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# Exploring habitat complexity in Mediterranean ponds: physicochemical heterogeneity and macrophyte community variation in inland Sicily

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## Abstract

Mediterranean ponds are small but valuable habitats, yet the relationships between environmental heterogeneity and vegetation remain poorly explored. We surveyed 10 sites in central Sicily, including permanent, semi-permanent and temporary ponds, recording 42 taxa and macrophyte communities in 31 vegetation units (*i.e.*, plots) and measuring water and sediment variables for each plot. Environmental clustering and PCA distinguished three vegetation typologies mainly defined by nutrients and sediment organic matter with significant differences in species turnover, whereas alpha diversity varied only marginally across clusters. The presence of differentiated plant communities sustains a relatively high baseline richness, while environmental heterogeneity is associated with macrophyte turnover rather than changes in cluster-level diversity.

**Key words:** macrophytes; biodiversity: small standing-water ecosystems; vegetation patterns; environmental gradients, pondscape.

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## Introduction

Ponds are small ecosystems of standing water characterized by a depth and surface area not exceeding 5 m and 5 ha, respectively, and with emergent vegetation cover of less than 30 percent (Richardson *et al.*, 2022). Temporary ponds are small, shallow seasonal water bodies characterized by a progressive exposure of bottoms as late-spring and summer rainfall diminishes (Zacharias and Zamparas, 2010). In the Mediterranean biogeographical region, they constitute a priority habitat designated by code 3170* under Directive 92/43/EEC and are extremely vulnerable, particularly given the fragmentation of the natural environment in areas such as inland Sicily (Salemi *et al*., 2025). Permanent and temporary ponds exhibit a high ecological and conservation value because, despite their limited surface area, they encompass complex hydrological, chemical and biological processes and act as local biodiversity hotspots (Céréghino *et al.,* 2014). Their relevance is particularly evident in Mediterranean landscapes, where wetlands are naturally fragmented by intense exploitation of natural resources and strongly constrained by seasonal water deficits, producing strong gradients in depth, conductivity, nutrient and habitat availability that influence aquatic communities (García-Girón *et al.,* 2019).

Habitat complexity is a multidimensional attribute encompassing physical structures (*e.g.*, macrophyte growth-form architecture, bathymetry) and fine-scale physicochemical gradients (Meerhoff and de los Ángeles González-Sagrario, 2022). This multifaceted complexity increases niche availability, functioning as the primary ecological template that shapes community composition and promotes high species turnover.

Permanent and temporary ponds are especially important in agricultural and semi-natural mosaics, where they can serve as stepping-stone habitats for aquatic plants, invertebrates, amphibians and birds, thereby contributing to pondscape connectivity and regional biodiversity (Biggs *et al.,* 2017). At the same time, they are highly vulnerable to pressures such as agricultural intensification, nutrient enrichment, habitat transformation, drainage, hydrological modification, alien species and reduced rainfall under climate change.

Studies on Mediterranean ponds have shown that community structure is shaped by both local environmental filters, such as pond morphometry, water chemistry and nutrient status, and broader spatial processes, including dispersal limitation and landscape isolation (García-Girón *et al.,* 2019). In macrophyte assemblages, for example, dispersal limitation may interact with species sorting, indicating that local pond conditions and regional connectivity should both be considered when interpreting biodiversity patterns. Recent evidence from Mediterranean farmland ponds

further emphasizes that individual ponds can make unique contributions to plant diversity, supporting the need for pond-scale conservation and monitoring (Cannucci *et al.,* 2025).

In Sicily, the largest Mediterranean island, the links between macrophytes and physicochemical features a fundamental facet of habitat complexity remains poorly understood, despite recent advancements in the study of local wetland plant diversity (Caldarella *et al.*, 2021; Panzeca *et al.*, 2021; Gianguzzi *et al.,* 2024).; Furthermore, little evidence is available regarding the contribution of intra-pond structural and physicochemical (*i.e.*, mesohabitat) variation to supporting macrophyte community diversification, despite its expected high relevance (Meerhoff and de los Ángeles González-Sagrario, 2022). To address these gaps, we conducted an exploratory field survey aimed at investigating macrophyte diversity in 10 ponds in central Sicily (Tab. S1; Fig. 1), including i) natural (normally found in small or large depressions); ii) artificial (constructed using mechanical equipment to support agricultural and livestock farming activities); or iii) semi-natural origin (artificial but of ancient origin and by now fully naturalized and integrated into the surrounding natural landscape). The main aim was to explore habitat complexity by examining the relationships between macrophyte vegetation and water and sediment features.

