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Diatom taxonomic diversity and functional ecology in temporary ponds: ecological shifts along a Mediterranean - Alpine elevational gradient

Diversità tassonomica delle diatomee ed ecologia funzionale nelle pozze temporanee: cambiamenti ecologici lungo un gradiente altitudinale alpino-mediterraneo

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RIASSUNTO

Le pozze temporanee rappresentano uno degli ambienti di acqua dolce più diffusi al mondo: il loro numero e la superficie totale supera quella dei laghi, rendendole a tutti gli effetti tra gli ecosistemi lentici più importanti dal punto di vista ecologico, capaci di sostenere un'elevata biodiversità. In letteratura, le pozze sono recentemente definite come corpi idrici di piccole dimensioni (< 5 ha), poco profondi (< 5 m) e con meno del 30% di vegetazione emergente. La loro natura temporanea è determinata dal carattere intermittente dell'idroperiodo, ovvero del periodo durante il quale l'acqua, allo stato liquido, è presente nel bacino. In questi habitat, l'idroperiodo è generalmente limitato a una parte dell'anno: nelle regioni mediterranee, infatti, l'autunno, l'inverno e la primavera corrispondono al periodo di "piena", quando le pozze sono colme d'acqua. Al contrario, durante l'estate, a causa delle elevate temperature e della scarsa piovosità tipica della bioregione mediterranea, le pozze temporanee attraversano una fase di secca. Sebbene questo schema sia generalizzabile alla maggior parte delle pozze temporanee, quelle d'alta quota, tipiche delle bioregioni alpine, mostrano un andamento opposto: in inverno, a causa della neve e delle basse temperature, l'acqua è presente ma non disponibile (in forma solida, come ghiaccio o neve); viceversa, in estate, lo scioglimento nivale rende l'acqua disponibile allo stato liquido, riempiendo il bacino e consentendo la ripresa dell'attività biologica. La biodiversità che popola questi ambienti è altamente specializzata e perfettamente adattata ai periodi di secca, più o meno ricorrenti. Tuttavia, numerosi disturbi di origine antropica, come inquinamento, modificazioni del suolo, urbanizzazione, incendi e pratiche intensive di allevamento e agricoltura, possono contribuire in modo significativo alla scomparsa di questi habitat. Tra tutti i fattori di minaccia, il cambiamento climatico rappresenta quello più preoccupante, poiché genera idroperiodi sempre più brevi e mette in seria difficoltà l'intera biodiversità che dipende da questi ecosistemi effimeri. Tra gli organismi più impattati vi sono i produttori primari, e in particolare le diatomee, alghe microscopiche ed ubiquitarie, responsabili di circa il 30% della produzione globale di ossigeno. Nonostante siano incluse nella Direttiva Quadro sulle Acque come bioindicatori per gli ambienti d'acqua dolce, il loro utilizzo negli ecosistemi temporanei rimane estremamente limitato. I cambiamenti che potrebbero interessare le pozze temporanee nei prossimi anni richiedono monitoraggi e azioni di conservazione mirate, in cui le diatomee possono rivestire un ruolo chiave, fungendo da indicatori sensibili per rilevare variazioni su scala locale e globale.

Lo scopo di questo progetto di dottorato è stato quello di fornire una base solida per le attività di mappatura e monitoraggio delle pozze temporanee in Italia, colmando un importante vuoto scientifico sull'uso delle diatomee come bioindicatori delle acque effimere.

1. Primo obiettivo (Capitolo I) — Revisione bibliografica internazionale

Il primo obiettivo è stato quello di realizzare una sintesi globale sull'uso delle diatomee come bioindicatori negli ambienti acquatici temporanei, analizzando la letteratura scientifica disponibile e le principali tecniche e approcci adottati. La ricerca bibliografica ha individuato 512 pubblicazioni, delle quali 48 articoli (dal 1982 in poi) trattavano specificamente di diatomee e ambienti effimeri. Per evitare discrepanze terminologiche, gli ambienti effimeri sono stati classificati in tre categorie:

- Intermittent Running Waters (IRW): fiumi, torrenti, ruscelli, sorgenti
- Temporary Large Wetlands (TLW): zone umide, laghi, lagune
- Temporary Small Waters (TSW): stagni, pozze, ventarole, fossi, acque termali, abbeveratoi

Studi sono stati riportati in tutti i continenti, inclusa l'Antartide. Gli ecosistemi più indagati risultano gli IRW (n = 683), seguiti da TSW (n = 239) e TLW (n = 96). La maggior parte dei siti studiati si trova al di sotto dei 1000 m s.l.m. (n = 27) e riguarda diatomee epilitiche (52%). I principali parametri fisico-chimici considerati sono stati conduttività elettrica (80%), pH (77%) e temperatura dell'acqua (75%).

2. Secondo obiettivo (Capitolo II) — Mappatura delle pozze d'alta quota in Italia

Il secondo obiettivo è stato la mappatura della distribuzione delle pozze d'alta quota (oltre 300 m s.l.m.) sul territorio italiano, creando il primo database nazionale. A tal fine, sono stati integrati dati spettrali e morfologici provenienti dai satelliti Sentinel-1 e Sentinel-2, elaborati su Google Earth Engine (GEE). Sono state identificate 2156 pozze, di cui 62% nelle Alpi (n = 1343) e 38% negli Appennini (n = 813). Le aree con la maggiore densità di pozze sono risultate le Alpi Centrali (n = 642), seguite da Alpi Occidentali (n = 479), Appennino Centrale (n = 412), Alpi Orientali (n = 222), Appennino Meridionale (n = 216) e Appennino Settentrionale (n = 185). La superficie complessiva coperta dalle pozze è stata stimata in 4.258.640 m², con una media di 1716 m². Il 20% delle pozze è risultato temporaneo (n = 445) e l'80% permanente (n = 1711). In termini di uso del suolo, l'83% (n = 1797) è stato classificato come naturale e il 17% (n = 359) come antropizzato.

3. Terzo obiettivo (Capitoli III, IV, V) — Analisi delle comunità di diatomee in pozze mediterranee e alpine

Il terzo obiettivo ha riguardato lo studio delle comunità di diatomee in pozze temporanee mediterranee e alpine, valutando come stagionalità, idroperiodo e parametri fisico-chimici

influenzino la diversità alfa e beta, sia tassonomica che funzionale. Sono state campionate 12 pozze temporanee nella Tenuta Presidenziale di Castelporziano (a livello del mare, 0 – 74 m a.s.l.) e 12 pozze nella piana di Campo Imperatore (Parco Nazionale del Gran Sasso e Monti della Laga, circa 1700 m s.l.m.). È emerso che, nelle pozze mediterranee, la ricchezza di specie non è influenzata dalla durata dell'idroperiodo o dalle variazioni stagionali. Le variabili più determinanti per la struttura delle comunità sono risultate pH e conduttività elettrica. Al contrario, nelle pozze alpine la diversità delle diatomee risponde principalmente a fattori locali ed ambientali, che prevalgono su eventuali gradienti geografici. L'altitudine gioca un ruolo chiave nel determinare l'unicità delle comunità di diatomee, favorendo lo sviluppo di comunità funzionalmente uniche alle quote più elevate.

4. Quarto obiettivo (Capitoli VI, VII, VIII) — Effetto del gradiente altitudinale sulle comunità di diatomee

L'ultimo obiettivo è stato quello di analizzare come la quota altitudinale e la stagionalità influenzino le comunità di diatomee, attraverso il campionamento di sei pozze lungo un gradiente da 0 a 2000 m s.l.m., a intervalli di circa 400 m, per un anno. Anche in questo caso, pH e conduttività elettrica si sono confermati come i principali fattori determinanti, insieme all'altitudine. La similarità tra le comunità diminuisce con l'aumentare della quota, dimezzandosi ogni circa 1059 m; la similarità iniziale (a 1 m di differenza altitudinale) è stata stimata in 0.286, con una distanza di dimezzamento di circa 289.56 m. Altitudine e parametri ambientali influenzano indipendentemente la struttura delle comunità, mentre gli effetti stagionali risultano secondari. Dal punto di vista ecologico, ciò suggerisce che le comunità di diatomee delle pozze temporanee sono fortemente modellate da gradienti fisico-chimici e altitudinali, risultando altamente sensibili ai cambiamenti ambientali.

In conclusione, questa tesi di dottorato affronta un tema poco studiato in letteratura: il monitoraggio dello stato di salute delle pozze temporanee in Italia, realizzato attraverso la mappatura della loro distribuzione nazionale e l'impiego delle diatomee come bioindicatori degli effetti di stagionalità, altitudine e variabili chimico-fisiche su questi fragili ecosistemi effimeri.

ABSTRACT

Temporary ponds represent one of the most widespread freshwater environments in the world. Their number and their total surface exceed that of lakes, making them among the most ecologically important lentic ecosystems, capable of sustaining remarkably high biodiversity. In the scientific literature, ponds have recently been defined as small (< 5 ha), shallow (< 5 m) waterbodies with less than 30% emergent vegetation. Their temporary nature is determined by the intermittent hydroperiod, i.e., the period during which liquid water is present within the basin. In these habitats, the hydroperiod is usually limited to part of the year: in Mediterranean regions, autumn, winter, and spring correspond to the "wet phase," when ponds are filled with water. Conversely, during summer, due to high temperatures and the typical low rainfall of the Mediterranean bioregion, temporary ponds undergo a dry phase. Although this pattern applies to most temporary ponds, high-altitude ponds, typical of alpine bioregions, show an opposite trend: in winter, due to snow cover and low temperatures, water is present but unavailable (frozen as ice or snow); in summer, snowmelt provides liquid water, filling the basin and allowing biological activity to resume. The biodiversity inhabiting these environments is highly specialized and perfectly adapted to recurring dry phases. However, numerous anthropogenic disturbances, such as pollution, land-use changes, urbanization, wildfires, and intensive agricultural and grazing practices, are significantly contributing to the disappearance of these habitats. Among all threats, climate change is the most alarming, as it leads to increasingly shorter hydroperiods and severely impacts the entire biodiversity dependent on these ephemeral ecosystems. Among the most affected organisms are primary producers, particularly diatoms, ubiquitous microscopic algae responsible for approximately 30% of global oxygen production. Although they are included in the EU Water Framework Directive as bioindicators for freshwater environments, their use in temporary systems remains extremely limited. The changes that temporary ponds may undergo in the coming years call for targeted monitoring and conservation actions, in which diatoms can play a key role as sensitive indicators capable of detecting ecological variations at both local and global scales.

The main aim of this PhD project was to provide a solid foundation for mapping and monitoring temporary ponds in Italy, filling a significant scientific gap concerning the use of diatoms as bioindicators of ephemeral waters.

1. First objective (Chapter I) — International literature review

The first objective was to provide a global overview of the use of diatoms as bioindicators in temporary aquatic environments, by analyzing existing scientific literature and summarizing the main methodologies and approaches adopted. The bibliographic search identified 512 publications, of which 48 papers (from 1982 onward) specifically addressed diatoms and ephemeral waters. To avoid terminological discrepancies, ephemeral waters were classified into three categories:

- Intermittent Running Waters (IRW): rivers, streams, creeks, springs
- Temporary Large Wetlands (TLW): wetlands, lakes, lagoons
- Temporary Small Waters (TSW): ponds, ponds, ventaroles, ditches, thermal waters, troughs

Studies were reported from all continents, including Antarctica. The most investigated ecosystems were IRW (n = 683), followed by TSW (n = 239) and TLW (n = 96). Most study sites were located below 1000 m a.s.l. (n = 27) and focused mainly on epilithic diatoms (52%). The main physicochemical parameters considered were electrical conductivity (80%), pH (77%), and water temperature (75%).

2. Second objective (Chapter II) — Mapping high-altitude ponds in Italy

The second objective consisted of mapping the distribution of high-altitude ponds (above 300 m a.s.l.) across Italy, creating the first national database. To achieve this, spectral and morphological data from Sentinel-1 and Sentinel-2 satellites were integrated and processed using Google Earth Engine (GEE). A total of 2,156 ponds were identified, of which 62% (n = 1,343) were located in the Alps and 38% (n = 813) in the Apennines. The regions with the highest pond densities were the Central Alps (n = 642), followed by the Western Alps (n = 479), Central Apennines (n = 412), Eastern Alps (n = 222), Southern Apennines (n = 216), and Northern Apennines (n = 185). The total surface area covered by ponds was estimated at 4,258,640 m², with a mean of 1,716 m² per pond. Approximately 20% (n = 445) of the ponds were temporary, while 80% (n = 1,711) were permanent. In terms of land use, 83% (n = 1,797) of ponds were classified as natural, and 17% (n = 359) as anthropogenic.

3. Third objective (Chapters III, IV, V) — Diatom communities in Mediterranean and alpine ponds

The third objective focused on analyzing diatom communities in Mediterranean and Alpine temporary ponds, evaluating how seasonality, hydroperiod, and physicochemical parameters influence taxonomic and functional alpha and beta diversity. A total of 12 temporary ponds were sampled in

the Presidential Estate of Castelporziano (sea level, 0–74 m a.s.l.) and 12 ponds in the Campo Imperatore plain (Gran Sasso and Monti della Laga National Park, ~1700 m a.s.l.). Results showed that, in Mediterranean temporary ponds, diatom species richness was not influenced by hydroperiod length or seasonal variation. The most important drivers of community structure were pH and electrical conductivity. Conversely, in Alpine ponds, diatom diversity mainly responded to local and environmental factors, which outweighed any potential geographical gradients. Elevation played a key role in shaping the uniqueness of diatom communities, promoting the development of functionally distinct assemblages at higher altitudes.

4. Fourth objective (Chapters VI, VII, VIII) — Effect of the altitudinal gradient on diatom communities

The final objective was to assess how elevation and seasonality affect diatom communities, through the sampling of six ponds along an altitudinal gradient ranging from 0 to 2000 m a.s.l., at approximately 400 m intervals, over one year. Again, pH and electrical conductivity were confirmed as the main driving factors, together with altitude. Community similarity decreased with increasing elevation, halving every ~1059 m; initial similarity (at 1 m elevation difference) was estimated at 0.286, with a halving distance of about 289.56 m. Elevation and environmental variables independently shaped community structure, while seasonal effects were minor. Ecologically, these findings indicate that diatom communities in temporary ponds are strongly structured by physicochemical and altitudinal gradients, making them highly sensitive to environmental changes such as climate shifts or pollution.

In conclusion, this PhD thesis addresses a largely understudied topic: the monitoring of the health status of temporary ponds in Italy, achieved through the nationwide mapping of their distribution and the use of diatoms as bioindicators of the effects of seasonality, elevation, and physicochemical variables on these fragile and ephemeral freshwater ecosystems.

INTRODUCTION

Globally, it is estimated that there are 547 million – 3,19 billion ponds distributed across all continents, covering a surface area larger than that of lakes (Hill et al., 2021). These ponds are extremely difficult to define scientifically, as their classification often overlaps with that of lakes or wetlands. Although this uncertainty remains widespread, Richardson et al. (2022) attempted to provide a standardized definition based on a comprehensive literature review that examined the most common terms used for these habitats. The outcome of that review was adopted as the reference definition in this thesis. Accordingly, ponds are defined as *waterbodies that are small (< 5 ha), shallow (< 5 m), and with < 30% emergent vegetation.* Temporary ponds represent one of the most peculiar and biodiversity-rich ecosystems on Earth. Temporary ponds are particularly widespread in Mediterranean regions, where they often represent the remnants of ancient wetlands that, in past decades, were much more extensive but were drastically reduced following land reclamation projects, leaving behind small, fragmented patches of wetland habitat (Van den Broeck et al., 2015). However, their distribution is not limited to the Mediterranean biogeographic region: temporary ponds also occur in a variety of other environmental contexts, including continental and alpine zones (Lamouille-Hébert et al., 2024).

The term *"temporary"*, when referring to ponds, indicates the variability of their hydroperiod, that is, the period during which water in its liquid state is actually present in the basin (Coccia et al., 2024). Unlike permanent ponds, whose hydroperiod remains stable throughout the year (except for extreme events that cause complete evaporation of the water) for at least 90% of the observation years, temporary ponds undergo one or more dry phases that generally correspond to the summer period in at least 90% of the observation years (Tinelli et al., 2012). Ponds are usually filled by rainfall, snowmelt (at higher elevations), or groundwater intrusion (Hayashi and van der Kamp, 2021). The permeability and slope of the substrates determine the amount of water reaching the pond through surface runoff and groundwater flow (Zacharias and Zamparas, 2010), which controls the duration of the hydroperiod. Therefore, the geological structure of the site in which a pond develops has a strong influence on its hydrological regime (Fernández-Ayuso et al., 2018): seasonal and annual variations in water volume mainly depend on the balance between water inflows and outflows. Inflows include rainfall, surface runoff, melting snow, and groundwater inputs. Outflows include infiltration, overflow, and evapotranspiration.

This alternation between wet and dry phases makes temporary ponds unique environments from a biodiversity perspective. They host some of the most distinctive organisms on the planet, many of

which have evolved highly specialized adaptations to cope with the temporary absence or scarcity of water (Bagella, 2023). Such adaptations include short life cycles, multiple reproductive strategies, or the formation of resistant cysts that allow individuals to enter a dormant phase corresponding to the dry season, during which metabolic activity is drastically reduced, enabling survival until the next wet phase (Guidetti and Møbjerg, 2019). Furthermore, certain species are found only in specific "types" of ponds that provide the optimal conditions for their survival. These conditions are primarily abiotic and include differences in the physicochemical properties of water, such as temperature, pH, salinity, oxygen availability, nutrient concentrations, light intensity, and water turbidity (Brönmark and Hansson, 2017). Most pond-dwelling organisms are *poikilothermic*, meaning their internal body temperature varies with the external environment. Therefore, extreme or rapid temperature fluctuations can significantly affect their survival. Similarly, *pH*, a measure of water acidity, is closely linked to the equilibrium processes of the carbon dioxide–bicarbonate system (involving free CO_2, carbonic acid H_2CO_3, bicarbonate ions HCO_3^-, and carbonate ions CO_3^{2-}). Variations in pH can negatively affect the reproduction and survival of numerous aquatic organisms, including mollusks, insects, and crustaceans, and can reduce species richness even at the base of the food web, within phytoplankton and zooplankton communities (Pinheiro et al., 2021). Lastly, water turbidity and the amount of light reaching the pond bottom (expressed in photons and wavelengths) have a strong influence on primary productivity, as they directly affect the photosynthetic activity of primary producers (Tanabe et al., 2019). These, in turn, regulate the availability of dissolved oxygen. Changes in hydroperiod and abiotic variability also shape the biotic interactions among organisms inhabiting temporary ponds (Cruz et al., 2025). For instance, during wet phases, temporary ponds provide abundant trophic resources for the resident biodiversity, leading to a general decrease in predation pressure. Conversely, during dry phases, trophic resources become limited and predation rates tend to increase. Predation, together with herbivory, represents one of the main sources of mortality for many freshwater organisms and therefore acts as a fundamental structuring force within ponds, affecting population dynamics and overall community structure (Brönmark and Hansson, 2017).

Temporary ponds, due to their inherently ephemeral nature, are among the most threatened habitats worldwide, mainly because of the direct and indirect effects of human activities (Zacharias et al., 2007). Increasing urbanization, particularly in the Mediterranean region, together with the construction of roads, buildings, and land modification, has led to a massive loss of temporary ponds (Keinath et al, 2023; Oertli and Parris, 2019). Furthermore, agriculture and intensive livestock farming contribute enormously to their disappearance (Erős et al., 2020). Water from ponds is often used for various purposes, such as irrigation of agricultural fields, watering of livestock, and domestic

use (Pulido et al., 2020). This may occur through direct extraction or by pumping groundwater near the ponds, frequently resulting in their premature drying (Zacharias et al., 2007). For this reason, temporary ponds are often artificially converted into permanent ones through water supplementation, to be used as reservoirs by landowners. The extension of the hydroperiod may sometimes have positive effects: ponds with a longer water supply can host both early- and late-breeding faunal species (Brönmark and Hansson, 2017). However, when water input is prolonged, processes such as competition among aquatic plants, algal blooms, and competitive exclusion can occur, particularly affecting rare species typical of ephemeral environments, while favoring taxa characteristic of permanent water bodies. Moreover, many ponds are artificially drained for mineral extraction, such as clay or fertile soils. In other cases, the surrounding land is cultivated with woody species whose root systems absorb large amounts of water, depleting both groundwater and, indirectly, the ponds themselves (Brönmark and Hansson, 2017). The disastrous impact of climate change must also be considered, as its effects are clearly visible in Mediterranean regions. Should rainfall decrease in frequency and intensity over the coming decades, temporary ponds could experience longer or even total dry phases, leading to their disappearance or causing significant shifts in the phenology of the biodiversity they support (Celewicz and Gołdyn, 2021). Wildfires, typical of the Mediterranean region and exacerbated by increasingly arid summers, along with unregulated tourism and rising pollution from intensive agricultural activities, pose serious threats to the survival of these environments (Zacharias et al., 2007). At the same time, many animals that rely on these ponds as watering sites may face severe water shortages, with consequent negative effects on ecological balance.

From a legislative perspective, the protection of temporary ponds falls within the broader framework of wetland conservation, as established by the Ramsar Convention (1971) (Hill et al., 2018). This intergovernmental treaty provides the basis for national action and international cooperation for the conservation and rational use of wetlands and their resources, and it remains the only global environmental agreement specifically dedicated to this category of ecosystems. However, despite this overarching framework, the conservation of temporary aquatic environments at the global and regional scales remains precarious. In particular, under the European Water Framework Directive (WFD; 2000/60/EC), small water bodies, including temporary ponds, are not considered “significant water bodies” and are therefore excluded from mandatory ecological status monitoring by environmental protection agencies. This regulatory gap has contributed to the long-standing neglect of temporary ponds in routine assessment and management programs, further limiting their effective recognition and conservation. Only recently, through the EU Habitats Directive (92/43/EEC),

Mediterranean temporary ponds have been acknowledged as priority habitats, due to their high biodiversity value (Bagella, 2023). They act as biodiversity hotspots, providing refuge for extremely rare and isolated organisms belonging to different taxa.

The classification as priority habitats identifies three types of ponds to be protected, based on the presence of specific plant species belonging to Isoëtion communities (Angelini et al., 2016):

- 3120 – Oligotrophic waters with very low mineral content on generally sandy soils of the western Mediterranean, with *Isoëtes* spp.
- 3130 – Standing waters, from oligotrophic to mesotrophic, with vegetation of L*ittorelletea uniflorae* and/or *Isoëto-Nanojuncetea*
- 3170* – Mediterranean temporary ponds

As defined by the Directive, these are small-sized environments whose surface area is rarely mappable (de Necker et al., 2025). Regarding water analyses, since they are small water bodies, the Directive specifies that it is not possible to fully apply the indicators and reference values proposed for large permanent water bodies under the Water Framework Directive (WFD), as predictive ecological models differ due to their distinct trophic potential (Masouras et al., 2021). Nevertheless, although this classification provides protection to certain pond types identified through the above criteria, it excludes many temporary ponds that do not host the specific plant species listed in the Directive. This exclusion represents a serious legislative gap, endangering key environments that are essential for the survival of numerous plant and animal species inhabiting these fragile ecosystems.

Among the various types of temporary ponds, high-altitude temporary ponds, often included within the Alpine biogeographic region, are among the most fragile and threatened. These ponds sometimes incorrectly referred to as “lakes,” are typically formed at high elevations by the melting of snow and ice during the summer season, which corresponds to their wet phase (Salerno et al., 2016). Above a certain altitudinal threshold, generally coinciding with the tree line, these ponds exhibit a hydroperiod complementary to that of Mediterranean temporary ponds. While the latter experience a dry phase in summer and a wet phase in winter, Alpine ponds undergo a “dry” or more precisely frozen phase in winter, when water is present only in solid form, rendering biotic activity impossible. During spring and summer, as temperatures rise, snow and ice melt, restoring liquid water and filling the ponds once again (Lamouille-Hébert et al., 2024). However, in recent years, due to the increasingly high temperatures that affect these environments even during summer, Alpine temporary ponds have been observed to experience a double dry phase: one during winter (when frozen), and another during the

warmest months of summer, caused by evaporation. In some cases, ponds may refill briefly during the early autumn months before drying again. This double alternation between wet and dry conditions makes these habitats exceptionally peculiar and even more fragile.

The short and intermittent hydroperiod, characterized by alternating phases within the same solar year, can strongly influence the ecology and phenology of the biocenosis, as well as the overall productivity of these systems. Many primary producers, which form the base of the trophic web and sustain all higher trophic levels, require a certain period of time to restore their populations following pond desiccation (Allan et al, 2021). This is the case for diatoms, ubiquitous unicellular algae that typically require about 15–20 days to fully regenerate after rewetting. Diatoms are brown-colored Bacillariophyceae, lacking flagella and enclosed within a siliceous shell composed of two overlapping parts, the epitheca and the hypotheca, resembling a box with a lid (Seckbach and Gordon, 2019; Menon and Minu, 2017). Generally autotrophic (with only a few saprophytic species), diatoms store energy in the form of oil droplets. During periods of drought or in the absence of liquid water, diatoms produce quiescent spores (endospores or aplanospores): thick-walled cells with drastically reduced metabolic activity that enable survival under adverse conditions or complete desiccation. These spores form within the frustule, the siliceous skeleton of the diatom, and can withstand prolonged periods of dehydration, freezing, or darkness. This represents a key survival strategy during unfavorable seasons, although not the only one. Even without forming true spores, some diatom species can persist as quiescent cells within the sediment, where microfilms of residual moisture allow individuals to maintain minimal metabolic activity until environmental conditions improve (Sanyal et al., 2022).

Diatoms represent a fundamental component of all freshwater and marine ecosystems, contributing to over 30% of global primary production and thus playing an important role in the formation of aquatic food webs (De Tommasi, 2016; Kamp et al., 2011). They show distinctive characteristics, such as ubiquity, species-specific responses to environmental parameters, ease of sampling, and short generation times, which have led to their inclusion in the Water Framework Directive as one of the four biological quality elements (together with macroinvertebrates, macrophytes, and fish) used for water quality monitoring. Although their use is strongly recommended, diatoms are still employed less frequently than other bioindicators. In temporary ponds, however, their application, though limited, represents an ideal solution for assessing changes in ecosystem health: fish are excluded since they are absent from ephemeral habitats; macrophytes often exhibit low coverage; and macroinvertebrates, while present, show a much more restricted distribution than diatoms, often with only a few families represented in each pond. Diatoms, by contrast, develop successfully even in

temporary environments, providing a robust bioindicator base for defining the ecological status of these ecosystems (Delgado et al., 2012).

The conservation of temporary ponds is therefore closely intertwined with the study of diatom communities. Protecting these habitats is of primary importance for safeguarding the rare species they host. A significant knowledge gap still exists in the scientific literature concerning temporary ponds, a gap that becomes even more pronounced when considering the use of diatoms as bioindicators for assessing their ecological condition. Climate change, extreme weather events, and anthropogenic pollution, together with land use changes, seriously threaten the current and future persistence of temporary ponds (Matthews, 2010). The systematic use of diatoms as bioindicators can provide a comprehensive and detailed picture of the ecological health of temporary ponds, the impacts of climate change, and the expected shifts in biodiversity in response to ongoing global environmental transformations. These changes may vary according to the geographical context, revealing differences among temporary ponds, similar habitats embedded in distinct regions, such as the Mediterranean and Alpine zones.

This work, along with the papers associated with it, represents a first step toward filling this substantial scientific gap concerning the conservation and management of temporary ponds on a national scale in Italy. The thesis has been organized into eight sections, providing a solid foundation for the conservation of both Mediterranean and Alpine temporary ponds in Italy, with approaches that can be extended and replicated worldwide. Specifically, the rationale underlying this doctoral thesis concerns the study of diatom communities in temporary ponds, used as bioindicators of environmental variations at local, regional, and biogeographical scales. Through remote sensing analyses for pond identification and the use of diatoms as bioindicators, also in relation to local and regional environmental parameters, this research investigates how and to what extent diatom assemblages vary in temporary environments and how the hydroperiod influences vegetative recovery in terms of both taxonomic and functional diversity. This type of analysis is conducted for the first time in Italy, and among the very few globally, representing one of the first broad-scale mapping and monitoring efforts of diatom assemblages that simultaneously considers factors such as seasonality, hydroperiod, environmental and biotic parameters, and altitudinal gradients.

The first objective of this work, addressed in the first chapter, is to investigate the global use of diatoms as bioindicators in temporary waters, through a literature review. These environments can include not only ponds but also wetlands, rivers, streams, lakes, vernal pools, and many other habitats, which share similar abiotic characteristics but are referred to by different names in different

geographical contexts. To date, diatoms have been largely used as bioindicators in permanent environments. Their application in temporary habitats remains very limited, and greater attention should be paid to these ephemeral and vulnerable ecosystems, especially in light of ongoing climate change. Moreover, diatoms represent a powerful tool capable of providing essential ecological feedback to managers and policymakers to assess the health status of ephemeral habitats (Agarwal, 2025). To achieve this objective, it is crucial first to understand the trends in the literature regarding the use of these bioindicators and to identify the gaps associated with this approach.

The second objective of this work concerns understanding the actual distribution of high-altitude ponds on a national scale, with a dual focus on both temporary and permanent ponds. The proper management of these habitats relies primarily on accurate geographic identification, risk and anthropization categorization, understanding of origin, and mapping. To date, no mapping of high-altitude ponds has been conducted in Italy, generating a significant gap in the active conservation and protection of these ecosystems. In the second chapter, a mapping of temporary and permanent high-altitude ponds in Italy is presented, conducted through satellite imagery and remote sensing, forming a foundational dataset for the management of these habitats. The focus on high-altitude ponds was set above 300 m a.s.l., where knowledge gaps are generally most pronounced.

The third objective is the analysis of diatom communities in Mediterranean and Alpine temporary ponds. The hydroperiod is one of the most important abiotic determinants influencing communities in temporary environments. Globally, studies on diatoms in ephemeral habitats are very scarce, and it is also likely that in similar environments with complementary dry periods, diatom communities may respond differently. Community responses are crucial and highly influential for the ecosystem because, being at the base of the food web, they strongly affect higher trophic levels. These responses can be examined at both taxonomic and functional levels: life forms, cell size, and guilds serve as indicators of ecosystem health, with each functional trait reflecting specific environmental conditions. Simultaneously, environmental filtering selects only certain functional traits, generating cascading effects throughout the ecosystem. Knowledge of the various levels of diatom diversity is scarce or nearly absent in the literature, and chapters three, four, and five of this work aim to address this gap by providing the first data on how physicochemical, geographical, and spatial factors may influence diatom diversity.

The fourth objective is to analyze how elevation influences diatom communities. For this purpose, temporary ponds along an altitudinal gradient in central Italy, from typically Mediterranean zones to Alpine regions, including intermediate elevations, were studied. Altitudinal gradients generally bring

structural diversity that translates into abiotic differences, which in turn shape biodiversity. Diatoms are highly sensitive to physical and chemical variations in their environment; thus, analyzing how their diversity is affected by elevation is key to understanding how, in the not-so-distant future, climate change might shape diatom communities, applying the same principles to habitats at different latitudes. In this regard, chapters six, seven, and eight of this work focus on the taxonomic and functional biodiversity of diatoms along an elevation gradient, from coastal areas to high Alpine environments. Through a one-year sampling protocol, we analyze how seasonal variations influence diatom communities, with a specific focus on the range of diversity between Alpine and Mediterranean temporary ponds.

Although Objectives III and IV address different ecological aspects related to diatoms and temporary ponds, and are presented in separate chapters, the methodologies applied throughout the thesis are fully comparable. In other words, while each chapter adopts distinct ecological perspectives to explore diatom diversity in relation to different environmental parameters, diatoms were consistently sampled, analyzed, and identified using the same standardized techniques and methodological approaches.

By employing uniform methods across all studies, it was possible to examine and compare α-, β-, γ- and ecosystem-level diversity among different environments, thereby minimizing potential sources of methodological bias and ensuring the robustness and comparability of the overall findings presented in the eight chapters that follow.

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CHAPTER I

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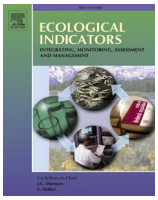

Review

Diatoms as bioindicators for health assessments of ephemeral freshwater ecosystems: A comprehensive review

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ABSTRACT

Ephemeral waters (EW) are sensitive and vulnerable ecosystems that, although the numerous ecosystem services provided, are poorly investigated and properly managed. Diatoms represent an important biological model largely used in aquatic permanent habitats but underused in ephemeral waters. The aim of this review is to address the knowledge gap, examining the current state of knowledge regarding the health of EW, with a focus on diatoms as bioindicators, specially (i) presenting research trends on EW ecosystem types investigated (ii) analysing the methodological approach, (iii) providing the influence of abiotic factors on the diatom assemblages in EW. A bibliographic search yielded a total of 512 papers. After removing duplicate records, 118 were eliminated. The screening of abstracts resulted in the selection of 48 articles on diatoms and EW since 1982. To avoid discrepancy between terminology we decided to classify the EW found in 3 different categories: Intermittent Running Waters (IRW: rivers, streams, creek, springs), Temporary Large Wetlands (TLW: wetlands, lakes, lagoons) and Temporary Small Waters (TSW: ponds, pools, ventaroles, ditches, thermal waters, troughs, pans). Studies are reported in all continents including Antarctic. IRW are the most investigated ecosystem (n = 683), followed by TSW (239) and TLW (96). Furthermore, studies were mainly conducted on sites located below 1000 m a.s.l. of altitude (n = 27). Studies mainly focus on epilithic diatoms (52 %). Electrical conductivity (80 %), pH (77 %) and water temperature (75 %) are the principal physical–chemical parameters considered. Moreover, electrical conductivity, pH, temperature, nitrate and nitrite resulted the best diatom community drivers. Future research is encouraged (1) to achieve a standardised protocol for monitoring EW using diatoms, (2) to focus on conservation projects for high-altitude temporary waters, and (3) to investigate the main driving forces influencing diatom diversity on EW.

1. Introduction

Ephemeral waters (EW) refer to both lentic or lotic ecosystems which water completely or partially stops flowing at any time of the year (Zharov et al., 2020). The presence and duration of EW are influenced by the fluctuating climate and ecological conditions throughout the year (Kulkarni et al., 2019). Abiotic factors like hydrogeological conditions (e.g., substrate type and permeability) and the annual precipitation regime are the main drivers influencing the consistency of these ephemeral ecosystems, while temperature, humidity, and hydroclimatic conditions contribute to the overall dynamic (Madaschi and Díaz-Villanueva, 2021). Despite their global distribution and the multitude of ecosystem services they offer, it is surprising how researchers and society have historically overlooked EW, probably due to their ephemeral existence and limited appeal for tourism and economic activities (Alonso, 2009).

In literature, EW are often identified with different names, lacking a unique nomenclature for these ecosystems. Behind the most common water bodies like ephemeral lakes, ephemeral ponds, intermittent rivers, intermittent streams, ephemeral wetlands there is a wide variety of EW, identified with different terms: ventaroles are Karst cavities in Miocene calcarenite, occasionally flooded (Bologna et al., 1994); vernal pools are

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EW not connected to river systems, but fed by shallow groundwater or by precipitation (Brooks, 2005); riverine pools are EW located in outer channels of rivers, not connected with the principal channel but usually fed by the alluvial water table (Schofield et al., 2018); ditches are part of wetlands created by human activities of natural phenomena, but also trenches occasionally flooded by rain (Al-Khudhairy et al., 2002). The diversity of terminology can lead to confusion regarding the precise definitions of certain terms or how to accurately interpret and compare various types of ecosystems.

EW ecological relevance resides in the support to life they give to biota inhabiting them and the surrounding habitats (Arumugam & Athikesavan, 2021): invertebrates dominate the fauna of EW, in terms of richness, abundance and rare taxa adapted to the natural hydrogeological modifications (Bird et al., 2019), besides uniquely specialised invertebrates (Strachan et al., 2015); birds use EW as a resource for foraging, nesting, bathing and water consumption (de Morais-Junior et al., 2019); moreover, EW represent a biodiversity hotspot for macroinvertebrates, fishes, macrophytes and diatoms supporting unique assemblages of vertebrates and invertebrates (Fritz et al., 2021). Among these, diatoms represent the main inhabitants of EW, due to their ability to colonize different substrates, their ubiquity and the rapid recovery time from the dry periods (Duong et al., 2007).

Furthermore, EW provide habitat and refugia for many species as well as contributing to human needs for freshwater in particular in arid and semiarid landscapes (Higgison et al., 2020). Under global change scenarios and widely anthropogenic pressure, EW threats tend to increase due to population increase, land use changes and declining precipitation (Grzybowski et al., 2019). Intermittent rivers and intermittent streams are among the most dynamic and complex freshwater ecosystems, occupying more than one third of the planet's land surface. However, they are also among the most threatened ecosystems (Karaouzas et al., 2018), for instance from human activities like wastewater treatment (Pascual-Benito et al., 2020) or acidification (Shah et al., 2021); ephemeral ponds are very vulnerable to human activities, due to their special physical and ecological characteristics and especially threatened by agriculture pollution, tourism, fire and water abstractions (Zacharias et al., 2007); ephemeral lagoons are often threatened by mining, pollution, biological disturbances (e.g., introduction of exotic species), and anthropogenically induced climatic and atmospheric changes. Ephemeral wetlands are threatened directly (i.e., agriculture) and indirectly by various human activities and pressures (i.e., reclamations) (Parra et al., 2021).

These ecosystems are very vulnerable to natural drought and anthropogenic water stress, as well as to nutrient enrichment from industrial and urban wastewaters, and organic pollution from agricultural activities. Furthermore, climate change affects mostly this type of ecosystems due to their ephemeral nature (Acuña et al., 2017; Zacharias et al., 2007). Despite significant advances in understanding their hydrology, ecology and management, many scientific and management gaps still remain, particularly in methodological and conceptual fields. This is especially evident regarding the use of ecological indicators for the assessment of the conservation status of EW.

The combined effects of abiotic, in particular anthropogenic (Akhtar et al., 2021; Vanderley et al., 2021; Gutiérrez-Cánovas et al., 2019) stressors on aquatic biotic assemblages, necessitate the use of biological models to assess the threat level to these ecosystems (Dubey et al., 2022).

Several efficient biological models are widely used to evaluate waters ecological status, including fishes, macroinvertebrates, macrophytes, and diatoms (Guerrero-Aguilar et al., 2022; Taurozzi et al., 2023; Marcheggiani et al., 2019;). Among these, benthic diatoms offer distinct advantages over other biological models in most habitats or for different environmental stressors, making them particularly valuable in routine environmental studies (Sharma et al., 2023). Benthic diatoms represent one of the most common biological quality elements (BQEs) used in surface water monitoring, adopted by the European Water Framework Directive 2000/60/EC (WFD) focusing on their advantages as bioindicators (Masouras et al., 2021). Diatoms are ubiquitous, easy to sample, and can be abundant in poor habitats, on hard substrates, or in rivers with high flow velocity where macroinvertebrates, macrophytes and phytoplankton (commonly used in lowland rivers), could be absent. Additionally, owing to their rapid growth rate, benthic diatoms exhibit swift responses to short-term hydrological changes, unlike macroinvertebrates (Pinheiro et al., 2020). However, their morphological and taxonomic identification is challenging and requires specialist expertise (Rimet et al., 2019).

The response of diatoms to multiple stressors in EW has received much less attention, leading to significant uncertainty on how natural and anthropogenic hydrological variation and physico-chemical pollution affect aquatic algae communities (Ganai et al., 2014). Furthermore, the use of diatoms for assessing the conservation status of EW is scarcely widespread. While pollution and water stress can have pronounced and well-documented effects on macroinvertebrate and fish species richness, abundance and community structure (Strungaru et al., 2021; Wheeler et al., 2020), only few studies have addressed this issue using diatoms as the primary tool for evaluating the ecological status of EW.

Given the global distribution of diatoms, their important role as ecological sentinels, and the critical conservation status of EW, this paper reviews and discusses the use of diatoms as bioindicators to assess the conservation status of EW worldwide. To address the knowledge gap, this review examines the current state of knowledge regarding the health of EW, with a focus on diatoms as bioindicators, specially (i) presenting research trends on EW ecosystem types investigated (including year, study area, author's affiliate Country), (ii) analysing the methodological approach (i.e., object of the study, and temporal and spatial effort of sampling), (iii) providing the influence of abiotic factors (i.e., altitude and physical–chemical parameters) on the diatom assemblages in EW.

2. Materials and methods

2.1. Bibliographic collection

This review follows the PRISMA guidelines for systematic reviews (Page et al., 2021; Moher et al., 2009). Data collection was carried out among peer reviewed international scientific articles via Scopus and Web of Science (without a lower time limit, until 24 March 2024). The review includes only peer reviewed material. For this search, only papers written in English were considered. Obviously, there is the possibility that some reports on this topic have been written in other languages. However, given that English is considered the universal form of communication in science and to uniform the results, articles in languages other than English, books, and other reports have been excluded. The keywords used to select the scientific material on the topic of interest were "diatoms", coupled with "waters", and "freshwaters", and "water basins" and "ponds", and "lakes", and "wetlands", "and "rivers", and "streams", all preceded by the adjectives and synonymous "ephemeral", "temporary" or "intermittent".

"Ephemeral", "temporary" and "intermittent" are used here as synonymous for waters that intermittently has standing water and that, once inundated, holds water long enough on occasion for some species to complete aquatic phases of their life cycle (Blaustein and Schwartz, 2001). A recurrent dry phase is the common feature shared by these ecosystems. However, there is not a unique definition regarding the hydroperiod characteristics in terms of duration and occurrence of the dry phase in the years (Williams et al., 2001). Here, we considered the definition from Wars (1992), considering temporary waters those which have a predictable annual dry phase, usually in the order of 3–8 months, predominantly during summer and autumn. They are also defined as "drying in most years" from Nicolet et al. (2004) adapted from the standard definition used for the UK National Pond Survey (NPS; Pond Action, 1998). Finally, it is important to consider that, according to

Williams (1997)) is difficult to predict the hydroperiod of EW and that, for this reason, there are two different types of EW: those following a seasonal cyclic pattern of dryness and flooding and those which will be flooded unpredictably.

Results were selected for including articles on the detection of epilithic, epiphytic, epipsammic, epipelic and planktonic diatoms, excluding papers on fossil remains of diatoms and paleolimnology. Firstly, duplicate articles were eliminated by a preliminary screening based on the title of articles. Not pertinent articles were excluded after verifying abstracts. Authors considered non-pertinent articles those referring to permanent freshwaters. The manuscript content of the remaining articles was subsequently checked for suitability as a final step. Thereafter, the list of results was obtained.

2.2. Collection of information data

Qualitative information were collected from all the articles about the year of publication, the ecosystem type (lakes, ponds, rivers, streams, wetlands…), the study area, the affiliation country of the authors, the topic of investigation and the diatoms type (planktonic, epiphytic, epipelic, epilithic, epipsammic), the study period (month and duration of the study), the global distribution of study sites, the altitude of the study area, and the chemical parameters taken into consideration (Table S2). Information about the study sites and relative coordinates were taken both from the paper body and from supplementary materials, where available.

2.3. Statistical analysis

A two sample *t*-test was performed comparing the number of articles and the relatives months involved in sampling for the northern and the southern hemisphere. Data normality and homoscedasticity were tested before performing the two sample *t*-test..

3. Results

3.1. Research trends on diatom indicators in ephemeral freshwaters

The initial output of the bibliographic research yielded a total of 512 papers. After removing duplicate records, 118 were eliminated. The screening of abstracts resulted in the selection of 48 articles for inclusion in the review (Fig. 1).

3.1.1. Bibliographic search – year of publication

Studies on temporary waters and diatom communities began in 1982 and followed a stable trend until 2012, where the number of papers increased until 2020, reaching a maximum of 7 publications per year (Fig. 1c).

3.1.2. Ephemeral freshwater ecosystem types investigated

Among the reviewed papers, 14 different types of EW were reported to be investigated using diatoms as indicators: lake, pond, river, stream, wetland, pool, spring, trough, ditch, thermal water, creek, lagoon, pan, ventarole. However, some of them are reported to be similar to others from an ecological point of view and are often described by different names, for example pond and pool, or creek and stream, or lake and lagoon (Krishnaraj, 2022). Ponds and wetlands are distinguished primarily by three variables, i.e., depth, surface area and water chemistry: Richardson et al., (2022) described ponds as small (<5 ha) and shallow (<5 m) ecologically distinct type of ecosystem respect to wetlands,

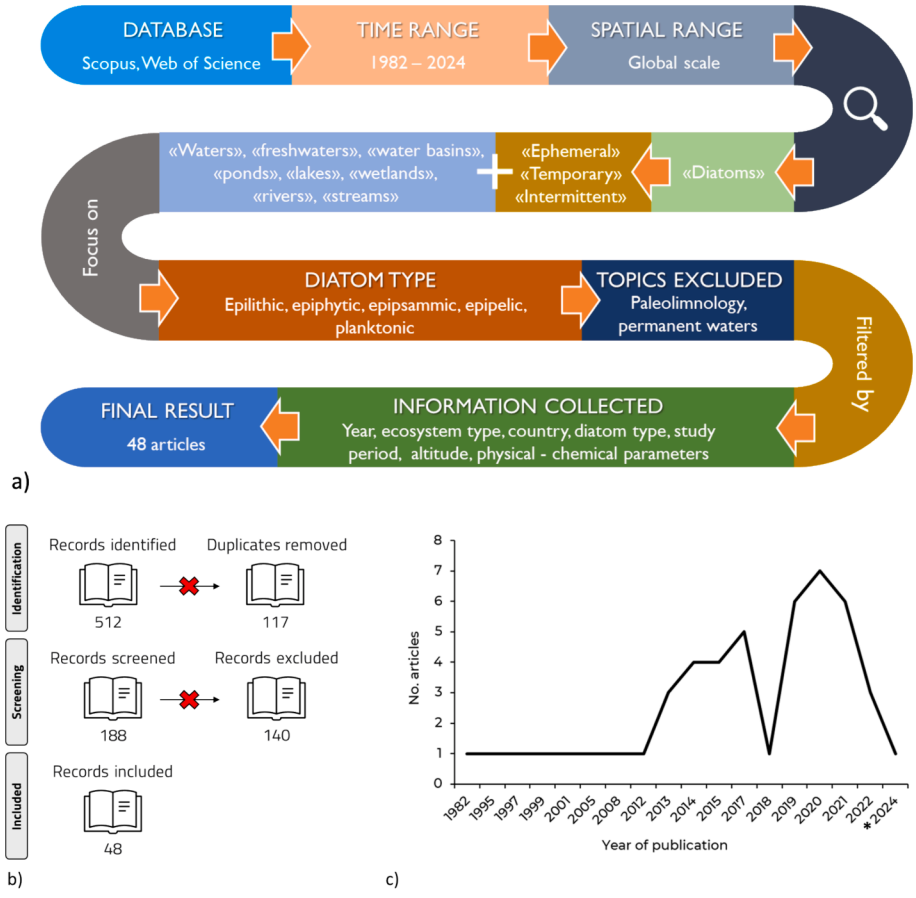

Fig. 1. Workflow of the bibliographic research (a), the PRISMA scheme followed for the selection of articles (b) and the temporal trend of literature about EW and diatoms (c). The asterisk (*) highlight that data available for 2024 are considered those until 24 March 2024 (last day of bibliographic research).

considering also chemical parameters like Total Nitrogen (TN) and water chemistry (pH) as important discriminant. Following Xu et al., (2019), wetlands can be considered a subset of lakes. Streams and rivers are distinguished by their flow and their fate: streams don't have mouths that exit into the oceans and seas, and they are more closely linked to wetlands in higher altitudes (Constantz et al., 2016). Instead, rivers are large, flowing bodies of freshwater that usually exit into the sea or ocean, with water sourced from several streams (Merz et al., 2021). Moreover, papers referring to diatoms in marine and brackish waters were not considered in the analysis. To avoid discrepancy between terminology and to simplify the categorization of different types of freshwaters we decided to unify and classify the EW found in 3 different categories: Intermittent Running Waters (IRW: rivers, streams, creek, springs) included in lotic EW ecosystems, Temporary Large Wetlands (TLW: wetlands, lakes, lagoons) and Temporary Small Waters (TSW: ponds, pools, ventaroles, ditches, thermal waters, troughs, pans) included in lentic ones (Fig. 2).

Studies on EW through the analysis of diatom communities are more frequent for IRW (26), followed by TSW (16) and TLW (15) (some papers take into consideration more than one ecosystem type). Considering the lentic-lotic character, studies on EW and diatoms are more frequent for lotic (39) than lentic EW (18).

3.1.3. Study areas overview

Overall, 20 countries are reported to be investigated, included Antarctica (Table S1). Obviously, ecological distributions of diatoms are not linked to the political division of countries: however, investigating their distribution among countries make comparisons easier to realize and understand. The papers selected show an important heterogeneity among the countries. The most studied countries are Portugal (9) and Spain (9), followed by South Africa (4), Antarctica (3), Hungary (3) and Italy (3) (Fig. 3). The number of papers varies among continents: Europe (29), Asia (2), Africa (7), North America (3), South America (2), Oceania (2), Antarctica (3). Some geographical regions are taken into consideration more than others, like Balearic Islands, Fuente de Piedra and the Carpathian Basin.

3.1.4. Author's affiliate country

Although the majority of studies have been conducted in the same country of the author's affiliate country, there are some exceptions: the researches conducted in Antarctica were performed by researchers from Poland (1) and Czech Republic (2) (see Kochman et al., 2018; Skacelova et al., 2014; Kopalova et al., 2013), a research conducted in Senegal was performed by authors with an affiliation belong to a France research institution (see Beauger et al., 2019) and a research conducted in Mexico was performed by researchers belonging to German research institutions (see Mora et al., 2015). Researchers from Portugal, Spain and Hungary are the most active on the assessment of EW condition through the use of diatoms in their countries, while researchers from Czech Republic are the most involved in studies in Antarctica.

3.2. Methodological approach

3.2.1. Topics of investigation: Diatom analysis,substrate type and main aims

Overall, 5 different types of diatoms were sampled. Epilithic diatoms were the most sampled (52 %), followed by epiphytic (19 %), planktonic (15 %), epipelic (10 %) and epipsammic (4 %) (Fig. 4). In IRW, epilithic diatoms were the most sampled (75 %), followed by epiphytic (11 %), epipsammic (7 %), epipelic (4 %) and planktonic (3 %); in TLW, planktonic (43 %) and epiphytic (43 %) diatoms were the most sampled, followed by epilithic (14 %); in TSW epilithic diatoms were the most sampled (35 %), followed by epiphytic (29 %), epipelic (24 %), epipsammic (6 %), and planktonic (6 %).

Sampling choice is strictly related to the substrate type and substrate availability. Overall, 85 % of the studies used benthic diatoms over planktonic ones. Benthic and planktonic diatoms show different seasonal variations in species richness and, while the former type is affected by spatial processes (Chen et al., 2019; Rimet et al., 2019), the latter type is affected the most by environmental processes (Pan et al., 2020; Bartozek et al., 2019); the use of benthic diatoms as biotic indicator group over planktonic ones is widely spread because they show stronger correlations with environmental factors (Hu et al., 2022). In some cases, due to the shallow depth of the EW considered, differentiation between benthic and planktonic communities could be difficult to recognize. The papers considered performed phytoplankton samplings at 25 or 30 cm deep. Overall, 85 % of the papers reviewed counted up to 400 valves for each slide.

Moreover, the main analysis (81 %) performed emerged is the taxonomic analysis of the diatom assemblages in EW. In most of articles (61 %) the taxonomic analysis was linked to the evaluation of the ecological status of the EW considered (including the environmental variables and environmental stressors); other articles (38 %) focused on the description of the study sites providing a checklist of the diatoms identified; one article (1 %) focused on the geographic variables influencing diatom communities.

The remaining articles (19 %) focused on diatom communities functional analysis (diatom traits). Biological traits represent the functional response of a community to changes in the environment (Wang et al., 2022).

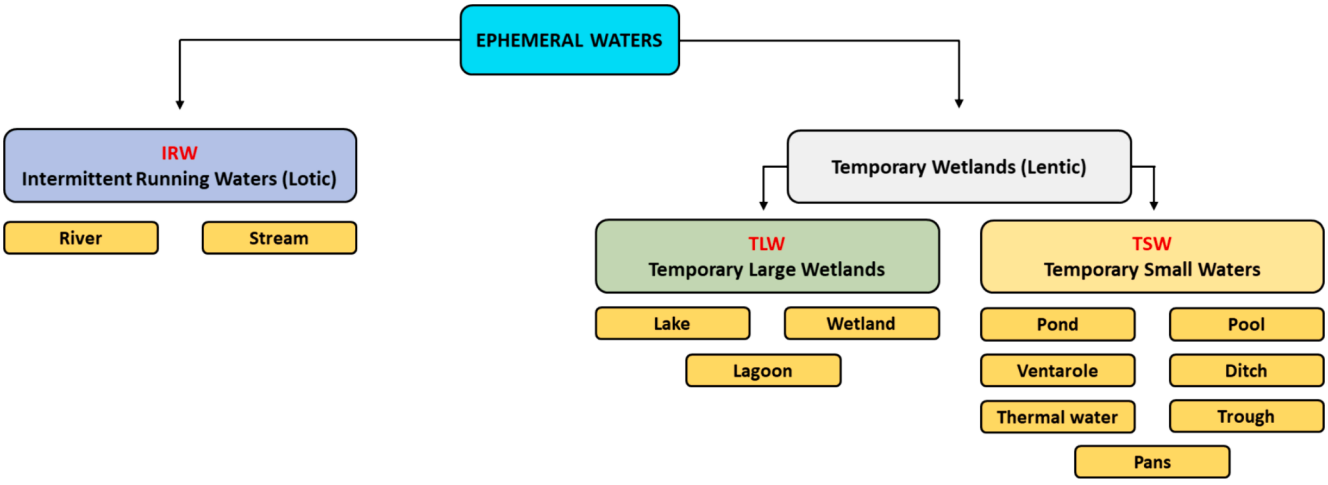

Fig. 2. The classification proposed for the EW found.

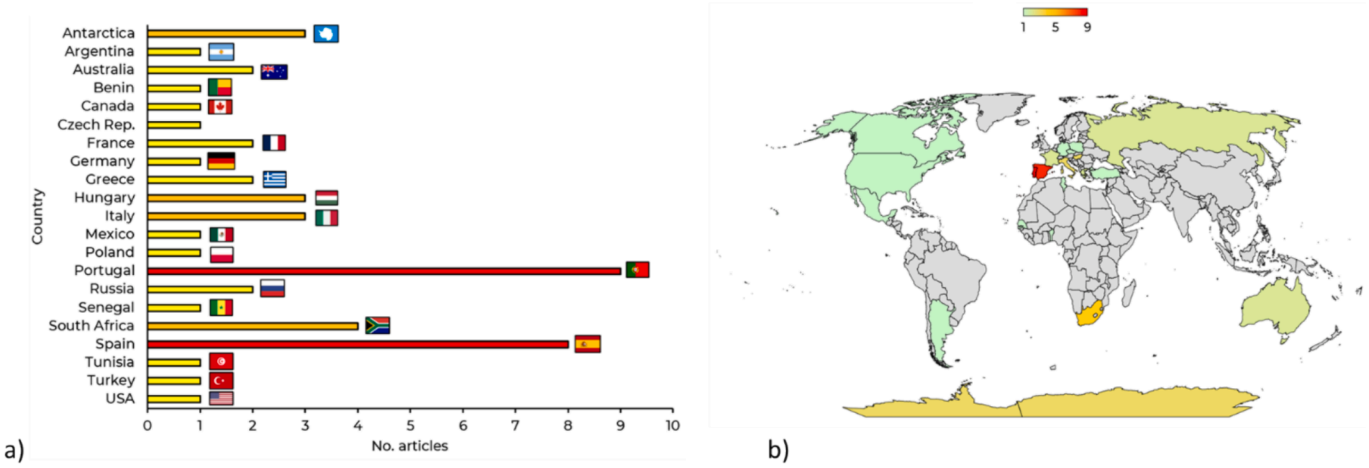

Fig. 3. The investigated countries with relative number of studies conducted (a) and the geographical representation of study sites (b) (No. articles = number of articles).

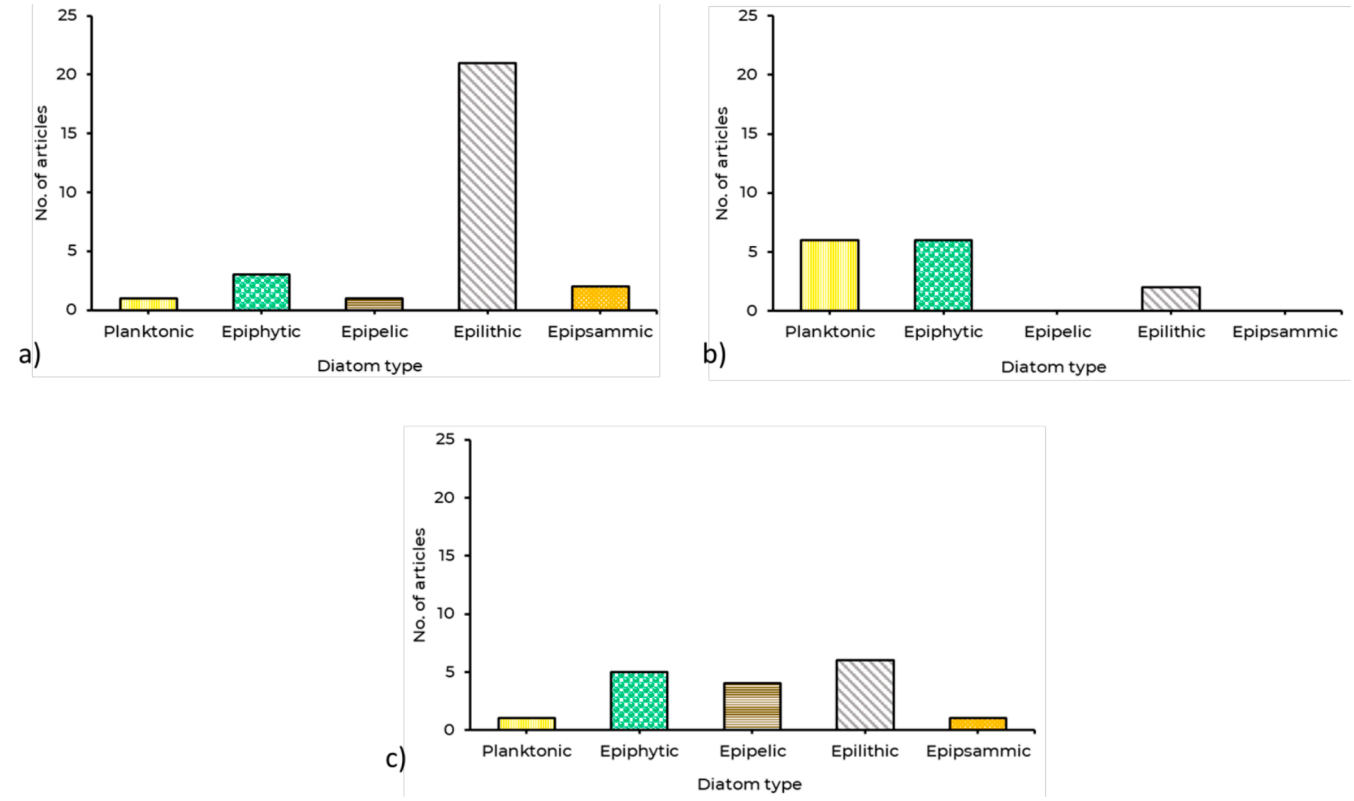

Fig. 4. Diatom types and related number of articles divided for Intermittent Running Waters (IRW, a), Temporary Large Wetlands (TLW, b), Temporary Small Waters (TSW, c) (No. articles = number of articles).

