisms. Given the growing pressures on alpine freshwater habitats from climate change, our findings highlight the importance of monitoring both local water chemistry and large-scale gradients (such as elevation and hydroperiod variation) when assessing biodiversity and developing conservation strategies. Future research should aim to incorporate these factors to fully capture the multifactorial drivers of diatom diversity in alpine ponds.

4.3. Environmental determinants of diatom β diversity

Diatom β diversity differed markedly between temporary ponds at different elevations, in agreement with Blanco et al. (2020) and Taxboeck et al. (2020), who found that diatom diversity is strongly influenced controlled by elevation and associated environmental factors. Our results suggest that greater elevational separation between sites is accompanied by higher species turnover, likely due to the combined effect of changing climatic, hydrological, and ecological conditions along the gradient. Elevation encompasses variations in temperature, UV radiation, precipitation, and ice-cover duration, all of which can alter community composition by favoring communities with different ecological tolerances (Sundqvist et al., 2013; Hillebrand, 2004). Similar elevation-related compositional shifts have been documented for diatoms, macroinvertebrates, and bryophytes (Bergamini et al., 2009; Jacobsen, 2003): for instance, Soininen et al. (2007) reported that diatom assemblages in boreal lakes became increasingly dissimilar to altitude, even when controlling for water chemistry, underscoring the structuring role of spatial and climatic factors. In our study, the elevational signal persisted even after accounting for measured physicochemical differences, indicating that β diversity is shaped not only by water chemistry, but also by broader environmental and spatial processes linked to elevation. Although the correlation with environmental distance was weaker, it remained significant, suggesting that local abiotic factors such as pH and EC also contribute, albeit to a lesser extent, to community differentiation. Overall, these findings demonstrate that both broad-scale (elevation) and local-scale (physicochemical) gradients act independently and interactively to structure diatom community composition in freshwater ponds.

However, unlike elevations, seasonality exerted comparatively weaker effects on diatom communities. Both LP and HP experience a typical dry phase during summer (Meerhoff and Beklioğlu, 2024), while HP can also undergo an additional “dry” phase in winter due to freezing or snow cover (Taurozzi and Scalici, 2024). Temporary ponds, beyond elevational and other geographical and ecological differences, are all characterized by restricted hydroperiods and dry phases (Pinto-Cruz et al., 2023; Parra et al., 2021); following Novais et al. (2020), it is possible that diatom communities reach a stabilization phase just before drying events. Diatoms can use cists to resist and regenerate communities after drought periods (Sabater et al., 2017). This strategy ensures continuity, so that post-drought assemblages resemble those present prior to desiccation, similar to patterns seen in permanent water bodies, contrasting with the shifts reported by Barthès et al. (2015). Diatoms typically require 10–20 days to colonize bare substrates (Taurozzi et al., 2023; Duong et al., 2007). In temporary aquatic habitats, lifeforms are forced to develop resistance strategies. In fact, in temporary habitats, organisms must develop different resistance and resilience strategies to cope with desiccation (Bogan et al., 2017) and understanding these strategies is key to explaining recolonization dynamics (Robson et al., 2008). Diatoms have a combination of strategies: they can resist and live in the dry sediments after rehydration or disperse to other wet habitats (Quevedo-Ortiz et al., 2024). We hypothesize that during drought, diatom communities enter a “frozen” state, resuming activity with the same species composition once water becomes available again. While one might expect post-drought communities to be dominated initially by pioneer species followed by secondary colonizers, our results do not support this scenario. Instead, they indicate that diatom assemblages display strong resistance, recovering rapidly and maintaining their pre-drought species composition.

4.4. Limitations of the study

Despite the robust design and analytical approach, our study has several limitations. First, the relatively small sample size and spatial extent may limit the generalizability of our findings to broader alpine landscapes. The restricted hydroperiod allowed us to sample the alpine ponds only for four summer months. It was not possible to collect data outside this period due to drought conditions in autumn and complete ice or snow cover in winter. Second, although we included a range of physicochemical variables, other important ecological drivers such as hydroperiod length, nutrient concentrations (e.g., P, N), or biotic interactions were not explicitly considered due to lack of instrumentation and funding. No water samples were collected for nutrient and cation analysis, despite their importance for diatom ecology and the spatiotemporal dynamics of benthic communities. Such measurements would also help quantify the influence of agricultural runoff and urban wastewater inputs, which can elevate sulphate, nitrate, and phosphate levels and strongly affect diatom assemblages. Third, given the relatively limited geographic extent of the study area and the discrete nature of the sampled ponds, we assumed minimal spatial autocorrelation among sites. The inclusion of elevation and season as covariates helped capture key spatial and temporal gradients that might otherwise induce spatial structure. However, we acknowledge that future studies should explicitly test for spatial autocorrelation (e.g., through Moran’s I tests) and potentially account for spatial structure using spatial eigenvector mapping or spatial autoregressive models. Finally, our analysis covered only a single year; Interannual variability may influence community dynamics and diversity patterns, particularly in highly seasonal temporary ponds. Long-term monitoring would be essential to capture such temporal fluctuations and to disentangle short-term variability from consistent ecological trends.

5. Conclusions

The ecological importance of ponds, particularly those at high altitudes, and their diatom communities is still overlooked, particularly in the Mediterranean region. Our study should be considered a starting point for investigating the main drivers of diatom diversity in ponds along elevational and seasonal gradients.