## Methods

The 10 ponds under study are located within three Special Areas of Conservation of the Natura 2000 network (ITA020007, ITA020008 and ITA020034), all of which fall within the Special Protection Area ITA020048. This is a vast inland area of Sicily where woodlands belonging to the *Querco-Fagetea* Br.-Bl. & Vlieger in Vlieger 1937 class, associated with the hilly and mountainous belts, alternate with agricultural areas, pastures, grasslands and garrigue.

The ponds were surveyed in early June 2018 (between the 1st and the 3rd). All the aquatic mesohabitats identified (31 in total; plots) were characterised using the phytosociological approach. Each community type was identified based on the dominant species distributed along a depth gradient, proceeding from the bank towards the deepest sectors of the water body. The vegetation gradient is illustrated in Tab. 1 and Tab. S2. Please refer to Caldarella *et al.* (2021) and Gianguzzi *et al.* (2024) for an in-depth phytosociological analysis of the plant communities studied.

In this study, we focused on plant communities dominated by hydrophytes and/or wetland plants capable of surviving for long periods while completely submerged. Vascular species identification, biological form, and nomenclature follow the "Portal to the Flora of Italy" (2026) and references therein. The vegetation surveys were supplemented by sampling of water and surface sediments at the plot scale.

Physicochemical parameters, including water temperature, pH, and electrical conductivity, were measured *in situ* using a multiparametric probe (YSI 556 MPS, YSI, Inc., Yellow Springs, OH, USA). Water samples were collected and immediately filtered on-site (0.7 µm glass fiber filters, GF/F Whatman). The filtered samples were stored at 4°C and analyzed within 72 hours for phosphate (SRP) and nitrate ($NO_3^-$) content. SRP was determined spectrophotometrically (Valderrama, 1977), while $NO_3^-$ was quantified using the sodium salicylate method (Rodier, 1978).

Sediment cores were collected at each sampling plot; the uppermost layer (top 5 cm) was homogenized and kept refrigerated until processing. Organic matter content was estimated as loss on ignition (LOI) after combustion at 450°C for 4 hours. Total phosphorus (TP) in the sediment was quantified spectrophotometrically following acid extraction of muffle-furnace combusted subsamples (450°C for 4 hours) according to Aspila *et al.* (1976).

Statistical analyses were performed using R software (v. 4.4.2; R Core Team, 2026) and the "vegan" package. Vegetation data were converted to percentage cover midpoints and Hellinger-transformed to account for the influence of rare species. The environmental dataset was standardized (z-score), and a Hierarchical Cluster Analysis (Euclidean distance, Ward's D2 method) was applied to identify distinct environmental typologies among the ponds. Average silhouette width (k=2-8) varied minimally, reflecting a continuous environmental gradient. Thus, k=3 was selected as a compromise, yielding ecologically interpretable groups with adequate sample sizes per cluster (n=31) for PERMANOVA and ANOVA tests.

A PCA was then used to visualize the main environmental gradients driving these groupings. To represent the environmental gradients on the ordination plot, environmental variables were explicitly fitted onto the PCA space using vector fitting (999 permutations). The direction and length of each vector indicate the direction of maximum change and the correlation strength with the ordination axes.

To test whether the floristic composition differed significantly between the identified clusters, a PERMANOVA was performed, followed by a "betadisper" test to assess the homogeneity of multivariate dispersions. Finally, differences in alpha diversity were evaluated by calculating the Simpson Diversity Index (1-D) for each cluster and comparing the means through a one-way ANOVA.