3.2.2. *Temporal effort of sampling*

The reviewed papers show a different distribution of the study periods throughout the year, with a heterogeneity in the studies conducted in the northern hemisphere and a homogeneity in the studies conducted in the southern hemisphere. The *t*-test performed show significant differences ($t = 6.03$, $p < 0.05$) between sampling months in the northern and the southern hemisphere, where March, April and May are the months most involved in this kind of studies in the northern emisphere (Fig. 5a).

Another important aspect to consider in research on EW is the duration of the research in terms of months (Fig. 5b). The reviewed papers showed a similar trend in the selection of samplings duration. Specifically, 28 % of the studies sampled for one month, 14 % for two months, 22 % for three months, while 24 % covered a sampling period of half a year (12 %) or an entire year (12 %).

3.2.3. *Spatial effort of sampling*

Overall, a total of 1018 temporary water-sites were sampled all over the world; the largest number of sites were considered for IRW (683), followed by TSW (239) and TLW (96) (Table 1).

The highest number of sites were sampled in Spain (317), Portugal (271) and Turkey (100). In IRW, the highest number of sites were sampled in Spain (266), Portugal (214) and Hungary (56); in TLW the highest number of sites were sampled in South Africa (38), Antarctica

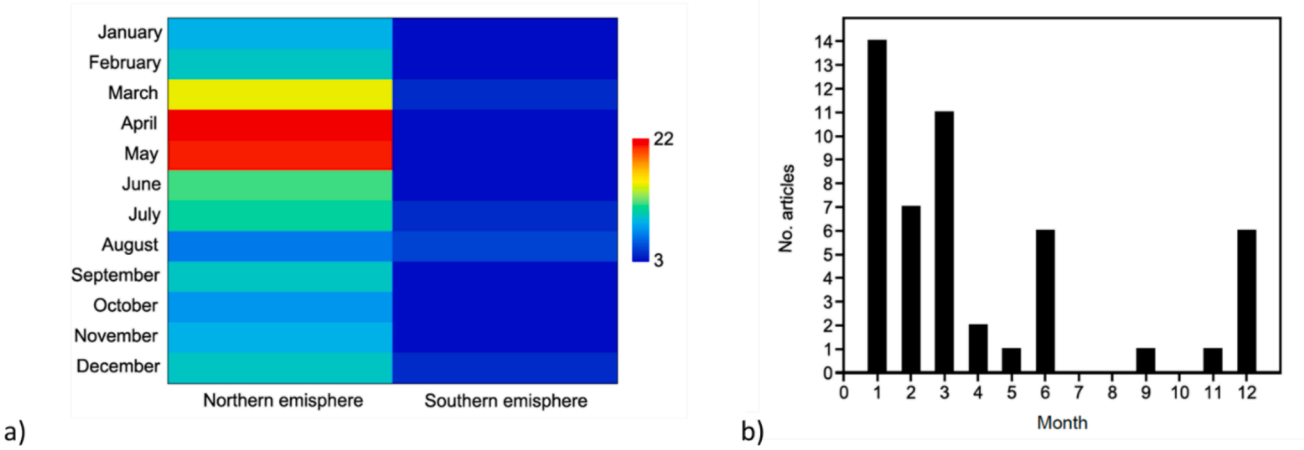

Fig. 5. Months coverage by articles (expressed in number) (a) and number of articles and relative number of months of sampling (b) (No. articles = number of articles; No. months = number of months).

Table 1
Distribution of study sites among countries and relative number of sites (IRW = Intermittent Running Waters TLW = Temporary Large Wetlands TSW = Temporary Small Waters).

Country	IRW	TLW	TSW	TOT
Antarctica	8	29	33	**40**
Argentina		4		**4**
Australia	23			**23**
Benin			78	**78**
Canada			1	**1**
Czech Rep.			4	**4**
France		3	4	**7**
Germany	3			**3**
Greece	8			**8**
Hungary	56			**56**
Italy	49	2		**51**
Mexico	6			**6**
Poland			1	**1**
Portugal	214	1	2	**217**
Russia	3		26	**29**
Senegal			1	**1**
South Africa		38		**38**
Spain	266	2	49	**317**
Tunisia		3		**3**
Turkey	47	13	40	**100**
USA		1		**1**
	683	**96**	**239**	**1018**

(29) and Turkey (13); in TSW the highest number of sites were sampled in Benin (78), Spain (49), Turkey (40) and Antarctica (33) (Fig. 6). As regards the continents, Europe shows the most sampled sites (725), followed by Africa (120), Antarctica (70), Asia (29), Oceania (23), North America (8) and South America (4).

3.3. *Influence of abiotic parameters*

3.3.1. *Altitude*

From the analysed literature emerge an altitudinal gradient, of the sampling sites considered. Overall, 47 % of articles refer to sites located on an altitude between 0 and 400 m a.s.l., 13 % refer to sites between 1600 and 2000 m a.s.l., 10 % refer to sites between 400 and 800 m a.s.l., only one site between 1200 and 1600 m a.s.l. and zero sites between 800 and 1200 m a.s.l., while 12 articles don't show any information about the altitude of sampling sites (Fig. 7).

3.3.2. *Physical-chemical parameters*

Ten studies over the 47 total studies didn't take into consideration any physical–chemical parameter of the water bodies. Physical-chemical parameters are taken into consideration in 78 % of the studies. The results of physical–chemical parameters analysis attest that electrical conductivity (37), pH (36) and temperature (35) are the parameters mostly investigated, followed by dissolved oxygen (27), phosphate (26) and nitrate (26) (Fig. 8). Low attention is given to water depth (1), turbidity (4) and chlorophyll *a* (6).

In EW, diatom communities are mainly influenced by electrical conductivity and pH (Novais et al., 2014). Other important abiotic factors are temperature (Celewicz and Goldyn, 2021; Dalkiran et al., 2020; Novais et al., 2020), nitrate (Witteveen et al., 2020; B-Béres et al., 2019; Beauger et al., 2019; Ben Naceur et al., 2013; Macedo et al., 2001), nitrite (Novais et al., 2020; Witteveen et al., 2020; Ben Naceur et al., 2013; Macedo et al., 2001), sodium (Luís et al., 2019; Kovaleva et al., 2014; Novais et al., 2014; Riato et al., 2014, dissolved oxygen (Novais et al., 2020; Karaouzas et al., 2017; Ben Naceur et al., 2013; Macedo et al., 2001), alkalinity (Beauger et al., 2019; Novais et al., 2014; Riato et al., 2014), chloride (Beauger et al., 2019; Kopalová and Van de Vijver, 2013; Riato et al., 2014) and sulphate (González-Paz et al., 2020; Kopalová and Van de Vijver, 2013; Luís et al., 2019), which were generally higher for taxa characteristic of temporary watercourses. In IRW, the main abiotic factors influencing diatom communities are electrical conductivity, pH and nitrate, followed by temperature, nitrite, sulphate e sodium. Similar positive correlations occurred in TLW, with electrical conductivity, pH, temperature, dissolved oxygen, nitrate, and nitrite influencing mostly diatom communities. In TSW, electrical conductivity, pH, temperature, sodium, and chloride result the main abiotic factors influencing diatom communities.

4. Discussions

This work represents a complete and global overview about the use of diatoms to evaluate the conservation status of EW. The scientific literature collected for this work includes all the articles based on field data from EW. Lentic EW ecosystems resulted less studied than lotic EW ones. The data we gain and transposed into this review highlight the importance of the temporal and spatial scale at which studies were conducted.

4.1. *Ephemeral freshwater ecosystem types*

In the last 25.000 years freshwater ecosystems have undergone a transitional environmental and morphological changes: some lakes dried up, some rivers transformed into small creeks and temporary pools get a permanent shape, while permanent ones translated into ephemeral

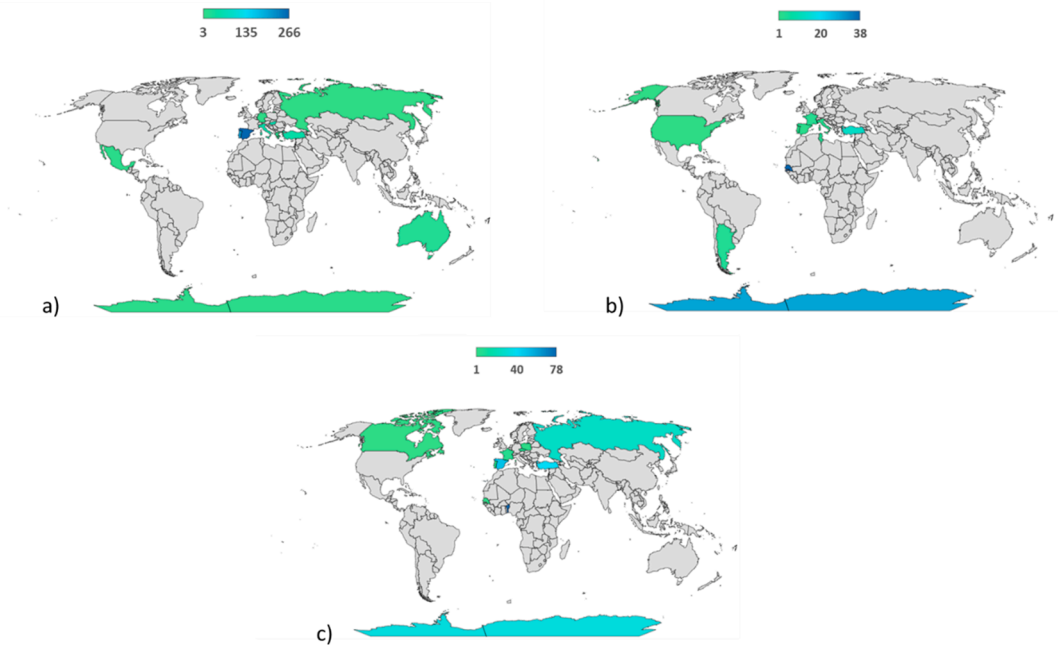

Fig. 6. Geographical distribution of study sites expressed in number for Intermittent Running Waters (IRW, a), Temporary Large Wetlands (TLW, b), Temporary Small Waters (TSW, c).

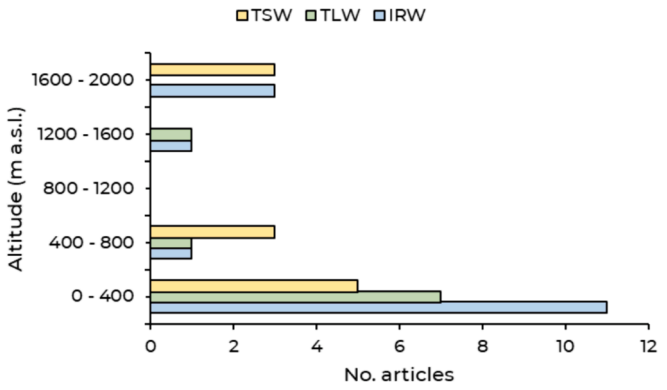

Fig. 7. Altitudinal distribution of study sites expressed in number of articles (IRW = Intermittent Running Waters TLW = Temporary Large Wetlands TSW = Temporary Small Waters) (No. articles = number of articles).

(Carpenter et al., 1992). In total, 67 % of literature analysed in this review refers to lotic EW ecosystems, while 33 % refers to lentic EW ecosystems. Historically, millions of humans across the globe depend on rivers and streams for numerous ecosystem services (Mammides, 2020); the ecosystem services provided by rivers and streams can support all the basic necessities for the survival and developmental needs of mankind (Ekka et al., 2020). This led into an important footprint of human on EW, that requested even more conservation projects. The emerging concern about the conservation of freshwater ecosystems through diatoms focused the most on IRW, for the historical reasons overquoted and also because, in literature, there are manuals, indexes and guidelines only referring to these ecosystems, while TLW and TSW conservation methods follow them, with some adaptation.

4.2. Global distribution of study sites

According to scientific literature, 55 % of the studies were conducted in the Mediterranean region. Based on our results, Portugal and Spain have the highest number of articles, showing a moderate sensitivity on this topic. South Africa, with its Mediterranean biogeographical region, follows Spain as regards the number of studies: following, there are Antarctica, Italy and Hungary. The results fit with data emerging from continents analyses: in Europe and Africa, both adjoining with the Mediterranean Sea, there is the highest number of sites. Antarctica, where climate change highly affects EW, follows Africa as regards the number of sampling sites, while Asia, the continent with the higher surface area (44.579.000 km^2) is only represented by Russia. In North and South America were considered only 12 sites, that is half of the sites considered for Oceania, which surface area is lower than 5 times respect that of the Americas.

Lotic EW sites are mostly studied in the Mediterranean region: 95 % of the stream sites (except for 5 % represented by Australia) and 91 % of the river sites. Lentic waters are less studied. There is a lack of studies about TLW through the use of diatoms as bioindicators: in particular, only 4 % of the sites are wetlands and, about these, more than 80 % are in South Africa. The distribution of TLW sites is highly heterogeneous, covering only three countries: South Africa, Turkey and France; there are no studies focusing on diatoms and TLW in the rest of the world. In addition, the data obtained show that 58 % of the lakes considered are in Antarctica, while only one site has been considered in North America and zero in Asia. According to Alonso (2009), there are numerous TLW in Asia, but they have never been inventoried. Instead, there are no data regarding the number of TLW in North America: this leads to an important gap of information regarding EW and their ecology.

TSW are mostly studied in the Mediterranean region, with 40 % of the study sites, followed by Africa with 33 % of the sites. However, TSW studied in Benin (78) are artificial sites, not referable to natural TSW. TSW, in general, are common ecosystems in the Mediterranean region, but also northern Europe, North America, Australia and South Africa (Zacharias et al., 2007). TSW are of particular concern in Spain, where, in Doñana National Park, most aquatic habitats are temporary (Díaz Paniagua et al., 2010). TSW are very important habitats and resources for people inhabiting rural areas in Africa: they provide numerous ecosystem services and are an important source of water for wild animals, domestic animals and human communities (Zongo et al., 2017).

The data obtained are not only referable to the countries found, but also to those countries which did not appear in the research. For

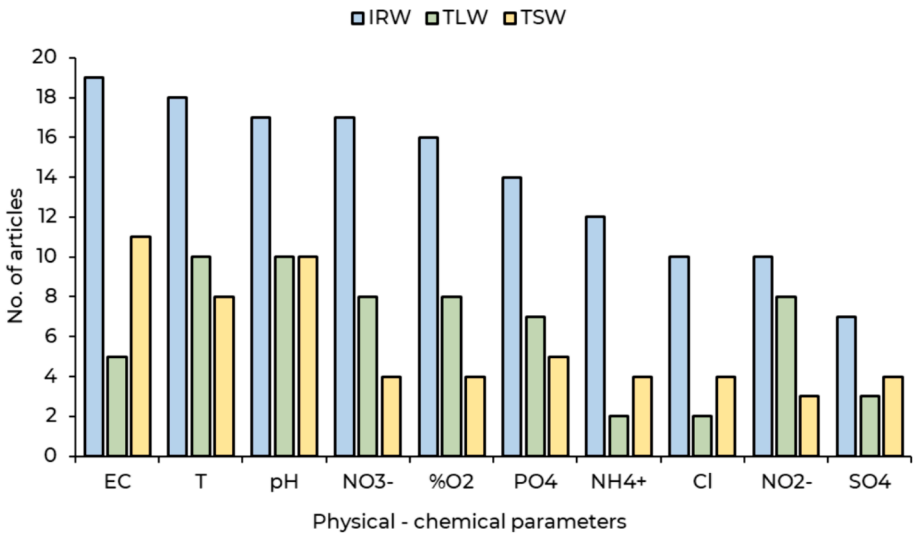

Fig. 8. Principal physico-chemical parameters taken during the studies (IRW = Intermittent Running Waters TLW = Temporary Large Wetlands TSW = Temporary Small Waters) (EC = electrical conductivity; T = temperature; No. articles = number of articles).

instance, China is the fourth largest country in the world as regards the surface size (9.596.961 km2) but become the first largest country without any study about diatoms and EW. In China there are over 3000 lakes, over 1500 rivers, while wetlands total area accounts for 10 % of global wetlands and 5.58 % of China's territorial area (Meng et al., 2017). Despite this, no studies have been conducted on EW in China using diatoms, highlighting the negligent scarce use of this biological model. However, the lack of studies could refer to the indexed journals used by the search engine. In this way, it could be possible that some studies were published in other languages and/or published in other platforms not recognized by the search engines used.

Moreover, assessing the reasons for the heterogeneous distribution of study sites between the two hemispheres represents a challenge. One reason could be found in the uneven distribution of EW worldwide. According to our results, most of sites are located outside of the "Torrid Zone", the portion of the Earth between the two "tropics". The high temperatures characterizing the equatorial region could generate higher evaporation rates as described by Trambauer et al (2014) for Africa. Arid and hyper-arid areas in Africa are characterized by a very low evaporation rate. In these conditions, EW hydroperiod often become lower or almost null. Moreover, global threats like climate change, could highly affect the EW hydroperiods: the subtropical zones, considered the most arid zones of the globe, will face decreases in precipitations amounts, affecting the water supply. It's important to consider that landmasses in the northern hemisphere are four times larger than those in the southern hemisphere (Box, 2002). Moreover, the southern hemisphere, unlike the northern one, includes a much wider band of equatorial and subtropical areas where, for the reasons mentioned above, the development of EW is more complex. The temperate zone, much larger in the northern hemisphere, is more suitable for the development of EW (Williams, 2006).

A relevant knowledge gap is that the worldwide description of the ecological status of EW ecosystems using diatoms is lacking. For instance, only 96 TLW over the globe have been studied through the use of diatoms as biological model; furthermore, in larger countries like USA and Canada, showing a cumulative surface area of 19.819.000 km^2, only one TLW and one TSW have been studied using diatoms, while zero IRW have been considered.

The largest number of sites considered for the Mediterranean basin can be related to the importance that this biogeographical region has from an ecological point of view: Mediterranean region is considered part of the first 25 Global Biodiversity Hotspots due to its unique biological and ecological diversity (de Figueroa et al., 2013). Moreover, the Water Framework Directive (WFD, 2000/60/EC) recognize diatoms as biological models for the evaluation of the quality of all water bodies at the river-basin level across Europe (Allan et al., 2006). The Europe, with the WFD, is at the forefront as regards the water quality biomonitoring using diatoms, which are particularly successful in detecting eutrophication, organic pollution and acidification. The implementation of the WFD lead also to an increase in the awareness about the importance of water quality assessment by countries all over the world, which started considering adopting similar legislations and assessment methods (Masouras et al., 2021), for instance: in North America, the US is using biomonitoring through the Clean Water Act; in South America, Argentina has developed its own diatom-based indices for assessing water quality (Pampean diatom index); In Australia, phytobenthos has been used for biological quality assessment for many years but without a precise legislative reference; in Asia, with the Asian Pacific Water Summit in 2007, water quality assessment became an ecological priority (Masouras et al., 2021).

4.3. *Types of diatoms,sampling methods and substrate selection*

In EW, although occasionally found in the water column as planktonic diatom cells, they are mainly considered benthic diatom species, i.e., attached on substrates such as aquatic plants (epiphytic), stones (epilithic), silt or clay (epipelic), sand (epipsammic), animals (epizoic) or plastics (epiplastic) (Taurozzi et al., 2023; Masouras et al., 2021). Benthic diatom assemblages are often associated with flow intermittency, which can influence variations in the diversity, composition, and trait patterns of diatom communities (Tornés et al., 2021). Moreover, the community structure of benthic diatoms is conditioned by physico-chemical parameters as dissolved oxygen, light, temperature and pH, which in turn may experience strong fluctuations in EW (Novais et al., 2020). With the typical dry periods which characterize EW, the planktonic diatom communities are often lost. Benthic diatoms show physiological adaptation to dryness, which can provide a quick recovery in the wet periods. Moreover, the analysis of benthic diatom assemblages in dry biofilm can be used as an indicator of ecological status during the dry-phase (Novais et al., 2020; Stubbington et al., 2019).

Diatoms can be sampled following different methodologies. Epilithic diatoms can be sampled scrubbing the upper layer of at least five cobble-sized rocks, stones, rocks, artificial substrates, collected at approximately 0.5 m depth (Kennedy and Buckley, 2021; Salmaso et al., 2019), according to European Committee for Standardization guidelines (CEN, 2003). Following Riato and Leira (2020) and Sanal and Demir (2018), epiphytic diatoms can be sampled pooling healthy established

submersed macrophyte stems from different individuals of the same species at 5–20 cm below the water surface, placing them into a zip lock bag with a small amount of distilled water and shake for two minutes to dislodge epiphytic diatoms (Zimba and Hopson, 1997). Epipsammic diatoms samplings can be performed pressing a petri dish lid into the top layer of sand to a depth of 5–7 mm and then cleaned of organic material using wet combustion with concentrated sulphuric acid following Bere and Tundisi (2010). Epipelic diatom samplings can be performed scraping sediments as deep as 0.5 cm in plots (Siregar, 2021) or using dissection corers (Hasrini et al., 2023). Planktonic diatoms are usually collected by a vertical net haul using a plankton net with mesh size of 20 μm from 0–30 m depth.

The literature reviewed didn't show important differences between diatom sampling methods among different ephemeral freshwater ecosystems. Most of articles regarding IRW refer to the standard methodology proposed by CEN (2003) for the routine sampling and pretreatment of benthic diatoms from rivers (Kelly et al., 1998):

i. A single substratum should be used at all sites included in a survey;
ii. Areas of the riverbed with naturally occurring moveable hard surfaces (large pebbles, cobbles and boulders) are recommended wherever possible;
iii. A segment of river that has substrata suitable for sampling should be selected: this should be about 10 m in length, but longer lengths may be appropriate, depending upon the physical uniformity of the river and the availability of substrata. Areas of heavy shade should be avoided. Zones of very slow current (approx. ≤ 20 cm s^{-1}) should be avoided. "Riffles" are preferred, as these tend to have a good variety of natural hard surfaces;
iv. At least five cobbles should be sampled. However, if cobbles are unavailable, then either 5 small boulders or 10 pebbles should be sampled. An area of approximately 10 cm^2 or more should be scraped.
v. In case of epiphytic diatoms, sampling should be performed during the flowing phase, from emergent macrophytes growing in the free-flowing parts of the riverbeds (B-Béres et al., 2019).

Also regarding TLW, the Water Framework Directive guidelines (WFD: European Union, 2000) represent the main reference for the monitoring of the ecological status of water bodies; the principal recommendations from WFD were summarized by King et al. (2006), highlighting that the guidance standard that is already available for rivers (CEN, 2003) was also applicable to TLW:

i. A single substratum should be used at all sites included in a survey;
ii. Epilithic diatoms are preferable to be sampled. At least five cobbles should be sampled, each of them covering an area of approximately 10 cm^2 or more. Five stones are sufficient to eliminate major sources of variability within a substratum. This sampling strategy avoids the problem of host specificity or substratum variability associated with macrophytes;
iii. Sampling should be performed preferable in the euphotic zone to avoid differences in diatom communities in function of different depths and consequent light quantity fluctuations. CEN (2003) recommends avoiding heavily shaded areas and selecting sampling stations in open water, with limited surrounding vegetation. Sampling should occur from permanently-submerged substrata within a wadable depth. Sampling within the eulittoral zone at a depth of 30–50 cm would be necessary to avoid exposure and/or movement of the substrata in heavily wave-washed shorelines and reduce the risk of photoinhibition caused by very high irradiance at the lake margins.
iv. Considering natural seasonal and successional dynamics, it is impossible to recommend any single period of the year as the best time for sampling. It is, however, important that both spatial and temporal comparisons are made only on samples collected from the same season in order to minimise these effects;
v. The use of composite samples (made of numerous samples from different microhabitats) can circumvent small scale variation (i.e. on a scale of a few m^2).

As reported by King et al. (2006), the overriding concern is to ensure comparability between lakes (or wetlands), which is best achieved by a set of simple guidelines applicable with only slight modification to all water bodies. However, there is considerable natural variability between water bodies. This variability is reflected in the WFD, which requires that ecological targets are 'type specific' (i.e. take the geological and hydromorphological nature of the water body into account). This allows for some flexibility within sampling regimes permitting, for example, the substratum that is sampled to differ between water body types so long as all samples from a single water body type are from the same substratum (King et al., 2006).

Lastly, regarding TSW, sampling activities can be performed referring to TLW guidelines, as suggested by Blanco et al. (2020). Indeed, often can be only subtle differences between TLW and TSW and, as described by the CEN (2003), although the methodologies for evaluating the diatom data vary, the sampling and pre-treatment processes are similar for the different ecosystems considered.

Suggestions from King et al. (2006) are in accordance with our results. All the literature reviewed refer to benthic diatoms. Furthermore, half of the studies mainly focus on epilithic substrates, considering also artificial ones. Stones, rocks and artificial solid substrates are easy to sample, to recognize and to withdraw and usually present in greater number in lotic and lentic waters (Afifah et al., 2021). This type of substrate can also make it easier scraping activities and recognize the green diatoms biofilm, which coloration stands out from the usually grey background of stones. This sampling strategy avoids the problem of host specificity or substratum variability associated with macrophytes (King et al., 2006). Moreover, diatoms are easier to sample on epilithic substrates than in epipelic or epipsammic substrates, which need to be processed before the isolation of diatom communities (Adl et al., 2020). On the other hand, bioassessment methods based on epiphytic diatoms has been proved to be particularly effective for tracking changes in hydrolimnological conditions of floodplain lakes and ponds, in particular temporal ones, because of the high presence of macrophytes in shallow water bodies (Wiklund et al., 2010). Nevertheless, in soft, muddy water bodies where epilithic habitats may be lacking or limited and do not represent a significant component of the phytobenthos, epiphytic samples should be collected (King et al., 2006). In any case, it is important that comparisons between water bodies are based on samples from the same substratum.

The main aims of the literature reviewed confirm the tendency and the large use of diatoms as bioindicators (Srivastava et al., 2016): the assessment of ecological conditions and the main environmental and geographic drivers influencing diatom communities represent the main focus of most of articles. Moreover, taxonomic analysis provides important data about biological diversity, which is generally considered an indicator of the quality of freshwater systems (Leira et al., 2009). Although the use of diatom guilds is limited, it could provide important information and responses to local ecological factors, such as current velocity and resource supply, and geographical connectivity (Jamoneau et al., 2017). Furthermore, explaining diatom distribution by guild rather than straight taxonomic unit would provide much more insights. Ecological guilds can be considered a proxy for describing responses to: (i) different stressors and disturbances (Stenger-Kovács et al., 2013); (ii) environmental and spatial mechanisms (Jamoneau et al., 2017); (iii) changing water regimes (B-Béres et al., 2014); (iv) nutrient and disturbances gradients (Passy, 2007) and its use is encouraged by the authors. The tendency to investigate mainly the ecological status of EW reflect both the underrated nature of EW in terms of scientific attention and the

evaluation of the increasingly anthropogenic impacts on these ecosystems (Chiu et al., 2017; Ortega et al., 2014; Zacharias and Zamparas, 2010).

4.4. Temporal effort of sampling protocols

The uniqueness of EW dwell in their characteristic dynamic nature, made of an alternance of wet and dry periods (Kulkarni et al., 2019). Despite the limited presence of niches because of the short lifetime of this habitats, restricted water availability forms a unique ecological condition influencing the evolution of an adapted biota community inhabiting them (Gilbert et al., 2021). The duration of hydroperiod length make important the definition of the study period while creating a protocol of analyses.

In the northern hemisphere, most of the studies focus on a temporal interval that goes from March to August, corresponding to the spring and summer seasons. The heterogeneity observed along the studies conducted in the northern hemisphere is compatible with the hydroperiod of EW, that is the most during the spring season (March, April, May): in these months the flow is at its maximum in low and high altitude TSW, because of the snow melting and rain (Pearce, 2018). Moreover, diatoms higher diversity is detectable in –spring and late summer (ISPRA, 2014), where EW flow is at its maximum.

On the contrary, the homogeneity observed for the southern hemisphere can be related to the different environmental features of the continents taken into consideration, in particular Antarctica and Africa, which show the maximum flow during different seasons: summer for the Antarctica (December, January, February) (Boxall et al., 2022), and late autumn/winter (May, June, July, August) for Africa (Ebodé et al., 2020).

Most of sites of the reviewed papers were sampled one month per year: this approach can give an important overview of the ecological situation and the diatom community structure in a precise time, but to give a complete panning of diatom community turnover and evolution over time is fundamental to repeat the samplings for longer periods (Adl et al., 2020). Diatoms spend 14 days to colonize new habitats and to give an exhaustive turnover overview of diatom species along a temporal gradient, sampling should be carried out for almost six or nine months (Taurozzi et al., 2023). However, given the restricted hydroperiod of EW, six months of sampling could be difficult to perform. Diatoms are ubiquitous and present throughout the year, so they can be sampled in all seasons. The greatest diversity of species is found in the periods May-June and September-October, periods with high light intensity and mild temperatures (ISPRA, 2014). The WFD, in the alpine zone, indicates summer as best period to sample diatoms in watercourses; however, the late spring- and early summer months in watercourses of glacial origin should be avoided because the high concentration of suspended solids can strongly alter the diatomic community, while, in the Mediterranean area, it is advisable to carry out sampling in the most suitable period from a biological point of view (ISPRA, 2014). Sampling protocols that cover an entire year can provide a comprehensive characterization of diatom communities. However, EW are characterized by restricted or prolonged dry periods which make impossible in most of cases sampling for 12 months. Moreover, only a few studies encompass a 12-month sampling period, primarily due to the ephemeral nature of EW, which typically allows for sampling periods of 3 or 6 months in most cases. Lastly, it is necessary to highlight that the existing standardized protocols mainly refer to permanent waters. From this emerges the need to investigate the applicable ecosystem health measures that may be transferable to ephemeral systems, as protocols to assess water condition has generally been developed from studies of permanent systems.

4.5. Altitudinal gradient

Hydroperiod is a key factor for EW, affecting its nature and the biota related with it (Madaschi and Díaz-Villanueva, 2021). As one ascends a mountain, environmental conditions change, and there are organisms that are commonly well adapted to the local conditions along an altitudinal transect. Literature address 'mountains' as any elevation of land mass from the plains 300 m or over above sea level (Kapos et al., 2000).

Considering abiotic factors influencing diatom communities, there are four primary atmospheric changes associated with altitude: (i) atmospheric temperature, which reduction can bring important implications for humidity; (ii) decreasing total atmospheric pressure and partial pressure of all atmospheric gases (in particular O_2 and CO_2); (iii) increasing radiation under a cloudless sky, both as incoming solar radiation and outgoing night-time thermal radiation; and (iv) a higher fraction of UV-B radiation at any given total solar radiation (Körner, 2007).

On average, air temperature drops by 5° C per kilometre of altitude (Gonfiantini et al., 2001); hence, across all plant life forms, there is no common altitudinal temperature trend to be expected. In this way, local and/or life form-specific, rather than global, patterns are likely to govern the degree of such departures from ambient conditions (Körner, 2007). Moreover, hydroperiod periods are shifted in mountain and lowland habitats: while precipitations are different in lowlands and highlands, affecting EW in different ways, the impact of snow on mountain habitats must be considered (Wickramagamage, 2016). Winter in mountain translate into presence of snow, ice and low temperatures, EW can be frozen for months, showing a long period of impossibility to be sampled; summer season means high-flow period for high-altitude EW, due to snow melting and following water availability (Lucianetti et al., 2020).

On low-altitude EW, seasons impacts are reversed: in winter EW are at their maximum flow, due to high precipitations and low temperatures and humidity, while during summer they show dry appearance and low flow (Ruiz Sinoga et al., 2011). On average, low altitude EW show a longer hydroperiod than high altitude ones (Eskinazi-Sant'Anna et al., 2020). For instance, in the northern hemisphere, EW on low altitudes can exist from September/October, until the end of May, while during the summer season they usually went dry. On the contrary, high-altitude EW fight a snow cover during winter and spring season and a partial wet period in summer, considering that also in mountain ecosystems, July and August high temperatures can seriously affect EW existence (Padula et al., 2021).

Regarding diatom communities, has been demonstrated that the altitudinal gradient hydrochemistry and different habitat features are the most important factors influencing their distribution than altitude and geographical position, but these factors are inter-correlated and very difficult to separate on their effects (Jüttner et al., 2010). A virtual limit on 2000 m a.s.l. has been described as important for species diversity: areas above and below this boundary show different species and should be considered separately when developing diatom – based monitoring projects (Juttner et al., 2010).

4.6. Physico-chemical analyses

Diatoms are important and sensitive bioindicators to assess water quality. The relationship between diatoms and environmental variables is often robust and quantifiable, and the sensitivity of diatom assemblages to environmental conditions did not differ between lotic and lentic habitats (Costa et al., 2022). Therefore, most of literature reviewed consider electrical conductivity, pH, and temperature as main parameters to relate with diatom assemblages. Electrical conductivity is a proxy for water salinity, that is strictly related to qualitative studies of diatom communities (Luostarinen et al., 2023). The total concentration of dissolved salts and the ionic composition of water are known to be important ecological factors influencing diatom distribution (Colla et al., 2022). The difference in salt content between marine and inland waters influence the local distribution of diatom communities and creates an important boundary that only a few diatom species can cross and in only one direction, from marine to freshwater habitat (Potapova, 2011).

Diatoms are found throughout inland waters in habitats spanning the full range of observed temperatures (B-Béres et al., 2022). Inland water temperatures are projected to increase over the coming century, with dramatic consequences for the biota: evidence from the field indicate temperature changes may lead to changes in diatom biogeography (Xiao et al., 2018). Each individual species and even strains within species have their own characteristic temperature performance curve, reflecting evolutionary constraints and past adaptations to its environmental regime; changing in the average temperatures can have heavy repercussions on diatom species assemblages. It is argued that diatom–temperature models are weaker than those developed for salinity, pH (Anderson et al., 2001), but is also clear that higher temperatures negatively affect diatom richness and also the composition of all functional groups (low-profile, high-profile, motile, and planktonic diatoms) (da Silva et al., 2019). Water temperature, which is highly dependent on altitude (Rivera-Rondón and Catalan., 2020), is also strictly related to hydroperiod length of ephemeral waters: air temperature is a key parameter, affecting many aspects of the ecology of aquatic biota (Carosi et al., 2022), increasingly frequent episodes of the early drying up of the ephemeral waters and, as a consequence, influencing the diatom community patterns.

The pH is also related with diatom richness: Wang, Strömgård, and Soininen (2019) surveyed subarctic ponds covering elevations from 10 to 1038 m a.s.l. and documented a unimodal richness–elevation trend, which was best explained by pH. It seems that variation in diatom richness along mountainsides is not only regulated by elevation and associated climatic gradients but may instead be more directly associated with local factors, such as the frequency of disturbances (Jüttner et al., 2010; Teittinen et al., 2016) or pH (Teittinen et al., 2017; Wang et al., 2022). The results of Bennet et al., (2010) suggest that pH can explain a large amount of variability, increasing with decreasing spatial scale: for instance, comparing the lake diatom pH optima in North America and Europe, they found that diatom pH optima were relative similar in the two continents, thus supporting niche conservation.

Comparing data from EW and permanent waters (PW), no differences emerged regarding the abiotic drivers for diatom diversity. In particular, as reported by Blanco (2014), temperature, pH, dissolved oxygen and alkalinity appears as the most important physiographical variable in terms of average variance captured in PW. These data are in accordance with our results, where electrical conductivity, pH, temperature, nitrate, and nitrite are the main abiotic factors influencing diatom communities. Climate has a profound effect on diatom dynamics, affecting diatom communities via its links with nutrient availability and temperatures; moreover, Blanco (2014) provides strong relationship between diatoms and pH in PW, due to the direct physiological influence of pH on diatom, especially in oligotrophic systems. On the other hand, most diatom assemblages appear to be unresponsive to a number of factors related to trophic status (e.g., nitrates, ammonia) in PW (Blanco, 2014). Nutrients also capture important fractions of variability in diatom assemblages across a large range of trophic levels (Blanco et al., 2014). However, the relative importance of nutrients depends on supply rates and on the order in which they are consumed (Blanco, 2014).

Even if diatom richness patterns are difficult to explain, some evidence for general environmental drivers exists. Among the most typical key variables are conductivity, temperature and pH (Benito et al., 2018a; Heino et al., 2010; Stenger-Kovács et al., 2013). Literature gained indicate different responses of diatom functional groups to electrical conductivity, temperature increase, changing in pH, showing the importance of considering ecological differences among functional groups to evaluate the possible consequences of variation in physiochemical variables. Given that abiotic factors are closely linked to the ecosystem considered, it is possible to conclude that diatom communities are strongly influenced by the ecosystem type.

5. Conclusions

Ecological and social importance of EW is widely proved and demonstrated. Last few years have seen an increase in conservation consciousness of EW, after many years of lacking approaches. Data from the scientific literature reveal that the assessment of diatom contribution to evaluate the ecological status of EW is poorly exploited, but highly sensitive and specific, providing exhaustive data about environmental alterations. Moreover, considering the large surface of land on earth and the relative surface extension of this type of ecosystems, studies on this topic are largely underrated.

Therefore, researchers are encouraged to engage an increasing data collection on diatom community structure, and on temporal and geographical turnover to their studies to implement the quality of the research. In this regard, chemical analysis of the water matrix can contribute to the characterization of the study site. EW are highly sensitive to climatic seasonal changes, like many diatom species: for this reason, physical–chemical parameters can differ among the seasons and can be highly suitable to associate precise chemical data to diatom species found to have a complete overview of the ecological situation. However, this methodology is based on chemical analysis which requires expensive equipment and generally long procedures. In this regard, the data sets provided here may help researchers conduct further research using metadata analysis in addition to (or by replacing) sampling and chemical analysis. Actually, regarding the application of diatoms in EW management programs recently increased but the lack of studies about high-altitude shallow waters is a further gap because it adds some difficulties in well understand how climate change affects mountain EW.

In conclusion, further investigations should be directed to the following:

- implement the study of EW worldwide using diatoms, which are very suitable bioindicators to evaluate the conservation status of EW;
- create a standardized protocol for EW management coupling data from diatoms and physical–chemical parameters, which could facilitate and enhance research on monitoring and risk assessment of EW;
- determining global threats to EW and harmonise the literature about the use of diatoms as bioindicators, in particular regarding benthic diatoms, which are generally easier to sample and to detect than planktonic ones.

In a world focused on the main themes of nature conservation, like climate change and anthropogenic pollution, the protection of EW is a central theme to investigate. Joint political and social focus on EW is the most important step to look forward to a flawless management of this threatened ecosystems, which ecosystem services provided can help to reach a better living for all mankind.

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Davide Taurozzi: Writing – review & editing, Writing – original draft, Investigation, Data curation, Conceptualization. **Giulia Cesarini:** Writing – review & editing, Validation. **Massimiliano Scalici:** Writing – review & editing, Validation, Supervision, Resources, Conceptualization.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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CHAPTER II

Environmental Management
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Mapping Italian high-altitude ponds

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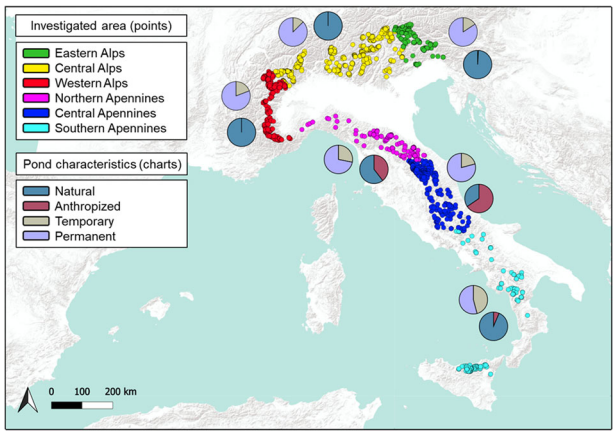
Abstract

Permanent and temporary ponds are considered peculiar ecosystems which provide important ecosystem functions, services, supporting biodiversity on small and large scales. Pond's conservation status is globally critical. Moreover, their ecological functioning and conservation status is frequently overlooked, because of the habitat small size, their seasonal occurrence and their unique appearance. While a certain attention is given to Mediterranean Temporary Ponds and, in general, to low altitude ponds, the ecological importance of high-altitude ponds is critically unrecognized, especially in the Italian peninsula. The main aim of this research is to create the first georeferenced checklist of Italian high-altitude ponds. In order to achieve this goal, we integrated spectral, spatial characteristics, and morphological operations based on Sentinel-1 and Sentinel-2 image data using the Google Earth Engine (GEE). Overall, 2156 ponds were identified: 62% ($n = 1343$) in the Alps and 38% ($n = 813$) in the Apennines. The highest number of ponds was detected in Central Alps ($n = 642$), followed by Western Alps ($n = 479$), Central Apennines ($n = 412$), Eastern Alps ($n = 222$), Southern Apennines ($n = 216$) and Northern Apennines ($n = 185$). For what concerns the Alps, the average altitude was estimated in 2428 m a.s.l., while in the Apennines the average altitude was estimated in 784 m a.s.l. The total area covered from ponds has been estimated in 4.258.640 m^2, with a mean of 1716 m^2. Ponds were described as 20% temporary ($n = 445$) and 80% permanent ($n = 1711$). Considering the land use, 83% ($n = 1797$) of ponds were described as "natural" and 17% ($n = 359$) as "anthropized". Identification and georeferentiation of high-altitude ponds are primary actions to the application of management plans and this research could be considered the first step towards the safeguard of these threatened ecosystems.

Graphical Abstract



Keywords Temporary · Permanent · Conservation · Anthropized · Climate change · Natural

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Introduction

Ponds are generally described as small (<5 ha) water bodies which are shallow (<5 m depth), with less than 30% of

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emergent vegetation (Olmo et al., 2022; Richardson et al., 2022). However, following this definition, ponds could overlap with other types of waterbodies: for this reason, their exactly definition is still debated in scientific literature. For example, The CEN standard defined lakes as water bodies characterized by surface areas greater than 1 ha (0.01 km^2) and maximum depths (at mean water level) greater than 1 m (Boon et al., 2019). Given the variety about those definitions, we adopted in this research the most recent and functional definition to distinguish ponds from lakes and wetlands from Richardson et al. (2022). Anyway, ponds are the most numerous freshwater environments globally (Biggs et al., 2017) and are considered peculiar ecosystems which provide important ecosystem functions (e.g., stepping stones) and services (e.g., nutrient retention, hydrological regulation) (Coccia et al., 2024). Ponds are not isolated systems. They are strongly linked to other aquatic systems and their surrounding terrestrial environment (Fehlinger et al., 2023). Temporary ponds (also known as vernal pools or ephemeral pools) are seasonally flooded aquatic habitats that support unique assemblages of fauna and flora (Taurozzi et al., 2024; Fritz and Whiles 2021). The term "temporary" refers to the hydroperiod of these ecosystems (i.e., the length of aquatic phase) (Fernández-Zamudio et al., 2016). Temporary ponds are characterized by a dry phase which occur, regularly, during spring and summer, when temperatures and scarce precipitations can reduce partially or totally the water supply (Boix et al., 2016). However, if anthropogenic disturbances like climate change occur, this natural cycle could shift, generating irregularities in hydroperiod length and water quantity (Celewicz and Gołdyn, 2021). Differently, permanent ponds are characterized by the presence of water throughout the year, without suffering a dry phase in the summer season (Obrador et al., 2018). Both temporary and permanent ponds are recognized as biodiversity hotspots, characterized by unique chemical, biological and physical features: these give them a crucial role in biodiversity conservation (Obrador et al., 2018; Bagella et al., 2016; Hassall, 2014).

The conservation status of ponds is globally critical (Bolpagni et al., 2019; Hill et al., 2018). Although the increasing awareness of temporary ponds in terms of ecosystem services and support to freshwater and terrestrial biota, their ecological functioning and conservation status is frequently overlooked (Oertli et al., 2010), because of the habitat small size, its seasonal occurrence and its unique appearance (Van den Broeck et al., 2015: Céréghino et al., 2010). In Europe, a particular conservation status is provided for the "Mediterranean Temporary Ponds": the European Community, recognizing the value of these environments, referred them to the priority habitat 3170*, listed in Annex I of the Habitat Directive (92/43/EEC) (Bagella, 2023). Near this habitat, there is a wide range of habitats, all characterized by permanent or temporary existence. The Habitats Directive focuses on the conservation of some target species and vegetation types, using their presence to categorize a pond into one of the habitats included in the Directive (e.g., *Littorelletalia uniflorae*, *Isoëtes spp.*, *Chara spp.*) (Campagnaro et al., 2018). In this way, it leaves out numerous ponds that do not show the presence of target species, vegetations type or habitats, but which represent the same, however, a fundamental ecosystem to preserve. In our study we will evaluate the effectiveness of Habitat Directive and National network of protected areas in the conservation of high-altitude ponds.

In fact, while a certain attention is given to Mediterranean Temporary Ponds and, in general, to low altitude ponds, the ecological importance of mountainous small waterbodies is critically unrecognized (Wissinger et al., 2016; Ilg and Oertli, 2014). Among all types of lentic waters, high-altitude ponds seem to be one of the most sensitive to climate change (Rosset et al., 2010). They are usually shallow, small, and temporary aquatic ecosystems located at an altitude above 300 m a.s.l., with a dry period recurring in late summer every year and lasting until the next year snow melting (Lamouille-Hébert et al., 2024; Wissinger et al., 2016). These ecosystems can show a double "dry" phase: the first during late summer, when water evaporates due to the high temperatures; the second during winter, when snow and cold temperatures make many of these ponds frozen or, in any case, without water reserves immediately available (Harrison-Day et al., 2019; Salerno et al., 2014). For this reason, high-altitude ponds show a wet phase restricted to few weeks, generally from early spring to early summer (Wissinger et al., 2016). To date, high-altitude ponds are considered biodiversity hotspots and, although these aquatic ecosystems represent a small part of the mountain region, they are a key component in safeguarding aquatic biodiversity (Blanco et al., 2020).

The Italian peninsula is largely characterized from both flat and hilly habitats and mountainous ecosystems. The Alps and the Apennines represent the two main mountain ranges. At the same time, the Italian peninsula is characterized by a large distribution of temporary and permanent ponds (Della Bella et al., 2007; Bazzanti et al., 2003), actually overlooked. In particular, high-altitude ponds in Italy show low concern and their distribution represent an important lack of knowledge, considering also that these lentic environments can represent key habitats for biodiversity conservation (Carchini et al., 2005). Some important questions to solve can be synthesized in:

- How many high-altitude ponds are present in Italy?
- Where are high-altitude ponds mostly distributed in Italy?

– How many of them can be considered natural and how many of them show anthropogenic origin?
– Are there more temporary or permanent ponds?

Mapping the distribution of high-altitude ponds is crucial for managing their dwindling quantities and quality (Malahlela, 2016). The geographical distribution of high-altitude ponds, considered a starting point for management plans, is often overlooked, because the low attention given to these ecosystems and the relative difficulty to identify their distribution. Moreover, it is considered particularly challenging to inventory these water bodies as they are generally small, numerous, temporary, and spread over large and often poorly accessible areas (Soti et al., 2009). The temporary hydroperiod and the restricted time available for the presence of water render the process, in some cases, even more difficult. Mapping natural resources like forests and water bodies using satellite image data have increased much importance and significance in the last decades (Wondrade et al., 2014). Data acquired by Satellite systems, covering a wide range of spectral, spatial, and temporal features, can be used for inventorying and monitoring high-altitude ponds and locating them over large areas (Soti et al., 2009; Ozesmi and Bauer, 2002).

The Italian peninsula currently lacks a checklist of high-altitude ponds. This could represent an important gap for conservation activities of these extremely threatened ecosystems. Identification and georeferentiation of high-altitude ponds are fundamental data to make a first step towards the application of management plans and the safeguard of these lentic ecosystems. The main aim of this research is to create the first georeferenced checklist of Italian high-altitude ponds. Given the numerous anthropogenic impacts which could impact ponds hydroperiod, in particular climate change (Lamouille-Hébert et al., 2024), we hypothesize that permanent and natural ponds show a larger distribution compared to temporary and anthropogenic ones. To achieve this goal, we integrated spectral, spatial characteristics, and morphological operations based on Copernicus Sentinel-1 and Copernicus Sentinel-2 image data using the Google Earth Engine (GEE) platform to develop a quick method for mapping high-altitude ponds in the Italian peninsula, providing information about geographical and altitudinal distribution and morphological features.

Materials and methods

Study area

The study focused on the Italian Peninsula, considering the two main mountain ranges: the Alps and the Apennines. The Alps and the Apennines represent part of a continuous Alpine orogenic system derived from a complex space-time interaction between the two major European and African plates (Molli et al., 2010). The political boundaries of the mountain ranges were taken from the European Soil Data Centre (Hiederer, 2013). The Alps have been divided into Western, Central and Eastern Alps (Brückl et al., 2010) following the Partition of the Alps from Comitato Geografico Nazionale Italiano (1926), showing a fundamentally different geological structure, geomorphology, and geological development (Neubauer et al., 2000). The system adopted covered the entirety of the Alps and not just that part of the Alps that lay on Italian soil. In our research, only the Alps laying on Italian political boundaries were considered.

The Apennines are a mountain range stretching from southern Piedmont to northern Calabria and Sicily with a total length of more 1000 km and a maximum width of 80/140 km (Di Pietro, 2009). Conventionally, the Apennines are divided in Northern (Mirabella et al., 2011), Central (Carafa et al., 2020) and Southern Apennines (Dichicco et al., 2019). Similarly to the Alps, generated from subduction of Europe "eastward–southeastward" underneath the Adriatic plate, the Apennines were generated by the "westward" subduction of the Adriatic plate (Carminati and Doglioni, 2012).

Geomorphological and hydrological features represent important environmental drivers influencing the composition of aquatic flora and fauna of ponds (Florencio et al., 2014) and their hydroperiod length. The Italian Alps are characterized mainly by crystalline magmatic rocks and carbonate platforms; the Apennines shows a soil system made of flysch and marly-arenaceous turbiditic facies on its northern side and carbonate and calcareous units in its central and southern sides (Bini, 2013). However, in this research, the division of the Alps and the Apennines in six zones follow the conventional partition adopted in literature; this partition was adopted instead to help the readers to clearly understand the results obtained. In this case, geomorphological characteristics will not be considered and discussed.

Data collection

Mountains are rugged structures in the landscape that are difficult to outline; whether an area belongs to mountains or not is a matter of definition and has substantial conservational and biogeographic implications (Körner et al., 2021). In our research, we adopted the mountain delineation by Kapos et al. (2000). This approach was developed for the United Nations Environmental Program (UNEP) through their World Conservation Monitoring Centre (WCMC). Following this approach, all lands with 300 m elevation above sea level (a.s.l.) are included in mountainous ecosystems. Given the necessity to standardize the research, our

study was restricted to ponds located at a minimum altitude of 300 m a.s.l. (Körner et al., 2021; Körner et al., 2017).

Data collection was performed with the adoption of the satellite images of Copernicus Sentinel-1 and Copernicus Sentinel-2, used to extract ponds area, perimeter, altitude and the relative coordinates of the ponds (Chitiga, 2018; Ogilvie et al., 2020). Sentinel-2 carries an optical instrument payload that samples 13 spectral bands: four bands at 10 m, six bands at 20 m and three bands at 60 m spatial resolution (Drusch et al., 2012). The orbital swath width is 290 km. The choice to use Sentinel satellites rather than Landsat ones, lies in the greater spatial (Sentinel = 10 m, Landsat = 30 m) and temporal (Sentinel = 5 days, Landsat = 16 days) resolution of the former (Phiri et al., 2020). Considering the small size and the short hydroperiod of some ponds, the Sentinel satellite has proven to be more effective in correctly identifying the site and calculating the spatial dimensions. Moreover, as described by Topaloğlu et al. (2016), image classification accuracies of Sentinel-2 are better than Landsat-8. In this work, we generated the referenced data by manual digitization of the presence and surface water with the assistance of Very High Resolution Satellite imagery from GEE (Google Developers, 2024) and ESRI World Imagery basemaps (ESRI, 2024). Accuracy (the distance between the actual geographic location of an object or detail compared to the position of the object in the image) and validation of the satellite images were also tested measuring points in situ with GPS following Topaloğlu et al. (2016) and then comparing them with satellite images from 16 ponds in the Apennines and 8 ponds in the Alps. Maximum Likelihood classification algorithm was used to classify Sentinel-1 and Sentinel-2 images. Overall accuracy of Sentinel-1 and Sentinel-2 images for water was estimated in 97%, in accordance with Topaloğlu et al. (2016).

We limited our area of interest within latitude 47°30'N–38°00'N and longitude 6°30'E–17°00'E. Data collection was performed with a standard virtual eye altitude of 300 m from GEE. Visualization, identification and references of ponds were performed moving along an S-shaped line inside the field of view from top to bottom, then moving from left to right to do not overlap with the previous field of view and then proceeding in the opposite direction. Geographical coordinates, altitude and the estimated area and perimeter of the ponds were recorded using the "surface calculator" tool from GEE. A unique code was then assigned to each pond, made up of two letters which indicates the partition of the Alps considered (WA = Western Alps; CA = Central Alps; EA = Eastern Alps) and a consecutive number; regarding the Apennines, the code was built with three letters (NAP = Northern Apennines; CAP = Central Apennines; SAP = Southern Apennines) followed by a consecutive number. Considering the historical image database provided from GEE, and to avoid the impact of pond drainage on the extraction effect, we selected the images from the most recent winter season (December 2023–February 2024) for the lower ponds (not subject to snow cover), when available, to search for ponds and estimate their surface area. On the contrary, for the very high-altitude ponds, which go frozen in winter, we selected images from the most recent late spring and early summer seasons (April–June 2023). In addition, we selected images with the lowest cloud coverage in the study area during the timeframe. For those ponds which are not covered by snow, winter is considered the season when the pond water supply is at its maximum (Hayashi and van der Kamp, 2021). Instead, for high-altitude ponds which go frozen during winter, the maximum level of the water is generally reached at the beginning of the summer (Thielke et al., 2023; Anhaus et al., 2021). Then, we collected all the images from the previous years (back to 15 years) to distinguish between "Temporary" and "Permanent" nature of the ponds. This approach also allows us to avoid errors in the assessment of the actual existence of ponds, which can otherwise be confused with shadow areas or geological features. The analysis of historical series therefore provides a validation of the method of identification of the pools through satellite data. To distinguish between temporary and permanent, we analysed images available from the summer season (June–August), considered, at these latitudes, the "dry" period. Ponds with at least one image where they appear dry, were considered as "temporary". High-altitude ponds can be identified and distinguished from other artificial or natural water bodies (such as lakes, reservoirs) with radar satellite data on the basis of different edges (not precise geometric shape) in combination with size, vegetation cover and shape features. Finally, all the water bodies identified with GEE labels as "lake", "lagoon" were excluded from the research. Following this criterion, some examples of excluded ponds are shown in Fig. S1. The land use was determined by CORINE Land Cover (CLC, 2018), a standardized methodology for producing continent-scale land cover, biotope, and air quality maps. Two land cover classes were considered: "Natural" and "Anthropized". Each pond was assigned to one class or another considering the high percentage of land cover in a buffer zone of 300 m: if at least 51% of the land cover was a natural habitat, the pond was considered as "natural". The same criterion was adopted for the "anthropized" classification, considering human activities (including agriculture and livestock) (Fig. 1). This division was adopted to simplify the ponds classification and make the results easier to interpret for the readers.

Results

The development of a method based on GEE enabled us to elucidate the national-scale pond distribution in the Alps and the Apennines of the Italian peninsula using Copernicus

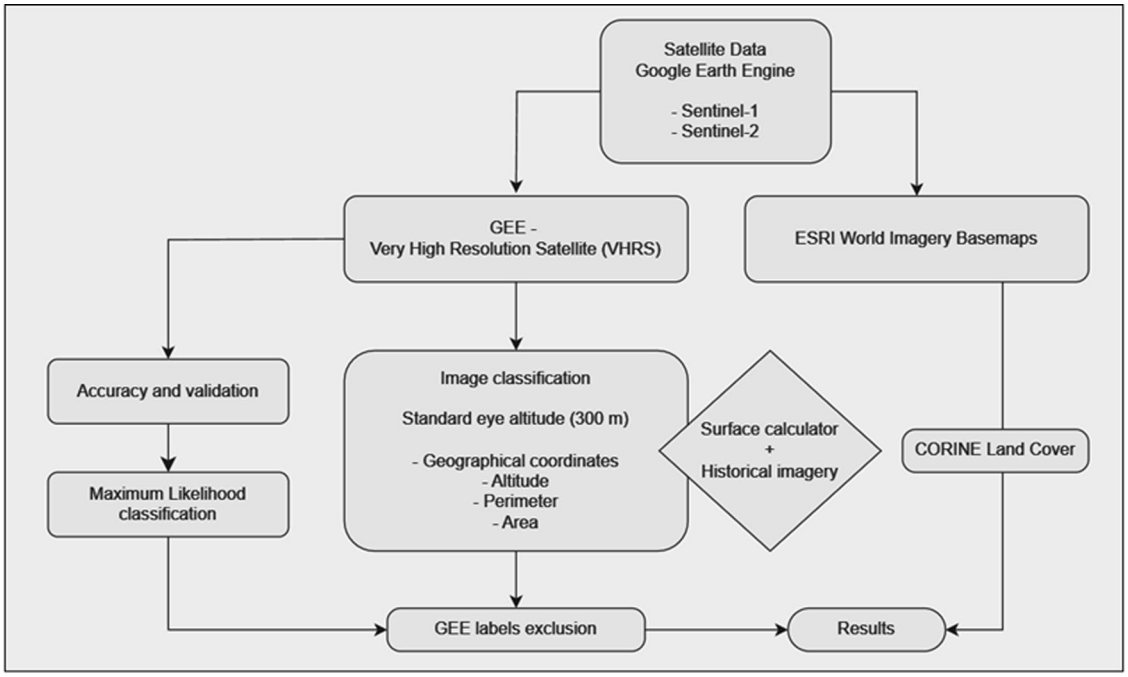

Fig. 1 The flowchart of the methodology

Sentinel-1 and Copernicus Sentinel-2. The results provide a comprehensive overview of the spatial distribution of ponds in the principal Italian mountain ranges. The total area of the Alps and the Apennines was estimated in 109.180 km^2. Overall, 2156 ponds were identified: 62% ($n = 1343$) in the Alps and 38% ($n = 813$) in the Apennines. The highest number of ponds was detected in Central Alps ($n = 642$), followed by Western Alps ($n = 479$), Central Apennines ($n = 412$), Eastern Alps ($n = 222$), Southern Apennines ($n = 216$) and Northern Apennines ($n = 185$) (Fig. 2).

In 2024, the total area covered from ponds was estimated in 4.258.640 m^2, with a mean of 1716 m^2, and the relative coverage of the ponds was 0.004% of the total surface of the Alps and the Apennines. Overall, the highest area covered by ponds was reported for CA (37%), followed by WA (23%), CAP (23%), EA (7%), NAP (5%) and SAP (4%) (Fig. 3). The largest area was recorded in EA (37091 m^2), while the smallest area was recorded in CAP (5.50 m^2).