This research showed, using both general criteria (environmental, local, and seasonal factors) and specific diversity metrics (relative diatom diversity, α and β diversity indices) that the structure of benthic diatoms developed on these habitats was strongly influenced by elevation, physico-chemical parameters and weakly from seasonal variables. Diatom α diversity analysis revealed no significant seasonal and elevational effects: species richness did not differ significantly between low (LP) and high (HP) elevation groups among seasons, while Shannon diversity was significantly lower in LP sites. Our models identified pH and electrical conductivity (EC) as significant predictors of species richness, while elevation emerged as the main driver of Shannon and Simpson diversity. β diversity analyses indicated a strong effect of elevation on community dissimilarity, with community similarity decreasing significantly with increasing elevational distance. In particular, the calculated exponential decay model revealed a halving distance of about 1059 m, indicating that similarity drops by half over relatively short elevational gradients, highlighting a pronounced nonlinear decay pattern.

These findings suggest that even modest elevational differences can lead to substantial biological divergence in pond diatom communities, underscoring the ecological sensitivity of these systems. The observed patterns reflect broader ecological processes, such as environmental filtering, dispersal limitation, and habitat heterogeneity, which operate across multiple taxonomic groups. In the context of global change, high-altitude temporary ponds represent particularly vulnerable ecosystems. Climate warming may alter hydroperiods, snowmelt timing and temperature regimes, thus modifying the composition and resilience of diatom communities. Shortened hydroperiods could severely restrict colonization windows, reduce habitat availability, and increase the frequency of desiccation events—leading to community simplification and biodiversity loss. Given their rapid response to environmental fluctuations, diatoms can serve as early warning indicators of climate-induced alterations in freshwater ecosystems. They can provide valuable insight into how aquatic communities respond to reduced water availability, increased drought frequency, and hydroperiod unpredictability. Protecting high-altitude ponds and integrating diatom-based assessments into climate monitoring frameworks are therefore essential steps to anticipate biodiversity losses and ecosystem instability under future climate scenarios. To fully capture the drivers of α and β diversity, future studies should extend monitoring over multiple years include ponds from different biogeographical regions, and sample multiple sites per elevation band A more comprehensive approach—incorporating additional environmental and local variables—will help confirm the reliability of diatom assemblages as bioindicators and strengthen their application in the conservation and assessment of temporary pond ecosystems.

CRediT authorship contribution statement

Davide Taurozzi: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Massimiliano Scalici:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Conceptualization.

Ethics approval

Not applicable.

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Declaration of competing interest

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.

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Dedicated to Lilly, for lovingly being a part of my family. May you live on in every gesture, thought, and achievement of your beloved sister.

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Appendix DSupplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2025.122630.

Appendices.

Appendix A. Model selection

We chose to use a GAM model over an LMM model for our analysis because the GAM allowed for flexible, non-linear relationships between the predictors and the response variable, which were essential when dealing with ecological data could not show a linear trend (Wood, 2017). Unlike LMMs, which assume fixed relationships between variables, GAMs enabled us to model smooth, non-linear effects for each environmental and local variable, accounting for complex interactions in a more flexible manner. This approach allows us to better capture the underlying ecological processes, which might not be apparent with a simpler linear model. Furthermore, while univariate analyses can identify individual variable effects, they do not consider potential interactions between predictors, which could lead to misleading conclusions (Wood, 2017; Zuur et al., 2009). In contrast, the GAM approach considered covariation among the predictors and their joint influence on species richness, leading to a more robust understanding of the system (Wood, 2017). Thus, the GAM model provided a more accurate and nuanced representation of the relationships between environmental variables and biodiversity in our study.

Appendix B. Physicochemical parameters

Across the six sampling sites, environmental variables exhibited spatial and temporal variability throughout the year. The water temperature showed a decrease from spring/summer to autumn/winter across all sites, with the recorded values that ranged from 3.8 °C (TP400, December) to 30.57 °C (TP0, July), while pH values varied from 7.37 (TP0, November) to 10.00 (TP800, August), with higher values generally recorded during summer. Oxygen reduction potential was highly variable, with maximum values observed in TP1200 and TP1600 in June (304,6 mV and 238.65 mV, respectively) and minimum values in TP1200 in October (−71.1 mV). Similarly, Electrical conductivity levels ranged from 1723 μS/cm (TP800, December) to 150 μS/cm (TP0, April), while Dissolved oxygen ranged from 8.51 ppm (TP0, May) to 1.07 (TP800, November) (Table S3).

Appendix C. Diatom summary

An investigation of the epilithic diatoms resulted in the description of 53 genera and 232 diatom species in the 6 ponds analysed throughout the year (overall, 41 samplings), corresponding to 50,400 individuals analysed. LP hosted 196 diatom species; HP hosted 150 diatom species.

Maximal richness was observed in TP400 (n = 122), followed by TP800 (n = 115) and TP0 (n = 93). For high-elevation ponds, TP1200 showed the highest number of species (n = 90), followed by TP2000 (n = 82) and TP1600 (n = 60). 14 species accounted for 60 % of total abundances; *Achnanthidium minutissimum* showed the highest records with 4202 individuals (8.3 % of the total population), followed by *Achnanthidium eutrophilum* with 4120 individuals (8.1 %) and *Denticula tenuis* with 3089 individuals (6.1 %).

Data availability

Data will be made available on request.

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