## Results

In the 31 different mesohabitats, each corresponding to a macrophyte community, a total floristic pool of 42 *taxa* was identified (Tab. 1 and Tab. S2). The vegetation was mainly represented by helophytic belts, hydrophytic communities and small floating or submerged stands. Recurrent dominant species included *Glyceria notata* Chevall. (n=6 plots), *Ranunculus rionii* Lagger (4), *Callitriche obtusangula* Le Gall (3), *Eleocharis palustris* (L.) Roem. & Schult. (2), *Sparganium erectum* L. (2), *Schoenoplectus lacustris* (L.) Palla (2), and *Ranunculus peltatus* Schrank (2).

The hierarchical cluster analysis based on water and sediment variables separated the plots into three main environmental typologies (Fig. 1F). This discrete partitioning was partially reflected in the PCA ordination, whose first two axes explained 60.9% of the total environmental variability (PC1=39.6%; PC2=21.3%). Vector fitting revealed that all environmental variables were significantly correlated with the ordination space ($p<0.002$).

PC1 mainly represented a trophic (SRP, $NO_3^-$) and sediment-enrichment (TP and LOI) gradient. Along this ordination space, Cluster 1 segregated clearly from the remaining groups, encompassing vegetation characterized by highly distinct physicochemical conditions that were partly related to $NO_3^-$ variation. In contrast, Clusters 2 and 3 exhibited a substantial spatial overlap. Cluster 2 included the largest number of plots and occupied a broader ordination space characterized by higher nutrient enrichment and greater organic accumulation, reflecting greater internal environmental heterogeneity and a wider range of physicochemi-