The total perimeter of ponds was estimated in 333.279 m, with a mean of 140 m. Overall, the highest perimeter covered by ponds was recorded for CA (34%), followed by WA (24%), CAP (21%), EA (8%), NAP (6%) and SAP (6%). The largest perimeter was recorded in CA (1799 m), while the smallest perimeter was recorded in CAP (9.46 m) (Fig. 3).

Overall, 42% ($n = 909$) of ponds are actually included in almost one Natura 2000 site, Special Area of Conservation (SAC), Site of Community Importance (SCI), Special Protection Site (SPA), Regional or National Park. In particular, WA shows the highest percentage of "protected" ponds with 62% ($n = 301$), included in almost one European Union or National protected area, followed by EA (48%, $n = 106$), SAP (44%, $n = 95$), CA (38%, $n = 243$), NAP (31%, $n = 57$), CAP (26%, $n = 107$).

For what concerns altitude, the highest value was recorded in WA (3345 m a.s.l.), while the lowest were recorded in NAP and CAP (300 m a.s.l.). Overall, the Alps show the highest altitude values, with the lowest value recorded at 693 m a.s.l. (EA). Regarding the Apennines, the highest altitude value was recorded in CAP (2048 m a.s.l.). The average altitude was estimated in 1577 m a.s.l. Regarding Alps, the average altitude was estimated in 2428 m a.s.l., while in the Apennines the average altitude was estimated in 784 m a.s.l. WA shows the highest average altitude (2580 m a.s.l.) for the Alps, while EA shows the lowest (2109 m a.s.l.).SAP shows the highest average altitude (990 m a.s.l.) for the Apennines, while NAP shows the lowest (603 m a.s.l.) (Fig. 4).

The nature of ponds was described as 20% temporary ($n = 445$) and 80% permanent ($n = 1711$) regarding the hydroperiod. In particular, the highest number of permanent ponds was recorded in CA ($n = 561$), followed by WA ($n = 387$), CAP ($n = 326$), EA ($n = 187$), NAP ($n = 133$), SAP ($n = 117$). The highest number of temporary ponds was recorded in SAP ($n = 99$), followed by WA ($n = 92$), CAP ($n = 86$), CA ($n = 81$), NAP ($n = 52$), EA ($n = 35$).

Moreover, considering the land use, 83% ($n = 1797$) of ponds were described as "natural" and 17% ($n = 359$) as "anthropized". WA and CA show 100% of "natural" ponds, while EA shows 99% of "natural" ponds. CAP represents the only zone where "anthropized" ponds show higher

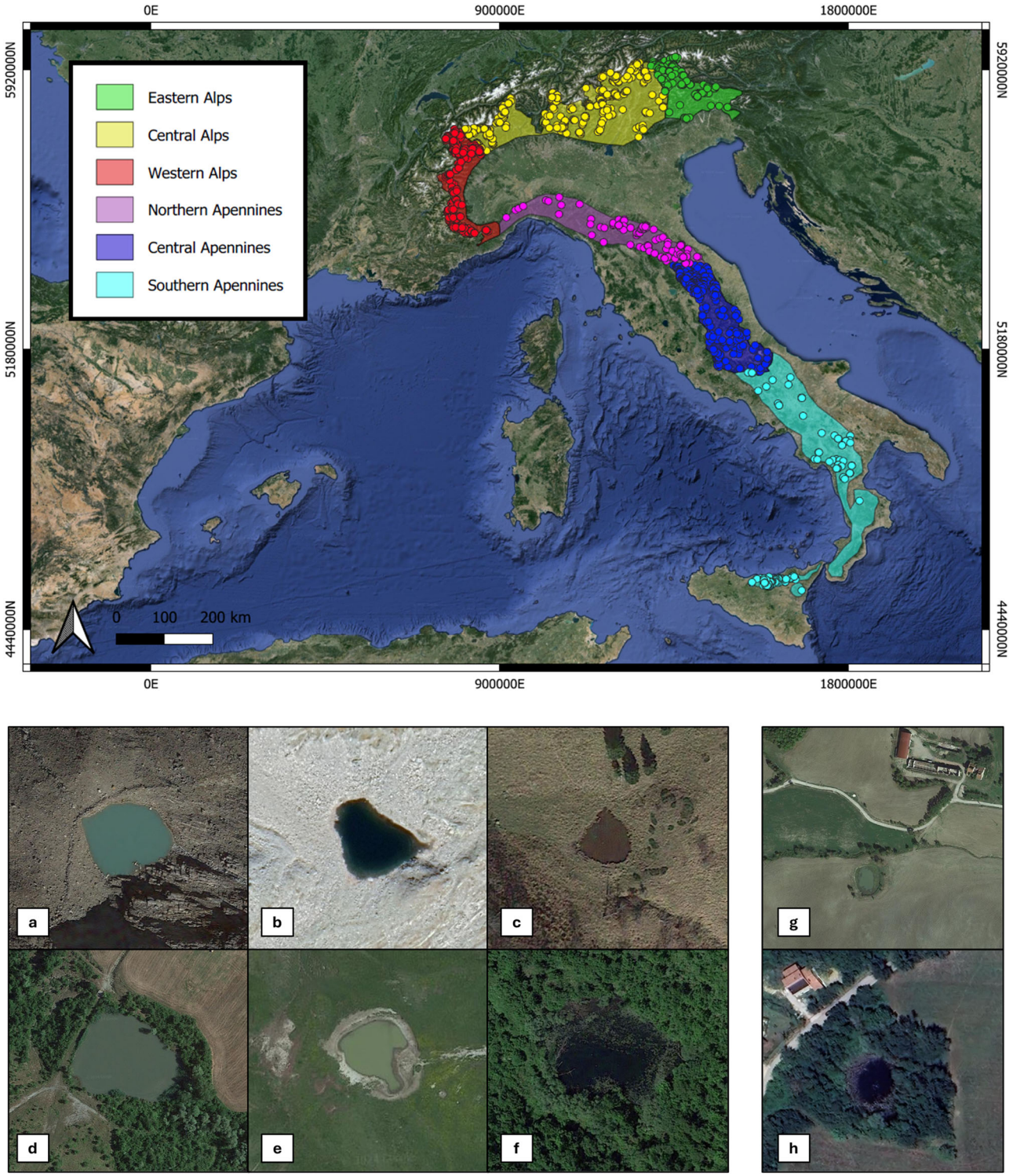

Fig. 2 Above: distribution of SW in the Italian Peninsula. The six zones considered are traced with different colors. Below: pictures of some different ponds detected (**a**–**f**: "Natural" ponds; **g**, **h**: "Anthropized" ponds). **a** WA91; (**b**) CA4; (**c**) EA98; (**d**) NAP56; (**e**) CAP37; (**f**) SAP19; (**g**) NAP61; (**h**) CAP271

abundances than "natural" ones. The highest number of "natural" ponds was recorded in CA ($n = 642$), followed by WA ($n = 479$), EA ($n = 220$), SAP ($n = 202$), CAP ($n = 142$) and NAP ($n = 112$). The highest number of "anthropized" ponds was recorded in CAP ($n = 270$), followed by NAP ($n = 73$), SAP ($n = 14$) and EA ($n = 2$) (Fig. 5).

Data of the ponds' areas were tested both for normality and homogeneity of variance. The ANOVA test calculated

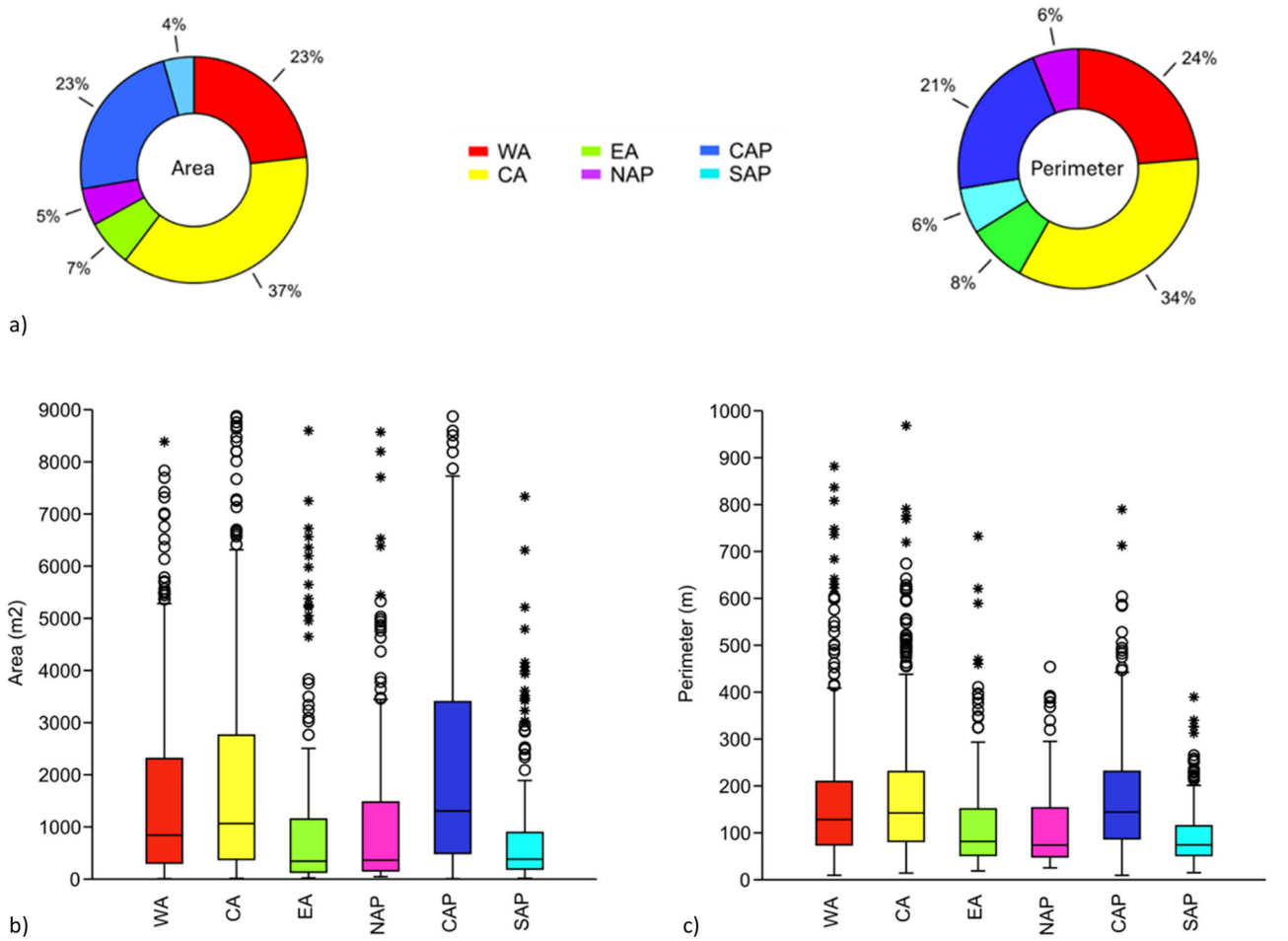

Fig. 3 **a** Total area and perimeter recorded for the six zones considered; (**b**) boxplot of ponds' area values; (**c**) boxplot of ponds' perimeter values. (WA = Western Alps; CA = Central Alps; EA = Eastern Alps; NAP = Northern Apennines; CAP = Central Apennines; SAP = Southern Apennines)

for the ponds' areas shows significant differences between groups ($F = 12.99$, $p < 0.05$). Tukey post-hoc test highlights differences between groups shown in Table 1.

Ponds' altitudes data were tested both for normality and homogeneity of variance. The ANOVA test calculated for the ponds' altitudes shows significant differences between groups ($F = 2011$, $p < 0.05$). Tukey post-hoc test highlights differences between groups shown in Table 2.

Correlation between "Area" and "Perimeter" were tested for all the six cases. All the zones show high correlation between these two variables (WA, $r = 0.88$, $p < 0.05$; CA, $r = 0.82$, $p < 0.05$; EA, $r = 0.83$, $p < 0.05$; NAP, $r = 0.97$, $p < 0.05$; CAP, $r = 0.92$, $p < 0.05$; SAP, $r = 0.94$, $p < 0.05$).

Linear regressions were performed to evaluate correlations between variables "Area" and "Altitude" for the six zones. Area and altitude result low correlated in all the six zones considered: in particular, average low positive correlation trends emerge for the Alps while average low negative trends emerge for the Apennines (Fig. 6).

Contingence tables were created to evaluate the relationship between the variables "Area", "Altitude", "Land cover" and "Hydroperiod" for the six zones considered.

Chi-squared tests were performed grouping altitudinal and area data in four classes (or quartiles, chosen in function of the altitudinal and area values of each zone). The results were shown in Table S1. Chi-squared tests show: (i) medium dependence between hydroperiod and land use ($V = 0.33$) and low dependences between altitude and land use ($V = 0.25$) and area and land use ($V = 0.27$) for NAP; (ii) strong dependences between altitude and hydroperiod ($V = 0.56$) and altitude and land use ($V = 0.72$), medium dependence between hydroperiod and land use ($V = 0.49$) and low dependence between area and hydroperiod ($V = 0.19$) for CAP; (iii) medium dependence between altitude and hydroperiod ($V = 0.33$) and between altitude and land use ($V = 0.30$) and low dependence between area and hydroperiod ($V = 0.26$) and between area and land use ($V = 0.26$) for SAP (Fig. 7).

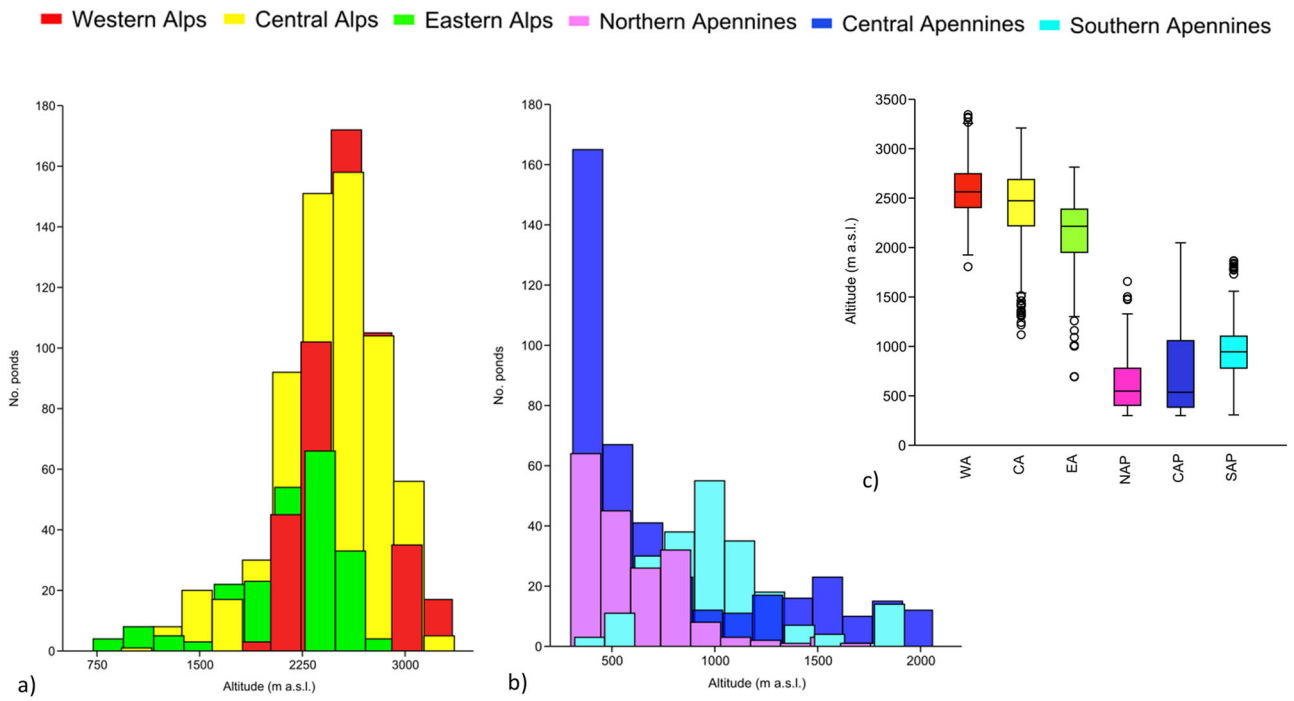

Fig. 4 **a** Barchart of altitude values of ponds in the Alps; (**b**) barchart of altitude values of ponds in the Apennines; (**c**) boxplot of mean altitudes recorded (WA = Western Alps; CA = Central Alps; EA = Eastern Alps; NAP = Northern Apennines; CAP = Central Apennines; SAP = Southern Apennines)

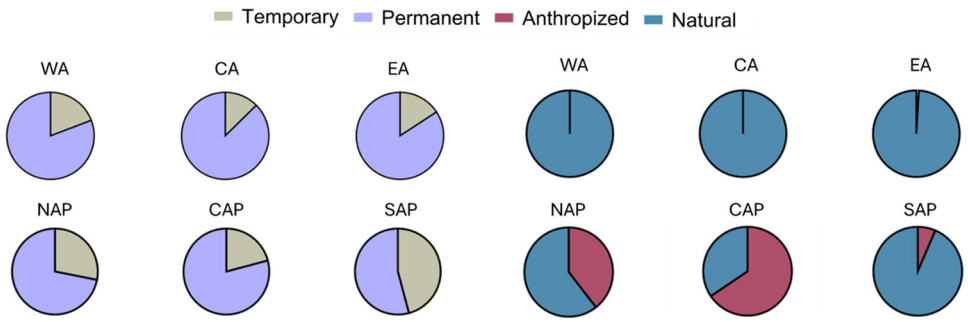

Fig. 5 Piecharts of the land use and hydroperiod of the six zones considered (WA = Western Alps; CA = Central Alps; EA = Eastern Alps; NAP = Northern Apennines; CAP = Central Apennines; SAP = Southern Apennines)

Table 1 Tukey's Q (below the diagonal) and p (same, above the diagonal) for area values; significant comparisons are bold (WA = Western Alps; CA = Central Alps; EA = Eastern Alps; NAP = Northern Apennines; CAP = Central Apennines; SAP = Southern Apennines)

	WA	CA	EA	NAP	CAP	SAP
WA		0.3392	**0.04478**	**0.04192**	0.6205	**0.0001471**
CA	2.837		**7.533E-05**	**0.0001175**	0.9999	**1.384E-08**
EA	4.089	6.463		1	**0.0006525**	0.7634
NAP	4.121	6.328	0.2489		**0.0007842**	0.8806
CAP	2.216	0.3541	5.776	5.713		**4.263E-07**
SAP	6.258	8.698	1.893	1.559	7.878	

Discussions

This study focused on the extraction of Italian high-altitude ponds from Copernicus Sentinel-1 and Copernicus Sentinel-2 images. Some small-scale ponds constitute the mixed pixels in the 30-m spatial resolution satellite images (Duan et al., 2020), thus, it is difficult to extract individual ponds, evaluate their precise area and perimeter while excluding clearings between them. Moreover, for the first time, the Italian Alps and the Apennines regions were taken into consideration in this study, and this consideration is consistent with the distribution of high-altitude ponds in the Italian peninsula and their underrated value. However, as high-altitude ponds represent a specific ecosystem,

Table 2 Tukey's Q (below the diagonal) and p (same, above the diagonal) for altitude values; significant differences are bold (WA = Western Alps; CA = Central Alps; EA = Eastern Alps; NAP = Northern Apennines; CAP = Central Apennines; SAP = Southern Apennines)

	WA	CA	EA	NAP	CAP	SAP
WA		**2,748E-10**	**6,415E-11**	**6,415E-11**	**6,415E-11**	**6,415E-11**
CA	9,609		**6,42E-11**	**6,415E-11**	**6,415E-11**	**6,415E-11**
EA	21,85	15,33		**6,415E-11**	**6,415E-11**	**6,415E-11**
NAP	85,97	82,23	56,94		**5,84E-05**	**6,421E-11**
CAP	102,1	99,53	61,13	6,54		**6,721E-11**
SAP	73,02	68,71	44,06	14,55	10,46	

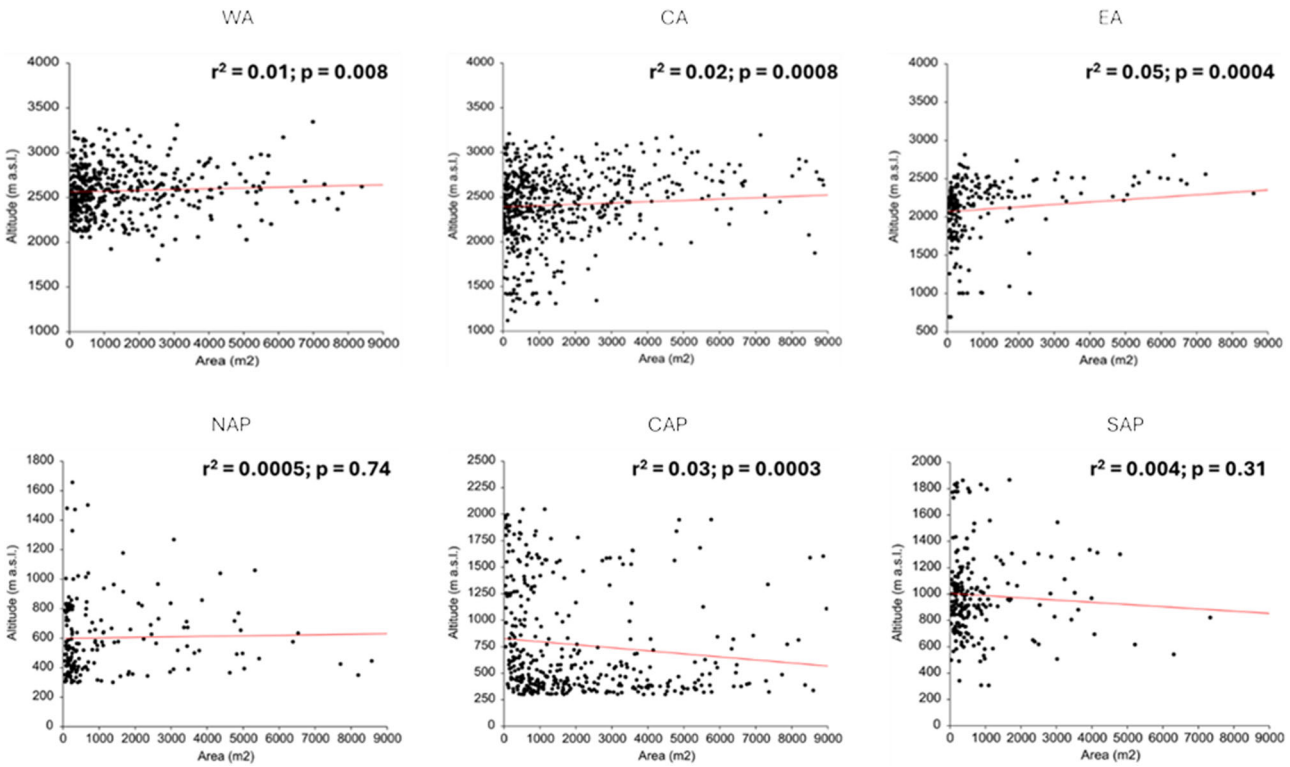

Fig. 6 Least square regressions for the variables "Area" and "Altitude". (WA = Western Alps; CA = Central Alps; EA = Eastern Alps; NAP = Northern Apennines; CAP = Central Apennines; SAP = Southern Apennines)

including several different small waterbody types (Taurozzi et al., 2024), it is difficult for traditional classification methods or a single spectral index to efficiently identify and classify high-altitude ponds at a large scale (Şerban et al., 2020). Therefore, we developed a method based on Copernicus Sentinel-1 and Copernicus Sentinel-2 images to enable the extraction of the whole high-altitude ponds on a national scale by combining spatial features, historical imagery, and morphological characteristics.

The Copernicus Sentinel satellite missions offer free access to continuous, timely, and routine Earth observation data (Jutz and Milagro-Perez, 2020) and thus offer enormous potential for large-scale and even national assessment of high-altitude ponds (Ottinger et al., 2021). Our research demonstrated the possibility to detect and map high-altitude ponds at the single-pond level for a national scale combining available data of essential time series information derived from high-spatial-resolution Sentinel-1 radar imagery in combination with Sentinel-2 optical time series information, as well as topographical and geometric features.

Overall, the highest number of ponds detected was in the Alps. This heterogeneous distribution can be related to the scarce tree coverage located on the Alps rather than Apennines. Leonelli et al. (2011) estimated the tree line for the Italian Alps at an altitude of about 2500 m a.s.l. 46% of the high-altitude ponds were detected above this altitude. In the last three centuries, the Italian Alps were extensively used for agriculture (Monzani, 2021; Chemini and Rizzoli, 2003), with many forests cleared for meadows and pastures (Tasser et al., 2007). Furthermore, grazing in subalpine forests created forest pastures with sparse tree coverage

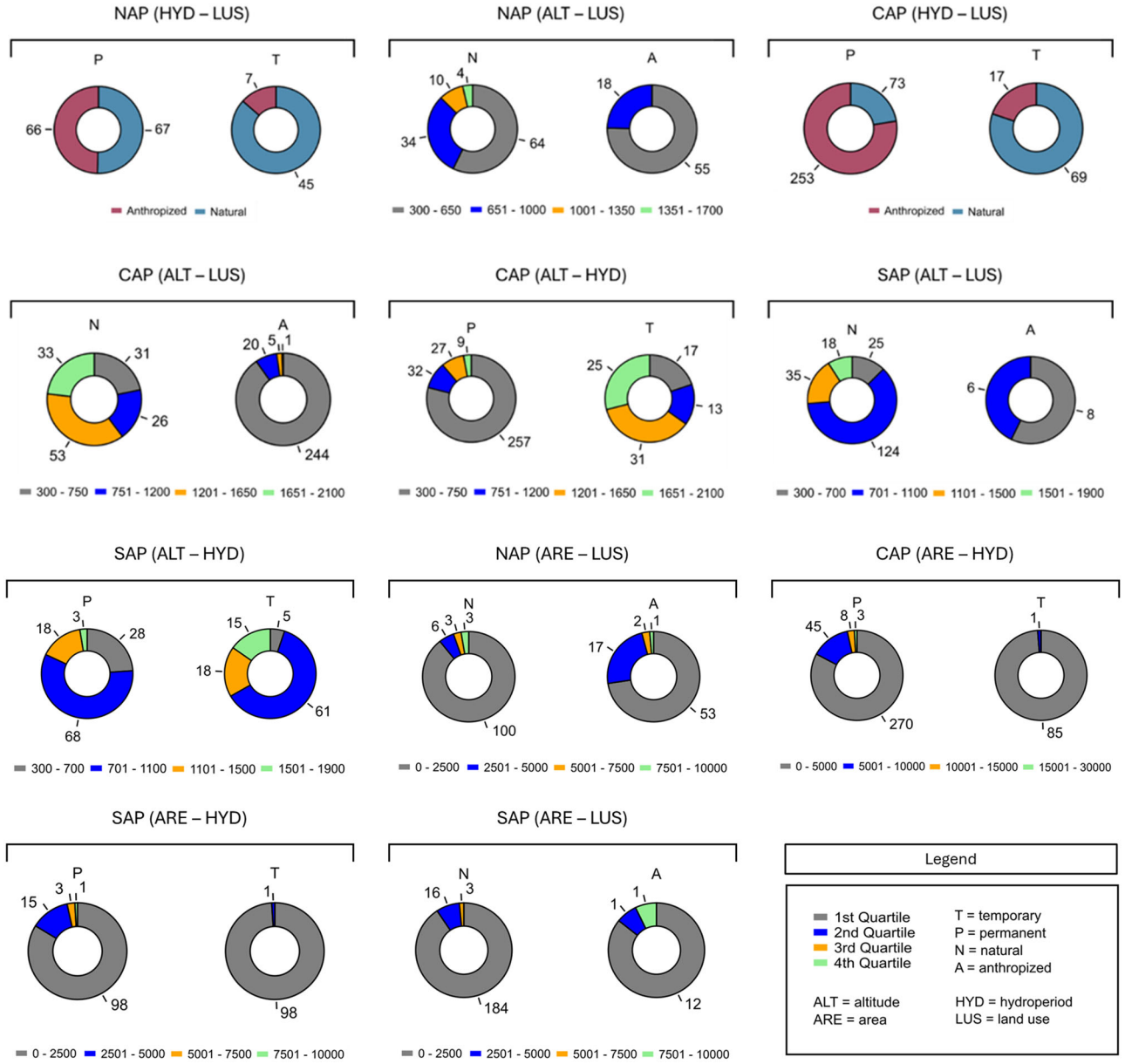

Fig. 7 Pie charts of the significant ($p < 0.05$) dependences calculated with chi-squared tests. The zone considered and the relative variables investigated are presented above each couple of pie chart. Label numbers represent the values emerged from dependence analysis. The variables considered are shown in the "Legend" box. Values below the pie charts represent the values of altitude (ALT) and area (ARE) found and organized in quartiles. (WA = Western Alps; CA = Central Alps; EA = Eastern Alps; NAP = Northern Apennines; CAP = Central Apennines; SAP = Southern Apennines)

(Stritih et al., 2023). On the contrary, the Apennines show a lower average altitude (Carminati et al., 2004), often below the tree line. These results translate into difficulties in identifying ponds in some cases using satellite images, due to the tree coverage. A widespread tree cover can be considered the limit of our research. In such cases, the only way to track down ponds is to do on-site inspections (Mahmoud et al., 2023). Indeed, as suggested by Boardman (2016), Google Earth is ideal for reconnaissance mapping and the discovery of sites missed during field work. The use of Google Earth "historical imagery" tool could provide an important help for detect ponds where an extensive tree coverage is present (Li et al., 2023; Duan et al., 2020). In the Anthropocene Era, characterized by numerous human influences and threats on environment, a 10 or 15-year look back could reveal the presence or absence of many environmental elements, now disappeared (Smith and Zeder, 2013). Moreover, as reported by Leonelli et al. (2011), also treelines are mainly influenced by human impacts and by geomorphological factors. Historical imagery can reveal the

existence of a pond where, nowadays, an extensive tree cover is present. However, to provide a complete overview of the pond distribution, only the most recent images from Copernicus Sentinel-1 and Copernicus Sentinel-2 satellites were considered in this study.

From the results emerges an estimated coverage of 0.004% of ponds respect to the total area under study. Considering the large portion of natural "pristine" environment available, especially on Italian Alps (Pastorino et al., 2024), and the critical status of conservation of ponds worldwide, this percentage represents an alarm bell for the conservation of these habitats. Although ponds represent a neglected habitat, scientific awareness about these ecosystems raised in the last years, in particular for Mediterranean temporary ponds (MTPs), considered as priority habitat (Bagella, 2023). However, in matters of legislation, the Habitats Directive (Council Directive 92/43/CEE) recognize only a small part of MTPs basing on the presence of target plant communities and excluding many other ponds, in particular high-altitude ponds; furthermore, the Water Framework Directive could represent an important tool for the protection of MTPs in Europe, but unfortunately the local legislation of many states fixed 50 ha as the minimum size of a waterbody for its application (Bagella et al., 2016). This limit emerges also from our research. We demonstrated that only 42% of ponds are included in, at least, one Natura 2000 site, Special Area of Conservation (SAC), Site of Community Importance (SCI), Special Protection Site (SPA), Regional or National Park. Natura 2000 network represents the most important conservation effort being implemented in Europe, increasing from 11% to 20% the area devoted to conservation in Italy and the coverage provided to areas with high biodiversity (Hermoso et al., 2019). However, as reported by Maiorano et al. (2007), if Natura 2000 is taken to represent the last point of all the EU conservation policies, it will inevitably fail. Given that Natura 2000 is not suitable alone for maintaining listed species and habitats in a "favorable conservation status" (Bastian, 2013; Maiorano et al., 2007), the high number of excluded ponds (58%) represent a critical situation for the conservation of these ecosystems. It is out of doubt that Natura 2000 represent, together with existing protected areas, an important component on which to build future conservation efforts. Nevertheless, further studies and management plans are necessary to expand the dimensions of protected areas to include and protect as many ponds as possible. Therefore, the data collected for monitoring water bodies at the European level cannot be used for the assessment of small ponds, as for other large water bodies (Bolpagni et al., 2017).

Data obtained acquire still more importance if related to ponds hydroperiod. The small percentage of temporary ponds detected (20%) suggest that, although the Alps show a dominance of permanent ponds, the Apennines, in particular SAP, are suffering a hydric crisis (Polemio and Casarano, 2008). Droughts and water shortage conditions, which occur frequently in southern Italian regions (Rossi and Peres, 2023; Buttafuoco et al., 2018; Mendicino et al., 2008), can affect ponds hydroperiod, favoring temporary ponds over than permanent ones. High-altitude ponds, although being characterized by a restricted dry phase (both for winter snow and summer drought), showed a predominantly permanent nature: these results can be addressed to the lower temperatures characterizing high altitudes. Temperature, precipitation, and evaporation rate represent the main factors influencing pond hydroperiods (Hayashi and van der Kamp, 2021; Florencio et al., 2020).

Moreover, as reported by Acquaotta et al. (2015), WA showed, in the last 20 years, an increase in temperature, particularly at high-altitudes sites (>2600 m a.s.l., Fahy et al., 2024), and a decrease in the number of cold periods due to anthropogenic climate change. In this regard, ponds are influenced by climate change in multiple ways, including increased rate of surface evaporation, reduced hydroperiods, alteration of chemical and biological processes, and increased temperatures (Lamouille-Hébert et al., 2024; Tasker et al., 2022; Ali et al., 2016; Matthews, 2010). As described by Rogora et al. (2018), all the three parts of the Alps considered here are suffering climate change, showing rapid responses at ecosystem level: changes in freshwater organisms were also reported, in terms of increases in solutes, decreases in nitrates and changes in plankton phenology and benthos communities. In the Alps, mean snow depth, snow cover duration and number of snowfall days have decreased since the early 1980s (Laternser and Schneebeli, 2003). Modifications in snow coverage, glacial extensions and frozen periods can affect mountain ecosystem hydrology, biogeochemical processes in soil and water, plant composition and phenology (Rogora et al., 2018). Trends from literature confirm that climate change is occurring in alpine environments, especially during the season of snow accumulation and vegetative growth (Dorji et al., 2020; Acquaotta et al., 2015; Casty et al., 2005).

Land use provide us important insights about the surrounding environment of detected ponds. In particular, the higher number of natural ponds (83%) over than anthropized (17%) found can highlight both the large distribution of pristine environments in mountainous ecosystems and the scarce consideration given to ponds from human activities (Oertli et al., 2010). However, although a pond could not be surrounded by agricultural areas or urban areas (i.e., what we describe as "natural ponds"), this does not mean that it is embedded in a totally natural and pristine ecosystem. In fact, also in high-altitude ecosystems, human direct or indirect disturbances can be present. In the

Apennines, ponds are frequently embedded in an agricultural context (Vitali et al., 2018). In the Alps, the presence of grazing cows moving through areas that are assessed as "natural" in the Corine Land Cover can be very impactful on the high-altitude ponds (Van den Broeck et al., 2019), changing the morphology of the shores and polluting the water with their droppings. For this reason, to assess the real conservation status of ponds it is necessary to perform in situ analysis. In this context, our research could represent an important starting point for conservation activities.

The large distribution of temporary ponds could be in accordance with the low affected nature of high-altitude ecosystems (Ballón et al., 2016); however, while the total number of ponds on the Alps are "natural", NAP and CAP are characterized by a large distribution of "anthropized" ponds. In the Apennines, anthropogenic pressure has historically taken the form of intensive grazing on high-elevation pastures and short-rotation coppicing in forests (Quaranta et al., 2020; Vitali et al., 2018; Caballero et al., 2009). The relatively recent decline of such traditional practices has progressively changed the mosaic structure of mountain landscapes, with the increase of high-elevation forest coverage (>1.500 m a.s.l.) (Vitali et al., 2018). NAP are historically characterized by burning and accelerated soil erosion, indicating widespread human activity (Branch and Marini, 2014); CAP show significant evidence of human impact on the forests and the development of grazing up to 2000 m a.s.l. since many centuries (Brown et al., 2013); SAP were characterized by the altitudinal expansion of the tree line, experiencing more intensive land uses in the past (Vitali et al., 2018). The widespread historical human impacts on the Apennines influenced distribution and morphological features of ponds. However, while "natural" ponds show with a certain probability to be not-related to human activities looking at satellite images, the nature of "anthropized" ones should be evaluated with on-field samplings.

The evaluation of the pond's areas highlighted the differences between the zones considered. WA, CA and CAP appear to share the highest values of area. On the contrary, EA, NAP and SAP show the lowest areas. WA and CA are characterized by higher altitudes and lower human activities. The Alps are instead characterized by a larger presence of snow coverage (Paul et al., 2007). The temperatures typical of the spring and summer seasons lead to the snow melting and the formation of ponds (Beniston, 2012). The hydroperiod of these ponds depends on many environmental and geographical factors. Generally, as demonstrated by our results, ponds generated by the snow melting are larger in size than the natural pools which generate at lower altitudes. Furthermore, at high altitudes, climatic variations interact with glacial processes and influence the dynamics of alpine ecosystems (Zemp et al., 2006). This is important also for social implications, considering that mountain glaciers are the major fresh water source for people living in or nearby the adjacent lowlands of mountain ranges (Giaccone et al., 2015). On the contrary, NAP and SAP ponds show statistically smaller dimensions. The difference may lie in the low altitude reached by the mountains present in these regions and the consequent almost total absence of snow coverage. Low altitude ponds are mostly generated and related to phenomena such as precipitation or the infiltration of groundwater (Hayashi and van der Kamp, 2021; Zacharias and Zamparas, 2010). On the contrary, CAP host the highest mountains in the Apennines, and the generation of ponds could be favored respect other Apennines districts from snow melting. Moreover, the highest area values could be explained also considering the influence of those which are supposed to be artificial ponds, showing average area values higher than natural ones. Finally, in EA, present-day glaciers cover an area of about 6.95 km^2 in this mountain group (Seppi et al., 2015; Cagnati et al., 2002). They are generally small cirque glaciers, persisting at low altitude thanks to high accumulation rates of avalanche snow. Also in this case, the low area of glaciers could explain the lower ponds area evaluated.

Comparing altitude, the difference between the six zones could be addressed to the different altitude reached by mountain chains. The Alps host the highest peaks in Italy like Monte Bianco (4806 m a.s.l.), Monte Rosa (4634 m a.s.l.), Monte Cervino (4478 m a.s.l.). The Apennines host, as highest mountains, the Gran Sasso d'Italia (2914 m a.s.l.), Maiella (2795 m a.s.l.) and Monte Velino (2487 m a.s.l.). Moreover, NAP are considered to reach a maximum altitude of 2165 m a.s.l., while SAP host, as highest mountain, the Pollino (2248 m a.s.l.). Altitudinal differences were expected and then confirmed by analysis. However, comparing altitude and area, only low correlation emerged. WA, CA, and EA showed low positive correlations, while NAP, CAP and SAP showed low negative correlations. These results can be explained considering land use and the altitudinal differences previously described: the Alps are characterized by an increase in area as a function of altitude. This correlation is probably influenced by the snow melting, especially at high altitudes, which has caused an increase in pond areas. Another reason can be identified in the vegetal coverage, which was proved to influence the area of the ponds. Water level and vegetation cover show a direct relationship, influencing each other. Water-level change in ponds is considered a disturbance to many aquatic plants (Hayashi and van der Kamp, 2021). Plant cover can instead greatly influence the availability of water in a pond due to continuous water capture (Oertli et al., 2023). Below the 'nival zone' where conditions are too cold to support vascular plant life (Verrall and Pickering, 2020), ponds area

and water volume are not affected by vegetation catchment. Below the climatic treeline, where vegetation cover increases as altitude decreases (Wang et al., 2023; Qanbari and Jamali, 2015; Wilson and Nilsson, 2009), plant biomass can significatively reduce water availability in ponds, especially during the growing season, which duration in high-altitude environment averages around 160 days (Verrall and Pickering, 2020). On the contrary, in the Apennines, ponds with the largest area were found at lower altitudes. This result is in line with land use, where ponds characterized by anthropic impacts (therefore also artificial) mainly appear at lower altitudes, showing larger dimensions than the natural ones at higher altitudes (but, in any case, not formed due to the snow melting). These results are also in accordance with the Chi-squared tests, where altitude results medium-high correlated with ponds hydroperiod and land use. As described before, especially in the Apennines, an increase in the anthropic impacts on the natural component of ecosystems appeared, especially at lower altitudes. Temporary ponds with larger areas are present at lower altitudes, especially in the CAP. The lower temperatures, typical of high altitudes, could favor a longer duration of ponds hydroperiod. On the contrary, at lower altitudes and lower latitudes, natural ponds are dominant. (Jacobsen and Dangles, 2017). Finally, a correlation emerged between “hydroperiod” and “land use”: the highest number of permanent ponds result also as “anthropized” ponds. Furthermore, permanent ponds are distributed mainly at lower altitudes, supporting the thesis that many of the ponds found and described as "anthropized" could be of artificial origin.

Our findings suggest that ponds are both widely distributed in the Italian high-altitude ecosystems and that their conservation status is highly threatened. Climate change and anthropic pressures can endanger the existence of small waterbodies, especially temporary ones (Xia et al., 2015; Rosset et al., 2010). Although all high-altitude ponds in the Alps are inserted in a natural context, the extreme temperatures of winter and summer can represent a significant risk factor, even for permanent ones (Ali et al., 2016). Considering the prolonged period in which ponds remain frozen and the high temperatures typical of the summer period, their hydroperiod becomes increasingly reduced. Given their value as a source of water for the flora and fauna of these extreme environments (Biggs et al., 2017), it is important to protect them throughout the year. Moreover, ponds support a larger proportion of rare, endemic and threatened freshwater species, and are key elements of blue landscape connectivity (Hill et al., 2018; Céréghino et al., 2010). In addition, ponds deliver multiple ecosystem services and Nature's Contributions to People, including carbon storage and climate regulation, water provision, flood control, groundwater recharge, pollution amelioration, recreation and tourism (Fu et al., 2018; Biggs et al., 2017). Their mapping therefore represents an important first step, especially for policy makers. Identification and geographical localization of ponds represent the basis for the design and implementation of long-term monitoring protocols by policy makers (Oertli et al., 2005). On this context, although it is not easy to intervene to maintain the water regime of high-altitude ponds, water can be made available through the installation of fountains for animals.

However, our results also demonstrate that many high-altitude ponds, especially in the Apennines, are far from being identified, certainly impossible by satellite due to the extensive tree coverage. It is therefore necessary to draw up on field surveys and monitoring plans through in situ censuses and subsequent conservation plans.

Conclusions

We evaluated the distribution of small waterbodies (ponds) in the main mountain ranges of the Italian Peninsula, the Alps and the Apennines, focusing on a crucial yet often overlooked aspect in the literature: the identification and characterization of permanent and temporary high-altitude ponds. Our results highlighted an unrepresented distribution of ponds, where the Alps host the highest number, and the highest total area compared to the Apennines. Moreover, ponds of the Alps can be considered mainly natural, while Apennines’ ponds show an important influence of human activities. Apennines ponds, showing lesser altitudinal distribution and lower area at higher altitudes, appear to be mostly artificial, or supported in their hydroperiod length from anthropogenic influences. Furthermore, a low positive correlation emerged between area and altitude for the Alps and a low negative correlation emerged between area and altitude for the Apennines. The different interactions found depending on the environmental and geographical variables, highlighted the importance to analyze ponds under multiple biological aspects. Moreover, this study raises the question of the importance given to these threatened ecosystems, as they may be more studied and protected. The main limit of this research is the tree coverage, which makes the pond identification from satellite images difficult and incomplete. To the best of our knowledge, this is the first study investigating the distribution of high-altitude ponds in the two principal mountain ranges of Italy. However, lacking an assessment error, mainly due to the large tree coverage, this research is to be considered incomplete. Field surveys are fundamental to determine the correct and complete distribution of ponds, especially for those not detectable by satellite images. Moreover, orthophotos can be an important tool to compare aerial photographed ponds and those found through satellite imagery. The comparison between these

data could provide important information about lacking ponds. Future studies are therefore necessary to correctly define errors and to make a complete checklist. The results found could have ecological implications and, in conjunction with assessments of pond presence with on-field surveys, can provide valuable suggestions on the effects of natural and anthropogenic threats on high-altitude ponds conservation and management activities.

Data availability

No datasets were generated or analysed during the current study.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s00267-024-02061-6.

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Author contributions DT: Investigation, Sampling, Visualization, Writing – original draft, Writing – review & editing. MS: Conceptualization, Resources, Supervision, Validation, Writing – review & editing

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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CHAPTER III

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Silent survivors: diatom resilience in Mediterranean Temporary Ponds

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ABSTRACT

Mediterranean Temporary Ponds (MTPs) are ephemeral freshwater habitats hosting a large number of aquatic and terrestrial organisms. Among these, diatoms represent the bioindicators that contribute mostly to Earth's global primary production. Herein, we focused our attention on the role of seasonality and hydroperiod on benthic diatoms of MTPs. We analyzed the diatom community turnover along one year in two MTPs in the Latium region, Italy. The two MTPs showed different levels of anthropogenic pressures. Our results demonstrated that hydroperiod length and seasonality showed a moderate influence on diatom richness of MTPs; in particular, although the highest number of species was found during spring, taxonomic diversity remained stable at seasonal and inter-annual level despite strong changes in community composition and hydroperiod length. Overall, sampling sites showed different ecological conditions and chemical drivers: protected site was dominated by species occurring in minimally impacted oligo- to mesotrophic waters; unprotected site with high EC and ORP was dominated by species occurring in euryhaline brackish waters. Moreover, physicochemical parameters showed a low influence on diatom assemblages in the protected site and a strong influence in the unprotected site. No significant correlation between diatom ecological guilds and physicochemical parameters emerged. While for all the seasons the dominant guild was the motile guild, no inter-season variations emerged: diatom communities and ecological guilds in MTPs are resilient to seasonal variations. To provide novel insights, future research should focus on additional environmental drivers such as nutrient availability, sediment composition, and hydrological variability, including long-term monitoring plans.

1. Introduction

Mediterranean Temporary Ponds (hereafter MTPs) are ephemeral freshwater habitats considered biodiversity hotspots and habitats of some biological importance because they can host a considerable number of rare and endemic species (de Necker et al., 2025). The definition of ponds is actually challenging researchers; here, we adopted the most recent definition by Richardson et al. (2022): small (<5 ha) and shallow (<5 m depth) water bodies with a maximum of 30 % emergent vegetation coverage. The appellative "Mediterranean Temporary Ponds" was firstly used in the scientific literature since the adoption of the Habitats Directive (Bagella, 2023): these ponds were categorized as priority habitats (Natura code: 3170*) included in Annex I of the Directive 92/43/EC (Dimitriou et al., 2009). MTPs are present in five biogeographical regions, mainly located in the Mediterranean biome, below 1500 m a.s.l. (Boix et al., 2016). MTPs are located in semi-arid and transitional regions (Pulido et al., 2020) with hot, dry summers and mild, wet winters. These habitats may experience seasonal fluctuations in water availability, with periods of drought and low water levels in the summer months (Koren et al., 2023). The term "temporary" refers to the hydroperiod of these ecosystems (Fernández-Zamudio et al., 2016). Hydroperiod, the length of aquatic phase, is a crucial environmental variable for these ecosystems (Taurozzi and Scalici, 2024a): MTPs show a significant variability regarding the length of their hydroperiod and the beginning of their inundation period (Zacharias and Zamparas, 2010). Furthermore, they play a significant role in the provision of ecosystem services, such as nutrient sinks, water sources for wildlife, reservoirs of biodiversity and cultural elements (Krivtsov et al., 2022).

MTPs are not isolated systems, but they actively interact with terrestrial environments (Fehlinger et al., 2023). MTPs host a large number of different aquatic and terrestrial organisms (Dimitriou et al., 2006), from viruses and bacteria to consumers and predators, showing strong and complex trophic interactions (Pille et al., 2023). However, despite an improving public sensibility to these habitats over recent years, they are still overlooked and exposed to threats or destruction

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(Boix et al., 2016). MTPs show an alarming rate of loss and degradation, and biodiversity rarefaction (Olmo et al., 2022). Vascular plants, amphibians, many microorganisms and macro invertebrates and phytoplankton are constantly threatened by numerous human activities (Zacharias et al., 2007): degradation and biodiversity loss (Beja and Alcazar, 2003), wastewater discharge and acidification (Zacharias and Zamparas, 2010), solid and liquid waste disposal (Yadav and Goyal, 2022), introduction of alien species (Van den Broeck et al., 2015) and climate change (Zacharias and Zamparas, 2010). Increased water temperature can decrease pH and dissolved oxygen (DO) concentration, as well as increasing levels of available phosphorous, of which could be stressful to pond inhabitants (Earl et al., 2023). In this sense phytoplanktic communities play an important ecological role, increasing primary production, providing oxygen and absorbing CO2, counteracting the effects of climate change (Widigdo et al., 2020; Häder and Gao, 2017).

Phytoplankton in temporary ponds are dominated by fast-growing, single-celled r-strategists and opportunists, which are adapted to variable conditions in rapidly changing environments (Celewicz and Gołdyn, 2021; Padisak and Naselli-Flores, 2021). Under climate change pressures, phytoplankton can show dramatic changes in population density (Diana et al., 2017), generating important shifts in distribution of species and compositional diversity (Domis et al., 2013; Hays et al., 2005). Among phytoplanktic communities, diatoms represent the group which contributes mostly to Earth's global primary production (20–40 %) (Rousseaux and Gregg, 2013); this characteristic make them fundamental for supporting global life forms, being primary producers and an important source of oxygen (Taurozzi and Scalici, 2024a). Diatoms are widespread in all the aquatic ecosystems and represent excellent indicators of environmental changes (Lobo et al., 2016): their response through compositional alteration was already applied in the understanding and management of lakes' surface water eutrophication and acidity, rivers' salinization studies (Stenger-Kovács et al., 2023) and water level change in wetlands (Wolin and Duthie, 1999). However, diatoms represent an important biological model largely used in aquatic permanent habitats but underused in ephemeral waters: the response of diatoms to multiple stressors (hydroperiod length, seasonal changes, physicochemical variations, droughts) in temporary ponds has received much less attention (Taurozzi et al., 2024a).

The diatom communities of MTPs are still overlooked; only a few studies considered the species temporal and spatial turnover of diatoms (Liu et al., 2025; Blanco et al., 2020; Remmer et al., 2019; Hill et al., 2017), none in MTPs consider a one-year monitoring. Different factors may affect species diversity at different spatial scales (Blanco et al., 2020). The scientific literature on this topic is highly contrasting. Some studies suggest that physicochemical water parameters do not play a fundamental role, unlike hydroperiod, which appears to be more influential (Blanco et al., 2020). Conversely, other research highlights the significant role of factors such as pH (Potapova and Charles, 2002) and electrical conductivity (Pestryakova et al., 2018). Similarly, Ferdoushi et al. (2023) demonstrated that temperature had a stronger influence on diatom diversity than pH and dissolved oxygen. Liu et al. (2025) further showed that the composition and relative abundance of dominant species varied significantly across seasons, with notable seasonal differences also observed in alpha and beta diversity, as well as in the relative influence of physicochemical parameters on both species- and trait-based communities. Examining elevational trends in diversity is crucial for identifying broad-scale distribution patterns (Soininen, 2007). Therefore, understanding the relative influence of various environmental factors on taxa diversity and their connection to different scale gradients is essential (Zaharescu et al., 2016).

Herein, we focused our attention on the responses of benthic diatoms to seasonal and hydroperiod variations in MTPs of Central Italy, one of the most threatened freshwater ecosystems of the Mediterranean region. For our purpose, we selected two MTPs located on different landscapes, altitudes and subject to different anthropogenic disturbances, focusing on the study of diatom communities along a one-year sampling. In our research, we considered anthropogenic disturbances as all directly or indirectly human-related activities that can alter ecological conditions (Battisti et al., 2016). This definition is based on the concept proposed by Pickett et al. (1985), where anthropogenic disturbances are defined as "environmental changes or disruptions directly or indirectly caused by human activities, which affect the structure, function, or composition of ecosystems". We further expanded this definition to include biological forms of disturbance. Specifically, we grouped under this term activities directly linked to human presence, such as vehicular traffic, tourism (White et al., 2000), and direct pollution, as well as biologically mediated processes (*sensu* Sousa, 1984), including agricultural practices and grazing. In this study, we acknowledge the presence of such disturbances based on direct qualitative observation, although we did not attempt to quantify them. These anthropogenic disturbances are conceptually and ecologically distinct from natural physical disturbances such as droughts, which in our case are interpreted as seasonal phenomena rather than being directly influenced by human activity.

Our aim was to explore.

i) whether benthic diatom richness responds to seasonal variations;
ii) the capacity of MTPs to host Red List species among different seasons;
iii) which physicochemical factors represent the main drivers for diatom diversity in MTPs;
iv) whether alpha and beta diversity indices and functional metrics (i.e. ecological guilds and life-forms) could be influenced by seasons and relative ecological and biological variations in MTPs, including droughts.
v) the influence of protection measures on diatom diversity.

Based on the scientific literature supporting our study, we hypothesize that both taxonomic and functional diatom communities respond significantly to seasonal variations, affecting both alpha and beta diversity, with a marked influence of the environmental variables considered. Moreover, following ISPRA (2014), we expect a higher number of species included in the Red List during the spring and summer months, which coincides with the peak of diatom floristic development. The variable related to the different management regimes of the two pond systems represents a novel aspect of this study. In the absence of directly comparable literature, we can hypothesize that protected and undisturbed sites will host a higher number of diatom species, as the absence of anthropogenic stressors (such as vehicular traffic, grazing, and tourism) allows diatom communities to structure themselves naturally, without external disturbances.

2. Materials and methods

2.1. Study area

The study focused on Central Italy, in Latium region. The ponds were identified using GEE images and field surveys, while their temporary nature was analyzed with the use of the GEE historical imagery tool, following Taurozzi and Scalici (2024b) (Fig. 1a).

- MTP(A): The first pond is included in the Castelporziano Presidential Estate (41°42′12"N, 12°23′45"E), a nature reserve along the Tyrrhenian coast near Rome and one of the residences of the Italian Republic President (Tinelli et al., 2012), covering an area of 59 km^2 (Sbarbati et al., 2022). The access is forbidden to the public and no direct anthropogenic threats are present (Taurozzi et al., 2024a). This pond will be considered the "protected site"
- MTP(B): The second pond is included in a grazing land in the Altipiani di Arcinazzo (41°50′39"N, 13°12′16"E), a typically alpine-looking plateau 60 km south-east Rome, included in an agricultural and grazing pattern (Taurozzi et al., 2024b). The pond is used

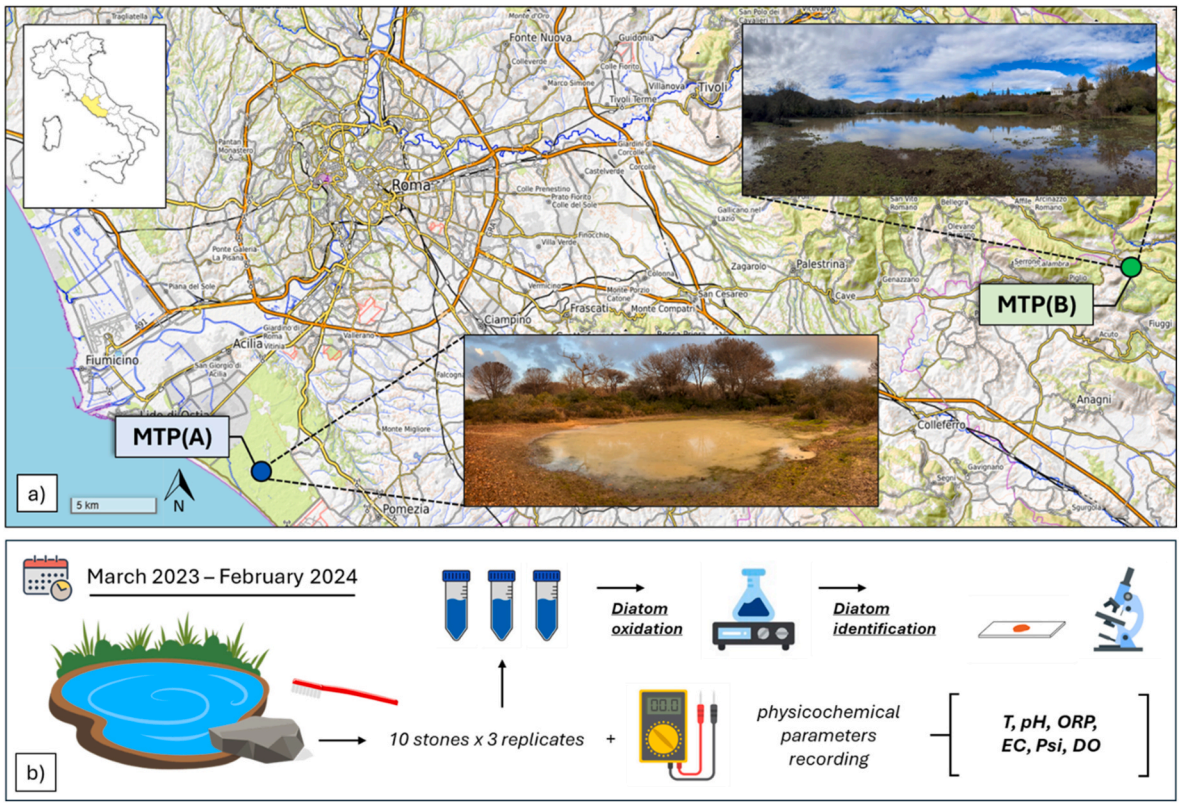

Fig. 1. a) Study area and sampling sites. MTP(A) is located inside the Castelporziano Presidential Estate; MTP(B) is located on the Altipiani di Arcinazzo plateau. b) Sampling activities and laboratory analysis.

daily for watering cattle. This pond will be considered the "unprotected site", where direct anthropogenic disturbances are present.

2.2. Sampling activities

The samplings were performed each month, one sampling per month, for one year, from March 2023 to February 2024. Samplings were performed by the same operator at the same hour of the day (±30 min) to avoid discrepancies in sampling methodologies. Diatom sampling was carried out according to ISPRA (2014) guidelines, with adaptations for pond environments as described in Taurozzi et al. (2024a). Samples were collected at multiple points and depths within each pond. 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 (Fig. 1a). Diatom oxidation operations followed in the laboratory (Fig. 1b). For each site, three different replicates were performed as suggested by Maitland et al. (2020). Before performing diatom sampling activities, 6 physio-chemical parameters of ponds were recorded using a multiparametric probe Hannah® HI98194 following Taurozzi et al. (2024a) Temperature (T °C), pH, Oxygen Reduction Potential (ORP, mV), Electrical conductivity (EC, μS/cm), Pressure (Psi), Dissolved oxygen (DO, ppm).