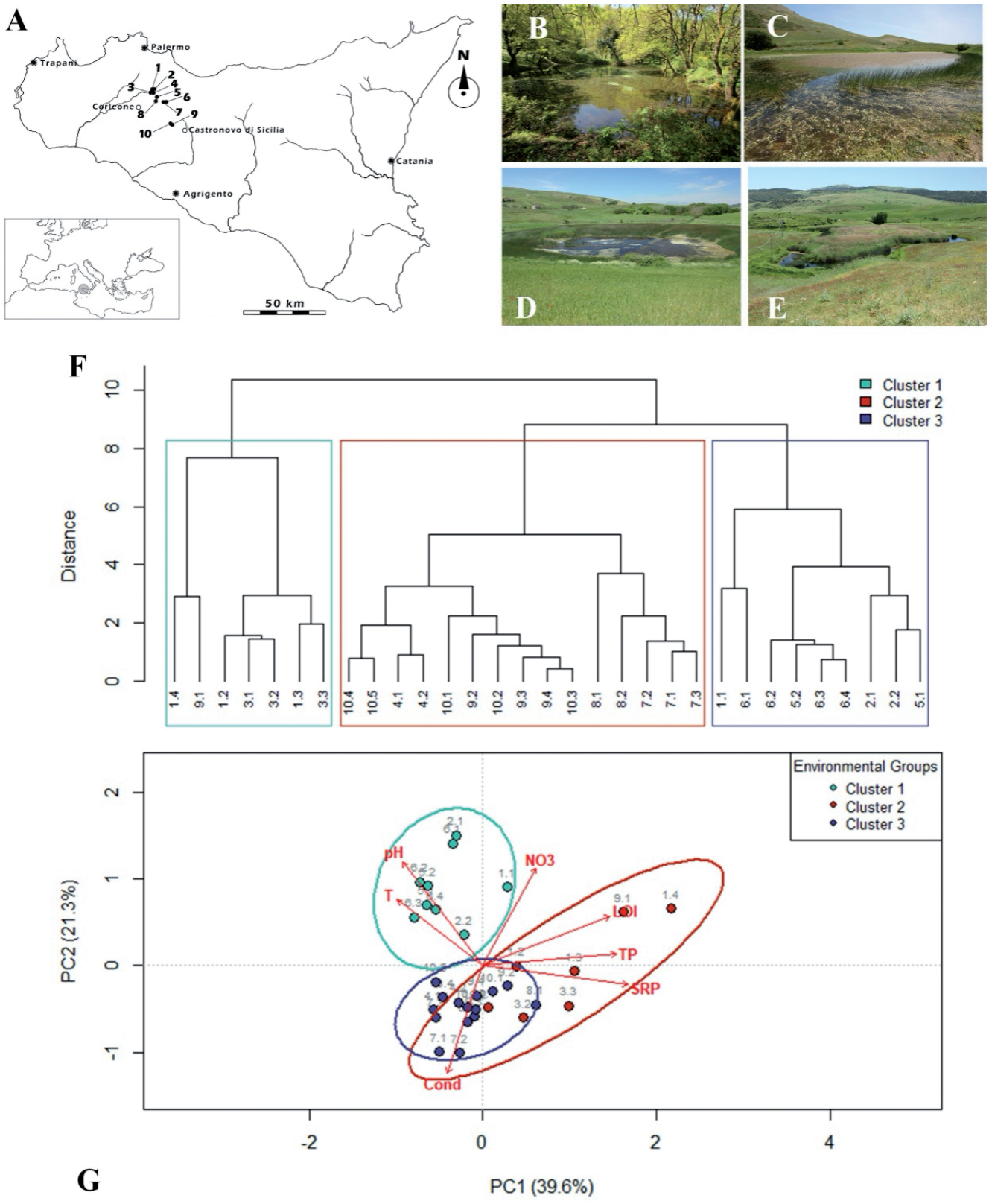


**Fig. 1.** A) Geographic distribution of sites: Gorgo Lungo (1), Gorgo del Drago (2), Gorgo Cerro (3), Laghetto Valle Maria (4), Laghetto Piano dei Prani (5), Laghetto Piano Guddemi (6), Pozza Piano Guddemi (7), Gorgo di Marosa (8), Gorgo Carcaci (9), Gorgo Carcaciotto (10). B) Gorgo Cerro. C) Laghetto Piano Guddemi. D) Gorgo Carcaci. E) Gorgo Carcaciotto. F) Hierarchical cluster analysis based on environmental data, G) PCA ordination.

cal settings. Conversely, the smaller Cluster 3 was environmentally nested within the broader variability of Cluster 2, yet it was distinctly differentiated by a strong relationship with water conductivity. Notably, several plots belonging to the same pond were assigned to different environmental clusters, demonstrating that significant environmental complexity occurs not only among different sites but also within individual pond systems.

PERMANOVA confirmed significant differences in floristic composition among the three environmental clusters (pseudo-F=2.18, $R^2$=0.13, $p$=0.003). Concurrently, the non-significant "betadisper" result (F=0.75, p=0.481) indicated homogeneous dispersion among clusters. This homogeneity of multivariate variance demonstrates that the detected compositional differences are robust and primarily reflect true species turnover among the environmental clusters, rather than artifacts of within-cluster heterogeneity.

Alpha diversity, measured using Simpson's diversity index, showed no significant differences among the environmental clusters (one-way ANOVA: F=1.74, $p$=0.193; Fig. S1). Overall, these results indicate that environmental gradients, particularly nutrient enrichment and sediment LOI, drive significant shifts in vegetation composition (species turnover). Conversely, plot-level alpha diversity remains unaffected by these environmental factors, showing no significant variation among clusters.

## Discussion

The dataset presented is of particular relevance in the Mediterranean context, where ponds are often small, isolated and subject to severe seasonal hydrological stress, exacerbated by human pressures and climate change, yet still serve as biodiversity hotspots within agricultural and semi-natural landscapes (Céréghino *et al.,* 2014; Cannucci *et al.*, 2025). Previous works on

**Tab. 1.** Dominant macrophyte species and environmental data for all the sampled plots.

| Site name | ID | Dominant species | Cl | T °C | pH | Cond µS cm$^{-1}$ | SRP µg L$^{-1}$ | $NO_3$- mg L$^{-1}$ | LOI % | TP µg g$^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| Gorgo Lungo | 1.1 | *Glyceria notata* | 3 | 24.0 | 6.3 | 83 | 35 | 40 | 16.2 | 494 |
| Gorgo Lungo | 1.2 | *Oenanthe aquatica* | 1 | 22.0 | 6.7 | 83 | 49 | 6 | 16.2 | 929 |
| Gorgo Lungo | 1.3 | *Callitriche obtusangula* | 1 | 18.6 | 6.3 | 100 | 88 | 13 | 23.7 | 763 |
| Gorgo Lungo | 1.4 | *Ceratophyllum submersum submersum* | 1 | 18.0 | 7.0 | 73 | 109 | 21 | 34.6 | 1851 |
| Gorgo del Drago | 2.1 | *Glyceria notata* | 3 | 28.4 | 9.3 | 159 | 26 | 19 | 24.9 | 398 |
| Gorgo del Drago | 2.2 | *Callitriche obtusangula* | 3 | 27.5 | 7.2 | 161 | 20 | 4 | 19.4 | 625 |
| Gorgo Cerro | 3.1 | *Glyceria notata* | 1 | 21.3 | 6.2 | 178 | 42 | 3 | 14.4 | 484 |
| Gorgo Cerro | 3.2 | *Callitriche obtusangula* | 1 | 19.5 | 6.4 | 168 | 78 | 2 | 13.7 | 655 |
| Gorgo Cerro | 3.3 | *Lemna gibba* | 1 | 20.3 | 6.5 | 148 | 111 | 2 | 16.8 | 1042 |
| Laghetto Valle Maria | 4.1 | *Ranunculus aquatilis* | 2 | 25.3 | 7.0 | 413 | 24 | 3 | 7.8 | 509 |
| Laghetto Valle Maria | 4.2 | *Glyceria notata* | 2 | 25.6 | 7.1 | 429 | 40 | 3 | 11.7 | 649 |
| Laghetto Piano dei Prani | 5.1 | *Glyceria notata* | 3 | 30.9 | 7.7 | 230 | 8 | 13 | 13.2 | 603 |
| Laghetto Piano dei Prani | 5.2 | *Ranunculus rionii* | 3 | 27.5 | 9.6 | 181 | 11 | 3 | 15.2 | 760 |
| Laghetto Piano Guddemi | 6.1 | *Potamogeton natans* | 3 | 22.0 | 9.4 | 174 | 16 | 36 | 13.8 | 405 |
| Laghetto Piano Guddemi | 6.2 | *Potamogeton trichoides* | 3 | 26.0 | 9.7 | 181 | 21 | 12 | 10.3 | 514 |
| Laghetto Piano Guddemi | 6.3 | *Eleocharis palustris* | 3 | 24.7 | 9.4 | 176 | 8 | 2 | 9.5 | 623 |
| Laghetto Piano Guddemi | 6.4 | *Ranunculus peltatus* | 3 | 24.2 | 9.4 | 171 | 18 | 3 | 13.6 | 651 |
| Pozza Piano Guddemi | 7.1 | *Typha angustifolia* | 2 | 19.8 | 7.2 | 629 | 30 | 8 | 6.9 | 311 |
| Pozza Piano Guddemi | 7.2 | *Ranunculus rionii* | 2 | 19.5 | 7.3 | 615 | 32 | 2 | 11.6 | 514 |
| Pozza Piano Guddemi | 7.3 | *Glyceria notata* | 2 | 22.8 | 7.4 | 620 | 25 | 12 | 8.9 | 380 |
| Gorgo di Marosa | 8.1 | *Oenanthe fistulosa* | 2 | 16.5 | 7.6 | 556 | 52 | 18 | 14.9 | 1241 |
| Gorgo di Marosa | 8.2 | *Ranunculus peltatus* | 2 | 18.0 | 7.3 | 520 | 8 | 6 | 18.2 | 468 |
| Gorgo Carcaci | 9.1 | *Sparganium erectum* | 1 | 23.4 | 7.1 | 486 | 77 | 29 | 34.8 | 1770 |
| Gorgo Carcaci | 9.2 | *Schoenoplectus lacustris* | 2 | 23.0 | 7.4 | 515 | 33 | 5 | 24.5 | 967 |
| Gorgo Carcaci | 9.3 | *Persicaria amphibia* | 2 | 22.8 | 7.2 | 551 | 23 | 3 | 16.4 | 843 |
| Gorgo Carcaci | 9.4 | *Ranunculus rionii* | 2 | 24.0 | 7.4 | 499 | 30 | 3 | 19.0 | 749 |
| Gorgo Carcaciotto | 10.1 | *Mentha aquatica aquatica* | 2 | 28.5 | 7.1 | 539 | 47 | 2 | 17.6 | 1190 |
| Gorgo Carcaciotto | 10.2 | *Eleocharis palustris* | 2 | 26.3 | 7.1 | 547 | 45 | 1 | 17.0 | 784 |
| Gorgo Carcaciotto | 10.3 | *Sparganium erectum* | 2 | 24.5 | 7.2 | 532 | 26 | 1 | 18.8 | 666 |
| Gorgo Carcaciotto | 10.4 | *Schoenoplectus lacustris* | 2 | 24.5 | 7.1 | 520 | 4 | 8 | 14.2 | 588 |
| Gorgo Carcaciotto | 10.5 | *Ranunculus rionii* | 2 | 27.2 | 7.2 | 529 | 7 | 9 | 14.2 | 588 |