The oxidation method used was taken from Taurozzi et al. (2024a) and Beltrami et al. (2007) for freshwaters: through the use of hydrogen peroxide, the organic matter was digested, and diatoms were mounted on permanent slides using Naphrax® (high resin power of refraction) to fix the coverslip. The morphological identification of diatom species was conducted under a microscope at 1000 × magnification (Leica), using taxonomic guides (DREAL Languedoc Roussillon, 2021; Bahls et al., 2018; ISPRA, 2014; Ector and Hlúbiková, 2010; Taylor et al., 2007a; Krammer et al., 2002; Krammer and Lange-bertalot, 1991b; Krammer and Lange-bertalot, 1991a; Krammer and Lange-bertalot, 1988; Krammer and Lange-bertalot, 1986). For each slide, the analysis was considered completed when identification at the species level of 400 valves was achieved (ISPRA, 2014). Counting 400 valves provides a reliable estimate of relative abundances, especially for detecting rare taxa and ensuring species richness and diversity indices are stable and representative of the true community. As reported in Battarbee (1986) and Taylor et al. (2007b), counting 400 valves is generally satisfactory for the calculation of relative abundance of diatom species.

2.3. Statistical analysis

Normality, homogeneity of variance and independence were tested before performing statistical analysis. Given all the assumptions checked, we performed both parametric and no-parametric tests in our analysis. Here, we considered two factors for our analysis: "season", expressed as division of the year based on changes in weather, ecology, and the number of daylight hours in a given region (spring, summer, autumn, winter) and "protection", considering the protected Nature of MTP(A), included in the Castelporziano Estate (expressed as the absence of potential direct anthropogenic disturbances following Taurozzi and Scalici, 2024a).

The hydroperiod of each pond was determined through in situ visual assessment, based on the detection of water presence and availability.

2.3.1. Physicochemical variables

Permutational Multivariate Analysis of Variance (PERMANOVA) is a non-parametric test of significant difference between two or more groups, based on any distance measure; here, physicochemical variables differences between sites and seasons were tested using one-way PERMANOVA with stratification: this tests whether diatom community composition differs by month, while only permuting within each pond. That respects the non-independence of samples from the same pond.

Ordinary least squares regressions were performed to highlight significative correlations between physicochemical parameters among seasons.

2.3.2. Diatom overview and Red List species

Repeated-measures analysis of variance (ANOVA) test, a statistical method that simultaneously compares means across several groups to determine if observed differences are due to chance or reflect genuine distinctions, was used to analyze significant differences in the number of species across seasons.

Here, we also considered Diatom Red Lists which provide an account of the diatom flora of a geographic area pointing out the distribution and threat status of individual species as determined by environmental factors and impacts (Cantonati et al., 2022). The presence of endangered diatom species was evaluated following diatom's Red List from Hofmann et al. (2018); for this purpose, 7 classes of risk were considered.

a) Endangered (EN)
b) Vulnerable (VU)
c) Near Threatened (NT)
d) Rare (RA)
e) Least Concern (LC)
f) Data Deficient (DD)
g) Not Evaluated (NE)

The repeated-measures ANOVA test was used to analyze differences between the distribution (number of species and relative code) of Red List species between sites. PERMANOVA tests with stratification were performed to highlight significant differences in Red List species abundances between seasons.

2.3.3. Environmental factors associated with diatom community variability

Redundancy Analysis (RDA) is a method to extract and summarize the variation in a set of response variables that can be explained by a set of explanatory variables, summarizing linear relationships between components of response variables that are "redundant" with a set of explanatory variables. Here, RDA was run to test statistical significance of each environmental variable in explaining species data structure and seasonal distribution of sampling sites.

2.3.4. Diatom alpha and beta diversity

Alpha diversity was calculated through the a) number of species and b) the Shannon diversity Index (H).

Linear multivariate multiple regressions were applied to test significant correlation between alpha diversity indices and physicochemical values. Multivariate linear regressions were applied to test for significant correlation between alpha diversity indices and the number of days after droughts. Beta diversity analysis, i.e. the comparison of dissimilarity in community composition between each pair of sites, was calculated using Bray-Curtis and Jaccard dissimilarity, based on occurrence data (abundance). One-way PERMANOVA with stratification based on Bray-Curtis and Jaccard dissimilarity matrices were set to investigate community differences among sampling sites. Two-way PERMANOVA with stratification was run to test variations in community composition among sampling sites and seasons and also for testing the influence of season and protection factors on diatom alpha diversity.

2.3.5. Diatom functional ecology

Then, three diatom ecological guilds were considered following Passy (2007).

- Low profile: species associated with environments characterized by low nutrient levels and high flow rates (Liu et al., 2025);
- High profile: larger species that tend to form colonies, and do not resist turbulence well but are enhanced by nutrient enrichment (Gelis et al., 2020);
- Motile: fast moving species not sensitive either to resource limitation or to disturbance (Stenger-Kovács et al., 2013).

The repeated measures ANOVA tests were performed to determine differences in species distribution among guilds and seasonal fluctuations. Polynomial regressions were performed to evaluate significant correlations between diatomic guilds abundances and physicochemical parameters.

Statistical tests were considered significant when p-value was <0.05. Statistical analysis were computed in PAST 4.02 software (Hammer et al., 2001), GraphPad Prism version 8.0.0 and R software (version 4.4.2) (R Core Team, 2021).

3. Results

3.1. Physio-chemical parameters

Ponds' hydroperiod was monitored throughout the year with 1 sampling per month. MTP(A) was facing 5 months of complete dryness (August, September, October, November, December), while MTP(B) was facing 1 month of dryness (July). Physicochemical parameters recorded were reported in Table S1 and Fig. S1.

PERMANOVA based on physicochemical values showed that abiotic characteristics statistically differed among the two sampling sites considering the protection variable ($F = 5.9$, $p = 0.02$), while no differences emerged between seasons ($F = 0.6$, $p = 0.7$).

For MPT(A) and MTP(B), PERMANOVA showed no significant differences between physicochemical parameters among seasons emerged ($F = 1.3$, $p = 0.3$ and $F = 1.4$, $p = 0.2$, repsectively). For MTP(A), significant correlations emerged between T and DO ($R^2 = 0.9$, $p = 0.008$) during spring, pH and EC ($R^2 = 0.9$, $p = 0.04$), pH and DO ($R^2 = 0.9$, $p = 0.006$), EC and DO ($R^2 = 0.9$, $p = 0.04$) during summer and between pH and DO ($R^2 = 0.9$, $p = 0.01$) during winter. For MTP(B), significant correlations emerged between pH and EC ($R^2 = 0.9$, $p = 0.006$) during summer, pH and ORP ($R^2 = 0.9$, $p = 0.03$) during winter and EC and DO ($R^2 = 0.9$, $p = 0.02$) during autumn.

3.2. Diatom overview and Red List species

The benthic diatom diversity in the two MTPs was represented by 146 species belonging to 44 genera, of which 24 genera were shared both from MTP(A) and MTP(B): in MTP(A) 93 species were found, in MTP(B) 115 species. The highest number of species was found in March ($n = 36$) and May ($n = 34$) for MTP (A) and in April ($n = 38$) and November ($n = 34$). Among 146 species, a total of 5 diatom taxa were classified as dominant (>5 %): *Achnathidium eutrophilum*, (number of valves counted, $n = 4045$), *Nitzschia palea* ($n = 2075$), *N. frustulum* ($n = 1406$), *Eunotia minor* ($n = 1139$) and *Navicula trivalis* ($n = 1113$) represent 45 % of the total number ($n = 21600$) of individuals counted ($n = 9778$).

Overall, 66, 52, and 38 species were identified in spring, summer and winter, respectively, in MTP(A); ANOVA test showed no significant differences in the number of species across seasons ($F = 1.4$, $p = 0.24$). 62, 42, 57, 43 diatom species were identified in spring, summer, autumn, winter, respectively, in MTP(B); ANOVA test showed no significant differences in the number of species across seasons ($F = 0.6$, $p = 0.63$).

Considering the Red Lists codes, MTP (A) hosted 70 % of LC species, 13 % NE, 9 % NT, 3 % VU, 3 % RA, 2 % DD and 1 % EN; MTP(B) hosted 70 % of LC species, 19 % NE, 4 % RA, 3 % NT, 2 % DD, 1 % VU, 1 % EN. Furthermore, no differences emerged between the distribution (number of species and relative code) between sites from ANOVA test ($F = 1.3$, $p = 0.29$). PERMANOVA showed that abundances for red list codes did not differ among seasons for MTP(A) ($F = 1.9$; $p = 0.13$) and for MTP(B) ($F = 0.2$, $p = 0.82$).

3.3. Environmental factors associated with diatom community variability

Statistical analyses conducted using the RDA method revealed statistically significant correlations between environmental factors and the structure of diatom assemblages (F = 1.7, p = 0.004) (Fig. 2). The RDA analysis showed that the first axis explained 23.7 % of the fitted variation, two axes explained 32.5 %, three axes accounted for 38.8 %, and four axes explained 44.1 %. The most statistically significant influence on the differentiation of diatom assemblages was attributed to Pressure (p = 0.001), explaining 9.1 % of the variability, followed by temperature (p = 0.011; 8.4 %). The influence of pH, ORP, EC and DO ions on the differentiation of diatom assemblages was smaller (p = 0.2–0.6; 5–3 %), and these factors were not also statistically significant. Temperature showed a very strong positive correlation with DO (r = 0.6, p = 0.003); this parameter correlated with the occurrence of, among others: *Gomphonema exillissimum*, *Gomphonema gracile*, *Nitzschia frustulum*, *Stauroneis phoenicenteron*. Electrical conductivity also demonstrated a weak negative correlation with Temperature (r = −0.4, p = 0.06), which in turn correlated with the occurrence of *Cocconeis placentula* and, to a lesser extent, *Anomoeoneis sphaerophora*. ORP demonstrated a strong negative correlation with pH (r = −0.5, p = 0.02), which in turn correlated with the occurrence of *Gomphonema pumilum*, *Fragilaria biceps* and, to a lesser extent *Denticula tenuis*, *Fragilaria capucina*, *Geissleria decussis*.

Based on the RDA findings, two distinct groups of sites were observed because of differences in species composition. The first group consisted of the protected site - MTP(A) - which is characterized by high dissolved oxygen, high temperature, high pressure and low pH. This site was dominated by diatom cosmopolitan species such as *Frustulia vulgaris, Gomphonema gracile, G. parvulum, G. zellense, Nitzschia frustulum, Pinnularia gibba*. The second group - MTP(B) - was composed of an agricultural and grazing site that was characterized by very low electrical conductivity and low Oxidation-Reduction Potential amounts. *Anomoeoneis sphaerophora, Cocconeis placentula, Denticula tenuis, Fragilaria biceps, F. capucina, Geissleria decussis, Gomphonema pumilum, Nitzschia filiformis, N. linearis* diatom species were abundant in this site.

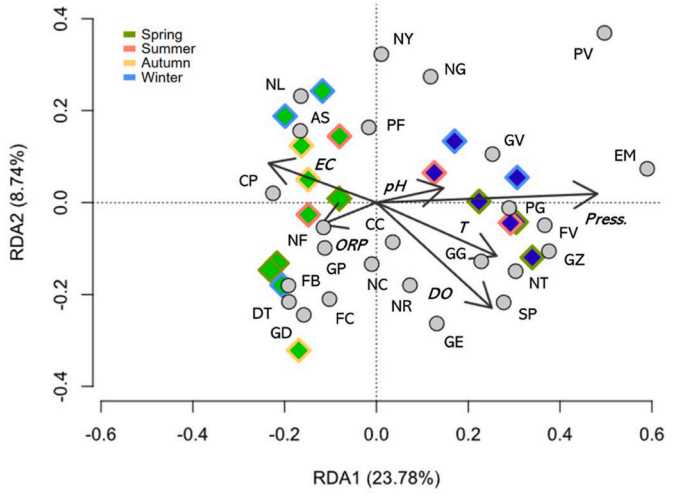

Fig. 2. Redundancy analysis (RDA) ordination showing environmental variables correlated with RDA1 and RDA2. Physicochemical parameters are shown in italic. Diatom species are shown with abbreviations and grey dots. (AS = *Anomoeoneis sphaerophora*; CC = *Craticula cuspidate*; CP = *Cocconeis placentula*; DT = *Denticula tenuis*; EM = *Eunotia minor*; FB = *Fragilaria biceps*; FC = *Fragilaria capucina*; FV = *Frustulia vulgaris*; GD = *Geissleria decussis*; GE = *Gomphonema exillissimum*; GG = *Gomphonema gracile*; GP = *Gomphonema pumilum*; GV = *Gomphonema parvulum*; GZ = *Gomphonema zellense*; NC = *Navicula capitatoradiata;* NF = *Nitzschia filiformis*; NG = *Navicula gregaria*; NL = *Nitzschia linearis*; NR = *Navicula reichardtiana*; NT = *Nitzschia frustulum*; NY = *Navicula cryptocephala*; PF = *Planothidium frequentissimum*; PG = *Pinnularia gibba*; PV = *Pinnularia viridiformis*; SP = *Stauroneis phoenicenteron.* Blue rhombuses represent MTP(A); Green rhombuses represent MTP(B). T = Temperature (°C), ORP = Oxygen Reduction Potential (mV), EC = Electrical conductivity (μS/cm), Press. = Pressure (Psi), DO = Dissolved oxygen (ppm).

3.4. Diatom alpha and beta diversity

Overall linear multivariate multiple models applied on alpha diversity indices and physicochemical values did not show significant correlations for MTP(A) (F = 1.6, p = 0.5), while showed significant correlations for MTP(B) (F = 3.7, p = 0.04). Moreover, the overall effect of each independent physicochemical parameter was not significant (ANOVA, p > 0.05). The number of species in MTP(B) was mainly influenced by T (R^2 = 0.0004, p = 0.04), pH (R^2 = 0.08, p = 0.01), ORP (R^2 = 0.02, p = 0.01), Press (R^2 = 0.06, p = 0.02) and DO (R^2 = 0.06, p = 0.01); Shannon index was influenced by DO (R^2 = 0.5, p = 0.01). Multivariate linear regressions applied on alpha diversity indices and the number of days after droughts did not show significant correlations both for MTP(A) and MTP(B) (p > 0.05).

One-way PERMANOVA based on Bray-Curtis dissimilarity showed that diatom structure differed among the sampling sites (F = 4.7, p = 0.001). Similar results emerged from one-way PERMANOVA based on Jaccard dissimilarity (F = 2.4, p = 0.0003). Two-way PERMANOVA highlighted that there was also seasonal variation in diatom community composition between the studied sites for Bray-Curtis dissimilarity (F = 1.9; p = 0.0032) but not for Jaccard dissimilarity (F = 1.1, p = 0.06). However, the interaction between seasonal and protection variables in defining diatom variation was not statistically significant (PERMANOVA, F = 0.5; p = 0.72). Furthermore, the influence of seasonal and protection variables on diatoms was reflected on species richness: two-way PERMANOVA highlighted the influence of protection factors on diatom alpha diversity (F = 9.8, p = 0.008) but not for season (F = 0.8, p = 0.6). Moreover, the interaction between seasonal and protection variables in defining diatom diversity was statistically significant (PERMANOVA F = 2.6; p = 0.04).

3.5. Diatom functional ecology

In this study, all categories of the diatom ecological guilds, including motile, low-profile, and high-profile were observed (Table 1). 25 among the 146 different species identified belong to the low-profile guild, 37 to the high-profile guild and 84 to the motile guild (Fig. 3a and b).

The results highlighted that the motile guild predominated among the diatom guilds (MTP (A), H = 7.2, p = 0.02; MTP(B), H = 8.1, p = 0.01) and displayed insignificant seasonal fluctuations (MTP(A), H = 0.5, p = 0.8; MTP(B), H = 1.2, p = 0.7). Polynomial regressions showed significant correlations between the number of species of high-profile guild and T (F = 6.4, p = 0.009), EC (F = 7.5, p = 0.005) and DO (F = 5.2, p = 0.01) (Fig. 3c, d and 3e).

Table 1
Frequencies (expressed in percentage %) of diatom guild for each season in the two ponds analyzed.

		Winter	Spring	Summer	Autumn
MTP (A)	Motile	63.1	69.7	53.9	–
	Low profile	13.1	7.5	15.4	–
	High profile	23.8	22.8	30.7	–
MTP (B)	Motile	74.4	50.0	69.0	59.7
	Low profile	13.9	17.7	9.6	8.7
	High profile	11.7	32.3	21.4	31.6

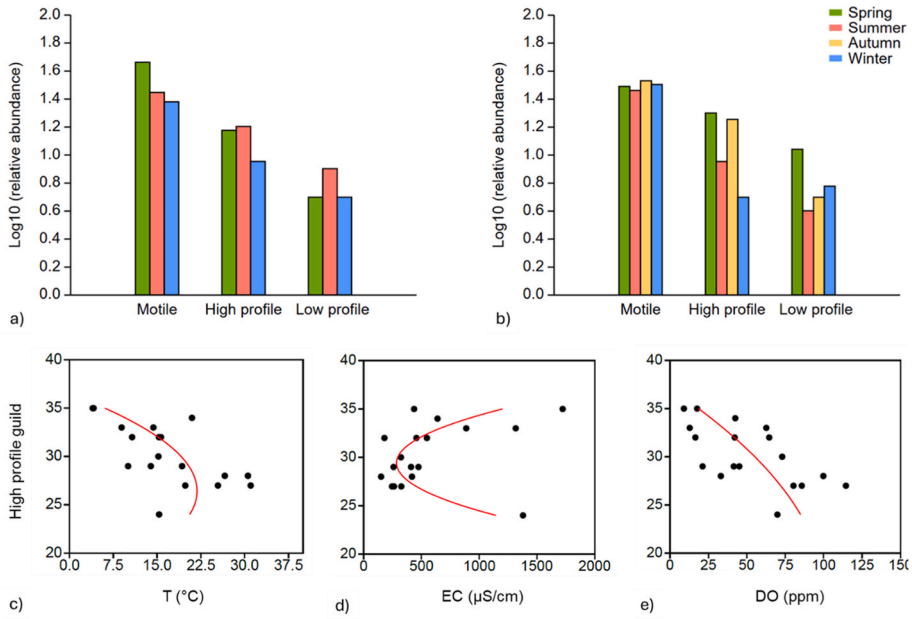

Fig. 3. Seasonal changes in the composition and relative abundance of the benthic diatom ecological guilds for a) MTP(A) and b) MTP(B); Polynomial regression for number of species of high-profile guild and c) Temperature (T), d) Electrical Conductivity (EC) and e) Dissolved oxygen (DO).

4. Discussions

This research represents, to the best of our knowledge, the first investigation of diatom community turnover and ecological responses to seasonality and hydroperiod fluctuations in MTPs. Our study demonstrated that hydroperiod length and seasonality showed, respectively, a low and moderate influence on diatom richness of MTPs.

4.1. Diatom overview and red list species

The highest number of species was found during spring months, March, April and May (for the northern hemisphere), confirming previous studies (Villa-Alfageme et al., 2024; Sassenhagen et al., 2023), while no significant differences emerged in the number of species across seasons, unlike our initial hypothesis. This is in accordance with Taurozzi and Scalici (2024a) and Virta et al. (2020), where taxonomic diversity remained stable at seasonal and inter-annual level despite strong changes in community composition and hydroperiod length. Hydroperiod can represent an important limit factor for many aquatic lifeforms (Pires et al., 2021; Schriever et al., 2014). Water availability can directly and indirectly affect seasonal assemblages of aquatic communities (Mdidimba et al., 2021): amphibians can show complex responses for survival and growth to variations in hydroperiod length (Thompson and Popescu, 2021), while macroinvertebrates communities can show important changes mainly due to physicochemical variations (Coccia et al., 2021). Here, we demonstrated that diatom richness was not affected by hydroperiod length and seasonal variations. This observation is consistent with the study by Ma et al. (2023) who showed that seasonal differences in planktic diatoms were more pronounced than those in benthic diatoms. Adhesion to the substrate leads to benthic diatoms to be more prone to resist dryness than planktic ones (Falasco et al., 2018). Then, we hypothesize that benthic diatoms respond to droughts with resistance cists (Kooistra et al., 2007), regenerating the same community during wet periods. Very few studies have investigated the relationship between ponds' hydroperiod and diatom communities. Cantonati et al. (2023) highlighted that the composition of diatom assemblages can provide information on the hydroperiod and waterflow variability; Blanco et al. (2020) demonstrated that diatomic diversity changes with hydroperiod. In our study we revealed that variations in diatom communities of MTPs are independent from hydroperiod length. Moreover, our findings suggest that MTPs are particularly suitable for hosting threatened species included in the Red List of diatom species (Padula et al., 2021). At the same time, no differences emerged between sites regarding the number of endangered species, raising awareness about the relative effect of protection measures on diatom communities and the consequent benefits for endangered species. In this sense, our study revealed that MTPs host similar Red List species regardless of the level of protection differently from our hypothesis. However, many variables could contribute to this homogeneity: aerial dispersion of diatoms (Stenger-Kovács and B-Béres, 2024), similarity in physicochemical conditions between sampling sites, dispersion through ectozoochory of freshwater diatoms via adherence to waterfowl feathers (Manning et al., 2021). For these reasons, considering the novelty of the research topic, further studies are necessary to deeply understand the potential biotic and abiotic influences on diatom communities and distribution of threatened species.

4.2. Environmental factors associated with diatom community variability

Based on RDA grouping of sites, physicochemical variables were determinant factors for diatom community structure for both MTP(A) and MTP(B). Sampling sites showed different ecological conditions and chemical drivers: our study revealed that substantial variation in physicochemical parameters emerged between samplings sites. Protected site with high Press., T and DO was dominated by *Frustulia vulgaris, Gomphonema gracile, G. parvulum, G. zellense, Nitzschia frustulum, Pinnularia gibba* species during the wet season. These species also occurred in minimally impacted oligo-to mesotrophic waters enriched with oxygen concentrations, with medium-low conductivity. Unprotected site with high EC and ORP was dominated by *Anomoeoneis sphaerophora, Cocconeis placentula, Denticula tenuis, Fragilaria biceps, Fragilaria capucina, Geissleria decussis, Gomphonema pumilum, Nitzschia filiformis, N. linearis* species during the wet season. These species also occurred in euryhaline, fresh and brackish waters with low to moderate concentrations of organic pollution. Our results align with previous studies that documented similar species assemblages in relation to physicochemical gradients. For instance, *Frustulia vulgaris*, *Gomphonema gracile*, and *Pinnularia gibba* have been commonly associated with well-oxygenated, oligotrophic to mesotrophic conditions in protected or minimally impacted aquatic environments (Cantonati et al., 2012; Rimet and

Bouchez, 2012). Similarly, *Anomoeoneis sphaerophora*, *Cocconeis placentula*, and *Fragilaria capucina* are known to occur in more disturbed habitats characterized by elevated conductivity and organic enrichment, including euryhaline and brackish systems (Lavoie et al., 2009).

Physicochemical variables are generally the main drivers of diatom species richness in lotic systems (Shibabaw et al., 2021). This study confirmed electrical conductivity, dissolved oxygen, T, and pressure were important in determining diatom community structure also in MTPs (Dalu et al., 2022). DO direct relationship with diatom richness is consistent with other studies (Shibabaw et al., 2021). However, our findings contrast with those of Guo et al. (2020) and Al-Imari et al. (2023) who reported that hydrology plays a primary role over physicochemical parameters in shaping diatom communities; on the contrary, our findings agree with Novak and Zelnik (2021) and Shetty and Gulimane (2023), where ecological conditions like temperature, pH, dissolved gases, were found to impact the growth and abundance of diatoms. pH was identified as a key factor for diatoms, significantly affecting their growth through direct physiological stress (Shibabaw et al., 2021). In the same study, electrical conductivity (EC) and dissolved oxygen (DO) also emerged as important predictors for diatom community composition. Similarly to Shibabaw et al. (2021), who compared urban and agricultural sites, we can draw parallels between our two study sites: a protected (=urban-equivalent) site and an unprotected (=agricultural-equivalent) site. Our protected site exhibited mean values of 8.52 for pH, 294 μS/cm for EC, and 69.3 mg/L for DO. In contrast, the unprotected site averaged 7.82 for pH, 760.7 μS/cm for EC, and 40.6 mg/L for DO. Notably, Shibabaw et al. (2021) reported quite different values: their urban site had a mean pH of 6.6, EC of 132.7 μS/cm, and DO of 3.29 mg/L, while their agricultural site had a mean pH of 7.5, EC of 99.2 μS/cm, and DO of 6.72 mg/L. These discrepancies may be attributed to the different aquatic environments studied (streams/rivers vs. our pond sites).

The importance of pH and conductivity for diatom communities was likewise highlighted by Della Bella et al. (2007) in their analysis of wetlands. However, even in their study, the mean values for pH (8.02) and conductivity (3.56 μS/cm) were lower than those observed in our sites. This suggests that diatom assemblages in freshwater ecosystems can respond differently to a broad spectrum of environmental conditions, which may influence community structure across diverse habitats (Soininen, 2007).

Given the absence in literature of studies involving MTPs as sampling sites, we could hypothesize that diatom communities are both influenced by hydrology, water availability and physicochemical parameters in many ways: the exact magnitude of these influences could be different for each pond considered, given the contemporary influence of different biotic and abiotic factors (Kumar et al., 2020).

4.3. Diatom alpha and beta diversity

Diatom alpha diversity indices did not show significative correlations with physicochemical parameters for MTP(A), while significative correlations emerged for MTP(B). Physicochemical parameters showed a low influence on diatom assemblages in the protected site: we could hypothesize that biotic and spatial factors may override the effect of physicochemical factors (Blanco et al., 2020), especially in anthropogenic disturbances-free environments. Differently, MTP(B) diatom communities resulted highly influenced by physicochemical parameters: all the parameters significantly influenced the number of species, with DO which was confirmed as one of the most important abiotic factors for shaping diatom communities (Schuch et al., 2015). Shannon diversity index was also driven by DO. These results are in line with previous studies reporting DO as a major environmental driver of taxonomic diversity in freshwater diatoms (Cui et al., 2023; B-;Béres et al., 2016). In addition, conductivity and redox potential (ORP), which significantly influenced assemblage structure in MTP(B), have also been identified as relevant predictors of diversity in various aquatic systems (Stenger-Kovács et al., 2023; Soininen, 2007), particularly in temporary or fluctuating habitats. Our study confirms the importance of seasonality as an important structuring force for diatom communities (Sun et al., 2022): however, protection factor showed a greater influence on diatom diversity. In fact, significative results for seasonality emerged only including the protection factor. The absence of anthropogenic stressors (vehicular traffic, grazing, tourism disturbances) lead to diatom communities to be structured without external disturbances. Comparable studies also showed similar patterns in diversity indices. For example, B-Béres et al. (2016) reported that in temporary or semi-permanent ponds, alpha diversity tends to be more variable in unprotected or human-influenced sites, where environmental filtering is stronger. This supports our findings, where MTP(B) sites, exposed to more variable and stressful conditions, showed significant correlations between alpha diversity indices and abiotic parameters such as dissolved oxygen.

Disturbed freshwater ecosystems generally show different diatom communities (Falasco et al., 2012), which differ from isolated ones (Blanco et al., 2020). Our results show this tendency: by comparing beta diversity, the two sampling sites showed different community assemblages, with MTP(A) showing general better water quality with high oxygen concentrations. The patterns of alpha and beta diversity observed in our study are consistent with those described by B-Béres et al. (2017) and Soininen (2007), who found that environmental heterogeneity and disturbance intensity can lead to high beta diversity via species turnover, especially in fragmented or temporary aquatic systems. This suggests that while local environmental filtering may be weak in protected areas, it becomes a stronger driver of diatom assemblage structure in more impacted or variable habitats. Moreover, the seasonal effect is amplified while comparing diatom communities, underlying the fundamental role of climatic and seasonal biotic and abiotic variations on diatom assemblages.

4.4. Diatom functional ecology

Our results were not consistent with Liu et al. (2025): although the ecological guild allows us to determine the magnitude of the change in the values of the investigated parameters, our research did not highlight the correspondence between diatom ecological guild and physicochemical parameters. While for all the seasons the dominant guild was, as expected, the motile guild, no inter-season variation emerged. Motile guild species are very sensitive to unfavorable ecological conditions, and very common in temporary ponds, given their ability to move to most suitable habitats (Passy, 2007). Their broad presence among all seasons reflects the unstable conditions of MTPs, often subject to alternating phases of floods and droughts (Van den Broeck et al., 2015). However, these results did not reveal a higher sensitivity of diatom ecological guilds to variations in physicochemical variations; high profile guild, sensitive to physical disturbance, including hydrological stress, showed, as hypothesized, a negative correlation with temperature and the relative DO, which represent the two main factors involved during MTPs droughts. These results imply that the diatom communities and the ecological guilds in the MTPs are resilient to seasonal variations. Furthermore, given the novelty proposed by our research, future investigations are therefore necessary to analyze the seasonal dynamics of diatom communities in MTPs.

4.5. Limitations of the study

Despite the robust design and analytical approach, our study has some limitations. First, the relatively small sample size and spatial extent may limit the generalizability of our findings to broader MTPs. Unfortunately, the restricted hydroperiod allowed us to sample the protected pond only for 7 months. It was not possible to collect data outside this period due to drought conditions. Second, although we included a range of physicochemical variables, other important ecological drivers such as nutrient concentrations (e.g., P, N), or biotic

interactions were not explicitly considered due to lack in instrument and fundings to perform such analysis. No water samples have been collected for nutrient and cation analysis, which are very important parameters for diatoms and for the spatio-temporal dynamics of the benthic diatom communities. The collection of these water samples would also indicate the effect of agriculture and urban wastewater treatment plants, with higher values of sulphates, nitrates and phosphates in the water. However, we used the same protocols adopted in other studies, analyzing an even higher number of valves (400), compared to many other studies that limit the count to 200 or 300 valves. Therefore, our study is fully replicable and follows established methodological standards. Finally, our analysis focused on a single year; temporal variability could influence community dynamics and diversity patterns, particularly in highly seasonal temporary ponds.

5. Conclusions

Our study confirms that benthic diatoms are reliable bioindicators for detecting both natural and anthropogenic disturbances in MTPs. The proposed research represents the first investigation of diatom community turnover among seasons, comparing protected and disturbed Priority Habitats. In particular, the results showed that diatoms were not affected by hydroperiod length and seasonal variations in benthic diatom richness were not statistically significant. However, MTPs have proven to be particularly suitable for hosting Red List species, although their unstable ecological and hydrological conditions. Temperature, dissolved oxygen and electrical conductivity emerged as the primary environmental drivers influencing the seasonal dynamics of the species-based diatom community. Furthermore, physicochemical parameters emerged as not significant for structuring benthic diatom communities in protected MTPs, while they represent the main driver for disturbed MTPs. Alpha and beta diversity described a complex community, with significant differences between sampling sites in function of anthropogenic and grazing disturbances. Moreover, protection and undisturbed MTPs showed different diatom assemblages, with alpha, beta and ecological diversity deeply influenced by the protection and, to a lesser extent, by seasonality. These findings are consistent with previous research highlighting the sensitivity of diatom communities to environmental gradients such as oxygen availability and conductivity. Importantly, we provide new insights into seasonal turnover and the differential impact of protection status on diatom diversity, expanding knowledge on ephemeral habitats in the Mediterranean region. Quantification of the disturbance levels and their effects on diatom communities is the main limit of our research: we therefore suggest, for future research, to integrate further studies taking into consideration precise data regarding the anthropogenic impacts in MTPs. As proposed by Salafsky et al. (2008), a structured system for classifying and evaluating threats to conservation should be based on two pillars: a standardized taxonomy of threats (e.g., pollution, land use, biological harvesting, etc.) and a systematic approach to quantifying each threat, considering three parameters: scope, severity, and timing. For quantifying and ranking anthropogenic disturbances on MTPs, with diatom as main target, for future research we suggest adopting a combination of field-based observations (e.g., signs of trampling, pollution sources, debris, or excessive algal growth), expert-based scoring systems (ranking the scope, severity, and timing of each threat or the creation of a composite disturbance index combining multiple stressor scores), GIS-based land use analysis and detection of nearby infrastructures or changes in pond hydrology. For this reason, this research should be understood as a starting point for analyzing diatom temporal turnover and ecological response in peculiar ephemeral ecosystems such as MTPs.

CRediT authorship contribution statement

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

Consent to participate

Not applicable.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.csr.2025.105547.

Data availability

Data will be made available on request.

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CHAPTER IV

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Environmental and elevational drivers of diatom diversity in alpine temporary ponds

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ABSTRACT

High-elevation temporary ponds (TPs) represent habitats of high ecological value, yet they are particularly vulnerable to ongoing climate change. Diatoms are excellent bioindicators, useful for detecting ecological shifts driven by natural fluctuations or anthropogenic impacts. This study investigates whether differences in the floristic composition, measured as diversity and uniqueness (i.e., the proportion of taxa occurring exclusively in one pond), of diatom communities at small spatial scales among alpine TPs (Central Apennines, Italy) are mainly driven by isolation, elevation, or environmental factors. We found that diatom diversity in alpine biomes primarily responds to local geographical gradients, which tend to obscure any detectable effects of environmental variables. Elevation plays a key role in shaping the uniqueness of diatom communities in TPs, as higher ponds host a greater proportion of exclusive taxa. Our results indicate that local environmental conditions associated with elevation override the effects of isolation (distance between ponds), leading to the development of functionally distinct diatom communities at higher altitudes. The diversity and uniqueness of diatom assemblages are influenced by a combination of geographic and, to a lesser extent, environmental factors, with elevation acting as a central driver, both enriching diversity and promoting floristically unique communities, especially in interaction with other variables. Furthermore, electrical conductivity appears to favour communities composed of similarly adapted species, likely reflecting specific ecological conditions or adaptive responses to environmental gradients. These findings highlight the importance of elevation and spatial gradients in shaping diatom community patterns in high-altitude ephemeral freshwater habitats.

1. Introduction

High above the world's forests and grasslands, where the air thins and temperatures plummet, lies a realm of extremes: the alpine biome (Myster, 2012; Woodward, 2008). Alpine ecosystems, i.e. high-elevation habitats above the climatic treeline, defined by the lack of trees due to cold temperatures, consist of a continuous range of shrubland and grassland habitats found above the climatic treeline across various latitudes (Pauli and Halloy, 2019; Nagy and Grabherr, 2009). The alpine biome, although its harsh climate and their complex history of climatic fluctuations (Körner and Hiltbrunner, 2021), is considered a biodiversity hotspot, including thousands of specialized plant and animal species (Vitasse et al., 2021). However, these ecosystems are particularly sensitive to climate change due to their specialized species and their restricted environmental tolerances. Indeed, with global temperatures rising, alpine habitats could face unprecedented modifications in temperature regimes, snow cover, and hydrological cycles, threatening their unique flora and fauna (Di Nuzzo et al., 2021; Haynes et al., 2021).

Alpine ecosystems supply freshwater to more than half of the human population and may stock up to 1 % of the global terrestrial carbon pool (Sharma et al., 2023; Schmeller et al., 2018). In this sense, the importance of temporary freshwater habitats, typical of this biome, is well documented. Temporary ponds (hereafter TPs, Richardson et al., 2022) represent one of the most particular environments that the Alpine biogeographical region host: they are critical freshwater habitats, supporting unique and specialized species adapted to their ephemeral nature (Fehlinger et al., 2025; Lamouille-Hébert et al., 2024). Despite their small size and often overlooked status, alpine TPs play a crucial role in maintaining regional biodiversity and supporting hydrological processes (Fehlinger et al., 2023). TPs networks can also enhance landscape connectivity, allowing populations to maintain genetic exchange and resilience by forming metapopulations, which is especially important in

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the face of climate change (Hyseni et al., 2021; Howell et al., 2018).

Among the aquatic communities inhabiting TPs, diatoms represent a widely used biological indicator to detect such alterations (Van den Broeck et al., 2015). Diatoms are unicellular, photosynthetic algae widespread in all the water habitats. They show rapid response to changes in water quality, nutrient availability, and hydrological regimes allowing for sensitive detection of ecological shifts caused by natural fluctuations or anthropogenic impacts (Hamed, 2023; Kumar et al., 2015). Moreover, the species composition and diversity of diatom assemblages reflect specific habitat characteristics, enabling detailed insights into the ecological status and successional dynamics of TP ecosystems (Gottschalk and Kahlert, 2012). However, as reported by Blanco et al. (2020), there are two main scientific perspectives regarding factors shaping diatom communities: the first suggests that diatom community composition is primarily determined by local environmental conditions such as nutrients, pH, and conductivity (Shibabaw et al., 2021). Supporting this, studies indicate that at narrow spatial scales, dispersal limitations have little effect, as diatom species occur wherever suitable habitats exist (Soininen and Teittinen, 2019). The second perspective emphasizes broader-scale influences, noting that freshwater diatom distributions exhibit biogeographic patterns shaped by dispersal processes, climate, and evolutionary history. In other words, climate and historical processes dominate at regional scales, while local environmental factors govern community composition at smaller scales (Benito and Fritz, 2020; Vyverman et al., 2007). Many studies alternately support one theory or the other, highlighting that factors like altitude, seasonality, isolation, and physicochemical parameters vary in importance when shaping diatom communities depending on spatial scale and context (Bhatt et al., 2025; Gonzalez-Saldias et al., 2025; Liu et al., 2025).

Investigating elevational patterns in diatom diversity helps identify large-scale distribution trends and clarify how environmental factors shape these patterns, providing key insights for future conservation of high-mountain temporary ponds. Furthermore, in an era of rapid and unpredictable climate change, understanding how these vulnerable ephemeral ecosystems respond to shifts in temperature, water availability, and seasonal cycles can be crucial for anticipating global impacts on freshwater biodiversity and for developing effective conservation strategies.

In their research, Blanco et al. (2020) investigated the role of geographical and environmental factors on diatom diversity inhabiting TPs in a mediterranean biome. Here, we applied a comparable research on diatom communities of TPs included into an alpine biome, aiming to investigate (i) whether the differences in the floristic composition of diatom communities between different high-elevation TPs are explained by geographical distance, altitude or environmental differences; (ii) if the spatial distribution of sites and their ecological characteristics (diversity and uniqueness) follows a specific pattern; (iii) which local environmental predictors and broader-scales patterns (like elevation and isolation) drive diatom community composition at smaller scales.

2. Materials and methods

2.1. Study area and sampling activities

The study was conducted in central Italy, on the Campo Imperatore plateau, which is part of the Gran Sasso and Monti della Laga National Park (Central Apennines, Italy), in the Alpine biogeographical region (European Environment Agency, 2020). The plateau stretches 18–27 km in length and 8 km in width, covering an area of about 75–80 km^2 and elevation range between 1300 and 2100 m above sea level (Cristofanelli et al., 2013). The landscape is shaped by glacial and alluvial processes, resulting in broad grassy meadows, small ponds, and scattered rocky outcrops. It is characterized by traditional summer grazing ground for sheep, cattle, and semi-wild horses.

Initially, TPs identification was carried out following the methodology from Taurozzi and Scalici (2024): we considered TPs as those showing a dry season during summer or those completely frozen and covered by snow during winter months. Out of the 22 ponds identified, only 12 contained water during the sampling campaign conducted during the wet season (July 2024). Consequently, all 12 available ponds were sampled (median elevation: 1690 m a.s.l., range: 1374–2007 m a.s.l.) (Fig. 1). All ponds were sampled once during the summer season (July 2024), without temporal replication. Therefore, temporal variation was not modelled, and the analyses focused exclusively on spatial differences in diatom community structure and environmental parameters among ponds. The sampling focused on epilithic diatoms collected from horizontal rock surfaces. Following the field protocol described by Balzano and Martone (2014), as referenced in Taurozzi et al. (2024), each rock was manually cleaned and thoroughly rinsed immediately before sampling to remove any deposited material or dead frustules. This procedure ensured that the biofilm collected represented only the living epilithic community present at the time of sampling. For each pond, three subsamples pooled into a single composite sample were collected, avoiding any implication of statistical replication at the pond level. Samples were oxidized in the laboratory following Balzano and Martone (2014), and 400 valves per slide (1200 per pond) were counted for community analyses. The morphological identification of diatom species was conducted under a microscope at 100 × magnification (Leica), using taxonomic guides (DREAL Languedoc Roussillon, 2021; Bahls et al., 2018; Taylor et al., 2007; Krammer et al., 2002; Krammer and Lange-bertalot, 1991b, 1991a, 1988; Krammer and Lange-bertalot, 1986).

To assess water physicochemical variables through the months, Temperature (T °C), pH, Oxygen Reduction Potential (ORP, mV), Electrical conductivity (EC, µS/cm), Dissolved oxygen (DO, ppm) were measured in each site during the two samplings. These parameters were recorded in the field using a multiparametric probe Hannah® HI98194 (Tab. S1).

2.2. Data analysis

All the statistical analyses were computed in R software (R Core Team, 2024). Alpha diversity was quantified for each site using the Shannon index (*diversity* function), which accounts for both species' richness and evenness. Site-level uniqueness (U) was calculated by weighting species' relative abundances by their inverse occurrence frequencies, providing a measure of how distinct each community is in terms of species composition: U values vary between 0 (all of the species of the sample are also found in all of the other ponds) and 100 (none of the taxa that were found occur in any other sample) (Ricotta, 2005).

The spatial distribution of sites was tested using the Nearest Neighbor index (R), which compares the observed mean distance between neighboring sites to the expected mean distance under complete spatial randomness. The null hypothesis was that sites were randomly distributed. Nearest Neighbour analysis was performed using *nndist* function (*spatstat* package), comparing the observed mean nearest-neighbor distance to that expected under spatial randomness (Baddeley et al., 2015). The R index (observed/expected) was used to infer clustering ($R < 1$), randomness ($R \approx 1$), or overdispersion ($R > 1$) in site distribution. Spatial autocorrelation in Shannon diversity and uniqueness was tested using Moran's I (*moran.test* function from *spdep* package), indicating whether similar diversity values cluster spatially (Bivand and Wong, 2018). A kernel-based isolation index was calculated for each site using a custom function that weighs distances from neighboring sites within a Gaussian kernel, with higher values indicating stronger spatial isolation. To assess the influence of spatial structure on diatom communities, we computed spatial eigenvectors (MEM, Moran's Eigenvector Maps) based on site coordinates (Longitude, Latitude). Positive MEMs were extracted and used as explanatory variables in redundancy analyses (RDA) together with key environmental factors (Elevation and Isolation) to test their combined effects on

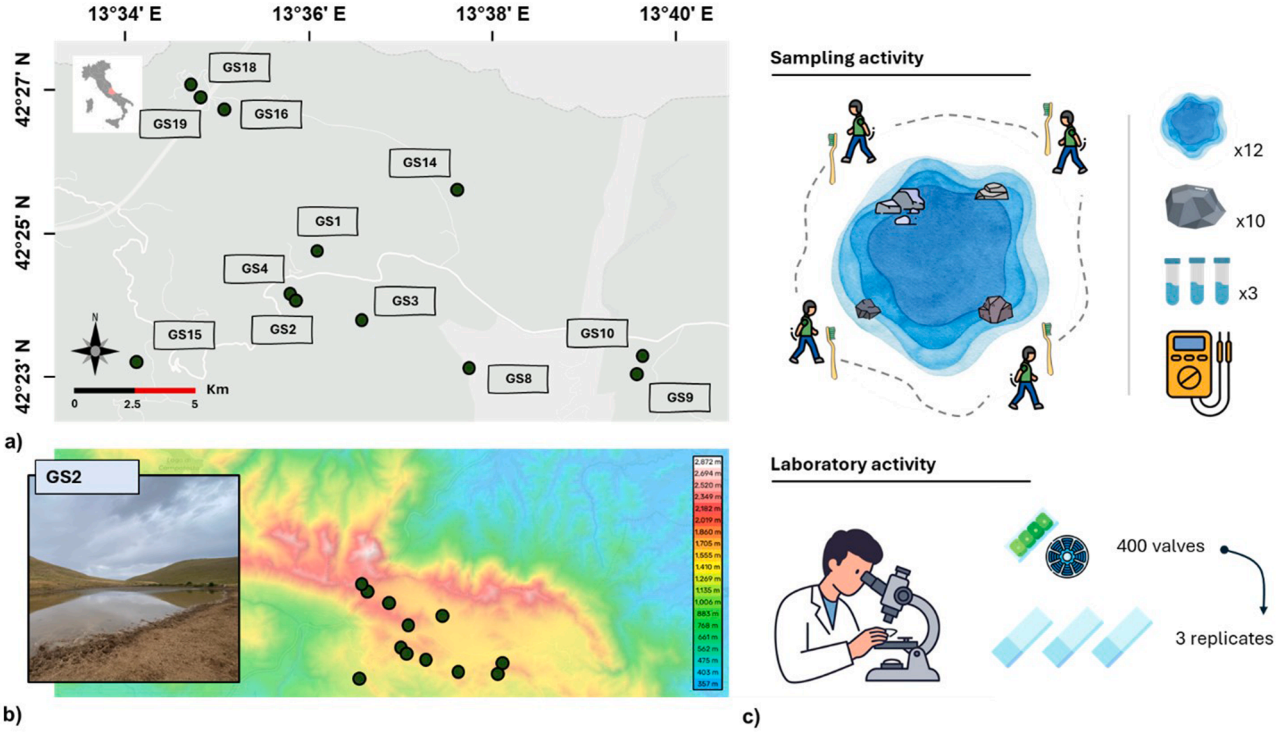

Fig. 1. a) Map of the sampling sites within the Campo Imperatore plain. Individual ponds are indicated by green dots. b) Below, the different colors of the map indicate elevation, expressed in meters above sea level (m a.s.l.). Satellite images are sourced from Carto® (https://carto.com/). c) On the right, sampling activities showing diatom sampling and laboratory activities showing diatom identification.

Shannon diversity and taxonomic uniqueness. Significance of the RDA models was evaluated using permutation tests with 999 iterations.

Then, we performed Generalized Linear Models (GLM) (*glm* function) to test the effects of elevation, environmental variables, and spatial isolation on Shannon diversity and uniqueness. Single-variable (additive) models were used to assess the independent effect of each predictor, while full (multivariable) models included all predictors simultaneously to evaluate their joint effects and account for potential covariation. Model fit and parsimony were assessed using Akaike's Information Criterion (AIC). This approach allows identification of both individual and combined contributions of environmental and spatial factors to diversity and uniqueness. Differences in significance or effect size between the two approaches do not indicate contradictions but reflect the influence of covariation among variables. To reduce multicollinearity among environmental predictors before fitting the models, we calculated the variance inflation factors (VIF) using the caret package in R. Variables showing high correlation (VIF $>$ 5 or pairwise correlation $>$ 0.5) were identified and removed from subsequent analyses. This procedure ensured that the selected explanatory variables in the GLMs for diversity and uniqueness were independent, improving model reliability and interpretability.

Sørensen dissimilarities (*vegdist* function, *vegan* package, method = "bray") on presence/absence-transformed data (*decostand* function, method = "pa") were calculated to assess taxonomic beta diversity between sites (Oksanen et al., 2025). Geographic distances between sites were calculated using the Haversine formula (distm function from the geosphere package), while environmental distances were derived using scaled Euclidean distances (dist function) across key physico-chemical variables (Temperature, pH, ORP, EC, and D.O.) (Hijman, 2024). Elevational distances were also computed separately. To assess distance–decay patterns, we performed partial Mantel tests (*mantel.partial, vegan* package) to evaluate the correlation between community dissimilarity and geographic distance while controlling for environmental distance (and vice versa) (Oksanen et al., 2025).

Taxonomic beta diversity analyses were performed using the *betapart* package (Baselga and Orme, 2012). Specifically, the functions *beta.pair* and *beta.multi* were applied to calculate pairwise and multiple-site Sørensen dissimilarity indices, respectively, partitioning total beta diversity into turnover and nestedness components. To evaluate the relationship between beta diversity and environmental gradients, we performed Mantel tests comparing Sørensen dissimilarity matrices with environmental distance matrices (elevation and isolation) using the vegan package (Oksanen et al., 2025). In this case, environmental distance, not geographic distance, was used as the explanatory variable. This dual approach allowed us to distinguish spatial effects independent of environmental variation (Partial Mantel test) from the overall association between beta diversity and environmental factors (Mantel test) (Oksanen et al., 2025). Pairwise Sørensen dissimilarity distances converted to a distance object and compared against geographic environmental distance matrices, specifically elevation and isolation distances.

Furthermore, to examine whether beta diversity dispersion differed among elevation groups, sites were categorized into three altitude classes based on elevation measurements: Low (1300–1600 m), Mid (1600–1800 m), and High (1800–2100 m). Ponds were grouped into three elevational classes to explore potential ecological gradients on environmental heterogeneity, as elevation in mountain ecosystems can affect environmental conditions over relatively short vertical distances (200–300 m) (Taurozzi and Scalici, 2025). Homogeneity of multivariate dispersion (PERMDISP) was then tested using the *betadisper* function from the *vegan* package, to evaluate whether the variance of beta diversity distances differs among the predefined elevational groups (Oksanen et al., 2025). An overall test of homogeneity of dispersions was performed with ANOVA, and pairwise group comparisons were conducted using Tukey's Honest Significant Difference (HSD) test.

Finally, to explore the relationship between species composition and environmental variables, we performed a distance-based redundancy analysis (db-RDA) using the *capscale* function from the *vegan* package. Variables with pairwise correlations above 0.5 were identified using the

findCorrelation function from the *caret* package and removed to avoid multicollinearity (Kuhn, 2008). The db-RDA was conducted using a Bray-Curtis distance matrix (which is mathematically equivalent to the Sørensen–Dice index for binary matrices), derived from the presence/absence community data (*dist* function), with the selected environmental variables as predictors. The significance of the overall db-RDA model and individual environmental variables was tested via permutation tests with 999 iterations using the *anova.cca* function. The proportion of variance explained by the first two canonical axes was calculated from the eigenvalues of the constrained ordination.

3. Results

We identified 123 diatom species belonging to 41 genera. The most occurring genera were Navicula (17 species), Gomphonema (14 species), Nitzschia (9 species), Pinnularia (7 species) and Achnanthidium (6 species). Maximal richness was observed in GS19 (32 species), GS3 (31 species) and GS4 (31 species). The most widespread species across the sampled sites were *Gomphonema parvulum* and *Navicula cryptocephala*, which were present in 11 out of 12 sites, followed by *Eolimna minima* (9 sites), *Encyonema minutum, Navicula cryptotenella, Nitzschia palea, Planothidium frequentissimum* each occurring in 8 sites. Conversely, 61 species were rare, occurring in only one site, indicating a large proportion of site-specific taxa. *Eolimna minima* ($n = 2051$), *Achnanthidium eutrophilum* ($n = 1791$), *Navicula cryptocephala* ($n = 1384$) and *Gomphonema parvulum* ($n = 1374$), were the most abundant species, capable of living from highly polluted (polysaprobic) and eutrophic environments with low dissolved oxygen, to moderately polluted and mesotrophic waters. Maximal uniqueness was observed in GS15, where 73 % of the individuals that were identified belong to taxa not found elsewhere.

The mean Nearest Neighbor distance observed was 0.01169 ($p = 0.36$), slightly higher (but not statistically significant) than the expected distance for complete spatial randomness (0.01087), resulting in an R index of 1.08. This value indicates that the sites can be considered randomly distributed, with no evidence of significant clustering or overdispersion.

However, while Shannon diversity did not show spatial autocorrelation (Moran's $I = -0.004$, $p = 0.256$), community uniqueness exhibited a significant positive spatial structure (Moran's $I = 0.181$, $p = 0.027$), suggesting spatial clustering of unique sites. Positive MEMs were then extracted and included in RDAs together with Elevation and Isolation to account for spatial structure. For Shannon diversity, the combined model (MEM1–MEM3 + Elevation + Isolation) did not significantly explain variation among sites ($F = 1.147$, $p = 0.433$), indicating that neither spatial structure nor these environmental variables had a strong effect on community diversity. For taxonomic uniqueness, the same model showed a marginally significant effect ($F = 3.931$, $p = 0.072$), suggesting that spatial structure and the two key environmental variables may contribute partially to differences in site uniqueness, although the effect was not strong.

The obtained results highlighted that the global GLM model better explained the variability in the data. Prior to fitting the models, we assessed multicollinearity among environmental variables using both correlation coefficients and variance inflation factors (VIF). Variables showing high pairwise correlations ($|r| > 0.5$) were removed to avoid redundancy (Table 1). Specifically, pH and ORP were excluded, while the reduced set of variables included Elevation, Isolation, Temperature, EC, and D.O., all with VIF values below 2, indicating low collinearity.

In the single-variable models, elevation showed a significant positive effect on Shannon diversity ($t = 2.42$, $p = 0.036$; AIC = 15.23) (Fig. 2b), indicating that higher elevation is associated with increased diversity. However, elevation did not significantly affect uniqueness ($t = 0.73$, $p = 0.480$; AIC = −8.08) (Fig. 2a). Isolation did not have a significant effect on either Shannon diversity ($t = 0.14$, $p = 0.890$; AIC = 20.74) or uniqueness ($t = -0.78$, $p = 0.454$; AIC = −8.16). In the global models including the reduced set of environmental variables (Elevation, Isolation, Temperature, EC, D.O.), elevation showed a marginally significant positive effect on Shannon diversity ($t = 2.21$, $p = 0.069$; AIC = 21.06) (Fig. 2c) and a non-significant positive effect on uniqueness ($t = 1.82$, $p = 0.118$; AIC = −9.52) (Fig. 2d). EC was marginally positively associated with uniqueness ($t = 2.41$, $p = 0.053$), while Isolation, Temperature, and D.O. did not show significant effects in either model.

DDS showed no significant trends. Partial Mantel tests revealed that neither geographic distance, elevational distance nor environmental distance significantly explained floristic dissimilarity among ponds after controlling for similarities (Table 2).

Taxonomic beta diversity among diatom communities across the elevational gradient was high, with a total Sørensen dissimilarity of 0.86. Pairwise taxonomic dissimilarities among the sampled sites, calculated using the Sørensen index, revealed moderate to high beta diversity, with values ranging from 0.42 to 0.91. The lowest dissimilarity was observed between sites GS18 and GS19 (β-sor = 0.42), indicating a relatively high similarity in species composition. In contrast, the highest dissimilarity was found between GS8 and GS16 (β-sor = 0.91), suggesting a pronounced turnover in community structure. Notably, sites GS14, GS15, and GS16 exhibited consistently high dissimilarity values (> 0.75) when compared with other sites, reflecting more distinct diatom assemblages (Fig. 3a). The partitioning of beta diversity revealed that turnover was the dominant component, accounting for 97.6 % of total dissimilarity, while nestedness contributed only 2.4 %. Pairwise comparisons between sites confirmed this pattern, with most dissimilarity values showing turnover rates above 90 %.

Mantel test analysis revealed a significant positive correlation between community dissimilarity and elevation (Mantel $r = 0.47$, $p = 0.005$), indicating that differences in diatom community composition increase with greater elevational separation (Fig. 2b).. Differently, Mantel test performed to examine the relationship between community dissimilarity and isolation distance among ponds did not reveal significant correlation (Mantel $r = 0.04$, $p = 0.38$). Results of ANOVA on the three elevational groups showed no significant differences in community dispersion among these altitude groups ($F = 0.48$, $p = 0.63$), indicating that within-group variability in diatom assemblages is relatively consistent across the elevational gradient.

The db-RDA based on Sørensen (binary Bray–Curtis) dissimilarities showed that environmental variables accounted for a substantial portion of the variation in community composition (Fig. 2c). The first two canonical axes (CAP1 and CAP2) captured 39.2 % and 26.3 % of the

Table 1
Pearson correlation matrix of the environmental variables measured across all sampling sites. Values represent the correlation coefficients between pairs of variables. Strong correlations ($|r| > 0.5$) were considered when selecting variables for inclusion in multivariate models to avoid collinearity.

Variable	Elevation	Temperature	pH	ORP	EC	D.O.	Isolation
Elevation	1.000	0.309	0.274	0.048	−0.380	−0.453	0.145
Temperature	0.309	1.000	0.332	−0.295	0.072	0.149	−0.050
pH	0.274	0.332	1.000	−0.660	−0.522	0.190	−0.420
ORP	0.048	−0.295	−0.660	1.000	0.482	−0.603	0.143
EC	−0.380	0.072	−0.522	0.482	1.000	0.071	−0.364
D.O.	−0.453	0.149	0.190	−0.603	0.071	1.000	−0.223
Isolation	0.145	−0.050	−0.420	0.143	−0.364	−0.223	1.000

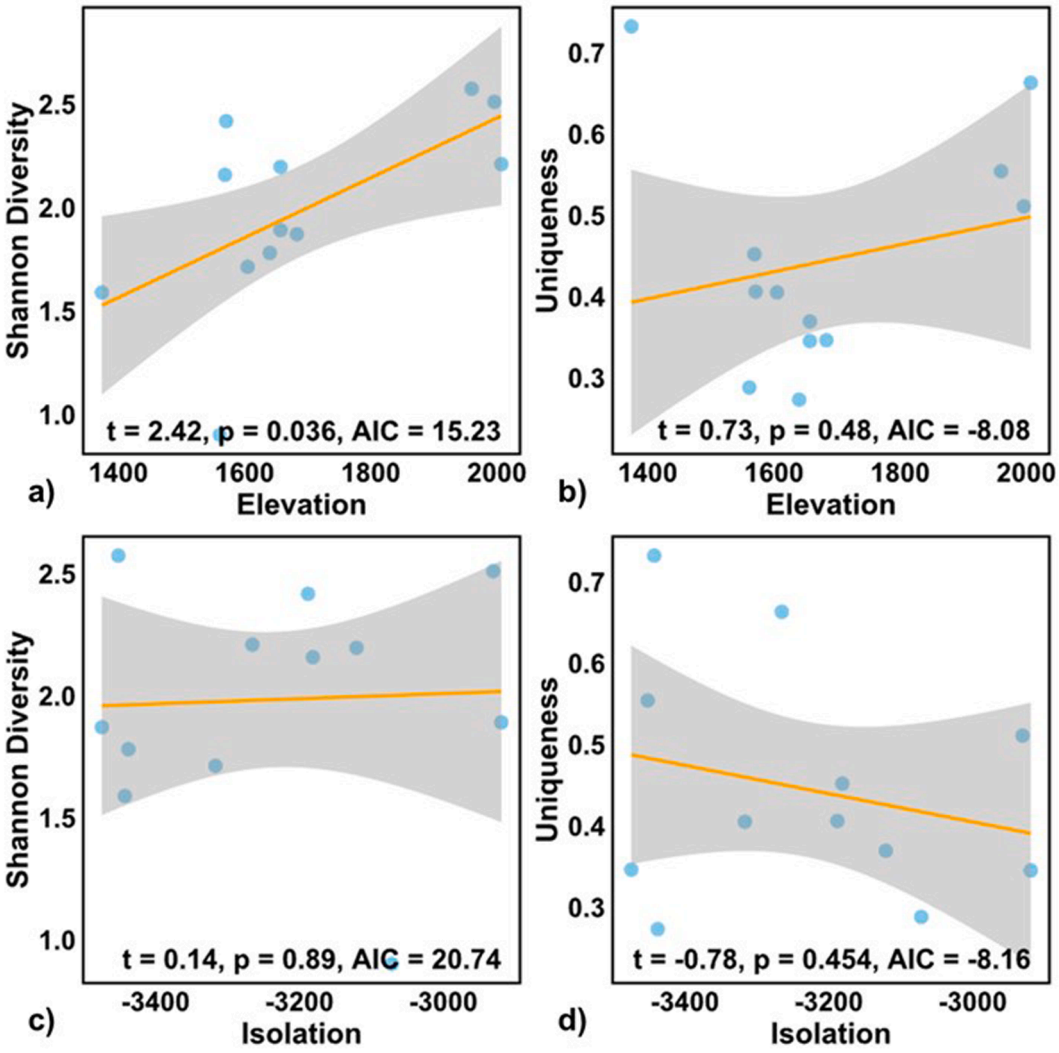

Fig. 2. Linear relationship between elevation and the biodiversity metrics: a) Shannon diversity and b) Uniqueness in single-variable GLMs. Each dot represents a sampling site, and the orange line shows the fitted values with 95 % confidence intervals. Coefficient plots representing standardized coefficients from global generalized linear models (GLMs) showing the effects of environmental variables, including elevation, isolation, temperature, EC, and D.O., on c) Shannon diversity and d) taxonomic Uniqueness across all sampled sites. Dots represent estimated effect sizes with 95 % confidence intervals. Orange points indicate statistically significant predictors ($p < 0.05$), while blue points are non-significant. The dashed red line indicates the null effect (estimate = 0).

Table 2
Partial Mantel test results from the comparison between floristic distance matrix and geographical, elevational and environmental distance matrices.

Compared matrix	Distance matrix tested for	r	p
Geographical	Environmental	−0.02	0.55
Geographical	Elevational	0.02	0.41
Elevational	Geographical	0.01	0.49
Elevational	Environmental	−0.05	0.62
Environmental	Elevational	0.18	0.17
Environmental	Geographical	0.18	0.17

constrained variance, corresponding to 22.5 % and 15.1 % of the total variance, respectively. A permutation test (999 permutations) confirmed that the overall model was statistically significant ($F = 1.61, p = 0.017$), indicating that environmental gradients structured species assemblages across ponds. Among the individual predictors, only elevation significantly explained variation in community composition ($F = 2.22$, $p = 0.010$). In contrast, temperature ($F = 1.29$, $p = 0.177$), electrical conductivity ($F = 1.26, p = 0.237$), dissolved oxygen ($F = 1.33$, $p = 0.154$), and isolation ($F = 0.96$, $p = 0.528$) did not show significant effects when tested individually. Overall, elevation emerged as the dominant environmental driver shaping community turnover across ponds.

4. Discussions

Understanding the factors that determine the diversity and uniqueness of aquatic communities in small high-elevation ecosystems is one of the main challenges of modern ecology. Recent studies, mainly conducted in Mediterranean biomes, have highlighted how community composition is often influenced by a combination of local environmental factors, geographic isolation and large-scale spatial processes (Blanco et al., 2020).

4.1. Diatom community drivers

In this study, elevational distance was the only factor significantly explaining differences in diatom community composition, while geographic and overall environmental distances did not show significant effects. Under the conditions and limited sample size of our study, we did not detect a clear spatial structure in community turnover; in other

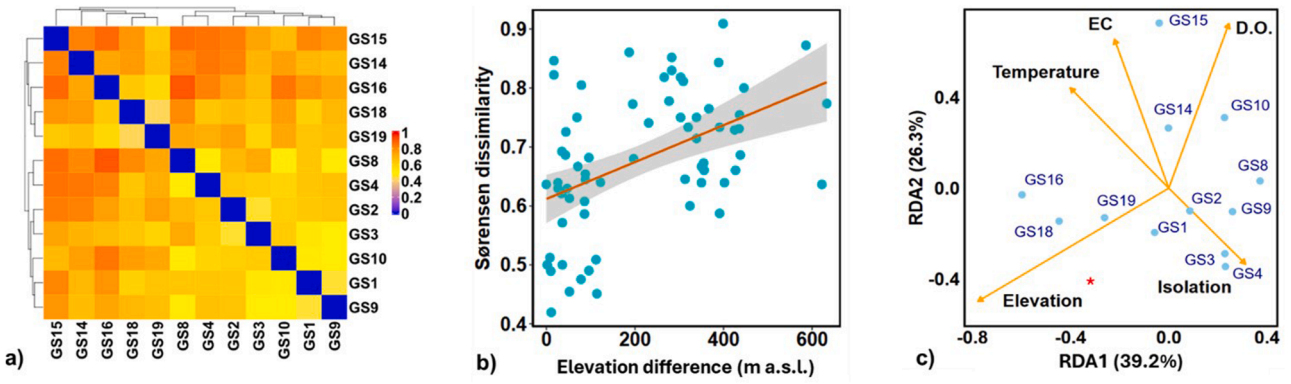

Fig. 3. a) Heatmap of pairwise Sørensen dissimilarity values (β-Sørensen) among sampling sites, illustrating taxonomic beta diversity with a color gradient from blue (low dissimilarity) through yellow to red (high dissimilarity). Row and column labels correspond to site codes; b) Scatterplot showing the relationship between elevation difference (m a.s.l.) and Sørensen dissimilarity, with a fitted linear regression line indicating increasing beta diversity with greater altitudinal separation; c) Redundancy Analysis (RDA) biplot illustrating the ordination of sampling sites (blue points) along the first two RDA axes, which explain 39.2 % and 26.3 % of the variance, respectively. Site labels are shown in dark blue. Orange arrows represent environmental vectors, indicating their direction and strength of influence on species composition. Asterisk represents significant variables.

words, community similarity did not show a pronounced decline with increasing geographic distance ("distance decay"). In contrast, Blanco et al. (2020) reported that the number of co-occurring taxa, measured as Sorensen similarity, decreased with increasing distance among Mediterranean high-elevation ponds. However, this absence of a statistically significant pattern should not be interpreted as evidence that spatial processes are unimportant in these systems. Given the relatively small number of ponds ($n = 12$), the lack of nutrient measurements, and the single sampling campaign, low statistical power may have limited our ability to detect subtle distance-related effects. Alternative explanations for the non-significant relationships include unmeasured variables that are likely important for diatom dynamics in temporary ponds, such as hydroperiod and nutrient availability. The lack of temporal replication also implies that communities integrate environmental conditions over weeks or months, whereas the physico-chemical measurements represent instantaneous snapshots; this temporal mismatch could attenuate detectable spatial signals. Within these constraints, two non-exclusive ecological interpretations remain possible: (a) local environmental filtering may be strong, with each pond's specific physicochemical conditions shaping community composition independently of spatial proximity or elevation (Schulze-Makuch et al., 2015); however, this hypothesis is only partially discounted by our results; and (b) stochastic processes may play a prominent role, generating turnover patterns that are weakly related to distance (Sun et al., 2018). The extent to which each mechanism contributes cannot be fully resolved with the present dataset, and future studies including temporal replication and nutrient and hydroperiod measurements will be necessary to clarify the relative importance of spatial versus local processes.

In practice, aquatic communities often result from the interplay between environmental filtering and stochastic processes (Moritz et al., 2013; Stegen et al., 2012). In highly selective or extreme environments, such as alpine biomes, environmental filtering tends to dominate, making community composition largely predictable from environmental parameters (Bello et al., 2013). In accordance with Szabo et al. (2018), where environmental factors played a relatively minor role compared to spatial ones, our results suggest that local environmental conditions do not have a strong influence on diatom diversity. In contrast, elevation appears to be a more relevant factor, although this effect could be partly confounded with spatial structure, as higher-elevation ponds tend to be more dispersed and may experience different disturbance regimes. This inference should be interpreted with caution given the limited sample size and the absence of nutrient data, as well as the single-season sampling.. Similarly, Teittinen et al. (2016) reported that diatom diversity along stream elevational gradients was shaped by interacting regional factors, such as dispersal and geographical variables, together with local environmental conditions. In our study, however, measured local environmental factors (e.g., pH, conductivity, temperature) appear to play a minor role in structuring diatom diversity, likely due to the limited set of variables considered and the spatial distribution of sites. While our results are consistent with general trends in the literature, such as those highlighted by Soininen (2007), we emphasize that the apparent influence of environmental factors can be context-dependent, and in our alpine temporary ponds, spatial and elevational patterns are more prominent than the measured local environmental parameters. Similarly, Van de Vijver et al. (2010) reported strong effects of local environmental parameters on diatom composition in subantarctic environments, but in our system, these factors seem less influential compared to elevation and spatial structuring.. In our case, however, the lack of temporal replication and the instantaneous nature of the physico-chemical measurements relative to the longer integration time of diatom communities limit our ability to fully disentangle the relative roles of environmental versus spatial processes. Therefore, although the supporting literature on this topic is still scarce, our findings support the idea that spatial predictors over local environmental factors could shape diatom communities in these systems, but the strength of this conclusion remains constrained by the data available and should be interpreted as a pattern detected under the specific conditions of this study

4.2. *Diatom spatial patterns*

Our results showed a random spatial distribution of sampling sites, with no evidence of clustering or significant dispersion among their physical locations. However, communities' uniqueness showed a significant positive spatial structure: sites with particularly unique communities tend to cluster in space, creating floristic clusters. In contrast, Shannon diversity did not show spatial autocorrelation, suggesting that species richness and uniqueness were homogeneously distributed among sites. These results agree with Blanco et al. (2020), where no spatial autocorrelation was detected for diatom diversity along the distance gradient. These findings indicated that, even in the absence of a spatial pattern in the arrangement of sites, there were processes which could favor the geographic concentration of floristic unique communities, probably linked to specific local environmental factors or to particular ecological dynamics (Khan et al., 2013; Jurasinski and Kreyling, 2007; Gentry, 1988).

Indeed, the value of "uniqueness" of communities (i.e., how different or special they are compared to others) was not randomly distributed in space but showed a tendency to cluster in certain areas. There is

evidence in literature that communities with unique characteristics are more often found close to each other, forming spatial clusters, rather than being randomly distributed in space (Lozada and Bertin, 2022; Altermatt and Holyoak, 2012). The occurrence of spatially clustered communities with unique characteristics represents an ecologically relevant phenomenon that can be interpreted through the lens of various environmental and ecological processes. Positive spatial autocorrelation indicates that similar values tend to occur in geographically proximate areas. This pattern suggests that shared environmental or ecological drivers, such as climatic conditions or environmental disturbances, may shape local communities, promoting the formation of clusters of floristically distinctive assemblages (Mathur, 2015). According to the literature, there are two ecological processes that could contribute to the spatial structuring of communities (Legendre, 1993): (i) large-scale processes, such as habitat connectivity and environmental gradients, which tend to generate positive spatial autocorrelation (Booker, 2023; Biswas et al., 2021); (ii) local-scale processes, such as biotic interactions (e.g., competition), which can instead lead to greater local inhomogeneity (negative autocorrelation) (Biswas et al., 2017). In our research, where unique communities form clusters, it is likely that factors such as habitat connectivity, environmental variability and the presence of natural barriers favor the concentration of these communities in certain areas.

From an ecological perspective, the presence of clusters increases the dissimilarity between communities of different areas (beta diversity), enriching the overall landscape variability (Wayman et al., 2021; Womack et al., 2021; Legendre et al., 2005). However, it may affect overall ecological resilience and vulnerability: if unique communities are concentrated, localized disturbance events (such as pollution or climate change) may simultaneously threaten many of these communities, reducing ecosystem resilience (Sankaran et al., 2019). Understanding where these clusters occur is critical for planning nature reserves and conservation strategies, as protecting key areas can safeguard a significant portion of diatom diversity.

In the single-variable models, elevation showed a significant positive effect on Shannon diversity, indicating that higher-altitude sites tend to host more diverse diatom communities (Blanco et al., 2020). In contrast, elevation did not significantly influence community uniqueness, nor did isolation have a significant effect on either diversity or uniqueness. However, when accounting for covariation among environmental predictors in the global model, elevation emerged as a significant positive driver of uniqueness, while its effect on Shannon diversity remained marginally significant. Among local environmental parameters, electrical conductivity (EC) was a significant predictor of uniqueness, whereas ORP (oxidation–reduction potential) showed a weak negative trend, and temperature, pH, and dissolved oxygen had no detectable effects. These results suggest that elevation acts as an integrative driver, shaping community composition both directly and indirectly through its association with other environmental gradients. This pattern aligns with Blanco et al. (2020), who found greater diatom diversity in high-elevation ponds, and is consistent with observations from Pescador et al. (2021) and Sreekar et al. (2018) that local environmental effects may be weak relative to broader integrative drivers. It is also important to consider that diatom communities integrate environmental conditions over extended periods, whereas our physico-chemical measurements represent instantaneous snapshots; this temporal mismatch may reduce the detectability of environmental effects, meaning that some variables that appear non-significant could still influence communities more strongly than our dataset allows to detect.

According to Blanco et al. (2020), diatom communities in Mediterranean biomes are mainly influenced by elevation: findings from our study are consistent with the above-mentioned research but, contrary to the latter, environmental variables did not mask this effect.. Contrasting findings were reported from Heikkinen et al. (2022), where subarctic ponds diatom communities were mainly shaped by a variety of local-scale environmental variables, some of which are in turn correlated with elevation. In this context, it is also important to acknowledge that key drivers such as nutrient concentrations and hydroperiod length, both known to affect diatom structure and function, were not measured in this study. Their omission, together with the limited sample size, could lead to underestimating the actual importance of local environmental conditions or to attributing effects to elevation that may instead be mediated by these unmeasured variables. Both the marginal effect of elevation on diversity and the weak influence of EC observed in our study have also been reported in other high-elevation systems, although findings across habitats and biogeographical regions remain partially contrasting: for instance, Teittinen et al. (2016) found no clear richness–elevation relationship in streams, with conductivity emerging as a more relevant driver; conversely, Taxböck et al. (2020) documented a monotonic increase in diatom richness with elevation in Swiss springs, and He et al. (2020) reported a similar positive trend in mountain streams. Likewise, Pestryakova et al. (2018) identified EC as the most important predictor of diatom diversity in high-elevation lakes. Despite these heterogeneous patterns, the broader evidence indicates that elevation often exerts a stronger and more consistent influence than individual physicochemical variables, acting as an integrative gradient that encompasses multiple environmental and spatial processes not captured by single measurements.

Comparable findings also emerged from β -diversity partitioning, highlighting how communities differ mainly due to species replacement, rather than nested loss or gain. This pattern is in line with ecological theories predicting that species turnover dominates in environmentally heterogeneous landscapes, such as elevational gradients (Kasel et al., 2017; Dufour et al., 2006). The strong pairwise dissimilarity values, especially between distant sites like GS8 and GS16, suggest that spatial filters act to shape distinct community compositions. That GS14, GS15, and GS16 consistently showed high dissimilarity relative to other sites further suggests that spatial conditions at these ponds may be particularly selective, hosting unique diatom assemblages. The positive Mantel correlation between beta diversity and elevation supports the role of elevation as a key structuring factor, probably due to differences in temperature, hydroperiod, or nutrient availability (Dodds et al., 2019). However, partial Mantel tests controlling for geographic distance revealed that the correlation between community dissimilarity and elevation was not significant (partial Mantel $r = 0.01$, $p = 0.49$), indicating that the apparent effect of elevation observed in the standard Mantel test may be partly confounded by spatial separation among high-elevation ponds. On the other hand, the lack of a significant relationship with geographic isolation in both standard and partial Mantel tests suggests that dispersal limitation alone was not a major determinant of diatom beta diversity in this system. Interestingly, no significant differences in community dispersion were found among elevation groups, suggesting that while community composition shifts strongly between elevation classes, the within-group variability remains relatively consistent. This could reflect a consistent level of environmental heterogeneity within each elevation group, or similar levels of ecological drift or stochasticity affecting communities regardless of elevation (Gilbert and Levine, 2017; Purves and Pacala, 2005). However, it should be noted that the lowest group (1300–1600 m a.s.l.) includes 4 ponds, the middle group (1600–1800 m a.s.l.) 5 ponds, and the highest group (1800–2100 m a.s.l.) 3 ponds. These numbers are relatively balanced, reinforcing the idea that the elevational classes, at least in our study, provide a reasonable framework to explore how elevational range may (or may not) influence diatom community diversity. The db-RDA results indicate that elevation is among the variables most strongly associated with community variation but given the moderate strength of correlations and the small number of ponds, this conclusion should be interpreted with caution.

In conclusion, unlike Blanco et al. (2020), where isolation was identified as a key factor influencing the ecological uniqueness of Mediterranean high-elevation ponds, our study provides, to the best of our knowledge, the first evidence that elevation may play a major role in

shaping the uniqueness of diatom communities in TPs within the alpine biome, although this effect appears partly confounded by spatial separation among high-elevation ponds.. Our results suggest that, in alpine TPs, elevational gradients appear to influence community composition independently of isolation but given the small number of ponds and the moderate strength of correlations, this conclusion should be interpreted with caution, leading to the development of functionally unique diatom communities at higher elevations. These findings highlight how the mechanisms structuring diatom communities can differ substantially depending on the biogeographical context.

The diversity and uniqueness of diatom communities are influenced by a combination of geographic factors, with elevation appearing as one of the strongest correlations of diversity and uniqueness within the limited set of variables measured, especially when considered together with other environmental variables. Electrical conductivity, in particular, seems to favor the presence of communities with similar adapted species, probably reflecting specific ecological conditions or adaptive responses to environmental gradients. Overall, the results highlight the importance of adopting an integrated approach when analyzing biodiversity determinants, as interactions between geographic and environmental factors can better explain the complexity and distribution of communities in high-elevation aquatic ecosystems. Furthermore, this emphasizes the importance of considering regional context and specific environmental gradients when investigating drivers of community diversity and uniqueness.

4.3. Limitations of the study

This study represents an initial attempt to explore the environmental and geographic drivers of diatom diversity and uniqueness in high-elevation temporary ponds, a type of habitat that remains largely understudied. However, some limitations should be acknowledged. First, our results are based on a single sampling campaign and a limited number of ponds, which does not allow for the assessment of temporal variability or interannual dynamics. Future studies including repeated sampling across different seasons and years would provide a more comprehensive understanding of community stability and environmental responses. Moreover, only a limited set of physicochemical variables was measured in situ; the inclusion of nutrient concentrations and additional hydrological parameters would likely refine the interpretation of environmental filtering processes. Despite these constraints, the patterns detected here highlight clear relationships between elevation, environmental conditions, and diatom community structure, offering a valuable baseline for future ecological and bioindicator research in alpine temporary ponds.

5. Conclusions

Although alpine biomes have received less attention than other ecosystems, they represent some of the most important and vulnerable environments in the context of climate change. The extreme conditions typical of high-elevation habitats foster distinctive biodiversity, and their sensitivity makes them among the first regions to exhibit the effects of global environmental change. In this regard, diatoms play a crucial role as bioindicators, providing valuable insight into the ecological responses of alpine ecosystems due to their rapid reactions to environmental fluctuations.

Our results show that the variability in diatom community diversity and uniqueness is best explained by geographic factors. Elevation and electrical conductivity emerged as significant predictors, whereas isolation did not significantly influence either community diversity or uniqueness. Although no clear spatial structure was observed among sites, floristic clustering (uniqueness) was evident, suggesting that specific local environmental factors—particularly elevation and conductivity—play a central role in shaping distinctive diatom assemblages rather than simple geographic distance. Furthermore, habitat connectivity, environmental heterogeneity, and natural barriers may contribute to the spatial concentration of floristically unique communities.

Understanding how alpine ecosystems respond to ongoing climate change is essential for predicting future biodiversity patterns and informing effective conservation strategies. Broader and long-term ecological research on alpine temporary ponds is therefore crucial to safeguarding their biodiversity and ecosystem functions in an increasingly changing world.

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

CRediT authorship contribution statement

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

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.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ecohyd.2025.100726.

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CHAPTER V

Similar, but not in functioning: investigating diatom traits in high-elevation ponds

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Abstract

1. On a planet constantly under siege from climate change, temporary ponds represent sentinels for the analysis of landscape and climatic changes at both local and regional scales. In this research, we investigated which local and regional factors shape diatom functional diversity in alpine temporary ponds.

2. We investigated twelve alpine temporary ponds located within the Gran Sasso e Monti della Laga National Park (Italy), sampled in July 2024 together with physicochemical, geographical (elevation), and spatial (kernel isolation) variables. We quantified functional α-diversity using five indices and assessed functional β-diversity from a presence–absence matrix using the Sørensen dissimilarity index.

3. Elevation emerged as a key predictor for functional richness and functional dispersion, temperature was associated with patterns in functional evenness, while, conversely, spatial isolation showed negative effect on functional evenness. A Mantel test revealed that community dissimilarity and elevational distance were correlated. Physicochemical factors and isolation were not.

4. Community similarity decreased as elevation difference between sites increased. Differently, community similarity did not vary with increasing isolation among sites.

Keywords: Alpine ponds; High-elevation mountain ponds; Physicochemical, geographical, spatial variables; Altitudinal diversity; Diatom functional diversity; alpha, beta diversity.

1. Introduction

On a planet constantly under siege by climate change, freshwater ecosystems represent sentinels for the analysis of landscape changes at both local and regional scales (Rose et al., 2023). Temporary ponds are ephemeral freshwater habitats characterized by a cyclic nature (i.e. by the alternance of wet and dry phases Celewicz and Gołdyn, 2021). These habitats, generally defined as shallow (< 5 m), small water bodies (< 5 ha) are distributed across most landscapes, from mediterranean regions to high-elevation alpine areas (Richardson et al., 2022; Lamouille-Hébert et al., 2024). Historically, these habitats have been largely overlooked in ecological research (Novak and Zelnik, 2021). However, recent studies have increasingly highlighted temporary ponds as highly vulnerable yet ecologically important habitats, crucial for maintaining biodiversity, landscape connectivity, and ecosystem services (Hill et al., 2021). Their role is particularly critical in extreme environments, where temporary ponds provide essential habitats for specialized and endemic species that cannot persist in larger or more dynamic water bodies (Meerhoff and Gonzalez-Sagrario, 2022).

Alpine biogeographical regions represent an emblematic example of this ecological connection; these habitats are particularly sensitive to changes in climate scenarios and represent extreme environments hosting highly specialized biodiversity (Dagnino et al., 2020; Dirnböck et al., 2003). High-elevation ponds often act as refuges for endemic species and stepping stones facilitating the movement of unique ecological communities (Lamouille-Hébert et al., 2024). In these regions, temporary ponds may experience two distinct dry phases:

one in late summer due to high evaporation and another in winter when ponds freeze (Taurozzi and Scalici, 2024b). For this reason, alpine ponds show a wet phase restricted to few weeks during summer that, considering their isolation and vulnerability, make them as valuable "field laboratories" to investigate the potential impacts of climate change on aquatic ecosystems.

Several bioindicators are commonly used to assess trends and modification on these extreme systems. Diatoms, unicellular microscopic algae widespread all over the globe, represent one of the main biological indicators used in biomonitoring studies. They are key organisms for water balances, playing a fundamental role in primary productivity and biogeochemical cycles (Benoiston et al., 2017). Diatoms are responsible for about 20–25% of the Earth's global primary production (Taurozzi et al., 2024) and, thanks to their sensitivity to environmental changes and local factors, are widely recognized as excellent bioindicators for assessing water quality and ecosystem health. Recent research has focused on diatom functional guilds, with functional traits providing insights into ecological variations, including cell size, shape, colony formation, motility, nutrient requirements, and physiological tolerances (Hansen and Visser, 2019; Passy et al., 2007). The use of functional approaches in monitoring studies increases understanding of ecological mechanisms and processes (Coccia et al., 2024), making these traits useful tools for ecological assessment and biomonitoring.

Debate remains about the relative importance of functional traits in community assembly and the environmental and geographical variables affecting them: trait selection can be enhanced by dispersal limitation by isolation or reduced by attenuation of environmental filters following disturbance events (Arim et al., 2023). If species traits do not affect performance, communities are neutrally assembled; differently, functional characteristics generate deterministic species composition in communities. Arim et al. (2023) reported community size and isolation as the main determinants of the relative importance of trait selection and drift in community assembly. In contrast, Blanco et al. (2020), described two main scientific perspectives regarding factors shaping diatom communities: a) community composition are primarily determined by local environmental conditions such as temperature, nutrients, and conductivity and b) diatom distributions exhibit biogeographic patterns shaped by dispersal processes, climate, and evolutionary history. However, none of the two perspectives precisely referred to diatom guilds: the first considered functional traits of different organisms, while the second one only considered diatom taxonomic diversity. Furthermore, to the best of our knowledge, literature investigating diatom communities in high-elevation temporary ponds have focused only on their taxonomic composition and the influence of environmental and regional factors such as elevation and water chemistry variables. There is still a lack of studies about the effects of small- and large scale drivers on diatom functional diversity. This study aims to address this gap by focusing on high-elevation temporary ponds.

The Apennines, and in particular the "Gran Sasso e Monti della Laga National Park", exemplify typical alpine ecosystems and serve as priority areas for biodiversity conservation in Central Italy. Besides direct climate change effects, conditions in temporary aquatic systems can depend on local and regional factors, such as isolation, elevation, or environmental variables. Given the lack of studies involving these ecological aspects, we investigated whether modifications at small and large spatial scales are associated with diatom functional diversity in alpine temporary ponds (Brosse et al., 2022). In particular, this research aimed to investigate: i) whether the differences in the functional composition of diatom communities between different high-elevation temporary ponds are correlated with isolation, elevation, or environmental factors and (ii) which local environmental predictors and broader-scale geographical patterns are associated with patterns in diatom community β diversity composition at small scales. In particular, following Arim et al. (2023), we hypothesized that (H1) diatom functional α diversity was most strongly associated with the degree of pond isolation and, following Blanco et al. (2020) we also hypothesized (H2) that spatial factors would be more correlated with diatom functional β diversity than any other abiotic variable.

2. Materials and methods

2.1 Study area and sampling activities

The study was conducted in the Campo Imperatore plateau, a high elevation plain with peculiar climatic characteristics, located within the "Gran Sasso e Monti della Laga National Park", in Central Italy. The area is located at the foot of the highest peak of the Apennines, the "Corno Grande" mountain (2912 m a.s.l.), above the tree line, and represents a tourist destination especially during summer (Cristofanelli et al., 2013). The area is characterized by a snow coverage during most months of the year, which generally remains expanded from November until May, when the melting of ice and snow provides water to the natural basins that fill up giving rise to temporary ponds.

There are 22 temporary ponds in the area (Taurozzi and Scalici, 2024b); of these, 12 ponds were full of water (Figure 1a – 1c) during the flood season, and therefore effectively sampleable; the remanent ponds were dry and were not sampled (Figure 1b). Consequently, all 12 available ponds were sampled during July 2024 (median elevation: 1690 m a.s.l., range: 1374–2007 m a.s.l.). Sampling focused on epilithic diatoms, which were collected and oxidized in the laboratory following the protocols described by ISPRA (2014) and Balzamo and Martone (2014), respectively (Figure 1d). For each pond, three replicates (samples) were collected: for each sample, at least 10 stones (dimensions between 64 mm to 256 mm) were scraped using a commercial toothbrush (taking care to use a new toothbrush for each sampling site) to cover a total surface area of at least 1 m²; then, the toothbrush was immersed in a 50 ml Falcon containing 70% diluted ethanol and distilled water. Diatom identification (400 valves counted per each slide) was performed using a Leica microscope at 100× magnification, with the help of specific taxonomic guides (DREAL Languedoc Roussillon, 2021; Bahls et al., 2018; Taylor et al., 2007; Krammer et al., 2002; Krammer and Lange-bertalot, 1991b; Krammer and Lange-bertalot, 1991a; Krammer and Lange-bertalot, 1988; Krammer and Lange-bertalot, 1986).

To assess water physicochemical variables, Temperature (T °C), pH, Oxygen Reduction Potential (ORP, mV), Electrical conductivity (EC, µS/cm) and Dissolved oxygen (DO, ppm) were measured in each pond, before diatom samplings, using a multiparametric probe Hannah® HI98194.

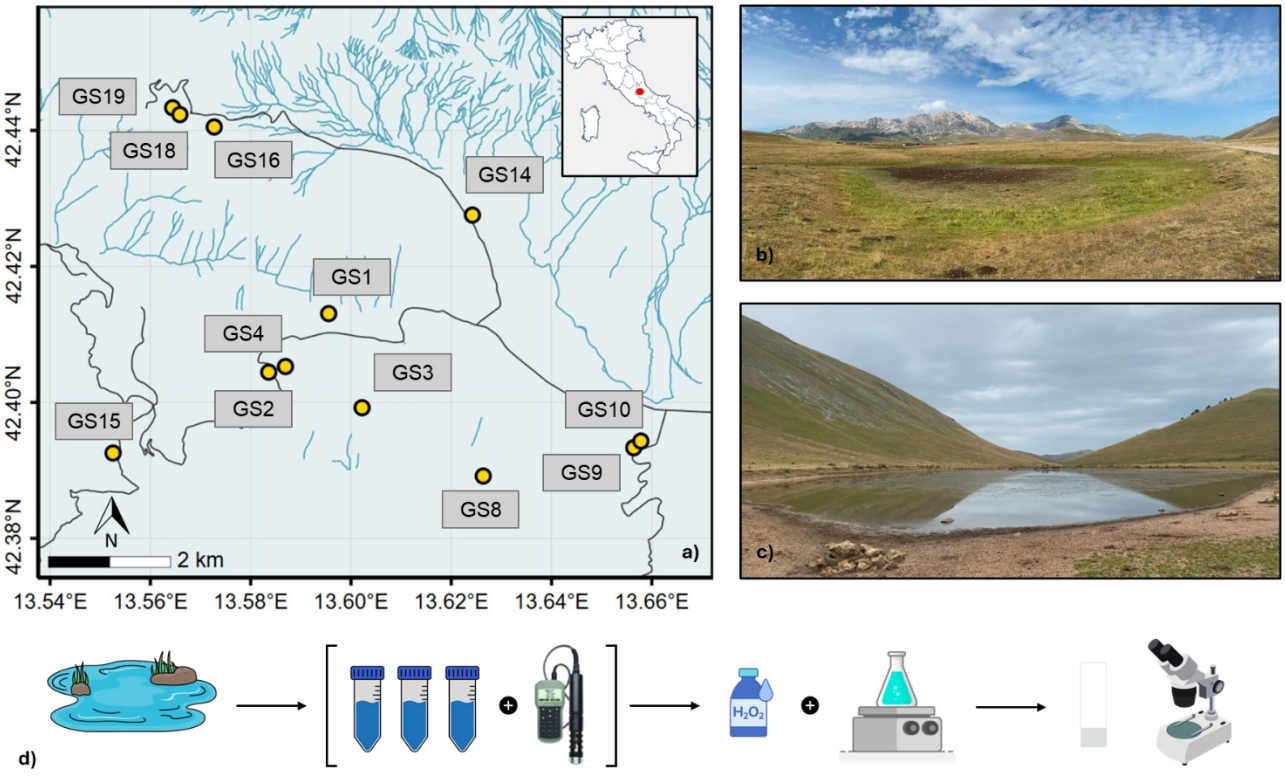

Figure 1. a) Map of the sampling sites into the Campo Imperatore plateau. Black lines are roads, blue lines are streams; b) example of a dry pond as detected during field surveys; c) example of a sampled pond (GS3); d) schematic representation of sampling (field) activities and laboratory activities (oxidation and microscope identification processes).

2.2 Data analysis

We calculated a Kernel-based isolation index to quantify the spatial isolation of each pond. This index was derived using a custom Gaussian kernel function applied to the Euclidean coordinates of the sites. For each site, distances to all other sites were computed, and their influence was weighted by a Gaussian kernel with a specified bandwidth parameter (r = 0.1) (Ting et al., 2023). In order to determine how isolated a site was inside the spatial network, the isolation index for each pond was then computed as one minus the weighted sum of impacts from nearby sites. Stronger spatial isolation was indicated by higher isolation values, indicating that the site's immediate neighborhood was influenced by fewer or more distant neighbors. By considering the contributions of several neighbors weighted by closeness, this method more thoroughly reflected the spatial structure than straightforward nearest-neighbor's metrics.

For testing the first hypothesis (i) we calculated functional α diversity indices using the *dbFD* function in R package *FD* (Laliberté & Legendre, 2010) on diatom matrix based on species composition and functional traits. Fifteen functional traits were selected for each diatom species (cell size, guild, and life forms). Trait data were transformed into a binary format (0–1) to indicate absence or presence, following Wu et al. (2024), which allows the computation of functional diversity metrics using the dbFD function Then, we calculated 5 α functional diversity indices: Functional Richness (FRic, measures the dimensional space occupied by diatom traits), Functional Evenness (FEve, represents the uniformity in the distribution of abundances across trait space); Functional Divergence (FDiv, captures the degree to which abundance is distributed toward extreme trait values); Functional Dispersion (FDis, reflects the mean distance of species from the community centroid weighted by their abundances); Rao Quadratic Entropy (RaOQ, a measure combining trait differentiation and relative abundances) (Li et al., 2022). All functional diversity indices were standardized to z-scores prior to statistical analyses. For each index, the z-score was calculated by subtracting the mean value across all sites and dividing by the standard deviation, resulting in a transformed variable with mean = 0 and standard deviation = 1. This procedure ensures comparability across indices with different scales and helps to meet the assumptions of parametric models used in subsequent analyses.

For testing the second hypothesis (ii) we calculated functional β diversity based on a presence–absence matrix where species abundances were transformed into binary values (0 = absent, 1 = present) to allow computation of the Sørensen dissimilarity index. Functional matrix was generated using the *pco* function from the *vegan* package (Oksanen et al., 2007). The decomposition of functional β diversity into turnover and nestedness components was achieved following the methodology proposed by Wu et al. (2024), using the *functional.beta.pair* function in the R package *betapart*.

To test our first hypothesis (H1), whether functional α diversity differed with abiotic factors, we ran generalized linear models (GLM) using geographical (elevation, isolation) and environmental variables (temperature, pH, ORP, EC, DO) as predictors. Before fitting the GLMs, we assessed multicollinearity among predictor variables using the *Variance Inflation Factor (VIF)* function (Akinwande et al., 2015) from the *car* package in R (Fox et al, 2012). Variables with VIF values above 5, indicating problematic collinearity, were removed from the models. The *relimp* function from the *relaimpo* package in R was used to assess the Relative Importance of Regressors in Linear Models, evaluating the contribution of each explanatory variable to the variation in diatom functional α diversity (e.g., FRic, FEve, FDiv, FDis, RaoQ) (Grömping, 2006). This approach estimates the relative contribution of each predictor variable to the total explained variance (R^2) of a

multiple linear regression model. We adopted the recommended LMG metric (Lindeman, Merenda, and Gold, 1980), which decomposes R^2 by averaging the marginal contributions of each variable across all possible orderings of predictors, ensuring non-negative and interpretable results. This method allowed us to assess the relative explanatory power of environmental factors (e.g., temperature, pH, ORP, EC, DO), spatial gradients (elevation), and spatial configuration (kernel-based isolation) in shaping patterns of functional α diversity across alpine diatom assemblages.

For testing our second hypothesis (H2), analyzing changes in community composition (β diversity) along environmental and elevational gradients, we performed Mantel test (Mantel, 1967), used as a distance-based approach to study diatom community (dis)similarities in relation to distance matrices (Wang et al., 2012). Prior to this analysis, biological data were transformed into a dissimilarity matrix using the Bray–Curtis index (on relative abundance data) to quantify pairwise differences between ponds. Similarly, environmental and elevation data were converted into distance matrices representing pairwise site differences. (Teittinen et al., 2016). To calculate the pairwise dissimilarities, we used the Bray–Curtis dissimilarity index on diatom relative abundance data, using the *vegdist* function from the *vegan* package in R (Oksanen et al., 2022). This index considers differences in species abundances and emphasizes dominant species. Mantel tests were run to assess the correlation between community dissimilarity and elevation, isolation, and environmental distances, using the *mantel* function in R package *vegan* (Oksanen et al., 2022). Then, to disentangle the individual effects of elevation, isolation, and environment, we used partial Mantel tests for: (i) testing the relationship between community dissimilarity and elevation while controlling for environmental differences, (ii) testing the relationship between community dissimilarity and environment while controlling for elevation and (iii) testing the relationship between community dissimilarity and isolation while controlling for environmental differences. 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.

Initial Compositional Similarities and Halving Distances of the distance decay relationships (Soininen et al., 2007) were calculated 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 (in this research the elevational distance, expressed as meters above sea level) in which compositional similarity has decreased to half of the initial similarity. We performed a linear regression analysis of pairwise Bray-Curtis compositional similarity between ponds against their elevational and isolation distances. We calculated the initial similarity (at 1 m distance) as $Sim1 = \beta \times 1 + \alpha$, and halving distance as $HD = (\beta - \alpha)/2$ β, where β and α are regression parameters (Teittinen et al., 2016). The regression intercept (α) represents the expected community similarity at minimal elevational or isolation separation (i.e., when ponds are very close in elevation or isolation) providing an estimate of the baseline similarity between communities at very small spatial scales. The slope (β) quantifies the rate at which similarity decreases with increasing elevational or isolation distance, indicating the turnover rate of species composition across the elevational or isolation gradient.

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). Ponds were grouped in low – medium – high elevation following quantile breaks of the elevation data to ensure balanced group sizes for the analysis. 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. (Teittinen et al., 2016).

In order to identify environmental variables significantly associated with diatom community composition, we calculated a non-metric multidimensional scaling (NMDS) on the community abundance data using the *metaMDS* function from the *vegan* package (Oksanen et al., 2022). 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 and their relative influence on functional β diversity (Anderson and Walsh, 2013). The response variable was a Bray–Curtis dissimilarity matrix representing functional β-diversity among samples. Environmental variables, namely water temperature, oxidation–reduction potential (ORP), and dissolved oxygen (DO), were included as continuous predictor variables. The analysis was conducted as a distance-based multivariate regression with a single model including only fixed continuous predictors. Statistical significance was evaluated using 999 permutations. 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 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 Environmental and geographical variables

Physicochemical variables showed substantial differences among sites. Temperature showed values ranging from 11.18 °C to 25.37 °C, with higher temperatures found at lower elevations (e.g., GS9, 1569 m, 13.62 °C; GS16, 1960 m, 25.37 °C). pH values ranged from acidic (5.59 at GS2) to alkaline conditions (8.87 at GS10), suggesting spatial heterogeneity across sites. ORP showed considerable variation, with values ranging from a low of 7.4 mV (GS18) to a high of 161.0 mV (GS2). EC ranged between 237 µS/cm and 871 µS/cm, with higher values generally recorded at lower elevations or in more isolated ponds (e.g., GS15 at 1374 m with 871 µS/cm). Dissolved Oxygen (DO) concentrations varied from 2.06 mg/L to 5.59 mg/L, indicating variability in oxygen availability among sites. *Variance Inflation Factor (VIF)* function identified pH (6.17) and EC (6.34) as possibly multicollinearity, so they were excluded from the analysis. Isolation values ranged from approximately -3475 m to -2919 m, which could reflect spatial positioning relative to other sampled sites or landscape features affecting connectivity (Table S1).

3.2 Functional α diversity

Functional α diversity varied significantly across the sites (Figure 2a) (Table S2). Regarding Functional richness (FRic), GS14 (2.31), and GS15 (2.97) showed the lowest values, while the highest values were observed at sites GS16 (16.40), GS19 (16.59), and GS18 (13.51). For FEve, the highest value was recorded at GS16 (0.49), while sites such as GS3 (0.23) and GS9 (0.28) exhibited the lowest values, highlighting a nonuniform trait distribution in most locations. FDiv consistently remained high (> 0.83 across all sites) suggesting that species with specialized traits tend to dominate the investigated communities. FDis varied noticeably across sites, ranging from a low of 0.83 at GS8 to a high of 2.38 at GS19. Similarly, RaoQ demonstrated substantial variation: the highest values were found at GS19 (6.06) and GS16 (5.75), indicating high functional diversity, while the lowest was observed at GS8 (1.63) (Figure. 2b). Overall, the results indicate marked contrasts among sites, with some hosting diverse, trait-rich communities while others are more functionally limited and clustered. The consistently high FDiv values underlined that communities were dominated by species with highly specialized traits across all sites.

The Community Weighted Means (CWM) values calculated for each site along the first five PCA axes clearly reflect the average functional composition of the communities. Sites like GS1 and GS2 showed negative values on PC1, indicating the dominance of adherent and sessile forms, while sites such as GS15 and GS18 showed higher values, suggesting the presence of mobile and loosely adherent species (Table S3). In terms of overall

contribution, the traits that most influenced functional variability in the first five PCA axes were found to be BioVol_c3, BioVol_c2, LifeForm_col, LifeForm_uni, and BioVol_c5, indicating the importance of morphology and life strategy in the functional structure of communities (Table S4). The first axis (PC1) was strongly associated with traits related to motility and substrate adhesion, with positive loadings for MotTax and ATT_Low, and negative loadings for ATT_Med, LowPro, and HighPro. This axis reflects a functional gradient from motile, poorly adherent species to sessile, well-anchored forms, suggesting a transition from opportunistic to specialized strategies in more stable environments. The second axis (PC2) was dominated by morphological traits, particularly cellular volume (BioVol_c1) and motility (LowPro, HighPro), as well as strong adhesion (ATT_Hig) (Figure 2c). This axis can be interpreted as a structural gradient related to size and morphological complexity, distinguishing species with compact structures and low adhesion from more developed, highly anchored forms.

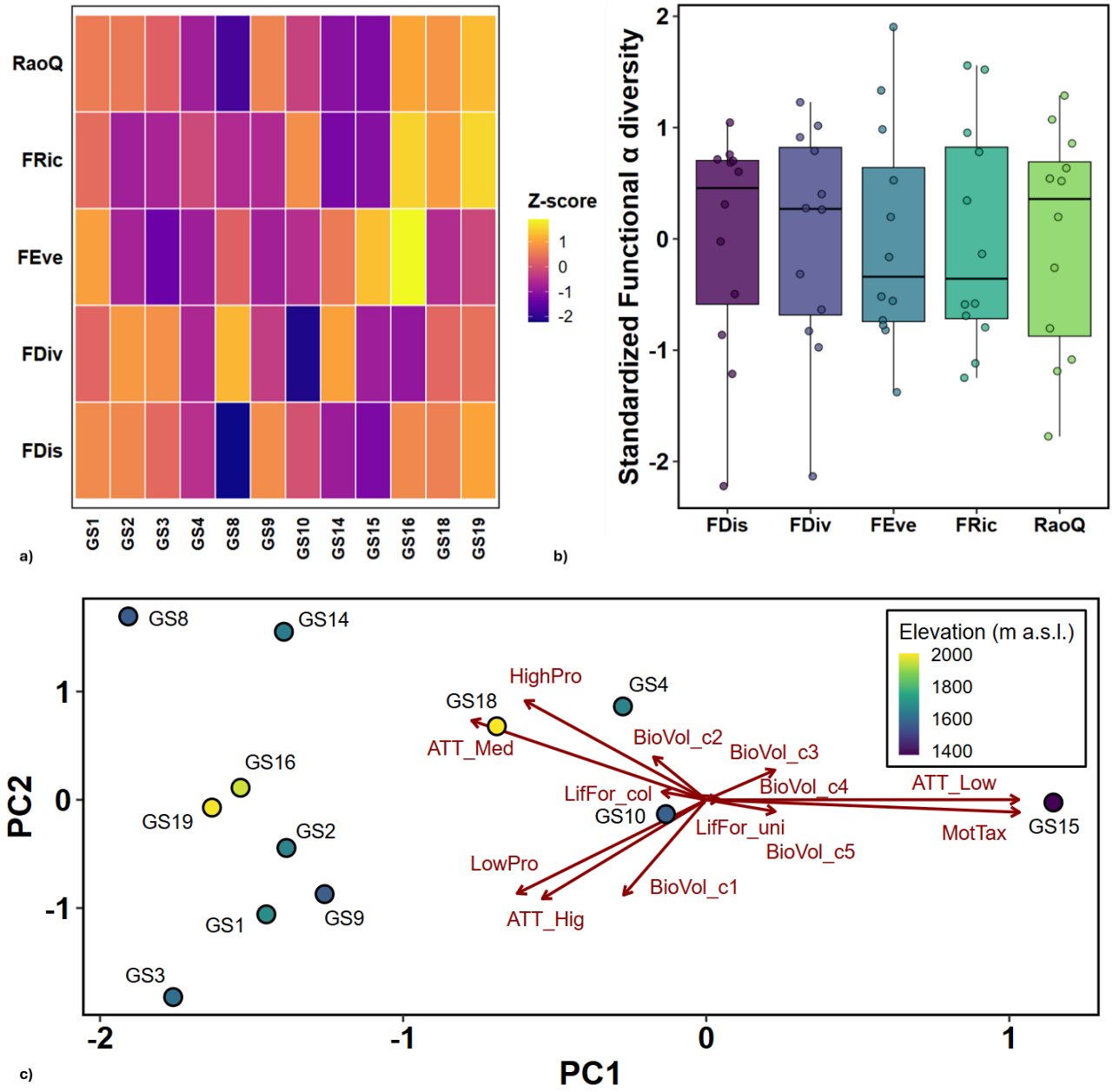

Figure 2. a) Functional α diversity values calculated for the 5 functional α diversity in the 12 sampling sites; b) Distribution of standardized functional α diversity for each index. Values were scaled to allow direct comparison between indices; c) Biplot of the Community Weighted Means (CWM) of the sampled ponds,

projected onto the first two main axes (PC1 and PC2) derived from the PCA analysis of functional traits. (FDis = Functional dispersion; FDiv = Functional divergence; FEve = Functional evenness; FRic = Functional richness; RaOQ = Rao Quadratic Entropy).

3.3 Environmental and geographical drivers of functional α diversity

GLMs highlighted the significant effects of temperature, isolation, and elevation on diatom α diversity (Figure 3a). Elevation emerged as a significant positive predictor for FRic ($t = 2.512$, $p = 0.046$) (Figure 3b), FDis ($t = 2.983$, $p = 0.025$) (Figure 3c), and RaoQ ($t = 3.013$, $p = 0.024$) (Figure 3d), suggesting that higher elevation sites support more functionally diverse diatom assemblages. This pattern may reflect changes in environmental filtering and niche availability along the elevation gradient, where harsher or more variable conditions select for a broader range of functional traits. Temperature significantly affected FEve ($t = 2.959$, $p = 0.025$), indicating that thermal regimes shape the relative abundances and balance of traits within communities. Conversely, spatial isolation had a significant negative effect on FEve ($t = -3.099$, $p = 0.021$), highlighting the role of connectivity and dispersal limitation in structuring community trait distributions. Isolated ponds may host less evenly distributed functional traits due to restricted colonization or local extinctions, resulting in functional homogenization. ORP and DO showed no significant effect on any of the functional diversity metrics, suggesting a lesser role of these chemical variables in shaping diatom functional assemblages in the investigated alpine pond system (Figure 3a).

Although some predictors showed collinearity according to the VIF (pH, EC), they were retained in the LMG models, as this approach can handle shared variance and provide a robust estimate of the relative importance of the predictor variables. Across all five indices (FRic, FEve, FDiv, FDis, RaoQ), a substantial proportion of variance was explained by the selected predictors, ranging from 37.9% for FDiv to 82.7% for FEve. Elevation emerged as strongest correlated with FRic (41.8%), FDis (33.1%), and RaoQ (42.5%), indicating a strong altitudinal structuring of multidimensional trait space (Figure 3g). In contrast, FEve was primarily associated with EC (24.4%), isolation (24.5%), and temperature (23.5%), suggesting a greater sensitivity to local abiotic conditions and spatial configuration. For FDiv, the most influential predictors were pH (10.1%), DO (9.3%), and EC (6.9%), though overall explanatory power was lower. ORP was a notable contributor to FDis (15.3%) and RaoQ (12.1%), while isolation contributed modestly (1.4%–3.8%) to most indices except FEve, where it played a more substantial role. These results highlight that different dimensions of functional diversity respond to distinct environmental and spatial gradients, with elevation and EC consistently influential across multiple metrics.

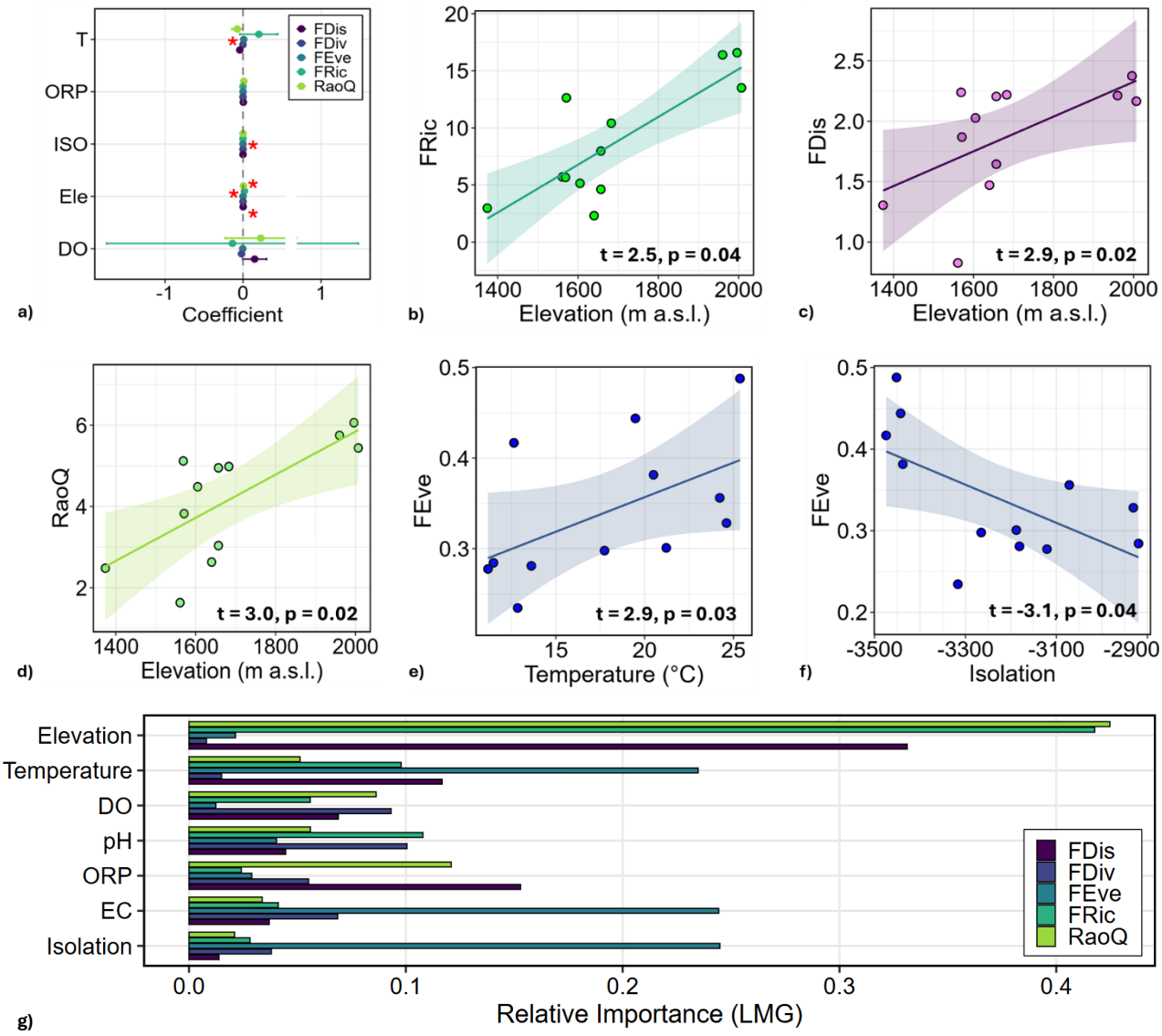

Figure 3. a) Standardized coefficients (± SE) of environmental predictors from GLMs explaining variation in functional diversity indices. Significant predictors are shown with the red asterisk, with coefficients centered on zero (dashed line) for interpretation. Color indicates the functional diversity index modeled; Linear relationships between b) Functional richness (FRic) and elevation; c) Functional evenness (FEve) and temperature; d) Functional evenness (FEve) and isolation; e) Functional dispersion (FDis) and elevation and f) Rao's quadratic entropy (RaoQ) and elevation. Lines show the linear model fit with 95% confidence interval. g) 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 colors indicating distinct α diversity indices.

3.4 Functional β diversity

Total functional β diversity among the ponds was 0.352, indicating a moderate level of functional differentiation in species composition. Turnover accounted for 51.3% (0.180) and nestedness for 48.7% (0.171) of the total, suggesting that functional differences among communities were driven by both species' trait replacement and patterns of trait loss or gain to a similar extent. Pairwise functional β diversity values between sites varied considerably, ranging from 0.03 to 0.20, indicating heterogeneity in functional composition among the sampled communities: some pairs exhibited low dissimilarity (e.g., GS8-GS9: 0.011)

while others showed considerably higher values (e.g., GS4-GS18: 0.199) (Table S5). Turnover percentages showed a wide spectrum, from nearly zero in some pairs (indicating minimal species replacement) to very high values above 90%. Differently, nestedness percentages showed an inverse trend respect to turnover, with some pairs displaying almost complete nestedness, suggesting that species compositions in one group are subsets of another, while others show much lower nestedness values, indicating more complex compositional differences. Interestingly, pairs involving GS1 and GS2 often exhibited high nestedness, while pairs involving GS3 and GS4 tended to show elevated turnover rates.

3.5 Environmental and geographical drivers of functional β diversity

Mantel tests revealed no significant correlation between community dissimilarity and the environmental distance matrix ($r = 0.094$, $p = 0.283$), so these environmental factors did not influence community turnover. In contrast, a significant positive correlation was found between community dissimilarity and elevational distance ($r = 0.541$, $p = 0.0004$), suggesting that elevational gradients play a major role in structuring diatom assemblages. No significant relationship was detected between community dissimilarity and isolation distance ($r = -0.159$, $p = 0.873$), implying that spatial isolation among ponds did not significantly influence community composition.

The partial Mantel test controlling for environmental variables confirmed a strong and significant positive correlation between community dissimilarity and elevational distance ($r = 0.524$, $p = 0.0002$), indicating that elevation shapes community structure independently from the influence of measured environmental variables. Conversely, when controlling for elevation, no significant correlations were observed between community dissimilarity and environmental distance ($r = -0.066$, $p = 0.621$), suggesting that environmental variation alone does not explain significant compositional β diversity. Additionally, isolation showed no significant relationship with community dissimilarity when controlling for environment ($r = -0.236$, $p = 0.978$), reinforcing the minimal role of spatial isolation in structuring diatom functional assemblages. Overall, these results underscore elevation as the primary geographic factor influencing functional β diversity, with environmental conditions and isolation contributing negligibly when elevation is considered.

Linear regression analysis (Initial Compositional Similarities) revealed a significant negative relationship between Bray-Curtis similarity and elevational distance ($\beta = -4.96 \times 10^{-4}$, $p < 0.001$), indicating that community similarity decreased as the elevation difference between sites increased. The intercept ($\alpha = 0.287$) represented the estimated initial similarity at a distance of 0, while the slope reflects the rate of similarity decay per meter of elevational distance. The model explained approximately 29% of the variation in similarity (adjusted $R^2 = 0.282$), indicating a moderate but meaningful pattern of distance decay. From the regression parameters, the initial similarity (Sim1) at 1 meter elevation difference was calculated as 0.286, and the halving distance (HD), the elevational distance at which similarity is reduced by half, was estimated to be approximately 289.56 meters. These results highlighted the spatial turnover of diatom communities along elevational gradients, with communities becoming less similar as elevational distance increased.

However, Linear regression analysis (initial compositional similarities) revealed no significant relationship between Bray-Curtis similarity and isolation ($\beta = 1.63 \times 10^{-4}$, $p = 0.201$), suggesting that community similarity did not systematically vary with increasing isolation among sites (Figure 4a). The model accounted for only about 2.5% of the variation in similarity (adjusted $R^2 = 0.010$), indicating a very weak explanatory power. The predicted similarity at 1 meter of isolation distance (Sim1) was 0.141, identical to the intercept. The halving distance (HD), calculated from the model parameters, yielded a negative value (-430.20 m), confirming the absence of a meaningful decay pattern, suggesting that isolation does not drive spatial turnover in diatom community composition across the studied ponds.

Both ANOSIM analyses showed no significant difference between ponds (R = 0.124, p = 0.144) or between isolation groups (R = -0.095, p = 0.776). The PERMANOVA analysis revealed that the combined physicochemical variables (T, pH, ORP, EC, DO) did not explained variations in community composition (R^2 = 0.247, F = 0.873, p = 0.67) (Figure 4b, 4c). Complementarily, the *envfit* analysis indicated that none of the investigated variables correlated with the NMDS ordination of community data (T, R^2 = 0.17, p = 0.39; pH, R^2 = 0.12, p = 0.59; ORP, R^2 = 0.05, p = 0.77; EC, R^2 = 0.38, p = 0.13; DO, R^2 = 0.14, p = 0.47). This suggests that, within this dataset, these environmental variables do not have a strong or significant structuring effect on community composition.

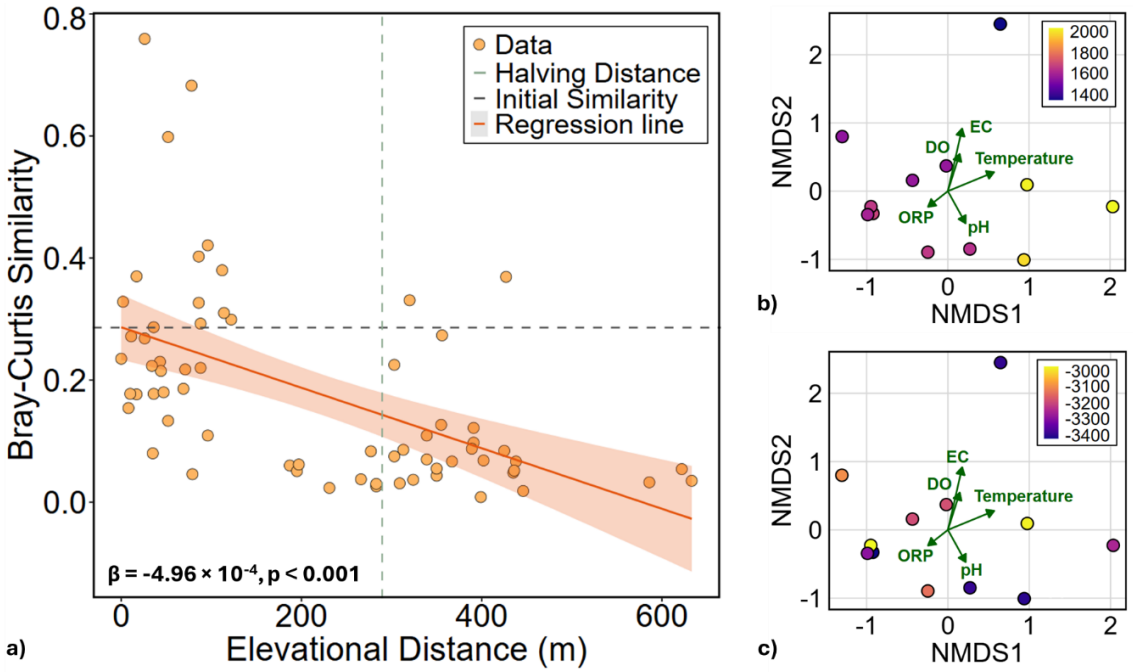

Figure 4. a) Distance decay analysis of diatom community similarity as a function of elevational distance between ponds. Points represent Bray-Curtis similarity values for site pairs, while the red line shows the linear regression of similarity against elevational distance. The dashed blue line indicates the initial similarity (Sim1) at minimal elevational separation (1 m), and the dashed light 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. Non-metric multidimensional scaling (NMDS) ordination of diatom community composition colored by b) elevation (expressed as m a.s.l.) and c) isolation (expressed as m). Environmental variables 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.