Cl, cluster based on cluster analysis; T, temperature; Cond, standard electrical conductivity; SRP, soluble reactive phosphorous, $NO_3$-, nitrate; LOI, loss on ignition as measure of organic matter sediment content; TP, sediment total phosphorous.

Mediterranean ponds have emphasized that these systems act as biodiversity "islands" and that their biological patterns are strongly shaped by site-to-site environmental heterogeneity, especially in relation to water chemistry, morphometry and habitat structure (García-Girón *et al.,* 2019; Cannucci *et al.,* 2025). However, there is often limited understanding of the role of habitat complexity in regulating diversity and function. Here we focused on the physicochemical characteristics of ponds, yielding preliminary indications of their predictive value for variation in macrophyte communities and *taxa* turnover. For future studies, however, it will be essential to combine the physicochemical perspective with detailed information on morphology and hydroperiods, to improve the understanding of the dynamics of macrophyte diversity in ponds.

The variation in flora and vegetation observed, therefore, suggests that individual Mediterranean ponds can support a complex mosaic of distinct mesohabitats, even within considerably small areas (Meerhoff and de los Ángeles González-Sagrario, 2022). The hierarchical clustering and PCA separated pond vegetation into three environmental typologies mainly structured by trophic and sediment-related gradients. Stands characterized by *taxa* such as *S. erectum*, *S. lacustris*, *Persicaria amphibia* (L.) Delarbre, *Oenanthe fistulosa* L. and *Mentha aquatica* L. subsp. *aquatica* can be interpreted as vegetation types able to persist under higher nutrient availability and sediment accumulation. Conversely, plots with *C. obtusangula*, *Ceratophyllum submersum* L. subsp. *submersum*, *Ranunculus aquatilis* L., *R. peltatus*, *Potamogeton natans* L. and *Potamogeton trichoides* Cham. & Schltdl. indicate the presence of permanent water bodies, with highly variable trophic requirements.

Furthermore, PERMANOVA indicates that floristic composition differs among environmental clusters, suggesting a role of the environmental filtering in structuring pond vegetation. Indeed, the three clusters correspond to macro-types of vegetation and sites documenting the existence of an interdependence between all the spatial levels involved: the meso-habitat level, the whole environment and the pondscape. A possible explanation may relate to the ability of the most common *taxa* to deviate from their growth optima, allowing a high degree of spatial overlap among species, thereby partly masking the significance of intra-site environmental gradients and amplifying the site-specific effect. These patterns are confirmed by the non-significant "betadisper" result, which indicates multivariate homogeneity of dispersion among clusters. Furthermore, alpha diversity showed no significant variation among clusters. This aligns with García-Girón *et al.* (2019), who argued that traditional alpha-diversity metrics alone cannot fully capture biodiversity patterns in Mediterranean ponds, and emphasizes that complementary measures of compositional turnover (beta diversity) are essential to reveal the true structure and resilience of these aquatic ecosystems.