4. Discussions

In light of ongoing climate and environmental change, our research is a first step in understanding whether diversity patterns of organisms at the base of the food chain are associated with environmental variations in alpine environments. Here, we showed that variation in functional diversity was strongly dependent on elevation, shedding light on how future global changes may affect the biodiversity of these fragile ecosystems. Elevation integrates changes in temperature regimes, hydroperiod duration, and oxygen availability, all of which are expected to be directly altered by ongoing climate warming and increased climatic variability, thereby potentially reshaping the functional structure and resilience of these fragile ecosystems (Jacobsen, 2020)

4.1 Environmental, geographical and spatial variables effects on functional α diversity

The results obtained in this study showed that the most functionally diverse diatom communities appeared in high elevation ponds. Furthermore, temperature, isolation and elevation emerged as significant predictors influencing diatom functional assemblages. The relationship between temperature and diatoms has already been documented in the literature: Virta and Hedberg (2024) reported that, in a marine environment, increasing temperature exerts stress on communities, leading to decreased functional α diversity of communities, in particular, FRic and FEve (rising ocean temperatures primarily drove the seasonal succession of diatom communities, following Wei et al (2024)). Similarly, Liu et al. (2022) reported water temperature and concentrations of silicate, nitrate, ammonium, and pH value as the main driving factors shaping the assembly of benthic diatom community of a river delta. In contrast, Shibabaw et al. (2021) reported no significant influence of temperature variations on diatom communities in rivers and streams in Africa, with EC, DO, and pH playing a major role. Hu et al. (2023) reported that diatom FRic and FEve in a delta system were significantly affected by temperature, with the direct influence of abiotic factors being more significant than that of biotic variables. Although the literature shows contrasting results, our outcomes suggest that higher water temperatures lead to communities with higher FEve. This indicates more resilient and stable communities, where ecological resources are exploited more uniformly and no function is dominated by a few species. Higher temperatures can influence the metabolic response of organisms and provide a greater variety of microhabitats available for settlement (Moisez et al., 2020; Miller et al., 2015), allowing coexistence of diatoms with different functional strategies. Furthermore, diatoms are generally favored by high temperatures (Anderson, 2000): in this case, temperature may act as a no-limiting abiotic factor, allowing the growth and development of many different species (Rühland et al., 2015), which present multiple specific functional traits. However, temporary ponds, given their shallowness (Richardson et al., 2022) and restricted hydroperiods, are generally rapidly affected by temperature variations (Celewicz, S., & Gołdyn, 2021; Williams, 2012), especially after a period of freezing; Higher temperatures may coincide with increased biological activity and faster life cycles, resulting in colonization by functionally diverse taxa (Precht, 2013).

An opposite (but ecologically similar) effect emerged for isolation and FEve, which were found to be inversely correlated. This result may reflect the role of filter dispersion in temporary alpine ponds: in difficult-to-reach environments, the flow of colonizers is limited, and community composition is more strongly influenced by local processes, such as environmental selection or stochastic colonization (Ord et al., 2017; Stegen et al., 2015 The absence of dominant species, often associated with greater connectivity, may favor coexistence of functionally diverse taxa and a more homogeneous occupation of functional space (Taurozzi et al., 2024).Alternatively, isolated ponds may have undergone historical selection that led to the persistence of functionally balanced communities, in which ecological strategies coexist without marked dominance (Mittelbach and Schemske, 2015; Fukami, 2015; Chase et al., 2003). Ponds far from frequent colonization may evolve stable communities less dominated by fast-colonizing species, promoting a balance of multiple strategies (De Bie et al., 2012; Leibold et al., 2004). Furthermore, if more isolated ponds are also less disturbed (e.g., by migration or species fluxes), they may have had time to accumulate diverse taxa, resulting in a more complete and balanced functional distribution, with less intense competition dynamics, oriented toward aggregation patterns (Mittelbach and Schemske, 2015). It was also possible that isolated ponds were colonized by a non-random mix of species, leading to a more functionally equitable combination of traits, perhaps due to historical selection or the absence of dominant competitors (Vanschoenwinkel et al., 2007). In this case, in isolated ponds, species with diverse traits are likely to appear, leading to a greater filling of the functional space and a regular distribution of traits, potentially due to a reduction in colonization rates (Hortal et al., 2015; Céréghino et al., 2008). Finally, although more isolated ponds may also be smaller, more ephemeral, or with more extreme hydrological regimes, which may act as an additional environmental filter, selecting a few optimal traits for survival under stressful conditions, it is also true that disturbance is often less pronounced, leading to a more even distribution of traits (Wellborn et al., 1996).

Different from Heikkinen et al. (2022) where elevation was not sufficient to explain the variation in diatom species richness and community composition, our findings showed that elevation emerged as a significant positive predictor for three functional diversity indices: FRic, FDis, and RaoQ. Our results therefore supported the idea that ponds located at higher elevations host more functionally diverse diatom communities (Teittinen et al., 2023; Blanco et al., 2020). Although partially founded and supported by our results, H1 proved false, as elevation, rather than isolation, played a fundamental role in shaping diatom functional communities. This result is consistent with the idea that high-elevation environments, often considered more ecologically hostile, can support a wide range of functional strategies (Hodkinson, 2005). At high elevations, more extreme and variable environmental conditions are generally observed (e.g., lower temperatures, more unpredictable hydrology, greater UV irradiance), which could select for greater functional heterogeneity within communities, favoring the occupation of diverse ecological niches (Tiberti et al., 2013; Catalan et al., 2009; Körner et al., 1999). Furthermore, elevation may be associated with greater microenvironmental variability (e.g., longer hydroperiods, presence of different substrates, variable pH), contributing to an expansion of occupied functional space. Lower human pressure may also allow coexistence of multiple functional strategies (Spehn et al., 2010; Nagy and Grabherr, 2009). The presence of rare or specialized species in high-elevation habitats, often characterized by unique traits, could also contribute to the increase in metrics such as FRic, FDis, and RaoQ. In our case, numerous low-elevation ponds are associated with grazing and tourism, which, although not considered as variables in this study, should be considered for further investigations.

Relatively few articles investigate these relationships, and they mostly focus on the taxonomic structure of diatom communities, rather than their functional traits. Consequently, the interpretation of our results with these few cannot be considered sufficient evidence on which to base decisions.; It is therefore essential replicate these analyses in multiple different ecological and geographical contexts to determine the main ecological variables correlating with functional aspects of diatom communities in extremely vulnerable habitats such as high-elevation temporary ponds.

4.2 Environmental, geographical and spatial variables effects on functional β diversity

Functional β diversity across these ponds showed intermediate functional differentiation among diatom communities. The nearly equal participation of turnover (51.3%) and nestedness (48.7%) indicates that changes in the functional composition of communities arise both from species replacement and from losses or gains of functional traits along the spatial gradient. The observed β diversity partitioning suggested that there was a series of ecological dynamics operating together on diatom communities of alpine ponds rather than a single predominant process. The observed variability in functional β diversity values between pairs of sites (ranging from 0.03 to 0.20) highlighted significant compositional heterogeneity, with some communities sharing numerous functional strategies, while others exhibiting important differences. Turnover values close to zero for certain pairs show reduced species replacement, likely caused by similar environmental conditions or geographic proximity. However, values above 90% in several pairs indicate active functional replacement processes, likely driven by marked environmental or elevation differences. The opposite trend in nestedness versus turnover highlights that in numerous pairs of sites, one community was represented as a functional subgroup of the other (e.g., GS1 and GS2), while in other situations the distinction was caused by a shift in operational strategies (e.g., GS3 and GS4). Spatial correlation analyses revealed that environmental distance was not significantly related to functional dissimilarity between communities, while elevation was an important geographic factor. The persistence of this relationship, even after accounting for environmental variables, supports the idea that elevation operates as an autonomous factor in the configuration of functional communities. Our findings indicated that elevational gradients were at the forefront as the most influential geographical variables associated with functional differences among diatom communities, probably due to indirect alterations in ecological factors (e.g., hydroperiod duration, temperature, light) (Thesome et al., 2020). These processes, typical of high-elevation environments, could act as selective ecological barriers, promoting

the spread of distinct functional strategies from that observable at lower elevations (Ferrara et al., 2024; Chakravarty et al., 2021). In contrast, the overall ecological distance showed no significant relationship with functional β diversity. Potentially the environmental variables examined were not sufficient to account for the observed differences among communities; in particular, it was possible that the variables recorded did not sufficiently capture the environmental complexity important for diatoms or that geographic and historical features, such as elevation or pond history, influence the structure more significantly (Heino et al., 2005; Potapova and Charles, 2002).

Conversely, isolation did not appear to be associated with the functional composition of the diatom community. This finding was consistent in both single-variable analyses and when controlling for elevation and environmental variables. Our results suggest that, even in a limited environmental context and a single sampling frame, dispersal limitation was not an ecologically significant mechanism in the formation of functional β -diversity. Diatoms are known for their remarkable dispersal capacity, which can occur through passive abiotic (wind, rain, runoff) and biotic (waterbirds, amphibians, insects) diffusion mechanisms (Souffreau et al., 2015; Vanschoenwinkel et al., 2008), as well as active dispersal via the secretion of mucopolysaccharide filaments (Urbani et al., 2012; Round et al., 1990). Thus, even in geographically fragmented high-elevation ponds, dispersal can maintain functional connectivity, allowing colonization by taxa with diverse traits. Secondly, the system may exhibit functional saturation, meaning that most ponds, regardless of geographic distance, contain a similar variety of functional traits. This situation may occur when environmental conditions favor a fairly coherent set of operational strategies widely distributed across the territory (Cottenie, 2005; Mouillot et al., 2005). Even if species composition differs (taxonomic diversity), communities can converge functionally thanks to functional redundancy, thus reducing the β variability associated with distance (Mouillot et al., 2013). Another possible interpretation concerns the spatial dimension of the study: it is known that the effects of isolation intensify at larger geographic scales (Kadmon et al., 2007; Quinn and Harrison, 1988). In this specific case, the distances between ponds may not be large enough to create significant functional isolation. In other words, communities may remain within a coherent biogeographic unit, where dispersal processes are efficient in preserving a certain functional homogeneity. In conclusion, the absence of a significant spatial signal could indicate a greater influence of historical and environmental processes in shaping the functional composition of communities. Although the environmental variables examined were not correlated with β diversity in this analysis, it is possible that other unmeasured factors (e.g., substrate properties, fine-scale hydrological variability, episodic disturbances) exert a more significant influence than geographic isolation alone.

Similar results also emerged from the initial compositional similarities model, where elevation, rather than isolation, was the primary geographic variable associated with diatom functional traits. Specifically, our results highlighted an inverse correlation between functional similarity and elevation. Although few studies examine this in alpine systems, evidence shows that elevational gradients generate environmental variation affecting functional composition. Elevational gradients often generate variations in environmental factors such as temperature, hydroperiod length, radiation exposure, and nutrient availability (Körner, 2007; Bruelheide et al., 2018), acting selectively at functional level. The moderate but significant decay in similarity observed with elevational distance also supports the idea that variations along the gradient are structured by a mix of deterministic and stochastic processes, as highlighted in recent studies on microbial and diatom communities (Wang et al., 2017; Heino et al., 2015). In particular, as reported by Herrera-Alsina et al. (2024), increasing elevational distance correlates with increasing habitat discontinuity, resulting in the diversification of available ecological niches. Although from a taxonomic point of view, similar results also emerged from Taurozzi and Scalici (2024a), where the influence of elevation was also demonstrated also for groups of Mediterranean ponds. This situation, specific to high-altitude environments (at least concerning functionality), could favor the presence of functionally diverse communities.

Our results also showed that there was no significant relationship between isolation and the functional composition of the community. Dispersal limitations did not appear important in shaping functional diversity in high-elevation ponds, consistent with literature on high dispersal capacity of diatoms. As reported in the literature (Schulte et al., 2022; Serôdio, 2021), diatoms exhibit a high capacity for passive dispersal due primarily to transport via wind, water, and animal vectors, which facilitate their transport even over large distances. Consequently, communities from different ponds can still maintain a certain functional homogeneity despite the spatial fragmentation of the landscape, with dispersal phenomena able to counteract the effects of local genetic drift, favoring rapid recolonization of the ponds (Fodelianakis et al., 2019; Leibold et al., 2004). It should be considered, however, that the spatial scale of this research may not be sufficient to reveal specific patterns of ecological differentiation as a function of isolation. In this regard, Blanco et al. (2020) have shown that isolation, in a larger number of Mediterranean ponds, can be a very important factor in shaping the diversity (albeit taxonomic) of diatom communities. Our study thus showed that elevation plays a main role in structuring the functional diversity of diatoms, establishing strong and progressive ecological filters (although not considered here), while horizontal isolation represents an ecological barrier that diatoms can overcome. These results underscore the importance of considering not only horizontal but also vertical spatial distances in ecological studies, especially in mountainous contexts, where they are crucial for understanding the processes that shape community assembly (Rahbek et al., 2019). Therefore, while elevation represents a vertical environmental gradient that imposes strong and progressive ecological filters, horizontal spatial isolation appears less decisive in shaping the functional and taxonomic composition of diatom communities in our temporary ponds.

Our results, however, show that the effect of elevation disappears when sites are grouped by elevation values. This suggests that, although the tendency of communities to differentiate along an elevational gradient has been demonstrated, this differentiation occurs primarily at small scales, with substantial changes in diatom functional structure observable across small elevational distances rather than at large scales (Grytnes and McCain, 2007). These results were consistent with previous studies demonstrating that, in highly dynamic and ephemeral environments such as alpine ponds, community structure can be influenced by multiple overlapping factors that attenuate the net effect of a single gradient (e.g., Heino et al., 2015; Lavoie and Hamilton, 2008). Furthermore, the non-significant differentiation between elevational groups may reflect the complexity of community assembly processes, in which local variability, stochasticity in colonization, and biotic interactions contribute to maintaining a certain degree of overlap between communities (Fodelianakis et al., 2019). Similarly, assessing the effect of isolation revealed the absence of systematic separation between communities grouped by isolation levels. This result further confirms that spatial fragmentation and distance between ponds are not determining factors in the structure of functional communities in this system, consistent with the literature highlighting that diatoms and other microorganisms are often not subject to severe dispersal limitations, especially at relatively small spatial scales (Liu et al., 2013; Logue and Lindström, 2008). High dispersal capacity and the existence of natural vectors may allow frequent turnover between ponds, thus mitigating the negative effects of fragmentation (Ord et al., 2017).

The analysis of environmental variables, aimed at assessing the combined impact of chemical and physical variables (temperature, pH, ORP, EC, DO), did not correlate with community composition. The fact that functional diversity appears independent of abiotic factors can be interpreted in several ways. On the one hand, it is likely that the measured environmental conditions may be relatively homogeneous within the sites under consideration, or the observed variations may not be sufficient to generate clear functional segregation of the community (Kraft et al., 2014; McGill et al., 2006). On the other hand, the literature has shown that diatom communities exhibit an extremely complex ecology, influenced by multiple abiotic and biotic factors not measured in our research, which can mask the predictive power of individual environmental variables (Heino et al., 2015). For example, Rivera-Rondòn and Catalan (2020) have shown that diatom communities, although not in mountain pond environments, were influenced by variables such as nutrient concentrations, ionic

composition, and physical factors (albeit not at a functional level). Furthermore, the temporary nature of these habitats and frequent disturbances can favor communities characterized by high resilience and ecological plasticity, reducing the impact of chemical and physical variables on their aggregation (Angeler, 2021; Angeler and Moreno, 2007). Our outcomes thus strengthen the hypothesis that other factors, such as historical processes, colonization dynamics, species-specific interactions, or unaccounted environmental variables (e.g., substrate characteristics, fine-scale hydrology), may play a more crucial role in the functional differentiation of diatom communities (Litchman and Klausmeier, 2008). These findings are consistent with previous work highlighting how, especially in temporary and high-elevation alpine environments, community composition is often governed by a complex interplay of forces, in which local conditions and metacommunity dynamics interact in a nonlinear manner (Soininen et al., 2018; Astorga et al., 2012). In summary, the lack of significant effects of isolation and physicochemical variables highlights the predominant role of elevation but also the need to consider more integrated models and abiotic variables not investigated here. Further in-depth studies are therefore needed to understand diatom community assembly in temporary alpine environments, where ecological complexity and temporal variability play a central role.

4.3 Limits of research and future perspectives

Despite the rigorous protocol followed, conducting sampling in the shortest possible time frame to minimize its influence on environmental variables (which can vary even during the same day) and sampling diatoms according to the ISPRA (2014) guidelines for alpine waters (e.g., samplings should be avoided after rainfall; after rain/flood events, it is recommended to wait at least 4 weeks to allow complete recolonization of the substrates. The most suitable period is summer, avoiding the late spring and early summer months in bodies freshwaters of glacial origin because the high concentration of suspended solids can severely alter the diatom community), our research had some important limitations. Firstly, the area under consideration was actively frequented by tourists during the period analyzed, but disturbance and human impact were not considered in this research ; secondly, the area was subject to free-range grazing by cows and sheep, which can influence the water chemistry through their feces and generate structural alterations to the ponds through trampling; finally, as proposed in other research along elevational gradients with regard to other organisms, this study did not take into account environmental variables that could be important for the functional assembly of diatom communities, such as: hydroperiod, sediment, nutrient concentration, photoperiod. In addition, we did not directly assess dispersal processes or experimentally test community reassembly. Manipulative experiments, such as mesocosm or transplant experiments (with target diatom species) designed to constrain or enhance dispersal and to force community reassembly under controlled conditions, would be particularly valuable to disentangle the relative roles of environmental filtering and dispersal limitation in shaping diatom functional diversity. Although our study represents a starting point for investigating the functional diversity of these precious bioindicators in ephemeral high-altitude environments, it is necessary to integrate the procedure proposed here with further analyses, especially of an abiotic nature, which we were unable to carry out due to organizational and financial limitations.

5. Conclusions

Diatoms represent a powerful bioindicator for assessing the ecological status of temporary ponds. Despite ongoing climate changes and the high ecological relevance of diatoms, few studies investigated the functional response of this taxon to abiotic, environmental, and geographic changes. In this study, we provided the first evidence that elevation was the primary driver of the functional variations observed in alpine temporary ponds. Isolation, although supported by scientific evidences from previous research, did not appear to play a key role, likely due to the high dispersal capacity (active but above all passive) of diatoms. Similarly, the environmental variables considered did not appear to significantly influence community functional composition, except for temperature, which played an important role in the equalization of functional traits, likely favouring the co-

occurrence of diatom guilds, also considering that diatom development is generally favoured by medium-high water temperatures. This study provides the first evidence of the influence of temporary pond diatoms. Further ecological research is needed to consider ecosystem variables not considered here (e.g. sediments, hydroperiod and nutrients) to understand how diatoms can vary their functional structure in a world increasingly subject to rapid and sometimes irreversible changes.

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Data availability statement
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval
Not applicable

Consent to participate
Not applicable

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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CHAPTER VI

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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

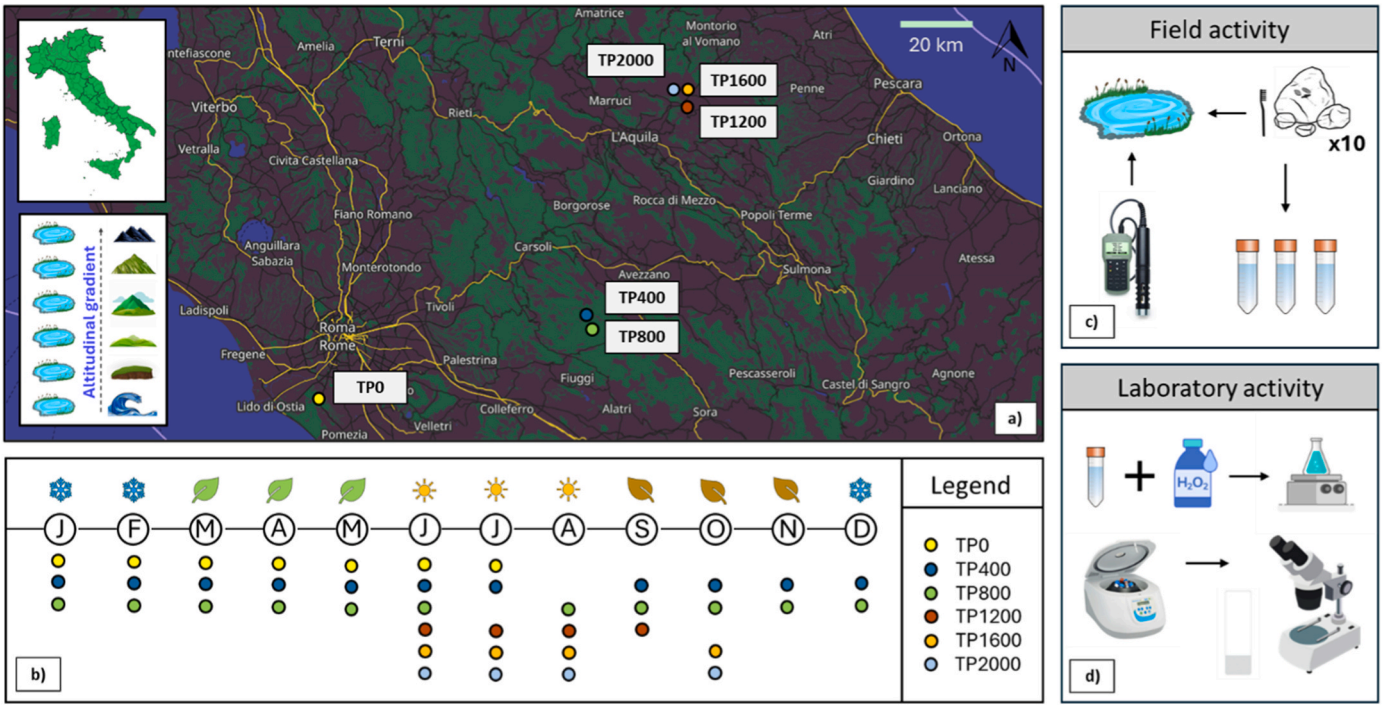

Fig. 1. a) Geographical distribution of the study sites considered: TP0, Castelporziano Presidential Estate; TP400, Simbruini National Park; TP800, Altipiani di Arcinazzo; TP1200, TP1600, TP2000, Gran Sasso and Monti della Laga National Park. b) Scheme of temporal distribution of sampling activities; 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:

$$\text{Diversity}_i = \beta_0 + s_1(T_i) + s_2(pH_i) + s_3(ORP_i) + s_4(EC_i) + s_5(DO_i) + s_6(\text{Elevation}_i) + \beta_7 \cdot \text{Season}_i + \varepsilon_i$$

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:

$$\text{Diversity}_{ij} = \beta_0 + \beta_1 T_{ij} + \beta_2 pH_{ij} + \beta_3 ORP_{ij} + \beta_4 EC_{ij} + \beta_5 DO_{ij} + \beta_6 \text{Elevation}_{ij} + u_j + \varepsilon_{ij}$$

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 Sim1 = b × 1 + a, and halving distance as 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.

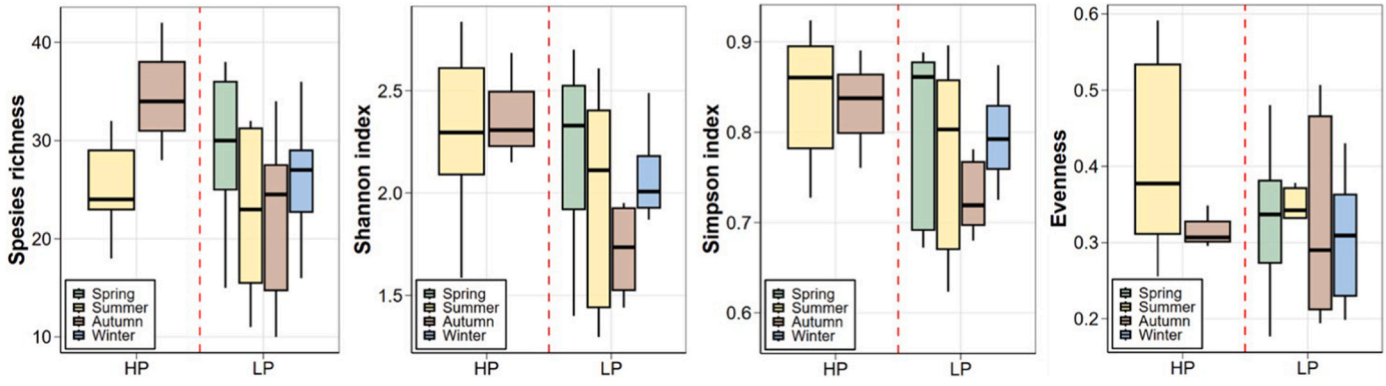

Fig. 2. Boxplot showing the species richness, Shannon index, Simpson index and Evenness across different groups (HP - LP) and seasons.

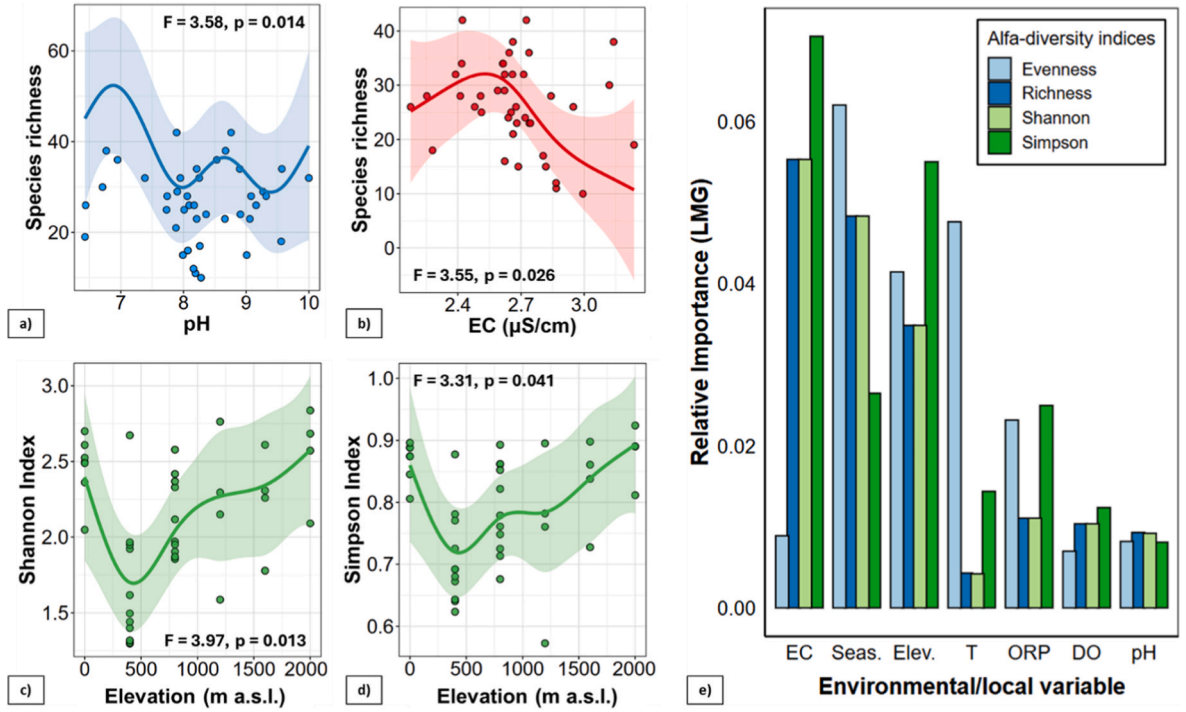

Fig. 3. Estimated relationship between a) pH and species richness, b) EC and species richness, Elevation and c) Shannon and d) Simpson diversity index, derived from GAM analysis. In all plots, observed data points are shown alongside GAM-fitted smooth curves representing the predicted relationships between 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)

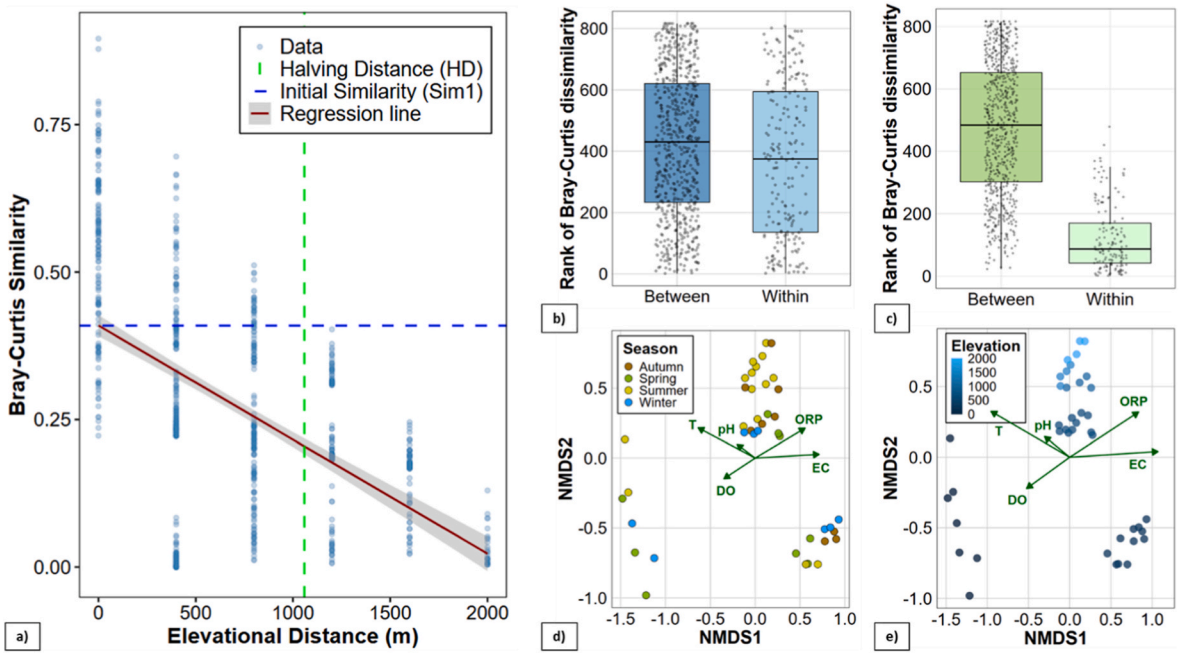

Fig. 4. a) Distance decay analysis of diatom community similarity as a function of elevational distance between ponds. Points represent Bray-Curtis similarity values for site pairs, while the red line shows the linear regression of similarity against elevational distance. The dashed blue line indicates 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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CHAPTER VII

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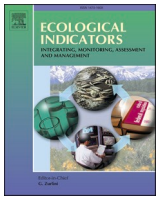

Game of traits: diatom functionality in a changing landscape

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ABSTRACT

Temporary ponds (TPs) are ephemeral freshwater habitats underlying seasonal drying creating highly dynamic environments. Here, for the first time, we focused on the trait analysis of benthic diatom communities along an elevational and seasonal gradient, aiming at: a) analysing taxonomic and functional α diversity indices variations; b) discerning whether or not seasonality and elevation affected taxonomic and functional β diversity; c) investigating the main physical and chemical factors influencing diatom communities. Among the seasonal changes, diatom guild-based communities exhibited significant variations only for high-profile guild. Seasonality appeared not influencing diatom taxonomic and functional α diversity, while the effect of elevation was statistically significant for taxonomic Shannon (TSha), Simpson (TSim) and Evenness (TEve). Furthermore, our results highlighted that neither seasonality nor elevation significantly influenced total taxonomic and functional β diversity, with the turnover component accounting for most of the observed variation. However, taxonomic β diversity showed substantial seasonal and elevational fluctuations, unlike functional β diversity, which was not driven by neither elevation nor seasonality. Physical parameters (Temperature, pH) exerted a significant influence on taxonomic α diversity, while Oxygen Reduction Potential and Total Dissolved Solids were identified as the main drivers of diatom functional α diversity. Differently, changes in Temperature, Resistivity and Dissolved oxygen emerged as important predictors for shaping elevational taxonomic β diversity. Our results emphasize the importance of considering multiple facets of biodiversity when investigating diatom communities in temporary ponds, as different aspects may exhibit distinct patterns and be shaped by different ecological drivers, particularly in high-elevation systems.

1. Introduction

Temporary ponds (TPs) are ephemeral freshwater habitats characterized by a cyclic nature, with recurring wet and dry phases (Celewicz and Gołdyn, 2021). These habitats, defined as shallow (< 5 m), small water bodies (< 5 ha) with less than 30% of emergent vegetation, are distributed across diverse landscapes, from low-elevation coastal regions to high-elevation alpine areas (Richardson et al., 2022). The hydroperiod cycle (described as the proportion of time a pond is inundated, Crase et al., 2013) is generally regular: under normal conditions, TPs fill with water every late winter and become desiccated with the onset of summer (Celewicz and Gołdyn, 2021). However, high-elevation TPs (typically present in high-mountain landscapes, > 1000 m a.s.l.) show a different hydroperiod, characterized by frozen waters during late autumn and winter, a wet phase during spring and early summer and a dry phase during late summer and early autumn (Taurozzi and Scalici, 2024b).

Both low- and high-elevation ponds are constantly under the siege of different anthropogenic direct and indirect threats, such as climate change, prolonged droughts, land-use changes, pollution, and habitat fragmentation, all of which can alter their hydrological regimes and compromise the ecological integrity of these sensitive aquatic systems (Bagella et al., 2016). Furthermore, monitoring programs frequently do not incorporate assessment techniques for temporary small waterbodies. In this perspective, the use of benthic diatoms as biological indicators is particularly valuable, as they are highly responsive to environmental changes and widely employed to assess the ecological health of aquatic ecosystems (Taurozzi et al., 2024).

Benthic diatom communities consist of ubiquitous, microscopic, photosynthetic algae that grow on submerged surfaces, such as rocks, sediments, and plants, in freshwater, marine, and brackish environments (Hamed, 2023). Currently, different methodologies including indicator species, diversity indices, and diatom indices have been developed according to the variations in the diatom species community

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(Liu et al., 2025). However, traits-based approaches can help increase our mechanistic understanding of how ecological communities assemble and function and how they change along different environmental gradients (Litchman et al., 2010). Ecological traits were firstly introduced by Passy (2007), categorizing diatom species on their responses to nutrient and disturbances gradients in rivers rather than their taxonomy (Ecological guilds; Liu et al., 2025; Jamoneau et al., 2018). This concept was further expanded by Rimet and Bouchez (2012), grouping diatom species based on their shared ecological strategies and functional traits, such as life-forms, mobility, cell-sizes and ecological preferences.

Intense modifications can occur in TPs regime hydrological and environmental variables along an elevational gradient, in relation with seasonality (Hayashi and van der Kamp, 2021). Benthic diatom communities of TPs are overlooked: only few research investigated the elevational and seasonal fluctuations of diatoms, with an almost complete knowledge gap regarding high-elevation ponds, in particular between 800 and 1600 m a.s.l. (Taurozzi et al., 2024) and above. Furthermore, the literature does not provide a universally accepted altitudinal range to define "high elevation" ponds; the elevational range considered here was taken from Krammer (2002) and Leuschner (2000), who suggested thresholds starting around 1000 m a.s.l. in temperate regions. The responses of benthic diatom communities appear to vary substantially depending on the type of freshwater habitat and the environmental gradients considered (Virta et al., 2020): temporary ponds, which represent extremely unstable habitats where diatom responses may substantially differ from those observed in more permanent water bodies, remain poorly investigated, making them key targets for advancing our understanding of diatom community dynamics under highly variable environmental conditions.

Finally, most of the very few existing studies have focused primarily on taxonomic (species-based) approaches, lacking functional trait-based analyses. Functional traits are morphological, physiological, or ecological characteristics of diatoms that reflect their ecological strategies, responses to environmental conditions, and roles in ecosystem functioning (Passy, 2007). Unlike purely taxonomic approaches (which focus on species identity), functional traits provide mechanistic insight into community assembly and ecosystem processes. By integrating both taxonomic and functional perspectives, this study provides a more mechanistic understanding of how diatom communities assemble and respond to environmental gradients in high-elevation temporary ponds, a context that remains largely unexplored. To translate these functional and taxonomic perspectives into measurable patterns of community structure, we examined both α and β diversity. α diversity captures local richness and functional attributes of communities, whereas β diversity reflects spatial and temporal differentiation (Arellano and Halffter, 2003), allowing the disentanglement of processes such as species turnover and nestedness. Turnover describes how species change between locations or time, involving the loss of certain species and the introduction of new ones: this process can be driven by ecological factors including spatial heterogeneity, species competition, and environmental filtering (Buckley and Jetz, 2008). Nestedness influences β diversity by explaining how species richness can change between sites: it indicates that some sites have a subset of species found in richer sites, which can lead to a more uniform distribution of species across habitats. Considering both alpha and beta diversity simultaneously provides a more complete understanding of diatom community dynamics, since local-scale patterns of diversity (α) are linked to regional or temporal differentiation (β).

In this study, we selected TPs along an elevation gradient to elucidate trends and potential causes of both taxonomic and functional diatom α and β diversity patterns. Assuming that hydrological regime cause variation in the biotic component, we expected differential structural and functional characteristics of the epilithic diatom community along an elevational gradient in different seasonality scenarios. To address this knowledge gap, our research, with a focus on diatoms as bioindicators, aimed to: (A1) Analyse guild-based diatom community variations across seasons and elevations; (A2) Investigate taxonomic and functional α diversity indices variations across elevation and season; (A3) Investigate the main physical and chemical factors influencing diatom communities. (A4) Whether or not seasonality and elevation affected taxonomic and functional β diversity patterns of benthic diatoms.

Following the taxonomic-based findings from existing literature (Virta et al., 2020; Cantonati et al., 2007), we hypothesized that: (H1) guild-based diatom community showed seasonal fluctuations; (H2) α diversity increased along the elevation gradient from low elevation to high elevation sites, with no effects of seasonality; (H3) Taxonomic and functional α and β diversity (and their components) showed different responses to the physical and chemical gradients; (H4) β diversity and its turnover component should be mostly influenced by elevation rather than seasonality.

2. Materials and methods

2.1. Study area and sampling activities

The study was conducted in the Italian Peninsula, considering six TPs along an elevational gradient through the two regions of Latium and Abruzzi (Table S1). The ponds were identified using GEE images and field surveys, while their temporary nature was analyzed with the use of the GEE historical imagery tool, following Taurozzi and Scalici (2024b) (Fig. 1a, 1b). The elevation of the six sites considered was chosen in the order of 400 ± 100 m a.s.l., from the Tyrrhenian coast to the Campo Imperatore plateau. The six ponds were named combining the abbreviation TP + the relative elevation (expressed as meters above sea level: TP0; TP400; TP800; TP1200; TP1600; TP2000) (Fig. 1c – 1i). The samplings were performed each month, one sampling per month for each pond, for one year (from March 2023 until February 2024), for a total of 41 samplings (Fig. 1j). Given the ephemeral hydroperiod, in some cases it was not possible to sample because of frozen or completely dryness conditions of the ponds (see Fig. 1j, red dots). In order to standardize the sampling methodology, samplings were conducted each time by the same operator at the same hour of the day (± 30 min). Summer was the only season where diatom data were available from all the ponds considered (i.e., at least one month sampled). High-elevation ponds were sampled only during summer and autumn.

Diatom sampling activities were performed following Beltrami et al. (2007): For each sample, at least 10 stones (dimensions between 64 mm to 256 mm) were scraped using a commercial toothbrush (taking care to use a new toothbrush for each sampling site) to cover a total surface area of at least 1 m^2; then, the toothbrush was immersed in a 50 ml Falcon containing 70% diluted ethanol and distilled water (Fig. 1 k). Diatom oxidation operations followed in the laboratory using hydrogen peroxide method (Taurozzi and Scalici (2024a). For each site, three different replicates were performed as suggested by Maitland et al. (2020). Diatom identification (400 valves for each slide) was performed using a Leica microscope at 100× magnification, with the help of specific taxonomic guides (DREAL Languedoc Roussillon, 2021; Bahls et al., 2018; Taylor et al., 2007; Krammer, 2002; Krammer and Lange-bertalot, 1991b; Krammer and Lange-bertalot, 1991a; Krammer and Lange-bertalot, 1988; Krammer and Lange-bertalot, 1986).

At each sampling site, 8 physical and chemical parameters of ponds were directly measured using a multiparametric probe Hannah® HI98194: Temperature (T °C), pH, Oxygen Reduction Potential (ORP, mV), Electrical conductivity (EC, μS/cm), Total Dissolved Solids (TDS, ppm), Resistivity unit (RES, MΩ/cm) Salinity (psu), Dissolved oxygen (DO, ppm) (Fig. 1 k) (Table S4).

2.2. Statistical analysis

2.2.1. Seasonal composition of species- and guild-based benthic diatom communities

Prior to all analyses, data were tested for normality (Shapiro–Wilk

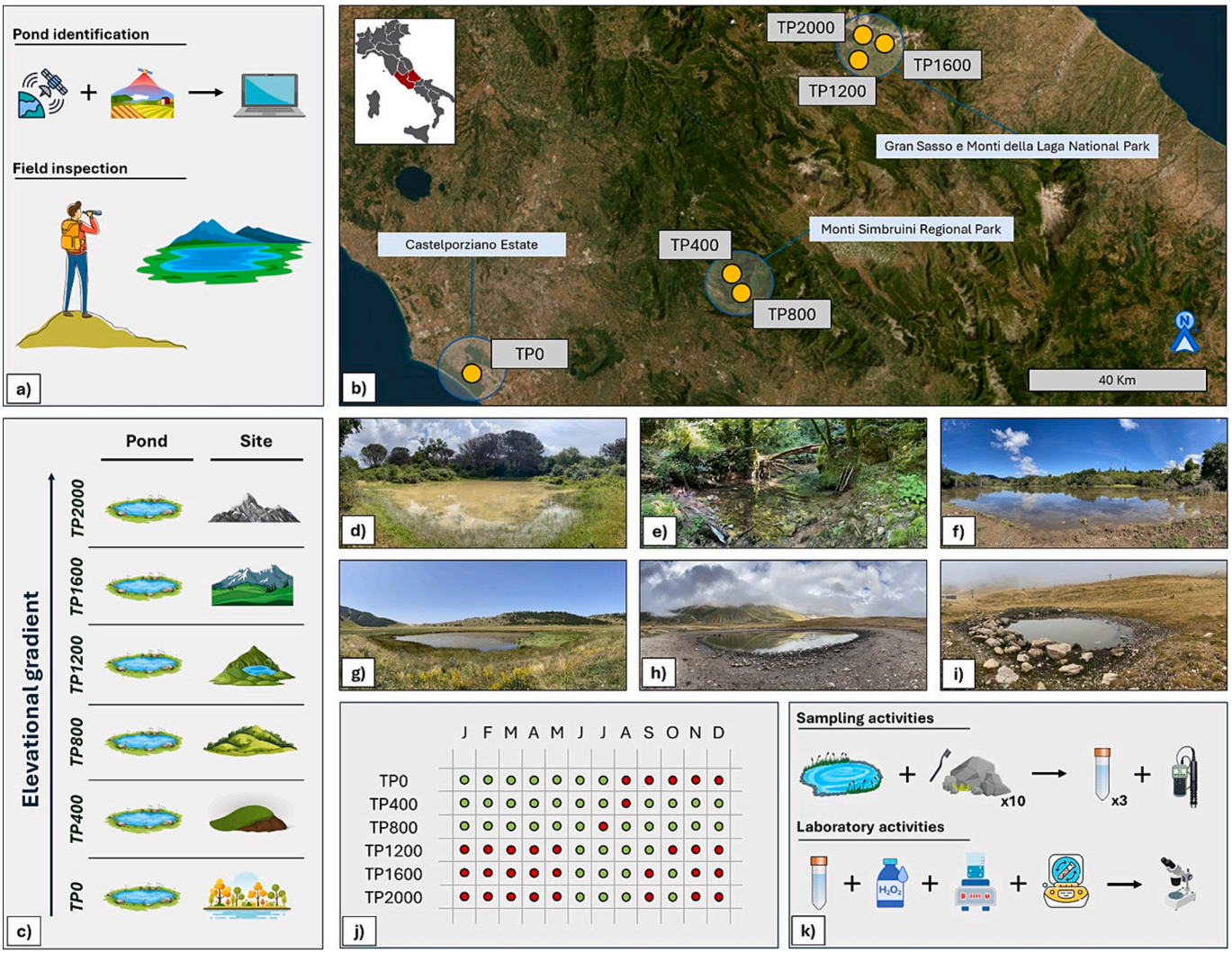

Fig. 1. a) Preliminary pond identification methodology and subsequent field inspection; b) geographical distribution of the study sites; c) schematic elevational distribution of the ponds and related habitat type; d) TP0; e) TP400; f) TP800; g) TP1200; h) TP1600; i) TP2000; j) Sampling activities calendar; green dots = sampling dates, red dots = unsampled dates; J – D = months of the year ordered from January to December; k) schematic representation of sampling (field) activities and laboratory activities (oxidation and identification processes).

test) and homogeneity of variances (Levene's test). Parametric tests were applied only when these assumptions were met; otherwise, non-parametric alternatives were used.

In the first part of our analysis, we focused on the principal ecological diatom guides proposed by Passy (2007): high profile, low profile, motile. The reported guilds represent the main functional traits generally used in diatom community ecology studies.

Normality and homogeneity of variances were tested prior to the analyses. When these assumptions were not met, non-parametric alternatives were applied. To test our first hypothesis (H1), ANOVA test was performed to identify seasonal fluctuations in the number of species for each guild. In instances of significance, subsequent pairwise Tukey's honestly significant difference (HSD) tests were performed to identify significant differences between ecological guilds. Similarly, ANOVA test and Tukey HSD were performed to identify significant seasonal differences between ecological guilds for each site. Kruskal-Wallis's tests and Dunn's post hoc tests were performed to identify significant differences in the distribution of guilds among ponds.

2.2.2. *Seasonal and elevational α diversity fluctuations in species-based and traits-based diatom communities*

In the second part of our analysis, we mainly focused on taxonomic and functional α diversity and physical and chemical variables. Taxonomic α diversity was calculated using the *diversity* function in vegan package (Oksanen et al., 2025) of R software (R Core Team, 2024). We calculated 5 α diversity indices: Richness (TRic); Shannon-Weiner (TSha); Simpson (TSim); Evenness (TEve); Margalef (TMar) (Description of the adopted indices was reported in Table S2). Similarly, functional α diversity was calculated using the *dbFD* function in R package FD (Laliberté and Legendre, 2010) on diatom matrix based on species composition and functional traits. 15 functional traits were selected (cell size, guild, and life forms), using 0–1 for presence and absence following Rimet and Bouchez (2012) (Table S3 and Table S5). Then, we calculated 5 α functional diversity indices: Functional Richness (FRic), Functional Evenness (FEve); Functional Divergence (FDiv); Functional Dispersion (FDis); Rao Quadratic Entropy (RaOQ) (Description of the adopted indices was reported in Table S2).

To test our second hypothesis (H2) and identify variability in the taxonomic and functional α diversity of diatom communities across seasons and elevations, we ran the non-parametric Kruskal–Wallis tests among the four seasons and the six elevations, followed by Dunn's post hoc tests. Non-parametric tests (Kruskal–Wallis and Dunn's post hoc) were selected because they are robust to unbalanced sampling designs, which are common in temporary pond datasets given the

unpredictability of hydroperiods. To test our third hypothesis (H3) and evaluate the key physical and chemical factors determining spatial patterns of taxonomic and functional α diversity, we performed Random Forest Regression (RFR) (Genuer et al., 2010) for α diversity indices. RFR is a widely used non-parametric machine learning algorithm characterized by higher flexibility and robustness compared to traditional linear regression models (Genuer et al., 2010). RFR constructs multiple decision trees (in this case, 1000 trees) to identify the most important predictor variables for the target α diversity index. The model's performance was assessed using mean squared error (MSE) and R-squared (R2) values: MSE quantifies the average squared difference between observed and predicted values and R^2 quantifies the proportion of variance in the observed data explained by the model Pearson's correlation test was performed to test the RFR model performance as an additional indication of agreement between observed and predicted values. RFR was performed using the *randomForest* function in the R package randomForest (Breiman, 2001), followed by the importance function from the same package to rank the variable importance.

2.2.3. Seasonal and elevational β diversity fluctuations in species-based and traits-based diatom communities

In the third part of our analysis, we mainly focused on taxonomic and functional β diversity and physical and chemical variables. Taxonomic and functional β diversities were calculated based on the presence–absence matrix, utilizing the Sørensen dissimilarity index. Functional matrix was generated using the *pco* function from the vegan package. The decomposition of taxonomic β diversity into turnover and nestedness components was achieved using the *beta.pair* function from the R package betapart (Baselga et al., 2023). Concurrently, the computation of functional turnover and nestedness components followed the methodology proposed by Swenson (2011), using the *functional.beta.pair* function in the R package betapart. Subsequently, taxonomic and functional β diversities, along with their respective components, were calculated both for seasons and elevation gradient.

To test our fourth hypothesis (H4) and identify variability in the taxonomic and functional composition of diatom communities across seasons and altitudes, permutational analysis of multivariate dispersions (PERMDISP) was employed, utilizing the *betadisper* function in the R vegan package on dissimilarity matrices generated with the *gowdis* function from vegan package. Principal Coordinate Analysis (PCoA) and the Euclidean distance were used to characterize seasonal and elevational β diversity variations in species-based and guild-based communities, which were statistically assessed by permutational multivariate analysis of variance (PERMANOVA). The combination of PERMANOVA and PERMDISP was adopted, as both methods are suitable for unbalanced designs and allow us to disentangle differences in community composition from differences in within-group dispersion. Finally, to test our third hypothesis (H3) on β diversity, we performed distance-based redundancy analysis (db-RDA) (Legendre and Anderson, 1999) for β diversity matrices. Distance-based Redundancy Analysis (db-RDA) is a multivariate statistical technique that allows for non-Euclidean distances, commonly used in community ecology to examine the relationships between species composition or guild-based community and environmental variables. Following Wu et al. (2024a), we first applied the *findCorrelation* function in the R package caret to remove the physical and chemical variables with strong intercorrelation (i.e., Spearman's rank $|r| > 0.8$) and reduce multicollinearity. Then, we performed a global test (by ANOVA at a significance level of $p < 0.05$) for each value of taxonomic and functional β diversity by using *capscale* function in the vegan package. *Capscale* function and ANOVA test were then performed for each physical and chemical variable, looking for significant influences of each variable on β diversity.

Statistical tests were considered significant when *p*-value was <0.05. Statistical analyses were computed in PAST 4.17c software (Hammer et al., 2001) and R software (v. 4.4.2, R Core Team, 2024). Figures were drawn using R software and GraphPad Prism 8.0 software (GraphPad).

3. Results

3.1. Seasonal composition of species- and guild-based benthic diatom communities

A total of 232 benthic diatom species, belonging to 53 genera were identified across four seasons and six sampling sites (Table 1).

Overall, 116 motile, 67 high-profile, 51 low-profile species were observed. ANOVA test highlights no significant seasonal fluctuations for motile ($F = 0.6$, $p > 0.05$) and low-profile guilds ($F = 0.02$, $p > 0.05$). Differently, seasonal fluctuations were observed for the high-profile guild ($F = 4.14$, $p < 0.05$) with significant differences emerged between winter and summer ($Q = 4.66$, $p < 0.05$) and between summer and autumn ($Q = 3.85$, $p < 0.05$) (Fig. 2a). Kruskal-Wallis test highlighted significant differences in the distribution of guilds among ponds only for TP800 ($H = 7.53$, $p = 0.02$) (Fig. 2b).

ANOVA tests indicated that, in general, there were no significant differences in the abundance of motile, high-profile, and low-profile guilds between ponds in summer or autumn (TP0 excluded). Similarly, no significant seasonal fluctuations were observed within most ponds, except for the high-profile guild at TP1600 ($F = 3.2$, $p < 0.05$) and TP2000 ($F = 5.3$, $p < 0.05$), which showed significant seasonal variation according to Tukey's HSD (Fig. 2c).

3.2. Seasonal and elevational α diversity fluctuations in species-based diatom communities

Taxonomic α diversity indices of diatom communities showed no significant variations across the seasons (Fig. 3a, Table S6).

The α diversity indices exhibited a distinct increasing trend from low-elevation ponds to high-elevation sites, as observed in Fig. 3b (Table S7). TSha ($H = 17.7$, $p = 0.003$), TSim ($H = 17.8$, $p = 0.004$) and TEve ($H = 15.1$, $p = 0.009$) showed significant differences in α diversity values (Table 2).

The random forest regression (RFR) models reliably predicted the taxonomic α diversity indices, as indicated by MSE (13.4, 0.04, 0.002, 0.002, 0.26) and R^2 (0.79, 0.74, 0.73, 0.72, 0.79) for TRic, TSha, TSim, TEve and TMar, respectively.

Results from RFR highlighted pH and EC as the primary factor influencing taxonomic α diversity (TSha, TSim, Teve, TMar), with fine-scale spatial factors (e.g., RES, ORP) following closely (Fig. 3c). The relationship between observed and predicted values using Pearson's correlation test yielded a strong positive correlation which was highly significant for all the α diversity indices considered (TRic, $r = 0.94$, $p < 0.01$; TSha, $r = 0.93$, $p < 0.01$; TSim, $r = 0.94$, $p < 0.01$; TEve, $r = 0.95$, $p < 0.01$; TMar, $r = 0.95$, $p < 0.01$).

Table 1
| Dominant species found for each season (we reported only the percentage for the season exceeding 5% following Liu et al., 2025).

	Winter	Spring	Summer	Autumn
Achnanthidium eutrophilum (Lange-Bertalot 1996)	–	11%	6%	–
Achnanthidium minutissimum (Kützing, 1994)	–	13%	5%	9%
Cocconeis placentula (Ehrenberg, 1838)	15%	–	–	6%
Denticula tenuis (Kützing, 1844)	9%	–	–	12%
Diatoma mesodon (Kützing, 1844)	–	–	6%	–
Ephitemia adnata (Kützing, 1838)	–	–	6%	5%
Eunotia minor (Kützing, 1881)	–	5%	–	–
Gomphonema micropus (Kützing, 1844)	–	–	6%	–
Gomphonema minutum (Agardh, 1831)	–	–	–	6%
Gomphonema parvulum (Kützing, 1849)	–	–	9%	–
Navicula tripunctata (Müller, 1822)	–	–	–	8%
Navicula trivalis (Lange-Bertelatot,1980)	5%	–	–	–
Nitzschia frustulum (Kützing 1880)	–	–	8%	–
Nitzschia palea (Kützing, 1844)	11%	–	–	–

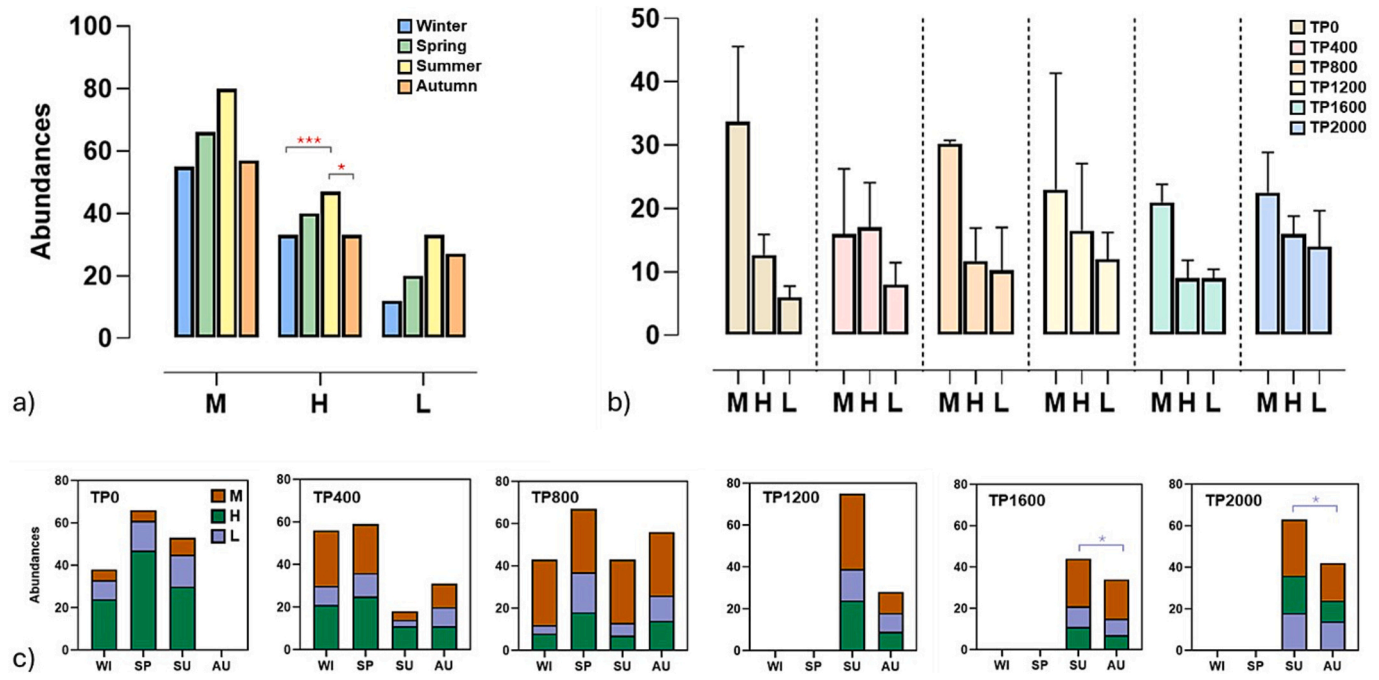

Fig. 2. a) Seasonal changes in the composition and relative abundance of the benthic diatom ecological guilds; b) Distribution of ecological guilds among ponds; c) Seasonal changes of guilds abundances among ponds. Asterisks indicate seasonal significant differences based on the ANOVA test and Tukey's pairwise (***$p < 0.001$; *$p < 0.05$). M = Motile guild; H = High-profile guild; L = Low-profile guild. WI = Winter; SP = Spring; SU = Summer; AU = Autumn.

3.3. Seasonal and elevational α diversity fluctuations in guild-based diatom communities

The results revealed that no significant differences were observed in functional α diversity among seasons (FRic, H = 1.851, $p = 0.6$; FEve, H = 1.312, $p = 0.7$; FDiv, H = 1.388, p = 0.7; FDis, H = 1.946, $p = 0.5$; RaoQ, H = 2.596, $p = 0.4$) (Fig. 4a) (Table S8 and Table S9).

Notably, functional α diversity indices exhibited a distinct increasing trend from low-elevation ponds to high-elevation sites, as depicted in Fig. 4b for FDiv (H = 13.15, $p = 0.02$), FDis (H = 14.4, $p = 0.01$) and RaoQ (H = 12.44, p = 0.02) (Table 3).

The random forest regression (RFR) models reliably predicted the functional α diversity, as indicated by MSE (12.16, 0.001, 0.001, 0.022, 0.34) and R^2 (0.77, 0.78, 0.77, 0.75,0.73) for FRic, FEve, FDiv, FDis and RaoQ, respectively (Fig. 4c).

Results from RFR highlighted ORP as the primary factor influencing functional α diversity (FDiv, FDis and RaoQ), with fine-scale spatial factors (e.g., pH, T) following closely (Fig. 4c). The relationship between observed and predicted values using Pearson's correlation test yielded a strong positive correlation which was highly significant for all the α diversity indices considered (FRic, r = 0.93, $p < 0.01$; FEve, $r = 0.92$, $p < 0.01$; FDiv, $r = 0.93$, $p < 0.01$; FDis, r = 0.96, $p < 0.01$; RaoQ, $r = 0.96$, $p < 0.01$).

3.4. Seasonal and elevational β diversity fluctuations in species-based diatom communities

Total elevational β diversity (0.697) was generally higher than seasonal total β diversity (0.542). Turnover and nestedness in the seasonal β diversity were 0.457 and 0.084. β diversity was mainly contributed by seasonal turnover (84.3% total) rather than nestedness (15.7% total) (Table S10). No significant differences between seasons emerged (F = 0.54, $p = 0.67$) (Fig. 5a and Table S11).

Similarly, turnover and nestedness in the elevational β diversity were 0.624 and 0.072. β diversity was mainly contributed by elevational turnover (89.6% total) rather than nestedness (10.4% total) (Table S12). No significant differences in dispersion among elevations emerged (F = 0.82, $p = 0.54$) (Table S13) (Fig. 5a).

PCoA showed that β diversity exhibited substantial seasonal (F = 2.05, $p = 0.003$) and elevational fluctuations (F = 3.94, $p = 0.001$). The first two axes (PCoA1 and PCoA2) together explain 30.66% of the variation in seasonal β diversity and 29.92% of the variation in elevational β diversity (Fig. 5b).

The db-RDA analysis revealed that the environmental variables explain 13.82% of the total seasonal variance and 17.96% of the total elevational variance. The first two axes accounted for 6.93% of the seasonal total variation (RDA 1 = 4.44%, RDA 2 = 2.49%) and 8.82% (RDA 1 = 5.39%, RDA 2 = 3.43%) of the elevational total variation, with no significant effects of physical and chemical parameters for seasonality (F = 0.9, $p = 0.862$) and significant effects for elevation (F = 1.24, $p = 0.006$) (Fig. 5c). Permutational analysis highlighted that T (F = 1.59, $p = 0.07$), RES (F = 1.62, p = 0.006) and DO (F = 1.37, $p = 0.047$) can be considered important predictors for shaping elevational β diversity.

3.5. Seasonal and elevational β diversity fluctuations in guild-based diatom communities

Total elevational β diversity (0.288) was generally higher than seasonal total β diversity (0.183). Functional turnover and nestedness in the seasonal β diversity were 0.068 and 0.11, respectively. Functional β diversity was mainly contributed by seasonal nestedness (62.8% total) rather than turnover (37.2% total) (Table S14), except for summer, with no significant differences between seasons (F = 1.98, $p = 0.14$) (Fig. 6a and Table S15).

Differently, turnover and nestedness in the elevational β diversity were 0.17 and 0.11, respectively. Functional β diversity was mainly contributed by elevational turnover (61.8% total) rather than nestedness (38.2% total) (Table S16), with no significant differences in dispersion among elevations (F = 0.74, $p = 0.59$) (Fig. 6a).

PCoA showed that β diversity exhibited no substantial seasonal (F = 0.8, $p = 0.4$) and elevational fluctuations (F = 0.6, $p = 0.07$). The first two axes (PCoA1 and PCoA2) together explained 71.61% of the variation in seasonal β diversity and 72.05% of the variation in elevational β diversity (Fig. 6b).

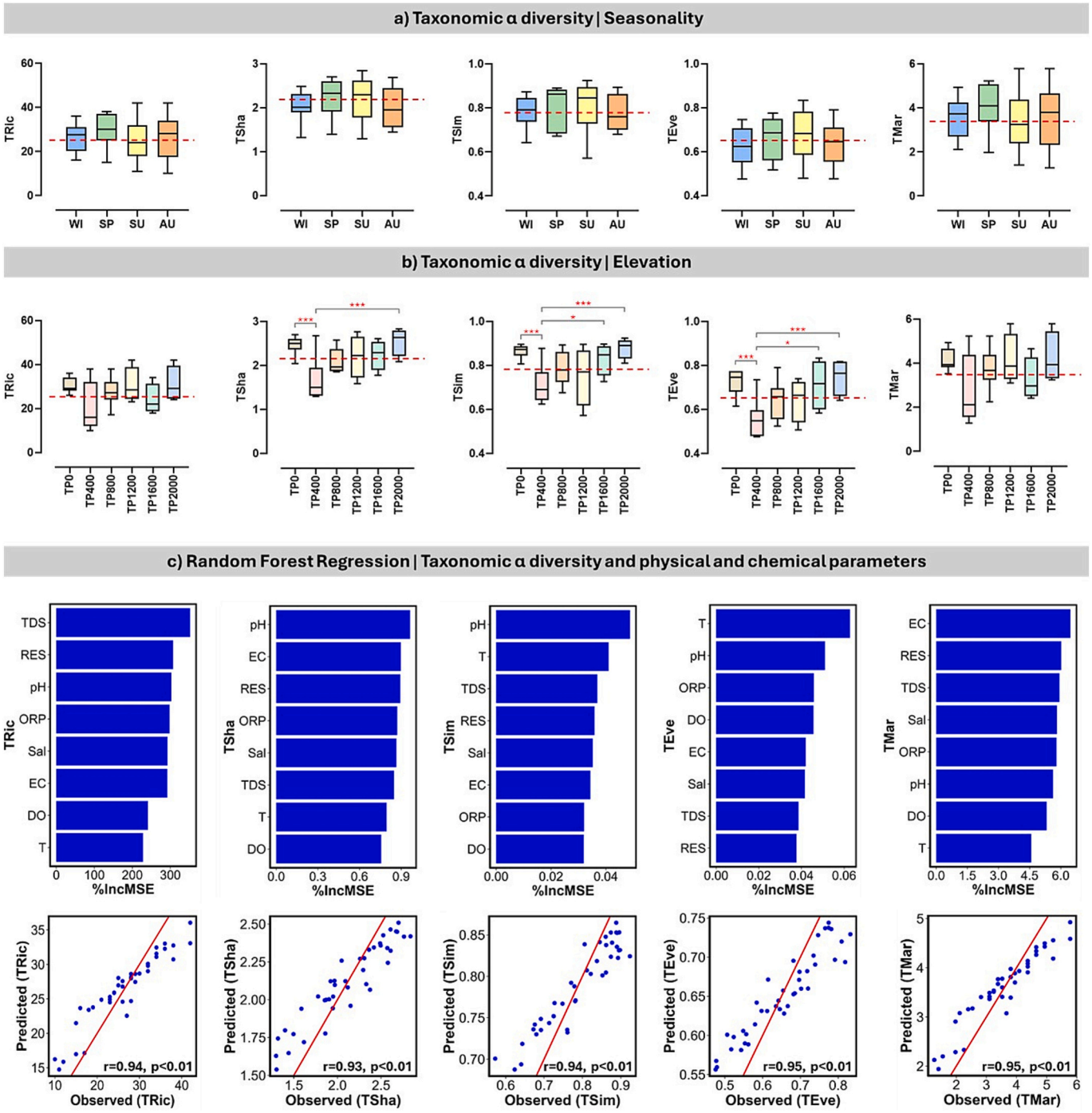

Fig. 3. Taxonomic α diversity values for the five indices: a) across seasons; b) across elevations. c) Above: Random forest regressions showing the 8 physical and chemical predictors for taxonomic α diversity indices; Below: Pearson's correlation tests. %IncMSE = increased in mean squared error (%). TRic = Taxonomic richness; TSha = Taxonomic Shannon; TSim = Taxonomic Simpson; TEve = Taxonomic evenness; TMar = Taxonomic Margalef. WI = Winter; SP = Spring; SU = Summer; AU = Autumn. DO = Dissolved oxygen; EC = Electrical conductivity; ORP = Oxygen Reduction Potential; RES = Resistivity unit; Sal. = Salinity; T = Temperature; TDS = Total Dissolved Solids. Asterisks indicate significant differences based on the Kruskal-Wallis test and Dunn's post hoc (***$p < 0.001$; *$p < 0.05$).

The db-RDA analysis revealed that the first two axes accounted for 10.74% of the seasonal total variation (RDA 1 = 7.43%, RDA 2 = 3.31%) and 10.49% of the elevational total variation (RDA 1 = 7.19%, RDA 2 = 3.3%), with no significant effects of physical and chemical parameters on functional β diversity (Season, F = 0.68, $p = 0.72$; Elevation, F = 0.66, $p = 0.70$) (Fig. 6c). Permutational analysis highlighted that no physical and chemical parameters can be considered important predictors for functional total β diversity.

4. Discussions

Our study aimed to assess, for the first time, the effect of two significant geographical factors, elevation and seasonality, on diatom communities in TPs, a field where existing research is limited (Taurozzi and Scalici, 2025). The novelty of our work lies in adopting an integrated approach that simultaneously considers taxonomic and functional α and β diversity in relation to both broad-scale geographical gradients and local physical and chemical conditions across contrasting

Table 2
| Dunn's post hoc pairwise z-values and *p*-values for variables with significant Kruskal-Wallis results.

Index	Comparison	z-value	p-value
TSha	TP0 – TP400	3.5	0.0004
	TP400 – TP2000	3.3	0.0001
TSim	TP0 – TP400	3.4	0.0006
	TP400 – TP1600	2.1	0.028
	TP400 – TP2000	3.3	0.001
TEve	TP0 – TP400	3.1	0.001
	TP400 – TP1600	2.4	0.01
	TP400 – TP2000	2.9	0.003

environmental contexts.

4.1. Seasonal composition of guild-based benthic diatom communities

Guild-based community analysis higlighted no significant variations across seasons both for motile and low-profile guilds, partially supporting our H1. Motile guild species show the ability to move actively, which helps them navigate through their environment and avoid sedimentation (Rimet and Bouchez, 2011). The observed similar occurrence of this guild among different seasons is consistent with the unstable conditions of TPs, which are subject to large daily and seasonal variations (Van den Broeck et al., 2015). Similary, low profile guild species are generally small and attached to the substrate by their entire valve area which helps them withstand high currents and physical disturbances. Their ability to resist to unfavorable conditions, generally present in all the season in TPs, make them suitable for inhabiting ephemeral waterboides, making their presence in all seasons predictable.

Differently, high-profile diatoms, often found in nutrient-rich environments, are sensitive to physical disturbances which can disrupt their growth and stability (Tapolczai et al., 2016). Given their affinity for nutrient-rich waters, our findings are consistent with their ecology: our results higlighted different abundances of high-profile guilds between summer and winter and between summer and autumn, confirming previous research (Snell et al., 2019). We could hypothesize that elevation amplified differences between seasons, given that nutrient enrichment during the summer season is more pronounced in high-elevation ponds (Gardner et al., 2008).

4.2. Seasonal and elevational α diversity in species- and trait-based diatom communities

Taxonomic α diversity did not reflect benthic guild-based outcomes: seasonality appeared not influencing diatom communities confirming our H2, differently from Liu et al. (2025), where spring showed significant lower values of diatom richness. Previous studies have observed bimodal patterns in diatom species richness, with peaks occurring during specific seasons, such as February–March and possibly another peak later in the year (Breton et al., 2021). However, the lack of statistical significance observed here suggests two main possible explanations: a) diatom communities in TPs reach taxonomic stability in a relatively short time, without the alternation of pioneer species and secondary and tertiary colonizers; or, in alternative, b) diatom communities are largely resistant to large-scale environmental changes (seasonal variations). The first hypotesis was supported by Taurozzi and Scalici (2024a), were no statistical differences emerged between TPs, permanent ponds and wetlands. Differently, although diatom communities exhibit resilience, their resistance to large-scale environmental changes might be limited, as they are influenced by factors such as nutrient supply and water temperature (Liu et al., 2022; Wang et al., 2023b).

Functional α diversity (considering all the 15 traits) showed similar patterns between different seasons. Our results partially agree with Liu et al. (2025), further confirming the limited influence of the succession of seasons on functional composition of diatom communities; on the other hand, taxonomic α diversity, here reported as being similar among seasons, was statistically different in Liu et al. (2025), where significant differences between spring and the other seasons were reported.

An interesting finding of our study was that taxonomic and functional α diversity differed significantly among elevations, confirming H2. Specifically, the most diverse diatom communities were observed in high-elevation ponds, in agreement with Blanco et al. (2020). This pattern is consistent with other studies reporting that elevation influences diatom diversity through changes in environmental conditions such as water temperature, ionic content, and dissolved oxygen (Şahin & Barinova, 2022; Feret et al., 2017). These environmental gradients can affect species distribution and abundance. For example, high-elevation conditions, which are more extreme and variable, may favor only a subset of well-adapted species. This can lead to decreases in evenness, as reflected by the Simpson index, and corresponding shifts in the Shannon index, which integrates both richness and evenness (Wu et al., 2024b). Differences in functional traits, such as motility and nutrient uptake strategies, may also contribute to these patterns, as species respond variably to environmental constraints (Padula et al., 2021). Overall, our results highlight that elevation acts as a key driver of both taxonomic and functional diversity. In our study, TSha, TSim, and TEve showed highly significant differences between low- and high-elevation ponds, suggesting that harsher conditions at higher elevations can filter species, altering community composition and functional diversity. On the contrary, fluctuations in functional diversity indices (FDiv, FDis, RaOQ) were observed in all the sites considered, regardless of elevation. FDiv was significantly higher in comparisons such as TP0–TP400 and TP400–TP1200, indicating that the dominant species in these ponds were functionally more divergent, suggesting the presence of specialized strategies among the most abundant diatoms. Analogous patterns have been observed in studies of diatom communities in stress-prone or highly variable systems. For instance in Central European soda pans, trait-based analyses showed that under harsh conditions functional diversity is reduced via filtering (Stenger-Kovács et al., 2019) In periphytic diatoms, microhabitat heterogeneity was shown to increase functional dispersion (FDis) and divergence (FDiv) (Nemes-Kókai et al., 2024). These patterns suggest that diatoms, due to their small size and rapid growth rate, are particularly sensitive to local environmental factors (Tapolczai et al., 2016), and that functional diversity in these ponds may respond more to local habitat variability than to general seasonal trends.

4.3. Diversity of benthic diatoms in relation to abiotic factors

Our results showed that taxonomic and functional α and β diversity, along with their components, responded differently to physical and chemical variables, supporting H3. Our findings are consistent with previous research showing that environmental factors such as T, pH, and conductivity (EC) shape diatom communities. In Mediterranean high-elevation lakes, differences in water chemistry were linked to distinct diatom assemblages (Padula et al., 2021), while other studies in coastal lagoons and rivers have highlighted both significant (Fernández-Moreno et al., 2024; Flower et al., 2012; Ali et al., 2015) and minor effects of EC on diatom diversity (Stefanidou et al., 2020). In the ponds analyzed here, variations in T, pH, and EC likely acted as filters, favoring species with traits adapted to specific conditions, which is reflected in both taxonomic and functional diversity indices. Functional traits, including motility and nutrient uptake strategies, further mediate these responses, allowing communities to cope with environmental heterogeneity.

It must be noticed that the physical and chemical parameters investigated explained very few variations of both taxonomic and functional β diversity components. This findings implied the main limit of our research, i.e. the possible influence of abiotic (nutrients, light availability, flow regime, trampling, pesticides and other contaminants) and biotic factors (competition, grazing and predation) not considered here. Indeed, the lack of significant relationships between physical and

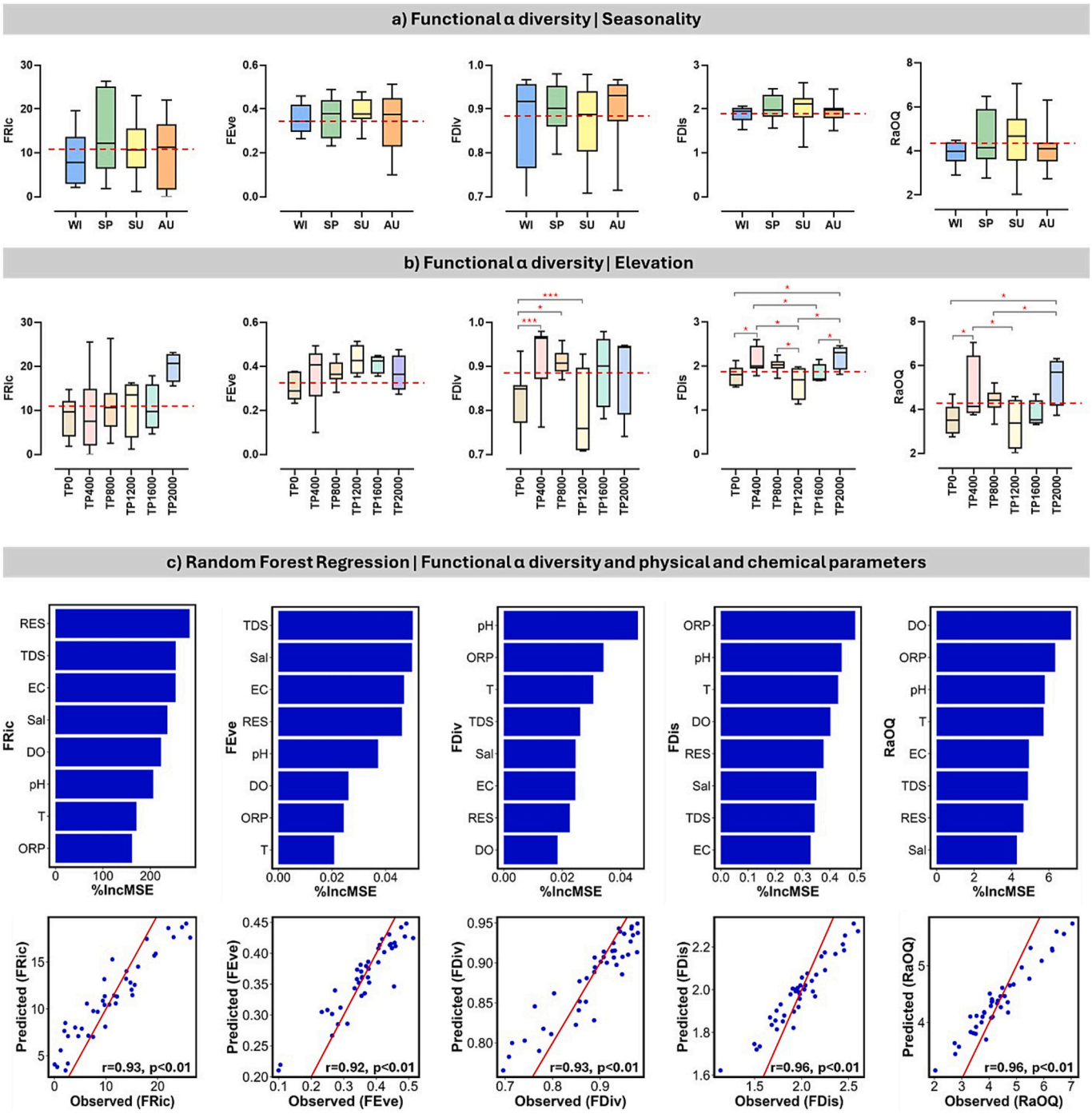

Fig. 4. Functional α diversity for the five indices: a) across seasons; b) across elevations c) Above: results of Random forest regressions showing the 8 physical and chemical predictors for functional α diversity indices; Below: Pearson's correlation. %IncMSE = increased in mean squared error (%). FRic = Functional richness; FEve = Functional evenness; FDiv = Functional divergence; FDis = Functional dispersion; RaOQ = Rao Quadratic Entropy. WI = Winter; SP = Spring; SU = Summer; AU = Autumn. DO = Dissolved oxygen; EC = Electrical conductivity; ORP = Oxygen Reduction Potential; RES = Resistivity unit; Sal. = Salinity; T = Temperature; TDS = Total Dissolved Solids. Asterisks indicate significant differences based on the Kruskal-Wallis test and Dunn's post hoc (***$p < 0.001$; *$p < 0.05$).

chemical parameters and functional β diversity suggests that other factors might play a predominant role in shaping diatom community structure. However, our outcomes are not consistent with previous studies: following Blanco et al. (2020), the lack of general trends in diatom communities pattern suggests that diatom diversity could be more affected by local environmental factors rather than by climatic variables that are associated with elevation. Here, although the db-RDA model explained low variability, physical and chemical factors emerged as significant drivers influencing diatom taxonomic diversity along the elevational gradient. Differently, physical and chemical parameters appeared not driving diatom functional β diversity along the elevational gradient, as they do in different seasons for taxonomic and functional diversity: these findings underscore the resilience of functional β diversity in diatom communities, with functional traits exhibiting stability despite environmental variation caused by both seasonal and elevational fluctuations.

In addition to the physical and chemical factors analyzed here, further spatial processes such as geographical distance among sites,

Table 3
| Dunn's post hoc pairwise z-values and *p*-values for variables with significant Kruskal-Wallis results.

Index	Comparison	z-value	p-value
FDiv	TP0 – TP400	3.1	0.002
	TP0 – TP800	2.0	0.04
	TP400- TP1200	2.7	0.007
FDis	TP0 – TP400	2.4	0.01
	TP0 – TP2000	2.3	0.02
	TP400 – TP1600	2.1	0.03
	TP800 – TP1200	1.9	0.04
	TP1200 – TP2000	2.4	0.01
	TP1600 – TP2000	2.1	0.03
RaOQ	TP0 – TP400	2.4	0.01
	TP0 – TP2000	2.3	0.02
	TP400 – TP1200	2.1	0.03
	TP1200 – TP2000	2.1	0.03

pond connectivity, isolation, and dispersal limitation can strongly influence diatom communities in TPs (Blanco et al., 2020). For completeness, we refer the reader to Taurozzi et al. (2025), which specifically addressed these aspects using the same set of samples and study sites. This allows our two studies to be read in a complementary way, highlighting both local and regional drivers of diatom diversity in TPs.

4.4. Seasonal and elevational β diversity fluctuations in species- and trait-based diatom communities

Unexpectedly, we found that the components of taxonomic and functional β diversity did not vary as a function of seasonality and elevation. Differently, as expected (H4), our results found that both taxonomic and functional total β diversity where mainly driven by the elevational gradient rather than seasonality, with the turnover component explaining most of the observed β diversity, except for seasonal functional diversity. As demonstrated here, nestedness contributes to β diversity by explaining variations in species richness between sites, but its contribution is typically smaller compared to turnover (Baselga, 2010). In diatoms, significant species composition shifts along environmental gradients, such as those seen in elevational gradients, can result in high turnover (Wu et al., 2024). Our findings are consistent with previous studies on benthic algae (Jamoneau et al., 2018) and diatoms (Wu et al., 2023) where taxonomic β diversity was predominantly attributed to turnover, while nestedness, was moslty influent in functional β diversity, Possible reasons behind this patterns include that: a) higher elevations may impose stronger constraints on functional traits (e.g., temperature tolerance, nutrient use), leading to a nested functional structure where only a subset of traits can persist in harsher conditions (Wang et al., 2023a); b) some species may be replaced taxonomically, but their ecological roles are maintained, leading to lower taxonomic nestedness but stronger functional nestedness; c) functional traits adapted to lower elevations may not persist at higher elevations, creating a nested structure where high-elevation communities are functionally filtered subsets of lowland ones.

However, these results, both and functional total, showed statistically significant shifts among seasons and elevations for taxonomic β diversity, and no statistically significant shifts among seasons and elevations for functional β diversity, challenging H4 but partially in line with previous findings. In recent studies, diatom taxonomic β diversity has been found to be relatively stable across seasons, despite changes in community composition, due to factors like functional redundancy and effective dispersal mechanisms (Virta et al., 2020). On the contrary, regarding elevation, while specific studies on diatoms and elevational β diversity are limited, research on environmental gradients suggests that β diversity can be high in areas with significant environmental changes, such as estuaries or mountainous regions (Virta et al., 2020; Eliasz-Kowalska et al., 2022). With different species assemblages linked to each season and location, our findings suggest that seasonal and elevational variations significantly influence the taxonomic diversity of diatom communities, while functional diversity is overall similar across seasons and elevations.

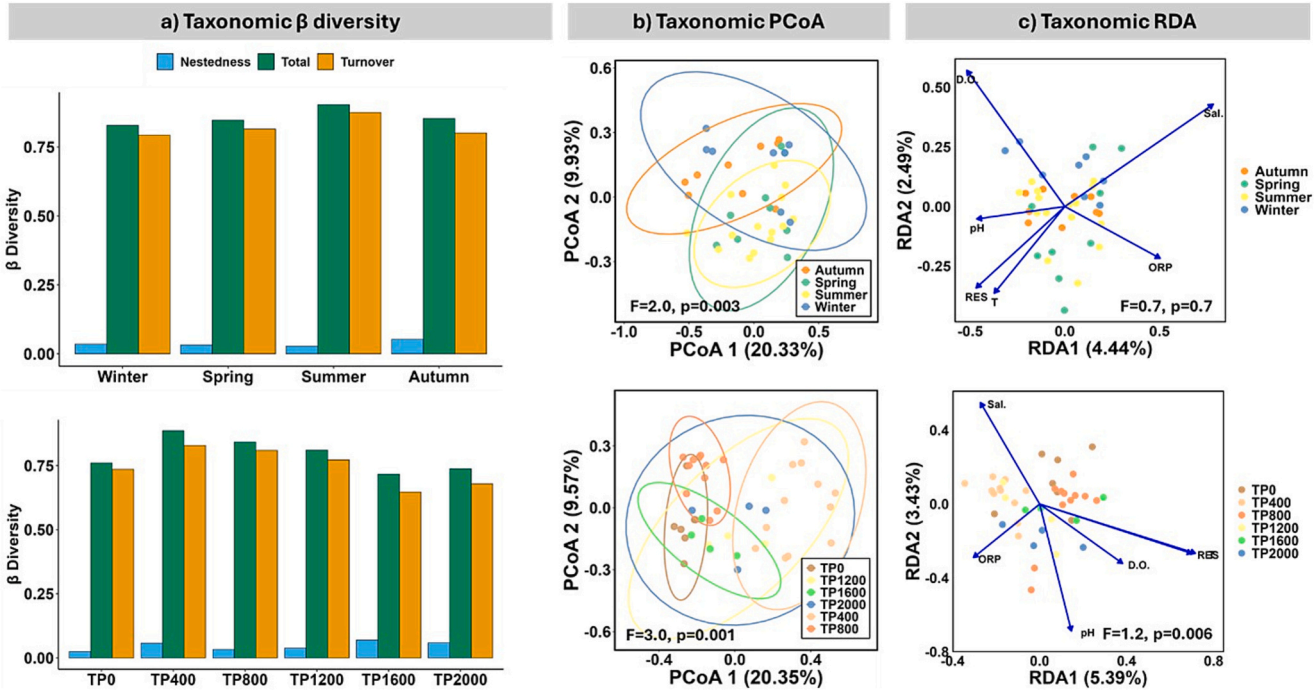

Fig. 5. a) Statistic description of taxonomic β diversity components (total, turnover, and nestedness) for seasons and elevational gradient; b) PCoA of seasonal and elevational β diversity of the taxonomic diatom communities; c) Plots of Redundancy Analysis for Seasonal and elevational taxonomic β diversity. DO = Dissolved oxygen; EC = Electrical conductivity; ORP = Oxygen Reduction Potential; RES = Resistivity unit; Sal. = Salinity; T = Temperature.

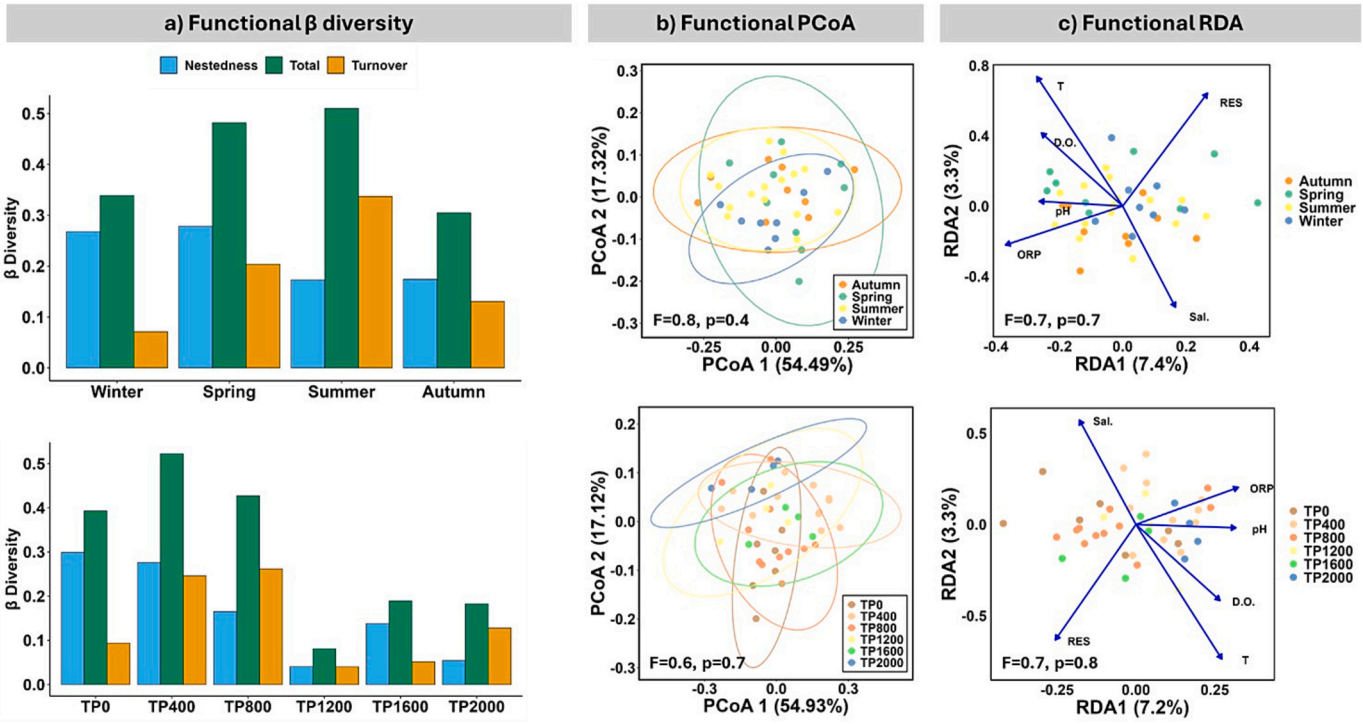

Fig. 6. a) Statistic description of functional β diversity components (total, turnover, and nestedness) for seasons and elevational gradient; b) PCoA of seasonal and elevational β diversity of the guild-based diatom communities; c) Plots of Redundancy Analysis for seasonal and elevational functional β diversity. DO = Dissolved oxygen; EC = Electrical conductivity; ORP = Oxygen Reduction Potential; RES = Resistivity unit; Sal. = Salinity; T = Temperature.

4.5. Limitations of the study

Despite the novel insights provided by our research, several limitations should be acknowledged. First, the lack of comprehensive nutrient data across all ponds and seasons restricts our ability to fully assess the role of trophic status in shaping diatom community composition. Parameters such as total phosphorus (TP), inorganic nitrogen forms (ammonium, nitrite, nitrate), orthophosphate, and proxies of primary productivity (e.g., chlorophyll-a and water transparency) were not consistently measured. These variables can be useful to understand algal growth potential and ecosystem productivity, and their absence limits interpretations on the nutrient–diatom diversity relationship. However, such analyses were not feasible due to budgetary constraints and limited access to the necessary instrumentation.

Additionally, the spatial and temporal resolution of our dataset was uneven, particularly in the high-elevation TPs (1200–1600 m a.s.l.), where only 4 out of 12 samples were collected, with 3 of them limited to the summer season. This limited dataset reduces our ability to draw robust conclusions about seasonal variation in those sites. It is important to highlight that, due to the unpredictable hydroperiod of temporary ponds, sampling was conducted whenever water was present and accessible, an inherent challenge when working in such dynamic and ephemeral environments. Moreover, the elevational gradient was represented by only six discrete levels, which inevitably reduces the generalizability of our conclusions.

Considering data analysis, although Hill numbers offer a more consistent and interpretable framework for biodiversity assessment, we used Shannon and Simpson indices to maintain comparability with the scarce previous studies on diatom communities in temporary ponds. Future research would benefit from the adoption of Hill numbers to provide a unified basis for biodiversity comparisons.

Finally, given the scarcity of studies on diatom communities in TPs, our interpretations were necessarily based on comparisons with other permanent freshwater systems, such as streams and lakes. However, these ecosystems differ markedly in terms of hydroperiod, connectivity, and ecological dynamics. Therefore, increasing the number of studies specifically focused on TPs, especially from different biogeographical regions, is crucial to enable more appropriate and ecologically meaningful comparisons.

5. Conclusions

In a world increasingly affected by anthropogenic pressures, climate change, and water scarcity, temporary ponds are becoming ever more exposed to intense, sometimes irreversible, stress. Our study represents a starting point for investigating the factors shaping diatom communities in these fragile ecosystems, offering valuable insights for future conservation efforts. We observed that elevation, rather than seasonality, was the main driver of diatom community structure. This suggests that local environmental gradients linked to altitude, such as changes in water chemistry or hydrological regimes, exert stronger control than short-term seasonal fluctuations. Functional diversity, in contrast, appeared relatively stable, indicating that diatoms may maintain similar functional strategies even under variable conditions, possibly reflecting resilience mechanisms typical of fast-growing organisms with diverse trait repertoires. The identification of specific chemical parameters, such as ORP and TDS, as significant predictors of functional α diversity further underlines the importance of fine-scale abiotic filters in shaping community assembly. This supports the view that temporary ponds are not homogeneous habitats but rather mosaics of environmental conditions where both local and regional drivers interact in complex ways.

From a broader perspective, our findings emphasize the value of using diatom communities as bioindicators for monitoring ecosystem health in temporary ponds. Conservation strategies should consider elevation-related variability as well as local water chemistry to better predict biodiversity responses under scenarios of climate change and water scarcity.

CRediT authorship contribution statement

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

Consent to participate

Not applicable.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2026.114696.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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CHAPTER VIII

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RESEARCH

Regional γ Diversity of Diatoms in Mediterranean and Alpine Temporary Ponds

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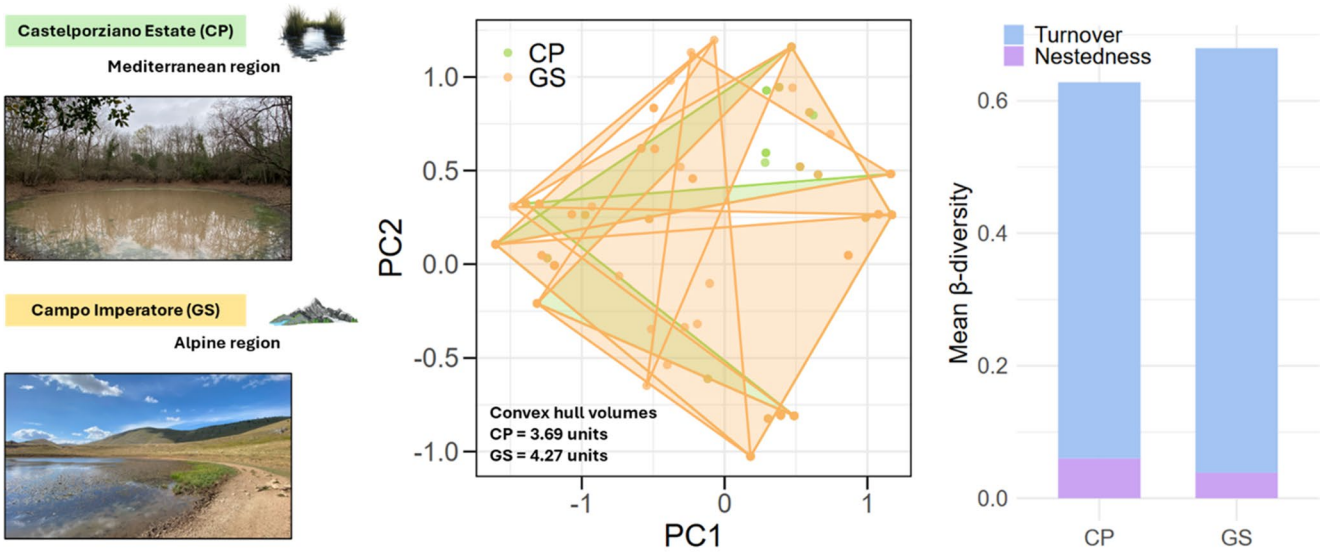
Abstract

Temporary ponds, characterized by periodic or intermittent hydroperiods, are globally widespread in all the biogeographical regions and host peculiar biotic communities. Here we investigated shifts in diatom community assemblages across two contrasting biogeographical regions in Italy, the Mediterranean and the Alpine. The study focused on 24 temporary ponds, with 12 ponds sampled at Castelporziano (CP) and 12 at Campo Imperatore (GS). Our results highlighted that γ diversity varied significantly between the two study sites, indicating a notably greater species richness in GS compared to CP. In GS, functional richness values were generally higher, whereas no significant differences were detected for functional distance and functional divergence. Species composition differed significantly between CP and GS indicating that the two sites host distinct communities, with species turnover (0.904) which contributed most to total beta diversity (0.926), while nestedness (0.021) was negligible. CP communities were characterized by pronounced functional clustering in specific sites while GS exhibited both clustering and slight overdispersion. However, although GS communities occupy slightly larger trait space, both regions shared most functional strategies, reflecting substantial redundancy in functional traits across the two environments. Overall, diatom communities in the GS were characterized by higher frequencies of small, mobile, low-profile, and mucilaginous-tube taxa, whereas CP ponds displayed relatively higher representation of larger or motile forms. Although our study is a starting point, large-scale analyses of diatom communities are crucial, as climate change may rapidly and irreversibly alter taxonomic and functional diversity, profoundly affecting the ecology of these temporary habitats and surrounding landscapes.

Graphical Abstract



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Introduction

Temporary ponds (hereafter TP) are unique and peculiar ecosystems, characterized by periodic hydroperiods, i.e. the alternance of wet and dry phases [1]. Although there is still no consensus on a single definition of ponds, we adopted one of the most recent definitions [2], which describes ponds as small (< 5 ha), shallow (< 5 m) waterbodies with < 30% emergent vegetation. Several factors, including light penetration, turbidity, dissolved organic matter, water supply and nutrients may influence the ecology and productivity of TP [3–5].

Hydroperiods in TP can be periodic or intermittent, depending on the presence or absence of water supply during a specific period of the year [6]. The summer season in Southern Europe, typically marked by drought and low precipitation, represents the most critical phase for TP, in particular in the Mediterranean biome [7, 8]. As a consequence, dryness excludes the more commonplace plant and animal communities, which are characteristic of more permanent waters [8]. However, not all TP show the same annual alternance of wet and dry phases. For instance, TP in Alpine biomes may experience two distinct "dry" periods: one in late summer, caused by evaporation at high temperatures, and another in winter, when ponds are either frozen or lack available water due to snow cover and low temperatures [9]. Alpine TP, also referred to as high-elevation ponds, are generally defined as shallow, small, and temporary aquatic habitats located above 300 m a.s.l [10].

Despite differences in seasonality, both Mediterranean and Alpine ponds undergo recurrent dry phases of variable duration. They are nevertheless considered key elements in landscape ecology, acting as biodiversity hotspots and reservoirs for rare species, as well as stepping stones that facilitate dispersal processes [10–13]. Furthermore, TP support entire trophic networks by providing suitable habitats for a wide range of organisms. In particular, they host primary producers, which form the basis of ecosystem structure and functioning [14–16].

Among primary producers, diatoms represent a driving force of primary production in aquatic ecosystems [17, 18]. Diatoms are unicellular algae capable of contributing approximately 20–25% of all dissolved oxygen to global net production [19]. Each species is characterized by a highly resistant silica skeleton, which enables accurate taxonomic identification [20, 21]. These characteristics, in addition to being ubiquitous, easy to sample, and each characterized by an optimum of nutrients and chemical-physical parameters, make them excellent bioindicators [22]. For these reasons, they were included in the Water Framework Directive as one of the five most recommended bioindicators for environmental monitoring [23, 24]. Despite their ecological importance, diatoms are still primarily studied in permanent aquatic systems, while their role in ephemeral waters remains largely underexplored [9]. Nevertheless, like other aquatic microorganisms, diatoms display unique adaptations to temporary habitats, including motility, specialized substrate attachment strategies, and the ability to withstand unfavorable seasons [25].

These adaptations are also shaped by larger-scale factors, including the physical, spatial, and geographical characteristics of the environmental context in which these communities occur. Comparing the same habitat across different biogeographical zones can help identify the main drivers of diatom diversity [26]. Gamma diversity (γ) represents the level of diversity observed when considering the set of communities within a broader area, linking local diversity (alpha, α) with differentiation among communities (beta, β) [27, 28]. In other words, it reflects the overall richness of species and functional traits within an ecological landscape [29]. For TP, which are often isolated and highly heterogeneous habitats [30], γ diversity provides insights into how local processes of colonization, extinction, and adaptation fit into a larger framework governed by climatic, geographical, and hydrological factors. This approach is particularly valuable when comparing Alpine and Mediterranean ecosystems. While algal communities in each pond reflect specific local conditions [31], their diversity patterns reveal the broader ecological and biogeographic variability of these regions. The Mediterranean biome is characterized by a warm, arid climate with strong hydrological seasonality [7, 32], whereas the Alpine biome features cold conditions and marked altitudinal gradients, which differentially influence the structure and composition of aquatic communities [33, 34]. In these contrasting biogeographical contexts, diatom communities are primarily shaped by a combination of abiotic and biotic drivers acting at both local and regional scales. While temperature and hydrological seasonality define the general ecological framework, other factors such as nutrient availability, conductivity, pH, light regime, and habitat stability could exert stronger and more immediate control on diatom distribution patterns [35–37]. In Mediterranean ponds, recurrent desiccation, high irradiance, and elevated ionic content often lead to community assemblages dominated by taxa tolerant to desiccation and osmotic stress [38]. Conversely, Alpine ponds are influenced by lower temperatures, reduced nutrient concentrations, and strong altitudinal gradients, which favor species adapted to oligotrophic and cold-water conditions [39]. Such differences in

community structure and function are expected to become even more pronounced under future climate change, driven by rising temperatures, shifting hydrological regimes and nutrient availability and the intensification of extreme events [40–42].

From a global perspective, the conservation of TP is critically overlooked, mainly because their ecological recognition has historically been neglected. Moreover, their degradation and disappearance have steadily increased over the past centuries [11]. Analyzing shifts in diatom community assemblages across different biogeographical contexts represents a crucial first step toward understanding how future climate change may reshape these communities under contrasting environmental conditions. To our knowledge, this study represents the first attempt to investigate variations in γ diversity, defined as the total species diversity within a region or landscape, of diatom communities across TP situated within Mediterranean and Alpine biomes. In particular, we focus on how differences in environmental and biogeographical settings shape community composition and ecological functioning. Highlighting these patterns is urgent, as TP are increasingly threatened by climate change and anthropogenic pressures, and understanding their ecological dynamics is essential to anticipate future biodiversity responses and to guide effective conservation strategies.

Materials and Methods

Study Area and Sampling Activities

The two study areas considered are the Presidential Estate of Castelporziano, representative of the Mediterranean biogeographic region, and the Campo Imperatore plateau, within the Gran Sasso and Monti della Laga National Park, representative of the Alpine region. At each site, 12 temporary ponds (TP) were sampled, for a total of 24 ponds (Table S1 (Fig. 1a, b, c and d)). Pond temporary hydroperiod was derived from both in situ observations and satellite analyses [43, 44].

The Castelporziano Presidential Estate (hereafter CP, 41°44’37"N 12°24’00"E) lies approximately 25 km from the center of Rome and covers an area of 60 km^2 (6,039 hectares), with altitude ranging from 0 to 78 m a.s.l. The estate is largely dominated by hygrophilous lowland forest, composed mainly of evergreen and deciduous oaks, together with more strictly hygrophilous species near wetlands. Other typical environments include coastal dunes, pine forests, cultivated fields and areas used for free-range livestock farming. Ponds of the estate represent remnants of ancient humid environments, flooded forests, and marshes that once extended across a much larger and continuous landscape (Fig. 1e and f, 1 g). Access to the estate is restricted to authorized staff and researchers.

The Campo Imperatore plateau (hereafter GS, 42°24’41"N 13°37’30"E) extends for approximately 20 km in length and 3–7 km in width, with elevations ranging from 1,500 to 2,000 m a.s.l., and is surrounded by the highest peaks of the Apennines, including Corno Grande (2,912 m) and Monte Camicia (2,564 m). The plateau is of glacial and karst-alluvial origin and is almost completely devoid of vegetation, due both to its high altitude and to centuries of deforestation and is currently characterized by extensive wild grazing. Annual precipitation averages around 900 mm, with peaks in autumn. Snow cover persists for most of the year, generally from November to May, and its melting supplies water to natural basins that give rise to TP (Fig. 1 h, i and j).

The wet hydroperiod of Castelporziano ponds extends from October to May, with peak precipitation in January and February; during the boreal summer, scarce precipitation leads to a prolonged dry phase. In contrast, Campo Imperatore TP exhibit two distinct “dry” periods: one during the winter period, from October to April, when ponds are covered by snow and ice, and another during August and early September, when high temperatures and limited precipitation cause complete desiccation. To ensure sampling during the most suitable periods, diatoms were collected in CP in February, corresponding to the maximum of the wet hydroperiod and in GS in July 2024, representing the late stage of the hydroperiod, when ponds are still water-filled but approaching desiccation. The choice of sampling in GS during summer was also constrained by logistical challenges (snow, closed roads) typical of high-altitude sites, which prevent access outside the summer season. This timing was chosen to allow diatom communities to reach a certain degree of stability while avoiding late spring–early summer, when high concentrations of suspended solids can substantially alter community structure [45].

Diatoms were sampled following standard methodologies [45]: For each sample, at least 10 stones (dimensions between 64 mm and 256 mm) were collected exclusively from the shoreline, at a maximum distance of 1 m from the water’s edge and scraped using a commercial toothbrush; then, the toothbrush was immersed in a 50 ml Falcon containing 70% diluted ethanol and distilled water (Fig. 1k). Once in the laboratory, diatom samples were oxidized following the hydrogen peroxide method [45]. Briefly, 5 ml of sample were centrifuged at 1500 rpm for 10 min, the supernatant was discarded, and the sediment was mixed with 20 ml of H_2O_2 and heated for 3 h to degrade organic matter (Fig. 1l). After complete oxidation, the mixture was centrifuged three times for 10 min each, and diatoms were mounted on permanent slides using Naphrax® (high refractive index resin)

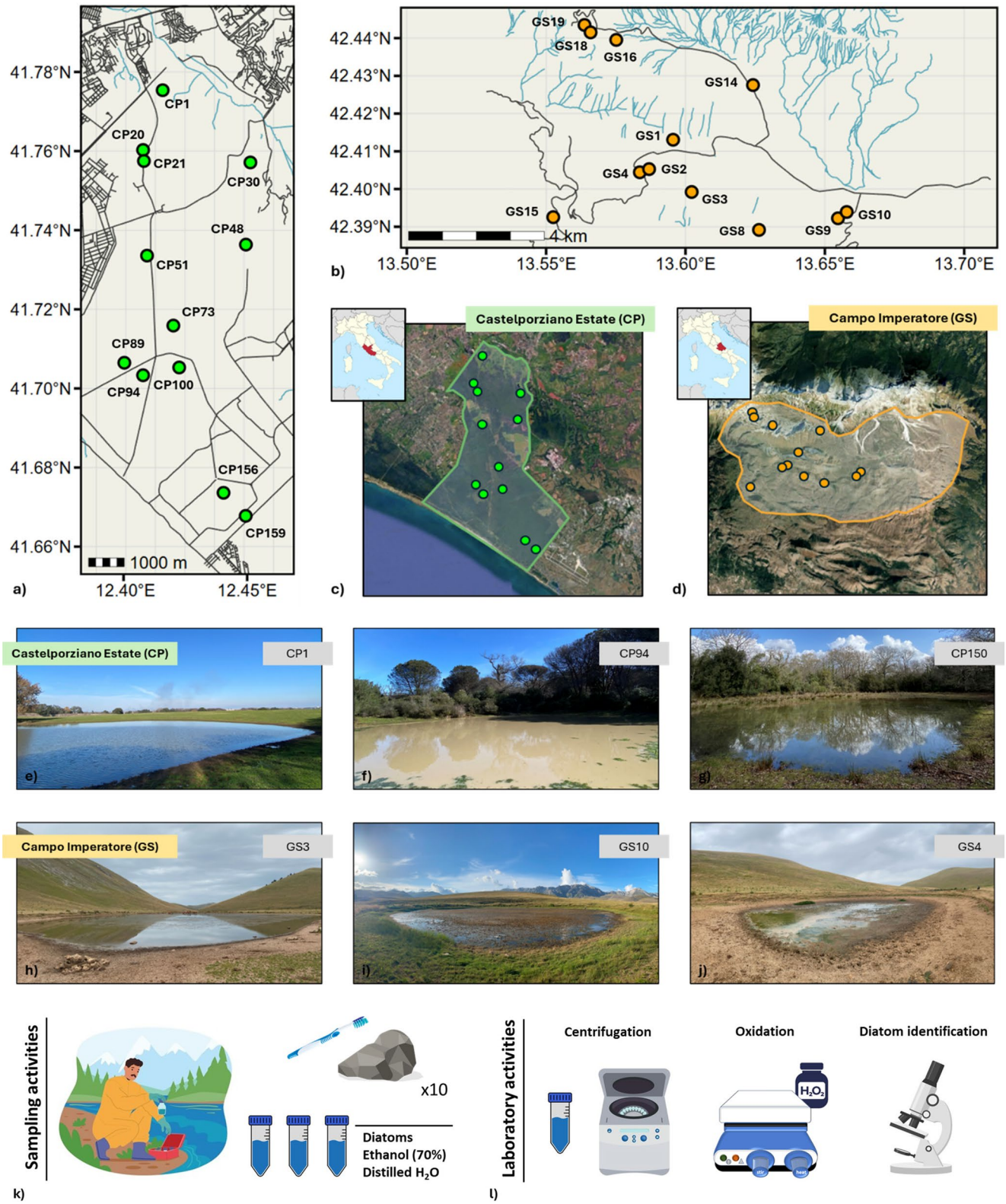

Fig. 1 **a**) Map of sampled ponds in the Castelporziano estate and **b**) in the Campo Imperatore plateau; **c**) geographical distribution of TP in the Castelporziano estate and d) in the Campo Imperatore plateau. Field photos taken by the authors of sampled ponds: **e** - **g**) Castelporziano estate; **h** – **j**) Campo Imperatore plateau. **k**) schematic representation of sampling activities and **l**) laboratory activities for diatom oxidation and identification

to fix the coverslip. For each site, three different replicates were performed [46]. Diatom identification was performed using a Leica microscope at 100× magnification, with the help of specific taxonomic guides [47–54]. For each slide, the analysis was considered completed when identification at the species level of 400 valves was achieved.

Data Analysis

Taxonomic and Functional α and γ Diversity

Taxonomic α diversity was calculated for each site and across all sites combined using the *specnumber()* function from the *vegan* R package [55]. Site-level species richness was then compared between Castelporziano and Campo Imperatore using a Wilcoxon rank-sum test (wilcox.test() in base R). To complement the statistical significance, we calculated the effect size using Cliff's delta (cliff.delta() function from the *effsize* R package) [56]. Cliff's delta is a non-parametric measure of effect size that estimates the probability that a randomly chosen value from one group is larger than a randomly chosen value from another group, minus the reverse probability [57]. In contrast, γ diversity was computed as the total number of species pooled across all ponds within each study area.

To evaluate species accumulation and assess differences in taxonomic richness between sites, species accumulation curves were computed for each study area using the *specaccum()* function from the *vegan* R package [55]. These curves describe the cumulative number of species observed as sampling effort (i.e., number of ponds) increases. To quantify overall species richness across all sampling units, we calculated the area under the accumulation curve (AUC) by summing the cumulative richness values for each site. Differences in cumulative richness between Castelporziano and Campo Imperatore were tested using a permutation approach: pond labels were randomly reassigned across both study areas 10,000 times, species accumulation curves were recalculated for each permutation, and the difference in AUC between permuted groups was recorded. This allowed us to test whether the observed difference in cumulative richness could arise by chance when ponds are freely shuffled between sites. The observed difference in AUC was then compared against the null distribution to obtain a one-tailed p-value, providing a statistical test of whether observed species accumulation differed significantly from random expectations.

Diatoms were classified according to three functional trait categories: size classes, ecological guilds, and life-forms [58–60] (Table S2). Size classes refer to the categorization of diatoms based on their cell size, which influences ecological processes such as growth rate and sedimentation. Ecological guilds group diatoms based on their adaptive strategies to environmental factors like nutrient availability and disturbance, reflecting their ecological roles within the community. Life-forms describe the morphological and behavioral traits related to the diatoms' mode of attachment or mobility, indicating their adaptations to specific microhabitats.

Functional α diversity was calculated for each site and across all sites combined using the *dbFD()* function from the *FD* R package [61]and. Prior to calculation, trait matrices were filtered to include only species present at each site. Principal Component Analysis (PCA) was applied to the trait matrices (*prcomp()* in base R) to reduce dimensionality, retaining up to 10 axes per site depending on species richness. The PCA scores were not interpreted directly but served as input for the subsequent computation of functional indices, ensuring that correlated traits did not bias the metrics. Site-level functional richness (FRic), functional dispersion (FDis), and functional divergence (FDiv) were then calculated for both sites. Functional richness (FRic) quantifies the volume of the functional trait space occupied by a community, reflecting the range of traits present. Functional evenness (FDis) describes the regularity in the distribution of species abundances and their functional dissimilarities within the trait space, indicating how evenly functional traits are represented. Functional divergence (FDiv) measures the degree to which species are spread out in trait space, capturing the extent of trait differentiation among co-occurring species. Total functional diversity across all sites was computed by combining site matrices. Differences between the two study areas for each functional metric were tested using Wilcoxon rank-sum tests (*wilcox.test()* in base R).

To evaluate γ diversity accumulation with increasing sampling effort, species rarefaction curves were generated for each site using the *specaccum()* function from the *vegan* package [55]. For functional rarefaction, cumulative functional diversity metrics, FRic, FDis and FDiv were calculated iteratively for increasing subsets of sampling units (ponds). At each iteration, only species present in the subset were retained, and functional diversity metrics were computed using the *dbFD()* function from the *FD* package [61] and. This procedure was repeated 100 times with randomized order of sampling units to obtain mean and standard deviation of cumulative FRic, FDis, and FDiv for each site. All functional diversity metrics were weighted by species abundance. Differences between Castelporziano and Campo Imperatore were statistically assessed by computing the area under the accumulation curve (AUC) for each metric and performing a permutation test with 10,000 randomizations, thereby testing whether the observed differences in cumulative functional diversity between sites were greater than expected by chance.

Compositional and Functional Analysis

Taxonomic dissimilarities among sites were calculated using the Bray-Curtis distance with the *vegdist()* function from the *vegan* package [55]. Non-metric multidimensional scaling (NMDS) was then performed with *metaMDS()* function from *vegan* package to visualize differences in community composition (k = 2). To statistically test for compositional differences between sites, we applied permutational multivariate analysis of variance (PERMANOVA) using *adonis2()* function from *vegan* package with 999 permutations. In addition, we assessed within-site dispersion (β dispersion) with *betadisper()* function from *vegan* package and tested for significant differences in variability among sites using *anova()* function. β diversity was further decomposed into turnover and nestedness components by converting the abundance matrix to presence-absence and applying functions from the *betapart* R package (*betapart.core()*, *beta.multi()*, *beta.pair()*), providing insights into the relative contributions of species replacement and loss to overall β diversity. To further explore the mechanisms underlying community assembly, we calculated Raup–Crick β-diversity (βRC) using presence-absence data. This metric provides a null-model framework to distinguish whether observed species turnover is primarily driven by deterministic (environmental filtering) or stochastic (neutral) processes. Presence-absence matrices for Castelporziano (CP) and Campo Imperatore (GS) were first aligned to include the same set of species across sites and then combined into a single matrix. Raup–Crick dissimilarities were calculated using the *raupcrick*() function from the *vegan* package with 999 randomizations. Resulting βRC values range from 0 to 1, where values close to 0.5 indicate turnover consistent with stochastic assembly, values near 1 indicate strong deterministic turnover, and values near 0 suggest lower than expected turnover. Mean and standard deviation of βRC were computed across all pairwise pond comparisons to summarize the degree of stochastic vs. deterministic influences on diatom community structure.

To explore functional community structure and assembly processes, we first calculated standardized effect sizes of mean pairwise functional distances (SES.MPD) using the *ses.mpd()* function from the *picante* R package [62], based on species abundances and functional trait dissimilarities computed with *gowdis()* from the *FD* package [61] and. Standardized effect sizes of mean pairwise functional distance (SES.MPD) were computed to quantify the functional assembly of diatom communities and to assess deviations from random expectations under a null model based on taxa labels. Negative SES.MPD values indicate functional clustering, suggesting environmental filtering or niche convergence, whereas positive values indicate functional overdispersion, potentially reflecting competitive exclusion or limiting similarity. This approach allowed us to test whether observed functional structure deviated from null expectations generated by a taxa-label randomization model (null.model = "taxa.labels", 999 runs). Functional dissimilarities among sites were quantified by calculating pairwise Gower distances between species traits (*gowdis()* function) and computing functional diversity indices, including FRic, FDis and FDiv, with the *dbFD()* function from *FD* package. To assess the extent of functional trait space overlap between sites, principal component analysis (PCA) was performed on all species traits using *prcomp()*, retaining the first three principal components. Convex hulls enclosing species positions in the 3D PCA trait space were computed with the *convhulln()* function from the *geometry* R package [63], and their volumes were used to calculate a functional overlap index:

$$Functional\ Overlap\ Index\ = \left(V_{cp} + V_{gs} - V_{combined}\right) / V_{combined} \quad (1)$$

where V_{CP} and V_{GS} are the convex hull volumes of Castelporziano and Campo Imperatore communities, respectively, and $V_{combined}$ is the volume of the combined species set. An overlap index of 0 indicates no functional overlap, while 1 indicates complete overlap. To test whether the observed overlap differed from random expectation, we performed a permutation test with 999 randomizations of species identities across sites and calculated a one-tailed p-value as the proportion of permuted overlaps equal to or exceeding the observed value.

Indicator Species and Traits Analysis

To identify species strongly associated with each site, we applied the indicator species analysis using the *multipatt()* function from the *indicspecies* R package [64], with 999 permutations. This method calculates the association between species presence-absence patterns and site groups, highlighting species that serve as indicators of a particular site. For functional traits, species-by-trait matrices were first converted to binary presence-absence form. Site-specific mean trait values were then calculated by multiplying the binary species occurrence matrices by the trait matrix and averaging across species. This procedure provides a summary of trait prevalence per site, allowing the identification of traits that are characteristic of each community.

Species-Trait Network Analysis

To explore the relationships between species and their functional traits at each site, bipartite species-trait networks were

constructed. In these networks, species and traits are represented as two distinct sets of nodes, and edges connect species to the traits they possess. Species-by-trait occurrence matrices were first converted to binary form and multiplied by the binary trait matrices to establish connections. Networks were then represented as graphs using the *igraph* R package [65]. Network structure was characterized by calculating node degrees (number of connections per species), modularity using the Louvain clustering algorithm (*cluster_louvain()* function), and network density (*edge_density()* function), providing measures of community connectivity and compartmentalization [66]. These metrics allowed the identification of highly connected species ("hub" species) and comparison of overall network structure between the two sites. To test whether observed modularity values were higher than expected by chance, a permutation procedure was applied. Specifically, species–trait matrices were randomized 999 times using the *r2dtable()* function (base R) to preserve row and column sums, generating null networks. Modularity was recalculated for each null network, and empirical p-values were obtained by comparing observed modularity to the distribution of null modularity.