Panzeca *et al.* (2021) found that artificial ponds in north-western Sicily hosted relatively few hydrophytes but included species of conservation interest such as *Potamogeton pusillus* L. and concluded that even artificial and floristically poor ponds may contribute to aquatic plant conservation. On the other hand, our investigation (including natural or semi-natural ponds) shows a greater physicochemical complexity and species richness, further supporting the relevance of natural ponds as refugia for wetland flora. Indeed, the occurrence of locally threatened species such as *P. trichoides*, *P. natans*, *C. obtusangula*, and *C. submersum* subsp. *submersum* highlights the role of these ponds in maintaining species and habitats that are often fragmented or declining in Mediterranean landscapes. In other words, despite the gradual abandonment and deterioration of inland areas in Sicily, coupled with the increasingly severe local impacts of the climate crisis, ponds continue to offer a variety of (physicochemical) conditions suitable for sustaining viable populations of rare macrophytes as well as *taxa* with more demanding nutrient requirements.

Overall, the results suggest that pond conservation should prioritize the maintenance of high environmental heterogeneity across the pond network while limiting nutrient enrichment and excessive organic accumulation. Because different environmental clusters support different plant assemblages, conservation strategies should not focus only on the richest plots, but also on preserving the full range of mesohabitat conditions. In this sense, the studied ponds represent small but complementary habitat units whose collective value is greater than that of individual ponds considered separately.

## References


Aspila KI, Agemian H, Chau ASY, 1976. A semiautomated method for the determination of inorganic, organic and total phosphate in sediments. Analyst 101:187-197

Biggs J, von Fumetti S, Kelly-Quinn M, 2017. The importance of small waterbodies for biodiversity and ecosystem services: implications for policy makers. Hydrobiologia 793:3-39.

Caldarella O, Lastrucci L, Bolpagni R, Gianguzzi L, 2021. Contribution to the knowledge of Mediterranean wetland vegetation: Lemnetea and Potamogetonetea classes in Western Sicily. Plant Sociol 58:107-131.

Cannucci S, Fanfarillo E, Maccherini S, Bolpagni R, Bonari G, De Simone L, et al., 2025. Mediterranean farmland ponds as unique habitats for plant diversity across different pondscapes. Hydrobiologia. Ahead of Print.

Céréghino R, Boix D, Cauchie HM, Martens K, Oertli B, 2014. The ecological role of ponds in a changing world. Hydrobiologia 723:1-6.

García-Girón J, Wilkes M, Fernández-Aláez M, Fernández-Aláez C, 2019. Processes structuring macrophyte metacommunities in Mediterranean ponds: Combining novel methods to disentangle the role of dispersal limitation, species sorting and spatial scales. J Biogeogr 46:646-656.

Gianguzzi L, Caldarella O, Sciandrello S, 2024. The natural vegetation of residual wetlands in the hinterland of Western Sicily (Italy). Land 13:2009.

Meerhoff M, de los Ángeles González-Sagrario M, 2022. Habitat complexity in shallow lakes and ponds: importance, threats, and potential for restoration. Hydrobiologia 849:3737-3760.

Panzeca P, Troia A, Madonia P, 2021. Aquatic macrophytes occurrence in Mediterranean farm ponds: preliminary investigations in North-Western Sicily (Italy). Plants 10:1292.

Portal to the Flora of Italy, 2026. Website. Accessed: 28 May 2026. Available from: http://dryades.units.it/floritaly

R Core Team, 2026. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. Available from: https://www.R-project.org/

Richardson DC, Holgerson MA, Farragher MJ, Hoffman KK, King KB, Alfonso MB, et al., 2022. A functional definition to distinguish ponds from lakes and wetlands. Sci Rep 12:10472.

Rodier J, 1978. [L'analyse de l'eau].[Book in French]. Dunod, Paris; 1136 pp.

Salemi D, De Luca R, Ilardi V, Napolitano T, Troia A. 2025. Episodic ponds as overlooked temporary habitats: the case of Lago Montagna in Sicily. Diversity 17:843.

Valderrama JC, 1977. Methods used by the hydrographical department of the national board of fisheries, pp. 13-40. In: K. Grasshoff (ed.), Report of the Baltic Intercalibration Workshop, Göteborg.

Zacharias I, Zamparas M, 2010. Mediterranean temporary ponds. A disappearing ecosystem. Biodivers Conserv 19:3827-3834.

*Online supplementary material:*

*Tab. S1. Location and physical features of the freshwater habitats investigated.*
*Tab. S2. Vegetation gradients.*
*Fig. S1. Boxplot alpha-diversity.*

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