All statistical analyses were performed using R software [67], and significance was assessed at $p < 0.05$.

Results

Taxonomic and Functional α and γ Diversity

Taxonomic α diversity varied significantly between the two study sites. In Castelporziano (CP) (Table S3) the mean α diversity per pond was 16.0 ± 3.77 species, while in Campo Imperatore (GS) (Table S4) it was higher, with 26.17 ± 4.49 species per pond (Table S5 - S6). Wilcoxon rank-sum test confirmed that this difference was statistically significant ($W = 3.5$, $p < 0.001$), indicating a notably greater species richness in GS compared to CP (Fig. 2a). When all ponds from both sites were combined, the total α diversity encompassed the full range of observed species across the study (23–32 species per pond). The effect size, calculated using Cliff's delta, was large and negative ($\delta = -0.95$), confirming that samples from CP generally had lower species richness compared to GS.

Species accumulation curves (γ diversity) revealed marked differences in taxonomic richness between CP and GS. The cumulative species richness across all samples, measured as the area under the accumulation curve (AUC), was substantially higher in GS (AUC = 1014.01) than in CP (AUC = 595.20), with an observed difference of −418.81 (Fig. 2b). A permutation test with 10,000 randomizations confirmed that this difference was highly significant ($p = 0.0002$), indicating that the higher species richness in GS is unlikely to arise by chance. These results suggest that the diatom community in GS is more diverse and exhibits faster species accumulation with increasing sampling effort compared to CP.

Functional α diversity metrics showed differences between the two study sites (Table S6). In CP, Functional Richness (FRic) ranged from 0.01 to 14.47 across ponds, Functional Dispersion (FDis) ranged from 0.63 to 3.01, and Functional Divergence (FDiv) ranged from 0.75 to 0.97 (Fig. 3). In GS, FRic values were generally higher, ranging from 0.02 to 7.40, FDis ranged from 1.16 to 3.04, and FDiv from 0.78 to 0.96. Wilcoxon rank-sum tests indicated that FRic differed significantly between sites ($W = 107$, $p = 0.04$), whereas no significant differences were detected for FDis ($W = 78$, $p = 0.76$) or FDiv ($W = 99$, $p = 0.13$). These results suggest that while the number of functional traits present per pond was higher in GS, the overall distribution and divergence of traits within ponds did not differ significantly between sites.

Functional γ diversity accumulation across sites was quantified using the area under the curve (AUC) of cumulative FRic, FDis and FDiv. The results indicated a marked difference in FRic between the two study areas: CP exhibited a substantially higher cumulative FRic (AUC = 70.06) compared to GS (AUC = 21.30), with an observed difference of 48.76. Permutation test confirmed that this difference was statistically significant ($p = 0.0059$), suggesting that the species in CP occupy a wider range of functional trait space. In contrast, FDis and FDiv showed minimal differences between the two areas (AUC FDis: CP = 28.28, GS = 29.10, $p = 0.7943$; AUC FDiv: CP = 10.93, GS = 10.56, $p = 0.2170$), indicating that the overall trait dispersion and divergence were similar. These results suggest that while the breadth of FRic differs significantly between the sites, the distribution and divergence of traits within the communities are largely comparable.

Compositional and Functional Analysis

Species composition differed significantly among sites within each study area (PERMANOVA, $R^2 = 0.179$, $F = 4.788$, $p = 0.001$), indicating that the two sites host distinct communities (Fig. 4a). Despite this, the dispersion of communities within sites did not differ significantly (betadisper, $F = 0.309$, $p = 0.584$), suggesting that the variability among ponds is similar within each site. β diversity decomposition revealed that species turnover (β SIM = 0.904) contributed most to total beta diversity (β SOR = 0.926), while nestedness (β SNE = 0.021) was negligible (Fig. 4b). Raup–Crick β-diversity (βRC) values, which account for stochastic assembly processes, averaged 0.56 (SD = 0.35)

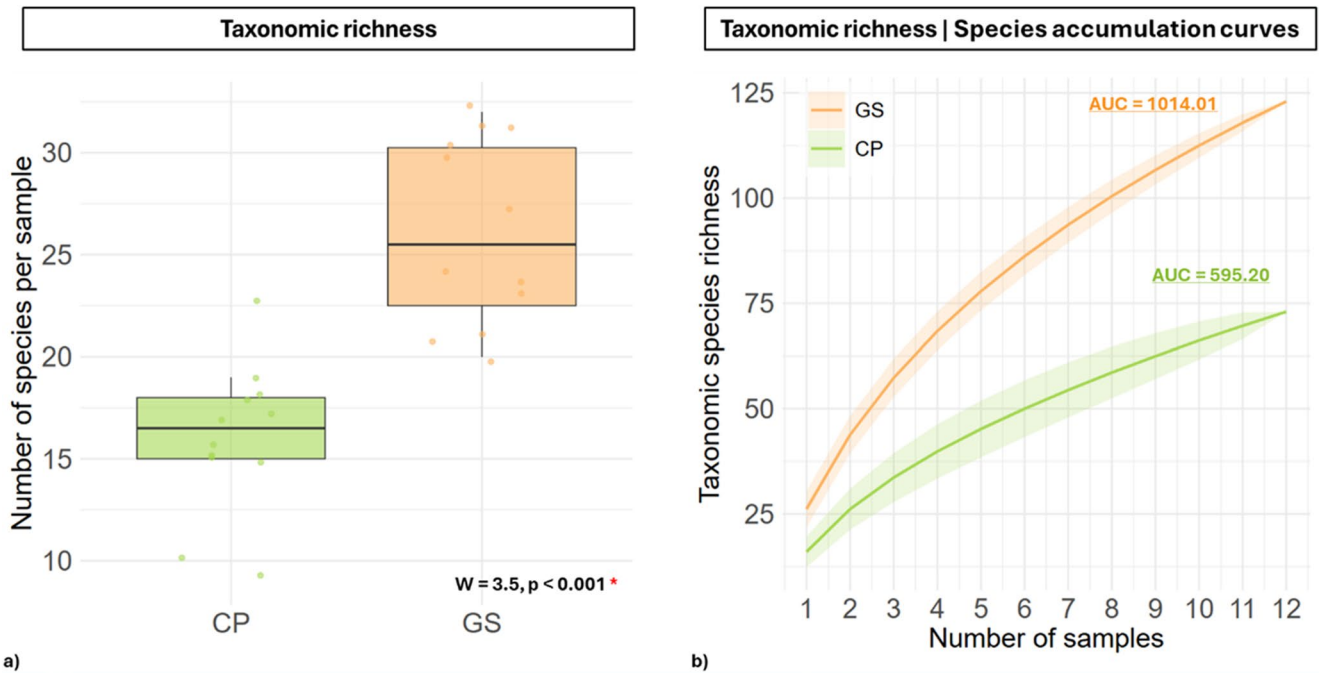

Fig. 2 Taxonomic αdiversity across the two study sites. (**a**) Boxplot of species richness per sample at Castelporziano (CP) and Campo Imperatore (GS), showing individual sample points. (**b**) Cumulative increase (γ diversity) of taxonomic species richness across 12 sampling steps at the two sites, with mean values represented by lines and variability shown as shaded ribbons (±SD). Colors correspond to sites: CP (green) and GS (orange)

between ponds across the two sites. These intermediate βRC values indicate that turnover is influenced both by deterministic environmental filtering, reflecting site-specific conditions and local adaptation, and by stochastic processes such as random dispersal or ecological drift. Ecologically, this implies that differences between sites are primarily driven by replacement of species rather than loss or gain of subsets of species, reflecting site-specific environmental conditions at CP and GS, while variability within each site is partly shaped by neutral assembly mechanisms.

In CP, SES.MPD values revealed pronounced functional clustering in specific sites (e.g., SES = −3,61), although only 1 out of 12 sites reached statistical significance (p<0,05) (Table S7). In contrast, Campo Imperatore exhibited a broader range of SES.MPD values, with both clustering (minimum SES = −2,74) and slight overdispersion (maximum SES = 1,72) observed across sites; 2 out of 12 sites showed significant functional clustering (p<0,05). Despite these local patterns, a Wilcoxon rank–sum test comparing SES.MPD values between the two regions did not reveal a statistically significant difference (W = 89, p = 0,347), indicating that, at the regional scale, the overall functional structure was comparable between CP and GS (Fig. 5a). These results suggest that functional trait distributions are largely similar across sites, but that local environmental constraints can produce site-specific clustering, highlighting the importance of fine-scale environmental heterogeneity in shaping community functional structure.

Functional trait space was compared between CP and GS using convex hull volumes calculated on the first three principal components of the species trait matrix. CP communities spanned a convex hull volume of 3.69 units, while GS communities covered 4.27 units (Fig. 5b). The combined convex hull volume was 4.37 units, resulting in a functional overlap index of 0.819, indicating a high degree of shared trait space between the two regions (Fig. 5c). A permutation test revealed that this observed overlap was not significantly different from random expectations (p = 0.786), suggesting that the functional trait spaces of coastal and mountain communities largely coincide, with only moderate differentiation (Fig. 5c). Overall, these results indicate that although GS communities occupy slightly larger trait space, both regions share some functional strategies, reflecting partial redundancy in functional traits across the two environments.

Indicator Species and Traits Analysis

Indicator species analysis revealed 19 species significantly associated with the two sites (Table S8, Fig. 6a). GS hosted 13 indicator species, including *Achnanthidium minutissimum* (0.764, p = 0.007), *Cocconeis placentula* (0.645, p = 0.033), *Encyonema caespitosum* (0.645, p = 0.044), *E. mesianum*

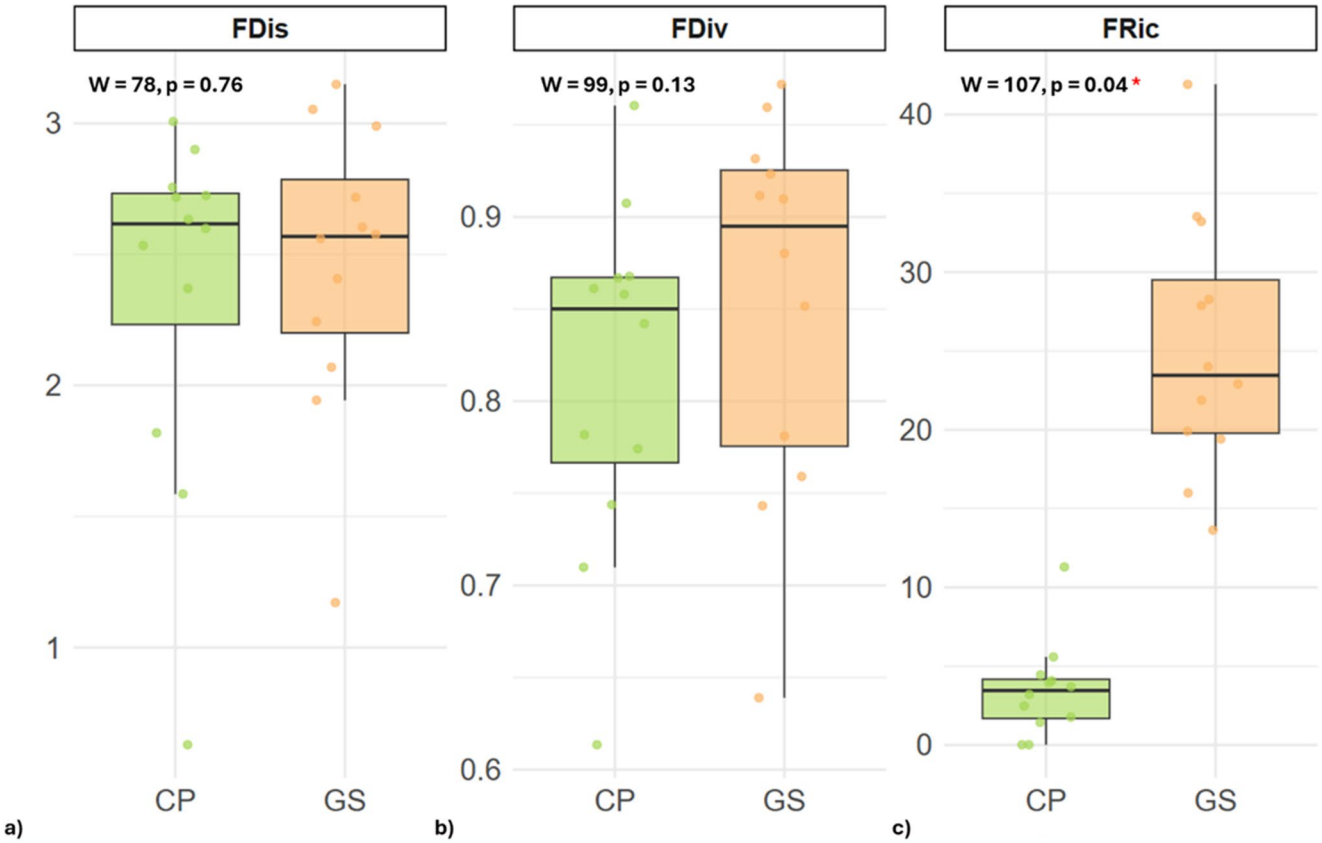

Fig. 3 Functional diversity metrics (**a**, FDis; **b**, FDiv; **c**, FRic) across the two sites, Castelporziano (CP) and Campo Imperatore (GS). Boxplots show the distribution of values for each metric, highlighting differences in functional richness, dispersion, and divergence between sites. Colors indicate site identity: green for CP and orange for GS

(0.707, $p=0.016$), *E. minutum* (0.816, $p=0.004$), *Staurosirella pinnata* (0.764, $p=0.005$), *Gomphonema micropus* (0.707, $p=0.016$), *Navicula cryptocephala* (IndVal=0.849, $p=0.003$), *N. cryptotenella* (0.770, $p=0.010$), *N. trivialis* (0.645, $p=0.034$). *N. veneta* (0.707, $p=0.014$), *Planothidium frequentissimum* (0.730, $p=0.046$) and *Fallacia subhamulata* (0.645, $p=0.037$). CP was associated with six indicator species: *Eunotia bilunaris* (0.645, $p=0.038$), *E. minor* (0.917, $p<0.001$), *Gomphonema zellense* (0.764, $p=0.008$), *Nitzschia filiformis* (0.714, $p=0.030$), *Pinnularia viridiformis* (0.764, $p=0.004$) and *Stauroneis anceps* (0.730, $p=0.041$).

The comparison of functional trait frequencies between CP and GS revealed significant differences for several key traits (Table S9, Fig. 6b). Low-profile forms (LowPro) were significantly more frequent in GS (mean frequency: 5.17 vs. 1.17 in CP; $p=0.003$), and taxa with mucilaginous tubes (MucoTub) were also more abundant in GS (2.00 vs. 0.08; $p=0.003$). Mobile taxa exhibited higher frequencies in GS (19.50 vs. 8.42; $p=0.004$), as did benthic taxa (19.50 vs. 8.42; $p=0.006$). Among attachment traits, high attachment (ATT_Hig) was more common in GS (3.50 vs. 1.00; $p=0.005$), as was ATT_Low (14.25 vs. 11.00; $p=0.005$). Biovolume classes showed notable differences: BioVol_c2 was enriched in GS (10.00 vs. 4.00; $p=0.005$), while BioVol_c5 was slightly more frequent in CP (2.42 vs. 2.33; $p=0.04$). Motile forms tended to be higher in CP (13.17 vs. 9.50; $p=0.08$), although this difference was marginally significant. Other traits, including medium attachment (ATT_Med), high-profile forms (HighPro), pedunculate forms, small biovolumes (BioVol_c1, c3), planktonic, colonial, and pioneer taxa, did not differ significantly between sites. Overall, GS communities are characterized by higher frequencies of small, mobile, low-profile, and mucilaginous-tube taxa, whereas CP shows relatively higher representation of certain larger or motile forms, reflecting distinct functional trait compositions between coastal and mountain environments.

Species-Trait Network

The bipartite species–trait networks revealed differences in the structural properties of diatom assemblages between CP and GS (Fig. 7a and b). In CP, the network exhibited a modularity of 0.32 and a low density of 0.079, indicating a moderately modular but sparse network. The most highly

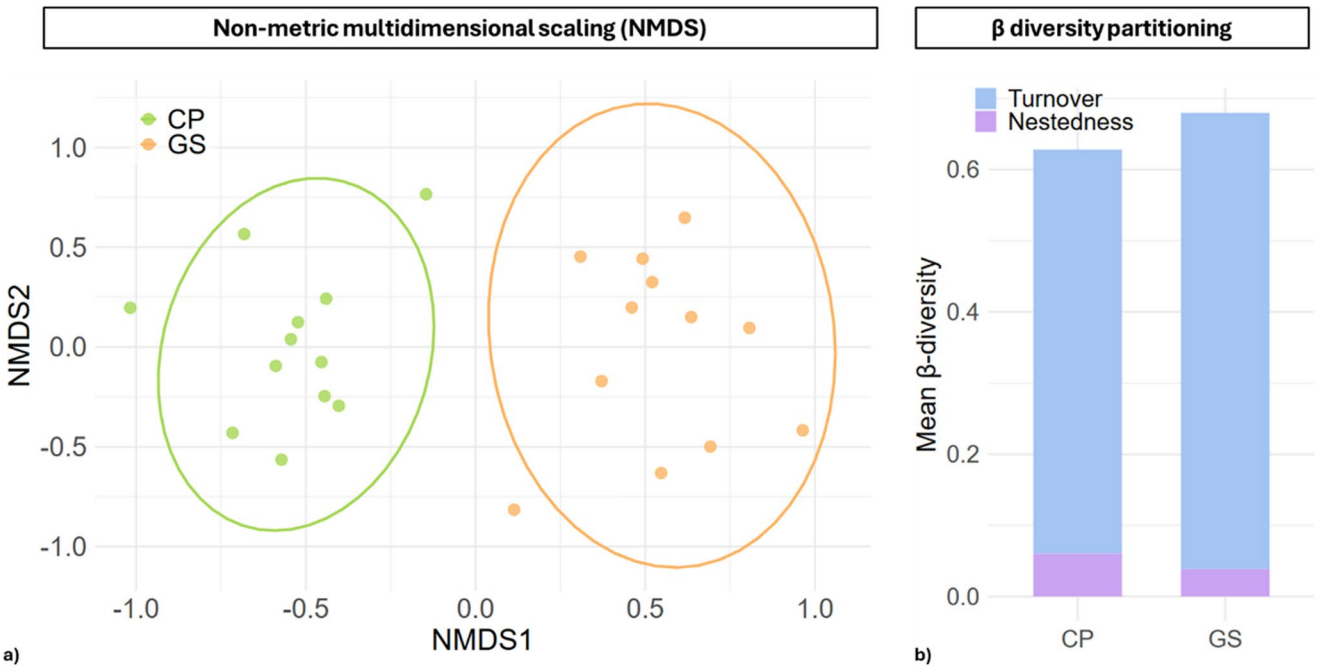

Fig. 4 (**a**) Non-metric multidimensional scaling (NMDS) plot showing the compositional differences in diatom communities between the two sites: Castelporziano (CP, green) and Campo Imperatore (GS, orange). Each point represents a sample, and the 95% confidence ellipses highlight the multivariate dispersion within each site. (**b**) Stacked bar plot illustrating the partitioning of β diversity within each site (CP and GS) into turnover (SIM, blue) and nestedness (SNE, purple) components. The values represent the mean pairwise dissimilarities among samples within each site

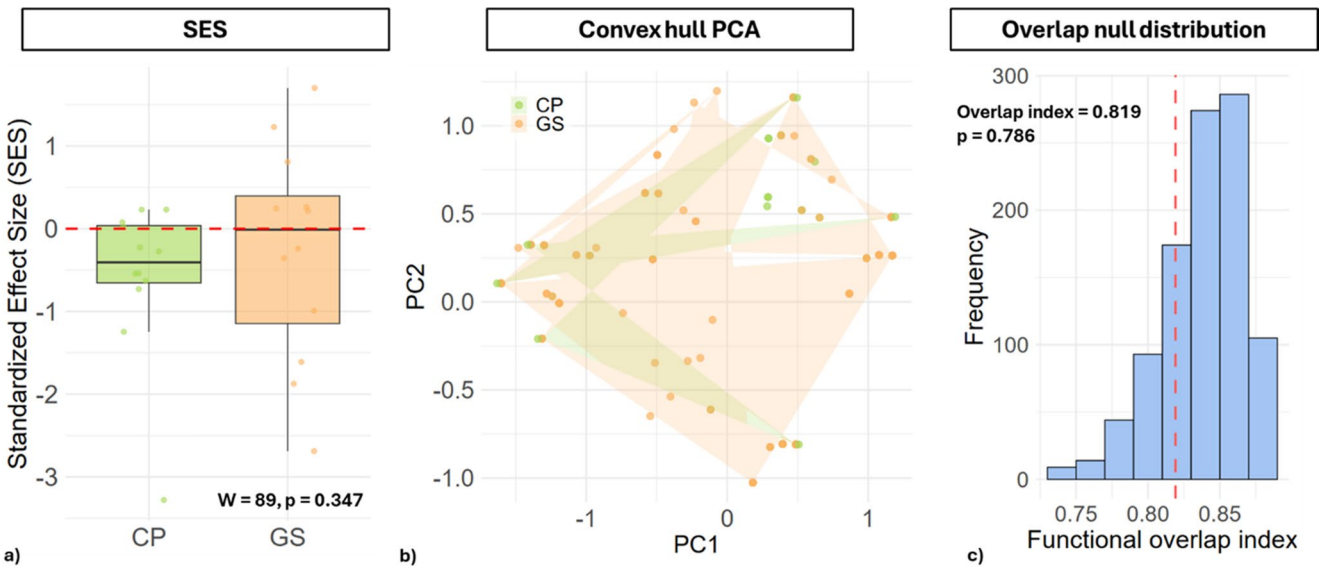

Fig. 5 (**a**) Standardized Effect Size (SES) per site, showing the deviation of observed functional diversity from null expectations. Points represent individual samples, and the dashed red line indicates SES = 0. (**b**) Functional trait space of species in Castelporziano (CP, green) and Campo Imperatore (GS, orange) based on the first two principal components of the PCA. Each point represents a species. Transparent polygons (convex hulls) show the functional space occupied by each site. Areas where the hulls overlap indicate shared trait combinations, while non-overlapping regions highlight functional differentiation between the sites. The size and shape of each hull reflect the diversity of trait combinations within that community. (**c**) Null distribution of the functional overlap index between CP and GS derived from 999 permutations. The dashed red line represents the observed overlap, indicating the degree of functional trait sharing relative to random expectation

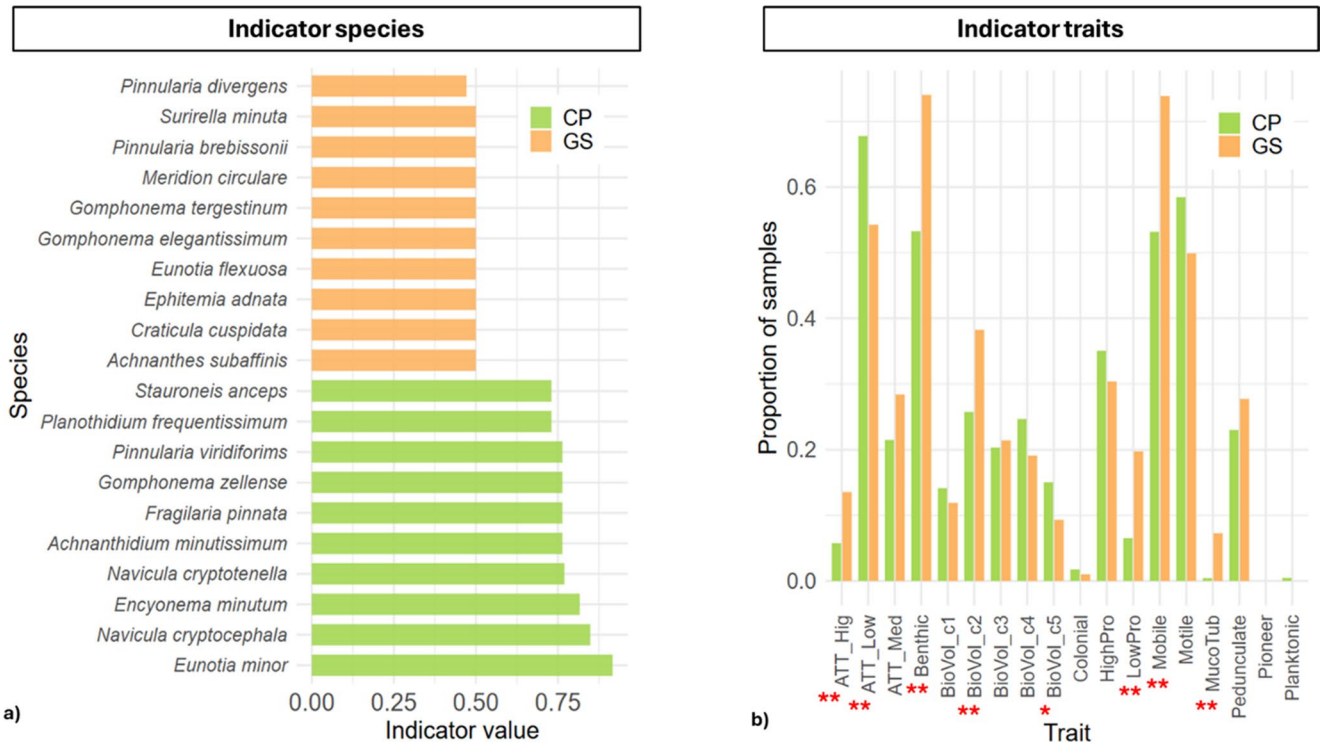

Fig. 6 (**a**) Indicator values (IndVal) of the top species for the two study sites (CP and GS); (**b**) Mean proportion of samples exhibiting each trait across the two study sites (CP and GS). *$p<0.05$, **$p<0.01$

connected nodes included traits related to low attachment (ATT_Low, degree = 48), benthic lifeform (Benthic, 42), mobility (Mobile, 42), motility (Motile, 41), and high-profile guild (HighPro, 20), suggesting that these traits are shared among many species. In GS, the network was slightly less modular (modularity = 0.29) and sparser (density = 0.056). Highly connected nodes included Mobile (77), Benthic (75), ATT_Low (75), Motile (65), and BioVol_c2 (38). Permutation tests based on 999 randomizations of the species–trait networks confirmed that the observed modularity in both CP and GS was significantly higher than expected by chance (CP: $p = 0.001$; GS: $p = 0.003$), demonstrating non-random clustering of species–trait associations in both regions. Overall, these results indicate that functional traits are structured in a non-random, modular manner, with certain traits acting as central connectors within the species–trait network, highlighting the presence of key functional hubs that potentially shape community assembly.

Discussions

The analysis presented here provides an important starting point for understanding and interpreting ecological differences in diatom communities inhabiting temporary ponds (TP). As shown in the literature for other organisms [68], elevation and the biogeographical settings of sites can strongly shape the composition of freshwater communities [69]. Although research on this topic remains limited, our findings highlight that environmental variation at the biogeographical scale could influence both the structure and functional organization of diatom communities. This underscores the ecological relevance of these fragile habitats which sustain unexpectedly diverse communities.

To better interpret our findings, it is important to consider the seasonal timing of sampling (February in CP vs. July in GS). During winter, productivity in Mediterranean ponds may be limited by lower temperatures, reduced solar radiation, and decreased hydrological stability, which could constrain diatom growth [31]. In contrast, Alpine ponds in summer are characterized by higher light availability and enhanced photosynthetic activity [10], stable conditions that coincide with seasonal peaks in diatom diversity [45], also favored by mineral inputs from snowmelt. Finally, the alpine sites are embedded in a high elevation context where species pools may be broader and ecologically distinct compared to Mediterranean regions [70]. Steeper environmental gradients in temperature, solar radiation, and photoperiod create a greater heterogeneity of available niches, potentially favoring the coexistence of multiple species [71].

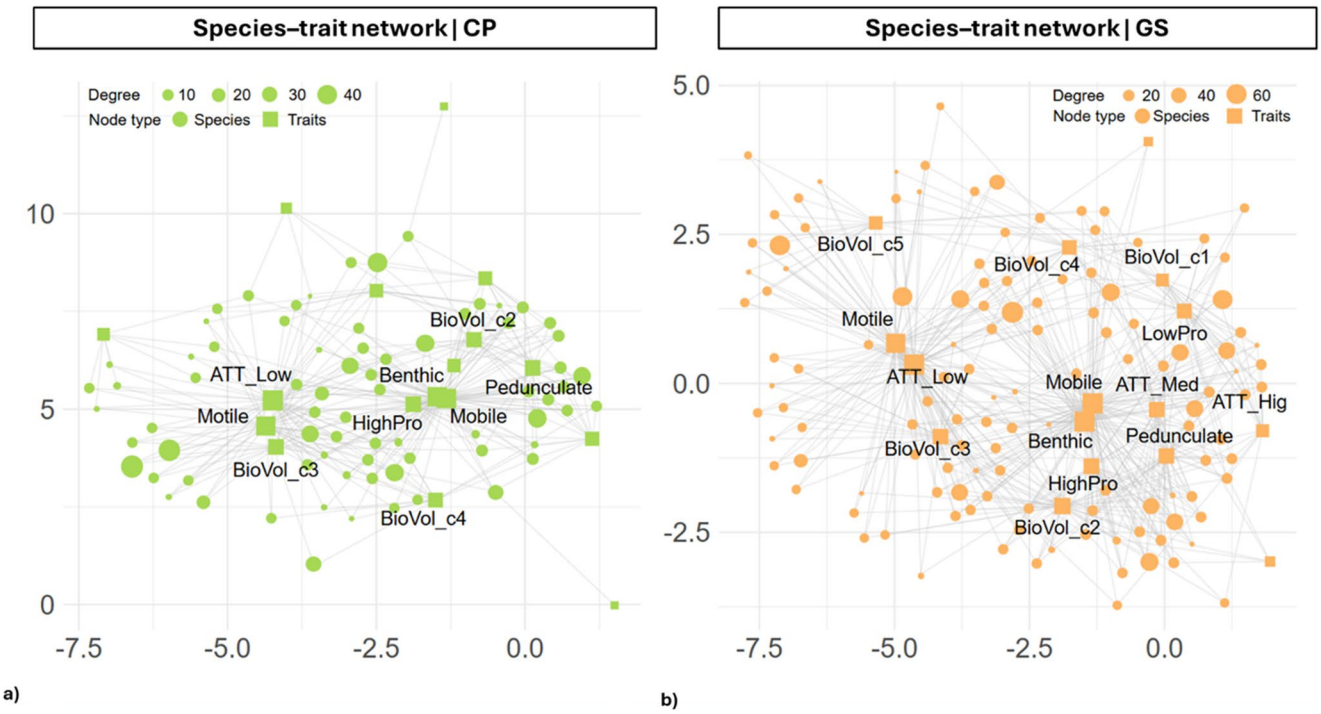

Fig. 7 Bipartite networks showing the relationships between species and functional traits for the two study sites. Nodes represent species (circles) and traits (squares), with node size proportional to the degree (number of connections). Edges indicate the presence of a trait in a given species. Only the most connected nodes are labeled for clarity. Networks are shown separately for (**a**) Castelporziano (green nodes) and (**b**) Campo Imperatore (orange nodes)

Taxonomic and Functional γ Diversity

The results obtained indicated clear differences in diatom γ diversity between the two sites, a pattern also observed in Mediterranean and Alpine regions for other biological groups [72]. Although TP from both study areas are defined under the same broad category of inland waters (*sensu* Habitat Directive, 92/43/EEC), they exhibited substantial differences in overall species richness. The current classification of TP, largely based on hydroperiod, overlook additional drivers such as climate, soil type, and geographic setting (both latitudinal and elevational), all of which can strongly influence freshwater bodies and their biotic communities [31, 73, 74]. In our case, samples from Castelporziano (CP) generally showed lower species richness compared to Campo Imperatore (GS). This result is unexpected: Mediterranean ponds, generally characterized by higher nutrient inputs [5], would be expected to support richer communities [75, 76]; Conversely, Alpine ponds are often subject to strong daily fluctuations in climatic, edaphic, and physicochemical conditions [10], together with reduced nutrient input, which typically make them less suitable for sustaining high biodiversity [77]. Furthermore, The Castelporziano estate, despite being fenced and inaccessible, receives substantial nutrient inputs, including anthropogenic ones, mainly from agricultural activities within the surrounding land [43]. This enrichment of soils and groundwater with nitrogen and phosphorus could elevate the trophic status of ponds, promoting higher diatom productivity and diversity [78]. By contrast, the mountain ponds analyzed are located in a markedly different context: although modestly affected by summer tourism [79], they remain distant from agricultural activities and direct anthropogenic pollution.

Furthermore, our results indicated that the diatom community in GS was not only more diverse on average per pond, but also exhibited faster species accumulation with increasing sampling effort compared to CP. This suggests that alpine ponds host less redundant communities, with higher variability between individual ponds, whereas Mediterranean ponds tend to host more similar assemblages. In February (CP), many species may be absent or occur at low abundance due to sub-optimal conditions, including limited light, lower temperatures, and unstable hydrology [80]. In July (GS), environmental conditions are more favorable and variable, allowing a wider range of species with differing ecological requirements to coexist, leading to greater spatial diversification [81]. The significantly higher AUC observed in GS indicates that the alpine metacommunity draws from a richer regional species pool. This pattern aligns with the biogeographic hypothesis, as mountain areas often support both specialized Alpine/Boreo-Alpine taxa and more generalist species, thereby increasing overall diversity [82–84].

These results were not confirmed by functional traits analysis, which revealed greater functional trait richness in the Mediterranean sites compared to the Alpine ones. Such decoupling between species richness and functional richness has been documented across taxa and ecosystems, generally occurring when additional species primarily occupy already-filled regions of trait space rather than expanding their boundaries [85]. Consequently, the higher species richness observed in GS does not necessarily translate into a broader functional spectrum.

In contrast, CP showed a less species-rich but more functionally diverse pool, potentially due to influences from multiple environmental contexts (coastal, brackish, and freshwater with strong gradients) [86]. High connectivity and dispersal, facilitated by the numerous animal species using TP as watering and breeding sites (birds, mammals, amphibians), could influence which species colonize the ponds and, consequently, the occupied functional space [43, 87]. If CP offers environmental conditions that support a wider range of strategies, such as more heterogeneous microhabitats, substrates, or chemical gradients, filtering may be less restrictive, allowing occupation of an expanded trait space. Meanwhile, GS may experience stronger environmental filtering, yet with many species that are functionally similar coexisting in overlapping niches. It is important to acknowledge that the observed differences in functional trait space between CP and GS may partly reflect our sampling strategy. Stones were collected exclusively along the shoreline, up to approximately 1 m from the water's edge, where diatoms are most abundant. Consequently, species associated with open-water habitats or more central zones of the ponds, which might include more planktonic or motile forms, could be underrepresented. This spatial restriction may lead to an overemphasis on traits common to shoreline communities, such as attachment structures or low-profile morphologies, and could influence measures of functional richness, dispersion, and overlap. Therefore, while the patterns observed provide valuable insights into community structure, they should be interpreted in the context of this sampling limitation, as inclusion of the inner zones could potentially increase the functional diversity captured, particularly in larger or more heterogeneous ponds.

However, seasonal composition of diatom taxa and the related expression of functional traits can vary substantially. Studies have shown that Functional Richness (FRic) and other facets of functional diversity fluctuate seasonally in response to temperature, nutrient availability, and hydrology [88, 89]. Therefore, some of the observed differences may reflect temporal effects: CP, sampled in February, could host species with more extreme traits than GS in July [90]. Although Functional Dispersion (FDis) and Functional Divergence (FDiv) are similar between sites, the most plausible explanation is that CP includes rare or occasional species occupying peripheral regions of the trait space (increasing total FRic), while the overall distribution and relative abundance of traits remain comparable across both regions.

Compositional and Functional Analysis

Our results showed that diatom communities differed significantly between sites (CP vs. GS), while internal variability within sites (i.e., dispersal among pools of the same site) was comparable. These findings indicate that observed differences were not due to greater heterogeneity within a site but rather reflect a distinct and consistent species compositions between the two environments. Furthermore, almost all of the observed β diversity arises from species turnover rather than nestedness. In other words, CP and GS hosted a distinct, site-specific community likely shaped by different environmental filters. This pattern underscores how temporary habitats in different biogeographic regions support unique diatom assemblages [91] Diatom diversity may be more influenced by local environmental factors than by broad-scale climatic variables such as elevation [39]. These differences may also reflect geographic separation rather than small-scale environmental variation [36, 92]. The absence of detailed physicochemical analyses in our study limits further interpretation in this context and highlights the need for more in-depth investigations.

Importantly, many studies on temporary or extreme habitats report that turnover is the dominant component of β diversity. Spatial patterns in freshwater microalgal communities are largely driven by turnover rather than nestedness, reflecting local environmental gradients rather than progressive extinction–colonization dynamics [93]. Furthermore, in diatom communities of temporary habitats diversity is primarily structured by environmental differences and ecological filtering rather than nestedness [94]. By contrast, in more stable systems such as permanent lakes or large rivers, nestedness often plays a larger role: stress gradients (e.g., nutrient availability, salinity) produce communities that represent predictable subsets of the regional species pool [95, 96]. The predominance of turnover in our study suggests local adaptation to divergent environmental conditions. In CP, communities are likely shaped by unstable hydrology, coastal organic inputs, and low seasonal temperatures [97]. In GS, communities are influenced by high-altitude conditions, intense radiation, and mineral inputs from snowmelt [98]. The comparable internal dispersal indicates that both systems exhibit similar local-scale variability: each pool differs from the others to a similar degree, yet the species pools themselves differ substantially between sites. The additional Raup–Crick β-diversity analysis indicates that community

assembly in our system is shaped by a combination of stochastic and deterministic processes. The mean βRC value (0.56 ± 0.35) suggests that stochastic mechanisms, such as ecological drift and random dispersal, likely play the predominant role in driving community turnover. However, the relatively high variability among pairwise comparisons shows that deterministic forces, including environmental filtering or niche differentiation, also contribute, particularly to a subset of pond pairs that deviate toward more extreme βRC values.

This pattern is consistent with a scenario in which species turnover dominates but arises from a balance between neutral processes and environmental constraints, rather than being driven primarily by deterministic filters. Internal βRC values indicate comparable levels of within-site variability in both systems, meaning that local-scale processes structure communities similarly at CP and GS. At the metacommunity level, the distinct species pools of Mediterranean and Alpine environments continue to contribute independently to regional diversity [99]. Therefore, conservation strategies should consider both systems, as each protects complementary components of taxonomic and functional diversity.

From a functional point of view, however, the investigated diatom communities showed some functional redundancy: at CP, there was a tendency towards functional clustering, while at GS, there was both clustering and a slight overdispersion. At the regional scale, therefore, no significant differences emerged between sites, suggesting that functional patterns were mainly driven by specific local conditions, rather than by systematic environmental filters at the regional level. The overlap index further highlighted that most of the functional trait space was shared between regions, indicating that both sites host species within a largely common "global functional space," with only minor differences attributable to traits unique to each location. Functional clustering (SES < 0) is often interpreted as a sign of environmental filtering [100]. In diatoms and phytoplankton, several studies have observed clustering in environments subject to strong physicochemical constraints, such as salinity, conductivity, water stress [58, 101], At local scale, diatom communities alternate clustering and overdispersion signals depending on environmental gradients, while at the regional scale they tend to converge on a common trait-space [102, 103].

In other freshwater systems, high functional overlap between regions has been interpreted as evidence of functional redundancy [104, 105], suggesting that similar ecological strategies are replicated across different environmental contexts. Functional redundancy in this case must be considered in light of the fact that, despite differences in environmental conditions, both systems consist of TP, where shallow water and highly variable ecological conditions select for similar functional traits. In other words, Alpine and Mediterranean TP draw from the same "catalogue of strategies," though implemented by different taxonomic compositions. Finally, the presence of clustering signals in specific sites indicates that some functions may depend on a few key species selected by the environmental filters è [106, 107]. This implies that these habitats could be particularly vulnerable to environmental changes, making them susceptible to the loss of specific traits and functional homogenization [108]. Consequently, monitoring and conservation efforts should aim to preserve key microhabitats within each site, safeguarding both species and the unique functional traits they support.

Indicator Species, Traits Analysis and Species-Trait Network

The results from the species- and trait-specific analyses align well with the characteristics of the studied sites. In GS, most species are typical of well-oxygenated, moderately productive, and stable benthic habitats, consistent with alpine conditions and with those reported in similar studies [45]. For instance, *Achnanthidium minutissimum* and *Planothidium frequentissimum* can be considered benthic generalists, tolerant but characteristic of fresh, oligotrophic waters [38, 109]; *Encyonema spp*. (*caespitosum*, *mesianum*, *minutum*) are epiphytic or mucilaginous species adapted to colonizing submerged surfaces and mobile substrates [110]; *Staurosirella pinnata* is a colonial diatom, often associated with oligotrophic conditions and fluctuating nutrient availability [111]; *Navicula spp*. (*cryptocephala*, *cryptotenella*, *trivialis*, *veneta*) are benthic species preferring fine substrates and perform well under moderate water stress [112, 113]; *Fallacia subhamulata* and *Cocconeis placentula* are typical of stable, well-lit substrates. Overall, these species describe GS as an environment dominated by small, specialized forms, highlighting the high ecological relevance and reliability of diatoms as bioindicators even in temporary Alpine habitats.

In CP, the analysis revealed greater heterogeneity, reflecting the more variable abiotic conditions of Mediterranean TP. Here, species were often associated with extreme or fluctuating environmental conditions and stress: *Eunotia bilunaris* and *E. minor* are typical of acidic or oligotrophic waters, indicating particular chemical stresses [114]; *Gomphonema zellense* is generally associated with substrates subject to environmental fluctuations and shows tolerance to variable conditions [115]; *Nitzschia filiformis*, a mobile species, is often linked to eutrophic or disturbed waters [116], while *Pinnularia viridiformis* and *Stauroneis anceps* are larger species, preferring oligotrophic environments and waters

with distinctive geochemical characteristics [109, 117]. Overall, the species composition of CP suggests a habitat more exposed to chemical and hydrological stress, colonized by tolerant taxa, with a predominance of large benthic or mobile forms.

The analysis of functional traits reflected a certain redundancy in functional traits, although with some important differences. For example, the higher frequency of low-profile forms (LowPro) represents an adaptive strategy to resist mechanical disturbance (e.g. turbulence, frost, partial desiccation) [58]; Taxa with mucilaginous tubes (MucoTub) favor anchoring in variable conditions and allow the colonization of stable microhabitats [118]; a higher frequency of small biovolumes (c2) is instead an adaptation to oligotrophic conditions, high efficiency in nutrient absorption [119, 120]. Overall, these traits describe resilient and plastic communities, capable of surviving in cold and oligotrophic environments, exploiting anchoring and miniaturization strategies. Conversely, species in CP showed a higher frequency (albeit marginal) of motile forms, which can represent an advantage in more variable and disturbed environments, where mobility allows for the exploration of different microhabitats and is typical of temporary habitats [25, 121, 122]. CP also showed a relatively higher occurrence of large biovolume species (c5), which, while less efficient under oligotrophic conditions, can confer competitive advantages in unstable or episodically nutrient-rich environments [116]. Overall, the CP community reflects a more opportunistic strategy, characterized by mobility and larger body size, enabling rapid responses to environmental fluctuations.

Network analysis confirmed that species–trait associations in our sites reflect a well-defined functional organization. In CP, species tend to cluster into distinct functional modules, but the connections between species and traits were relatively few, indicating a more "compartmentalized" structure. Such structuring suggests that environmental conditions select for specific niches, resulting in clearer and more separate functional subgroups. In contrast, the network in GS was less modular and sparse, producing a more fragmented community in which traits and species are distributed less homogeneously. This pattern indicates that species share traits more redundantly, potentially as a resilience strategy under extreme climatic conditions [123, 124]. Node analysis further revealed that, in CP, the functional structure revolves around mobile forms with weak attachment (low attachment) and a notable proportion of high-profile species, consistent with an opportunistic strategy that allows rapid responses to environmental variability [58, 125]. In Alpine sites, by contrast, traits are concentrated in small, mobile species, reflecting low nutrient availability and the need for active movement to exploit favorable microhabitats. Diatom communities of extreme environments are dominated by small, mobile forms adapted to nutrient-poor and physically extreme conditions [126]. Although the application of network analysis to diatom communities is relatively novel, studies in other systems support our findings: functional traits in communities are not randomly distributed but tend to form clusters or modules shaped by environmental conditions and ecological filters [105]. Furthermore, in ecological networks generally reflect adaptive functional strategies, highlighting the ecological significance of non-random trait organization [127].

Limitations of the Study

Although this study was conducted rigorously following established protocols for both field sampling and data analysis, several limitations should be acknowledged for scientific transparency. First, a relatively small number of TP were sampled in the Campo Imperatore region. However, all accessible and water-containing ponds were included, based on repeated field surveys conducted from May to October, which did not reveal additional suitable sites. Second, due to geographical and ecological constraints, sampling was carried out in different seasons for the two regions: winter for Mediterranean ponds and summer for Alpine ponds. Seasonal differences in water availability influenced accessibility; in summer, many ponds in Castelporziano were dry, whereas in winter, ponds in Campo Imperatore were often frozen or covered by snow, making field access logistically challenging. Finally, environmental and abiotic parameters such as physical and chemical variables, chlorophyll concentrations, nutrient levels, hydroperiod, and soil properties were not measured. These factors can substantially affect diatom biodiversity and functional organization. Due to financial constraints and limited resources, these analyses could not be included in the present study.

Conclusions

Temporary ponds, fragile and understudied ecosystems, actually host remarkable biodiversity and play a crucial role in maintaining ecological balance. In this study, we showed that TP in different biogeographical regions can host distinct communities, focusing specifically on diatoms. Our results revealed that γ diversity differed markedly between the two study sites, with alpine ponds exhibiting higher taxonomic and functional richness. These differences between

biogeographic regions were also reflected in the composition of diatom communities, indicating that each site supports unique assemblages. Despite these regional differences, within-site community dispersion did not differ significantly. Community-level analyses further indicated that, although functional trait distributions were largely similar across sites, local environmental constraints can generate site-specific clustering, underscoring the importance of fine-scale environmental heterogeneity in shaping functional community structure. Notably, a certain degree of trait space was shared between the two regions. Overall, diatom communities in the mountain sites were characterized by higher frequencies of small, mobile, low-profile, and mucilaginous-tube taxa, whereas coastal ponds displayed relatively higher representation of larger or motile forms, highlighting distinct functional compositions between mountain and coastal environments.

While our study represents an initial exploration and did not cover all environmental features, it provided an important base for future research on diatom communities in temporary habitats. In the context of ongoing and rapid climate change, understanding these patterns is essential, as community structures may shift or decline, potentially leading to irreversible losses in both taxonomic and functional diversity.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00248-025-02670-6.

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Author Contributions **Davide Taurozzi** : Conceptualization, Investigation, Visualization, Formal Analysis, Data curation, Project administration, Writing – original draft, Writing – review & editing. **Massimiliano Scalici: ** Conceptualization, Resources, Supervision, Validation, Funding acquisition, Project administration, Writing – review & editing.

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Data Availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

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DISCUSSION AND CONCLUSIONS

The integrated and multi-level approach adopted in this project provided a solid foundation for the monitoring and management of temporary ponds, especially those located at high altitudes, which are among the least studied and most threatened ecosystems under ongoing climate change. The results obtained from this Ph.D. research substantially enhance current knowledge on the distribution, management, and conservation status of temporary ponds in Italy. By employing diatoms as bioindicators, this project identified, for the first time on regional scale, the main abiotic drivers shaping the biodiversity of primary producers in these ephemeral environments.

The methodological framework developed throughout this work can serve as a replicable operational flow for future research on ephemeral ecosystems. In particular, it involves:

a. an initial bibliographic review to assess the global use of diatoms, understand the rationale behind its application, and how to adapt existing methodologies to the local context;
b. large-scale mapping and geographic identification of habitats, at least at the regional level, to obtain a comprehensive overview of temporary ponds distribution;
c. the targeted selection of representative sites for detailed field analyses, based on the insights gathered in the previous steps.

Until now, the actual distribution of temporary ponds across the Italian territory had remained largely unknown, hindering effective management and conservation planning. However, studying the current distribution of ephemeral water bodies is particularly challenging due to their small size, temporal variability, and the scarcity of long-term monitoring data (Borg Galea et al., 2019; Datry et al., 2016). Water availability is an increasingly critical issue (Rossi and Peres, 2023; Benedini and Rossi, 2021), and in the case of temporary ponds, the alternation between wet and dry phases represents a defining ecological feature. Nevertheless, prolonged periods of water absence can greatly complicate both monitoring and the accurate geographical mapping of these habitats, which represents an essential step for their effective conservation (Parra et al., 2021). The first outcomes of this project, namely the mapping and characterization of ponds through satellite imagery, therefore, provide a solid foundation for developing future management and conservation strategies for these fragile and understudied ecosystems (Riley et al., 2017). The unexpectedly high number of ponds detected underscores the urgency of implementing protection and monitoring plans for these ephemeral systems (Bagella, 2023). The total area covered by high-elevation ponds (approximately 425 ha within an Alpine region exceeding 20 million ha) confirms that these systems are spatially small habitats; however, they are far from marginal in ecological terms, as they host a disproportionately high and distinctive

biodiversity compared with the surrounding, largely homogeneous alpine landscapes. Their size, combined with a high degree of naturalness (83% of the mapped ponds are described as natural), highlights the persistence of relatively undisturbed environments, although indirect pressures such as climate change and atmospheric pollution continue to threaten their ecological balance (Celewicz and Gołdyn, 2021; Paredes et al., 2020). In this context, preserving the natural character of these environments is crucial to ensure the survival of species adapted to ephemeral waters and to prevent these little-known habitats from being altered by human activities, as has occurred in many other ecosystems, through the introduction of alien species, water abstraction, and urbanization (Hill et al., 2021).

Once temporary ponds are geographically identified, the next step for long-term monitoring involves selecting reliable and ubiquitous bioindicators capable of detecting both natural and human-induced changes over time (Beccarisi et al., 2024). In this context, this project demonstrated and reinforced the reliability of diatoms as biological indicators of water quality in temporary ponds. The initial bibliographic review on the global use of diatoms in temporary water bodies revealed a concerning knowledge gap: only about 15% of studies focused on ephemeral water habitat, and even fewer on high-altitude temporary ponds (>800 m a.s.l.). Although the use of diatoms in ephemeral ecosystems is still limited and not yet standardized, our findings suggest a slowly growing scientific interest in this direction. However, significant progress is still needed to fully implement diatom-based bioassessment in ephemeral systems (Novais and Morais, 2024; Masouras et al., 2021). One of the major limitations remains the taxonomic expertise required for accurate identification, which restricts widespread application (Zimmermann et al., 2015). Nonetheless, this research provides an important framework to approach the use of diatoms as indicators. It delivers an updated and comprehensive overview of the existing literature, revealing that most studies have been conducted in Mediterranean regions, while alpine and high-altitude ponds remain severely underrepresented. Our meta-analysis also highlighted consistent methodological trends: epilithic diatoms are the most frequently studied assemblages, and electrical conductivity, temperature, and pH are the most commonly measured environmental parameters.

However, the identification of pH and conductivity as key drivers of diatom diversity, particularly in high-elevation ponds, should be interpreted with caution, as these parameters are among the few variables consistently measured across studies. Both pH and conductivity can act as integrative proxies of underlying processes that are rarely quantified, notably nutrient availability in the water column. These nutrients can trigger cascading effects on diatom communities and biogeochemical processes, which in turn modulate pH and ionic composition, and thus conductivity (Giri et al., 2022).

The strong correlation often observed between pH and conductivity further supports their role as indirect indicators rather than primary drivers. This highlights a gap in current research and underscores the need for future studies to explicitly incorporate nutrient dynamics and sediment properties to better disentangle direct and indirect controls on diatom assemblages in alpine ponds.

Building on this knowledge, the field-based component of this project analyzed diatom communities from ponds across different geographical and physicochemical settings, applying the methodological insights gained from the global review. Our results confirm that benthic diatoms are reliable bioindicators for detecting both natural and anthropogenic disturbances in temporary ponds (Costa and Schneck, 2022). Clear compositional differences emerged between Mediterranean and alpine communities, reflecting variations in environmental conditions and geographical constraints. In particular, elevation and isolation were found to exert much stronger influences at high altitudes than in Mediterranean systems. This pattern, observed here for the first time, suggests that even modest elevational differences can drive substantial biological divergence in pond diatom communities, emphasizing the ecological sensitivity of these ecosystems.

The observed trends mirror broader ecological processes such as environmental filtering, dispersal limitation, and habitat heterogeneity, which collectively shape community assembly across multiple taxonomic groups. In the context of global change, high-altitude temporary ponds emerge as particularly vulnerable ecosystems (Lamouille-Hébert et al., 2024). Climate warming is expected to alter hydroperiods, snowmelt timing, and temperature regimes, with cascading effects on diatom composition and resilience (Sudlow et al., 2023; Rühland et al., 2015). Shortened hydroperiods may limit colonization opportunities, reduce habitat availability, and increase the frequency of desiccation events, leading to community simplification and biodiversity loss (Pujol-Buxó and Montori, 2025; Tournier et al., 2017). Given their rapid response to environmental fluctuations, diatoms can serve as early-warning indicators of climate-induced alterations in freshwater ecosystems (Rose et al., 2023). They can provide valuable insight into how aquatic communities respond to decreasing water availability, increased drought frequency, and hydroperiod unpredictability (Riato et al., 2017). Integrating diatom-based assessments into conservation and climate monitoring frameworks is therefore an essential step to anticipate biodiversity loss and ecosystem instability under future scenarios (Méndez-Zambrano et al., 2024; Cantonati et al., 2022).

Beyond their diagnostic value, our results highlight the potential of diatom assemblages and functional trait compositions as a foundation for the development of diatom-based assessment methods and biotic indices specifically tailored to ponds, including temporary and small water bodies that are currently underrepresented in monitoring frameworks. The strong sensitivity of diatom

communities to hydroperiod dynamics and habitat heterogeneity suggests that both taxonomic and trait-based metrics could be integrated into multimetric indices to assess ecological status and detect anthropogenic pressures, such as climate-driven hydrological alteration, nutrient enrichment, or land-use impacts (Stenger-Kovacs et al., 2020; Karaouzas et al., 2019). By identifying diatom indicators linked to environmental filtering and dispersal constraints, this study contributes empirical evidence for refining pond-specific bioassessment tools, thereby supporting more accurate evaluation of ecological quality and anthropogenic impacts in these vulnerable ecosystems.

Finally, this study also highlighted the positive influence of protection levels on community composition and the occurrence of rare or threatened species. Different levels of protection, such as nature reserves, national parks, or integral conservation areas, play a fundamental role in safeguarding natural biodiversity (Haq, 2016). As expected, ponds located within protected areas displayed higher ecological integrity and greater representation of sensitive taxa, reinforcing the importance of continued and enhanced conservation efforts.

In conclusion, this Ph.D. project provides one of the first integrated, multi-scalar analyses of temporary ponds in Italy, combining remote sensing, ecological assessment, and bioindicator-based monitoring. Beyond its scientific contributions, it offers a replicable methodological framework for studying and managing ephemeral aquatic ecosystems, supporting future strategies for their conservation in the face of accelerating environmental change. Although this project primarily focused on diatom communities, it is crucial to emphasize that these organisms, as primary producers, form the foundation of the entire trophic network. Therefore, protecting temporary ponds inherently means protecting diatoms and, by extension, all higher trophic levels that depend on them. Understanding the spatial distribution of these ponds and employing diatoms as biological indicators represents a concrete step toward safeguarding essential habitats, preserving rare and specialized species, and maintaining water availability and quality even in remote and sensitive regions. Protecting these systems now represents a key opportunity to conserve one of the last refuges of natural aquatic biodiversity within rapidly changing mountain landscapes.

Looking forward, several key knowledge gaps remain to be addressed. Future research should integrate diatom-based assessments with detailed measurements of nutrient dynamics, particularly within sediments, and microbial processes, in order to disentangle direct and indirect drivers of community structure and ecosystem functioning. Long-term (> 10 years) and seasonal monitoring is also needed to capture the pronounced temporal variability of temporary ponds under ongoing climate change. Moreover, combining high-resolution remote sensing with multi-trophic biological indicators would allow a more comprehensive evaluation of ecosystem resilience and thresholds of degradation.

Addressing these challenges will be essential for translating ecological knowledge into effective conservation and management actions for temporary ponds across mountain regions.

From a management perspective, the conservation of temporary ponds requires the definition of both ordinary and extraordinary actions, supported by clear organizational frameworks. Ordinary management should focus on the systematic inventory, protection, and periodic monitoring of ponds, ensuring the maintenance of natural hydroperiods and preventing gradual degradation caused by trampling, water abstraction, nutrient enrichment, or land-use changes in surrounding catchments. Extraordinary management actions may instead be necessary in response to acute disturbances, such as extreme droughts, climate-driven hydrological alterations, or physical damage, and should be guided by ecological thresholds identified through long-term monitoring. At an organizational level, effective conservation would benefit from coordinated governance across local administrations, protected areas, and research institutions, integrating scientific monitoring into existing management plans and conservation frameworks. The adoption of standardized protocols, scalable from local to regional levels, would facilitate comparability among sites and support adaptive management strategies. In this context, temporary ponds should be explicitly recognized not only as biodiversity hotspots but also as strategic natural infrastructures that contribute to water regulation, landscape heterogeneity, and ecosystem resilience in mountain regions.

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SUPPLEMENTARY MATERIALS

Chapter I - Diatoms as bioindicators for health assessments of ephemeral freshwater ecosystems: A comprehensive review.

Supplementary materials: https://doi.org/10.1016/j.ecolind.2024.112309.

Chapter II - Mapping Italian high-altitude ponds.

Supplementary materials: https://doi.org/10.1007/s00267-024-02061-6.

Chapter III - Silent survivors: diatom resilience in mediterranean temporary ponds.

Supplementary materials: https://doi.org/10.1016/j.csr.2025.105547.

Chapter IV - Environmental and elevational drivers of diatom diversity in Alpine temporary ponds.

Supplementary materials: https://doi.org/10.1016/j.ecohyd.2025.100726.

Chapter V - Similar, but not in functioning: investigating diatom traits in high-elevation ponds.

Supplementary materials: https://doi.org/10.6084/m9.figshare.31325743.

Chapter VI - Climbing the elevational gradient: Diatom diversity patterns across temporary ponds.

Supplementary materials: https://doi.org/10.1016/j.envres.2025.122630.

Chapter VII - Game of traits: diatom functionality in a changing landscape.

Supplementary materials: https://doi.org/10.1016/j.ecolind.2026.114696.

Chapter VIII - Regional γ diversity of diatoms in Mediterranean and Alpine temporary ponds.

Supplementary materials: https://doi.org/10.1007/s00248-025-02670-6.